

ROMA TRE UNIVERSITY



DEPARTMENT OF SCIENCE

XXXVIII DOCTORAL PROGRAMME IN
BIOMEDICAL SCIENCES AND TECHNOLOGIES

**Transition to the viable but non-culturable state as an
adaptive strategy of bacterial pathogens to survive
desiccation in hospital settings**

PhD SUPERVISOR
Prof. Paolo Visca

PhD CANDIDATE
Cinzia Spagnoli

PhD CO-SUPERVISORS
Dr. Daniela Visaggio
Prof. Eric P. Skaar

Academic Year 2024/2025

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PhD Thesis

Author

Cinzia Spagnoli, Department of Science, Roma Tre University, Rome, Italy

 [0009-0006-6215-9481](https://orcid.org/0009-0006-6215-9481)

Supervisor

Prof. Paolo Visca, Department of Science, Roma Tre University, Rome, Italy

Co-supervisors

Dr. Daniela Visaggio, Department of Science, Roma Tre University, Rome, Italy

Prof. Eric P. Skaar, Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

Coordinator of the PhD program

Prof. Alessandra Di Masi, Department of Science, Roma Tre University, Rome, Italy

Thesis reviewers

Prof. Paola Salvatore, Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy.

Prof. Amalia Porta, Department of Pharmacy, University of Salerno, Salerno, Italy.

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Preface

This thesis reports the results of a three-year PhD project in Biomedical Sciences and Technologies carried out between January 2023 and December 2025. The project was supervised by Prof. Paolo Visca, with co-supervision by Dr. Daniela Visaggio (Roma Tre University) and Prof. Eric P. Skaar (Vanderbilt University, USA).

Most of the experimental work was carried out at the Department of Science, Roma Tre University, Rome, Italy. Between September 2024 and August 2025, research was conducted during a visiting PhD period at the laboratory of Prof. Eric P. Skaar at Vanderbilt University Medical Center in Nashville, Tennessee, US. This international experience enriched the project by providing access to additional expertise and research facilities and fostered a new scientific collaboration.

Summary

Microorganisms are continuously exposed to environmental fluctuations. Desiccation represents one of the most severe challenges in many natural environments and is particularly relevant in hospital settings. In hospitals, which are typically dry and characterized by controlled humidity, desiccation represents a major selective pressure to bacterial colonizers. Under dry conditions, certain bacterial pathogens can persist on abiotic surfaces for prolonged periods, with significant implications for healthcare-associated infections (HAIs). In this context, *Acinetobacter baumannii* represents a paradigmatic healthcare-associated pathogen, as it combines antimicrobial resistance with remarkable desiccation tolerance. Despite its clinical and epidemiological relevance, the molecular mechanisms underlying desiccation resistance and dormancy in *A. baumannii* have remained largely unexplored.

This PhD thesis aims to elucidate the physiological and molecular strategies that enable *A. baumannii* to withstand desiccation through entry into the Viable But Non-Culturable (VBNC) state and the reverse processes governing resuscitation. The first part of the experimental work (Publication I, Chapter 3) is focused on the dynamics of *A. baumannii* VBNC entry under conditions mimicking desiccation on hospital surfaces. Severe cellular damage, including membrane injury, ATP depletion, and oxidative stress, determines the loss of culturability within 24 hours. Remarkably, cells regained growth when resuspended in a carbon-free buffer, revealing a reversible transition from the VBNC to the culturable state. Transcriptomic profiling after prolonged desiccation uncovered extensive transcriptional remodelling, notably the activation of the glyoxylate shunt *via* upregulation of *aceA* gene, encoding isocitrate lyase. Deletion of *aceA* resulted in a pronounced reduction in both desiccation tolerance and resuscitation efficiency, providing direct evidence for a role of the glyoxylate shunt pathway in desiccation resistance and highlighting the relevance of metabolic rerouting for the entry into the VBNC state.

The subsequent section (Manuscript II, Chapter 4) investigated the molecular basis of VBNC cells resuscitation. Comparative transcriptomic analyses revealed a marked enrichment of genes involved in DNA repair, replication, and recombination. Among these, a primary role is played by the *uvrA* gene, encoding a key component of the UvrABC nucleotide excision repair system. Deletion of *uvrA* severely impaired survival under oxidative and genotoxic stresses and impaired the recovery of culturability after desiccation stress. Furthermore, in a murine pneumonia model, resuscitated WT bacteria achieved significantly higher pulmonary burdens than the $\Delta uv rA$ mutant strain, thereby confirming that DNA repair is indispensable not only for exit from the VBNC state, but also for the recovery of virulence.

Subsequent investigations were aimed at assessing whether the transition into a VBNC state represents a common survival strategy among major nosocomial pathogens exposed to desiccation stress (Publication III, Chapter 5). For this purpose, attention was focused on the ESKAPE group, namely *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., which are recognized for their prominent role in HAIs and antimicrobial resistance. The results revealed that VBNC induction under desiccation is not exclusive to *A. baumannii*. Although previous evidence indicated that several ESKAPE pathogens can enter the VBNC state when exposed to environmental stresses, their response to desiccation on the surface of materials commonly found in hospitals had not been studied yet. Evidence is provided that all ESKAPE species exhibit a marked reduction in cultivability following desiccation. Gram-negative species entered the VBNC state under conditions mimicking hospital settings and regained culturability after resuscitation, whereas Gram-positive species did not recover culturability under the test conditions. This finding unveils a critical blind spot in current surveillance practices, as conventional culture-based environmental monitoring fails to detect VBNC cells, systematically underestimating viable bacterial burden on hospital surfaces. Notably, a pilot environmental sampling experiment on various surfaces within the Department of Science at Roma Tre University demonstrated that incorporating a resuscitation step prior to culture markedly enhances the detection sensitivity, underscoring the urgent need to revise existing environmental monitoring protocols.

Finally, the thesis translates these insights into a practical solution (Patent IV, Chapter 6) by introducing MORECOVERY, a novel patented method for environmental contamination control that incorporates a resuscitation step before plating samples on solid media, thereby restoring the culturability of VBNC cells and enabling accurate quantification of viable microorganisms on abiotic surfaces.

In conclusion, this thesis provides a comprehensive understanding of the *A. baumannii* response to desiccation and clarifies the VBNC state as a key survival strategy in hospital environments. It identifies metabolic rerouting via the glyoxylate shunt as central to VBNC entry and DNA repair as essential for resuscitation and virulence restoration. It demonstrates that VBNC induction under desiccation is widespread among Gram-negative ESKAPE pathogens, revealing the limitations of current environmental monitoring approaches. Lastly, it proposes an innovative solution to enhance the detection of VBNC cells, with possible implications for infection control.

Riassunto (Italian summary)

I microrganismi sono continuamente esposti a fluttuazioni ambientali, e l'essiccamento rappresenta uno degli stress più severi in ambienti naturali, con rilevanza anche in ambito ospedaliero. Negli ospedali, tipicamente secchi e caratterizzati da umidità controllata, l'essiccamento è un'importante pressione selettiva. In condizioni di bassa umidità, alcuni patogeni possono persistere su superfici abiotiche per lunghi periodi, con importanti implicazioni per la trasmissione ambiente-uomo delle infezioni correlate all'assistenza sanitaria. In questo contesto, *A. baumannii* rappresenta un esempio paradigmatico di patogeno associato all'ambiente ospedaliero, combinando resistenza agli antimicrobici con una notevole tolleranza all'essiccamento. Nonostante la sua rilevanza clinica ed epidemiologica, i meccanismi molecolari alla base della resistenza all'essiccamento e della dormienza in *A. baumannii* sono rimasti in gran parte inesplorati.

Questa tesi di Dottorato si propone di colmare, almeno in parte, tali lacune studiando le strategie fisiologiche e molecolari che consentono ad *A. baumannii* di sopravvivere all'essiccamento attraverso l'ingresso nello stato Viable But Non-Culturable (VBNC) e i processi che governano il percorso inverso, ossia la rianimazione. La prima parte della tesi (Pubblicazione I, Capitolo 3) si concentra sull'analisi delle dinamiche di ingresso di *A. baumannii* nello stato VBNC in condizioni che mimano l'essiccamento su superfici potenzialmente contaminate in ospedale. A seguito dell'essiccamento, *A. baumannii* subisce danni cellulari severi, tra cui lesioni di membrana, deplezione di ATP e stress ossidativo, che determinano la perdita di coltivabilità entro 24 ore. Sorprendentemente, le cellule recuperano la capacità di crescere (formare colonie) su un terreno solido quando risospese in un tampone isotonic privo di fonti di carbonio, in una transizione reversibile dallo stato VBNC allo stato coltivabile. Il profilo trascrittomico dopo l'essiccamento prolungato ha evidenziato un ampio rimodellamento trascrizionale, in particolare l'attivazione del ciclo del glicolizzato tramite sovra-regolazione del gene *aceA*, che codifica l'enzima isocitrato liasi. La delezione di *aceA* ha determinato una marcata riduzione sia della tolleranza all'essiccamento sia dell'efficienza di rianimazione, fornendo prove dirette del suo ruolo nella resistenza all'essiccamento e sottolineando la rilevanza del rimodellamento metabolico durante l'ingresso nello stato VBNC.

Nella sezione successiva (Manoscritto II, Capitolo 4) sono state indagate le basi molecolari della rianimazione delle cellule essiccate nello stato VBNC. Analisi trascrittomiche comparative hanno rivelato un arricchimento significativo nell'espressione di geni coinvolti nella riparazione, replicazione e ricombinazione del DNA. Tra i geni sovraespressi è stata posta l'attenzione su *uvrA*, che codifica un componente chiave del sistema UvrABC di riparazione del DNA per escissione nucleotidica. La delezione del gene *uvrA* compromette gravemente la sopravvivenza sotto stress ossidativo e genotossico e non consente il recupero della coltivabilità dopo stress da essiccamento.

Inoltre, in un modello murino di polmonite, i batteri *wild-type* rianimati hanno raggiunto cariche polmonari significativamente più elevate rispetto al ceppo mutante $\Delta uvrA$, confermando che la riparazione del DNA è indispensabile non solo per l'uscita dallo stato VBNC ma anche per il ripristino della virulenza.

Successivamente, si è voluto valutare se la transizione nello stato VBNC rappresenti una strategia di sopravvivenza comune tra i principali patogeni nosocomiali esposti a stress da essiccamento (Pubblicazione III, Capitolo 5). A tal fine, l'attenzione si è concentrata sui batteri del gruppo ESKAPE, ovvero *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* ed *Enterobacter* spp., ben noti per la loro importanza nelle infezioni correlate all'assistenza sanitaria e nella resistenza agli antimicrobici. L'analisi ha rivelato che l'induzione dello stato VBNC sotto stress da essiccamento non è esclusiva di *A. baumannii*. Sebbene evidenze precedenti indicassero che diversi patogeni ESKAPE possono entrare nello stato VBNC a seguito di stress ambientali, il loro comportamento in risposta all'essiccamento su superfici ospedaliere non era mai stato caratterizzato. In questo studio, tutte le specie ESKAPE hanno mostrato una marcata riduzione della coltivabilità dopo essiccamento. Le specie Gram-negative sono transitate nello stato VBNC in condizioni che mimano gli ambienti ospedalieri e hanno recuperato la coltivabilità a seguito di rivitalizzazione in tampone isotonico, mentre le specie Gram-positive non erano in grado di recuperare la coltivabilità. Questo risultato evidenzia un cruciale punto cieco nelle pratiche di sorveglianza attualmente utilizzate negli ospedali, poiché il monitoraggio basato sulla coltura convenzionale non rileva batteri Gram-negativi nello stato VBNC, sottostimando sistematicamente una componente rilevante della carica batterica vitale e potenzialmente infettiva sulle superfici ospedaliere. In particolare, un esperimento pilota di campionamento ambientale su varie superfici all'interno del Dipartimento di Scienze dell'Università Roma Tre ha dimostrato che l'inclusione del passaggio di rianimazione aumenta la sensibilità di rilevamento, sottolineando la necessità urgente di rivedere i protocolli di monitoraggio esistenti.

Infine, il lavoro di tesi è stato tradotto in una nuova soluzione pratica applicabile alla sorveglianza ambientale in ospedale (Brevetto IV, Capitolo 6) proponendo un protocollo operativo denominato MORECOVERY. Si tratta di un metodo brevettato per il controllo della contaminazione ambientale che incorpora una fase di rianimazione prima della semina convenzionale su terreno solido, ripristinando la coltivabilità delle cellule VBNC Gram-negative e consentendo una quantificazione accurata dei batteri vitali su superfici abiotiche.

In conclusione, questa tesi rappresenta un significativo avanzamento delle conoscenze della risposta di *A. baumannii* all'essiccamento ed evidenzia la transizione nello stato VBNC come strategia chiave

di sopravvivenza in ambienti secchi, particolarmente in ambienti ospedalieri. Evidenzia il ruolo centrale del riadattamento metabolico, attraverso l'attivazione del ciclo del glicilato, per l'ingresso nello stato VBNC e quello della riparazione del DNA per la rianimazione e il ripristino della virulenza. Inoltre, la tesi dimostra che l'induzione dello stato VBNC a seguito di essiccamento è una strategia diffusa tra i patogeni Gram-negativi del gruppo ESKAPE, rivelando alcuni limiti delle procedure di monitoraggio ambientale attualmente in uso. Infine, l'attività di ricerca è stata tradotta in una soluzione pratica innovativa che migliora il rilevamento delle cellule VBNC indotte da essiccamento, con potenziali ricadute nel controllo delle infezioni ospedaliere.

List of abbreviations

ACB	<i>Acinetobacter calcoaceticus-baumannii</i> complex
ACN	Aconitase
AP	Antibiotic Persistence
AV-T	Avirulent Translucent
a _w	Water activity
CDC	US Centers for Disease Control and Prevention
CS	Citrate Synthase
ECDC	European Centre for Disease Prevention and Control
EPS	Extracellular Polymeric Substances
FUM	Fumarase
GS	Glyoxylate Shunt
HAIs	Hospital-acquired infections
HPII	Hydroperoxidase
ICL	Isocitrate Lyase
ICUs	Intensive Care Units
IDH	Isocitrate Dehydrogenase
LOS	Lipooligosaccharide
MDH	Malate Dehydrogenase
MDR	Multidrug-Resistant
MS	Malate Synthase
ODH	2-Oxoglutarate Dehydrogenase
OSF	Open Science Framework
OxyR	LysR-Type Transcriptional Regulator
PDR	Pan Drug-Resistant
PolyP	Polyphosphates
PPK	Polyphosphate Kinase
PPX	Exopolyphosphatase
ROS	Reactive Oxygen Species
SCS	Succinyl-CoA Synthetase
SDH	Succinate Dehydrogenase
SOD	Superoxide Dismutase
TA	Toxin-Antitoxin
TB	Transport Buffer
TCA	Tricarboxylic Acid

ToxR	Toxin Transcription Activator
UV	Ultraviolet
VAP	Ventilator-Associated Pneumonia
VBNC	Viable But Non-Culturable
VIR-O	Virulent Opaque
WHO	World Health Organization
XDR	Extremely drug-resistant

Chapter 1. Introduction

1.1. Xeric stress: the burden of water limitation in bacteria

Water constitutes the cornerstone of life. Being the most abundant molecule in almost all eukaryotes and prokaryotes, it typically accounts for nearly 80% of the total cellular mass, and it is essential for the existence of all living organisms (Ball, 2017; Lebre *et al.*, 2017; Esbelin *et al.*, 2018). Moreover, it provides protons, hydrogen, and oxygen for biochemical reactions, sustains the structural integrity of macromolecules, and enables molecular diffusion. By stabilising proteins, lipids, and nucleic acids, water maintains a cellular microenvironment in which vital metabolic and enzymatic reactions occur (Potts, 1994; Ball, 2017). The amount of available water is defined by the parameter of water activity (a_w), expressed as the ratio between the vapour pressure of a material or a solution (p) and the vapour pressure of pure water (p_0) at the same temperature: $a_w = p/p_0$ (Grant, 2004; Zeidler and Müller, 2019). Therefore, pure water has an a_w of 1, whereas all aqueous solutions display values lower than 1 (Grant, 2004). Except for spore-formers, most prokaryotes cannot survive at $a_w < 0.91$, and most fungi become metabolically inactive at $a_w < 0.7$ (Esbelin *et al.*, 2018). With decreasing a_w , microbial metabolism becomes progressively inhibited until conditions are no longer permissive for growth, and all microbial multiplication ceases below a_w 0.60 (Esbelin *et al.*, 2018). Despite these constraints, numerous microorganisms have adapted to survive in environments with low a_w . These organisms are generically termed xerophiles, from the Greek “dry lover”, as they can thrive under stringent xeric conditions (Lebre *et al.*, 2017). However, it is essential to note that obligatory xerophiles do not exist; therefore, the more accurate term is xerotolerance (Lebre *et al.*, 2017). Indeed, xerotolerant organisms can withstand the xeric stress, but they do not require or prefer low a_w conditions for growth.

Xeric stress arises either from water scarcity, referred to as matric stress, or from excessive solute concentrations in the surrounding cellular environment, defined as hypertonicity (Lebre *et al.*, 2017; Bosch *et al.*, 2021). The mechanistic distinction between these stresses is frequently blurred by their physical similarities, both implying loss of cellular water, causing terminological confusion and methodological inconsistencies. Specifically, during hyperosmotic stress, cells are suspended in an aqueous solution where water is physically present but largely unavailable for biological processes (Potts, 1994; Bosch *et al.*, 2021), whereas during matric stress (*i.e.*, desiccation), cells are exposed to air-drying and are not immersed in a solution. Nevertheless, several studies aimed to investigate the bacterial response to matric stress have been carried out by challenging cells with highly concentrated solutions in order to reduce water content (Potts, 1994; Bosch *et al.*, 2021). Despite the inaccessibility, the presence of water, even if it contains high solute concentrations, can still allow the movement of microorganisms and enable nutrient diffusion. Conversely, matric stress arises when the atmosphere constitutes the immediate environment of the cell, whereby reduced a_w results in intracellular water

loss (Potts, 1994). Hence, in truly desiccated (air-dried) cells, the loss of a substantial fraction of bulk water halts enzyme-mediated metabolism (Bosch *et al.*, 2021), impairing bacterial growth (Billi and Potts, 2002).

For years, the scientific community has assumed that the presence of water is synonymous with life, whereas its absence inevitably leads to death. However, exceptions to this paradigm are represented by anhydrobiotic organisms, which can persist in a nearly completely dehydrated state after losing almost all their internal water (Bosch *et al.*, 2021). The term *anhydrobiosis*, derived from the Greek roots *an-* (“not”), *hydro* (“water”), and *biosis* (“a way of living”), literally means “life without water”. Closely related to this phenomenon is desiccation tolerance, defined as the ability to undergo nearly absolute dehydration through air drying without being killed (Billi and Potts, 2002). Traditionally, anhydrobiosis has been defined as a desiccation-induced state in which organisms exhibit no visible signs of life and their metabolic activity becomes hardly measurable or comes reversibly to a standstill. Anhydrobiosis comprises three key phases: induction by desiccation, absence of observable metabolism, and reversibility (Figure 1) (Bosch *et al.*, 2021).

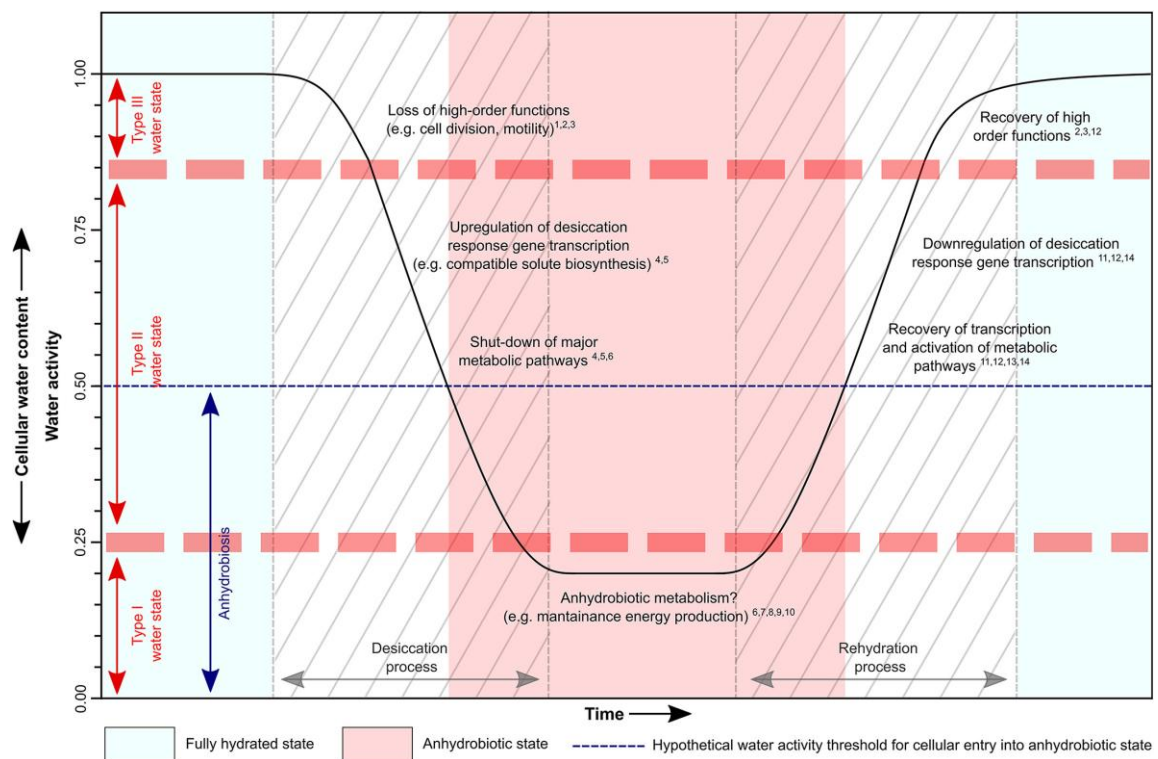


Figure 1 | Conceptual view of microbial cells undergoing a desiccation/hydration cycle. During desiccation, transcription of genes involved in stress responses (e.g., biosynthesis of stress protectants) is upregulated, while major metabolic pathways become inactive. With the further decrease in a_w , cells enter the anhydrobiotic phase, characterized by the maintenance of basal metabolism. The onset of rehydration is triggered by the increase in a_w , leading cells to exit the anhydrobiotic state. Transcriptional pathways silenced during desiccation are reactivated, accompanied by the downregulation of genes associated with the desiccation response (adapted from Bosch *et al.*, 2021)

More recent perspectives refine this definition, describing anhydrobiosis as a “steady state” between desiccation and rehydration, where cellular functions are restricted to basal metabolism (Bosch *et al.*, 2021). Therefore, since some enzymes and metabolic pathways retain activity during this steady state, anhydrobiosis implies that some enzymes are still active despite severe dehydration (Bosch *et al.*, 2021).

1.1.1. Cellular damages associated with desiccation

In hydrated conditions, bacterial cells are surrounded and permeated by water molecules, both extracellularly and intracellularly (Potts, 2004). During dehydration, cells undergo a dramatic loss of water, which disrupts essential molecular interactions. This multifactorial process can be divided into two distinct phases (Grefe and Michiels, 2020). Initially, the removal of extracellular unbound water leads to an increase in solute concentrations in the surrounding environment of bacterial cells, thereby generating an osmotic imbalance between the external environment and the intracellular compartment. These changes drive further water efflux across the plasma membrane, resulting in a progressive loss of turgor pressure and cellular shrinkage. As cytoplasmic water content declines, intracellular ions and metabolites become increasingly concentrated, leading to hyperosmotic stress. This condition intensifies macromolecular crowding, which elevates the density of cellular components and reduces the mobility of macromolecules, thereby impairing essential biochemical interactions (Lebre *et al.*, 2017; Grefe and Michiels, 2020). During the second stage of drying, macromolecules partially lose their hydration shell (França *et al.*, 2007). The extensive loss of water and the increment in ion and metabolite concentrations alter cellular metabolism and biochemical reactions. In aerobic bacteria, water depletion alters ionic concentrations, which in turn leads to metabolic impairments that compromise the activity of enzymes in the electron transport chain. This dysfunction results in oxidative stress, which leads to the accumulation of reactive oxygen species (ROS) consisting of free radicals [*e.g.*, superoxide anion ($^{\circ}\text{O}_2^-$), hydroxyl radical ($^{\circ}\text{OH}$), hydroperoxyl radical ($^{\circ}\text{OOH}$)] and non-radical forms [*e.g.*, hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$)] (França *et al.*, 2007; Lebre *et al.*, 2017; Grefe and Michiels, 2020; Giovannini *et al.*, 2022). It is noteworthy that ROS are considered as major drivers of the harmful alterations by which dehydration induces cellular damage (França *et al.*, 2007). Their impact contributes to the complexity of the desiccation process, which can simultaneously target multiple cellular structures, including membranes (lipids), proteins, and DNA (Figure 2).

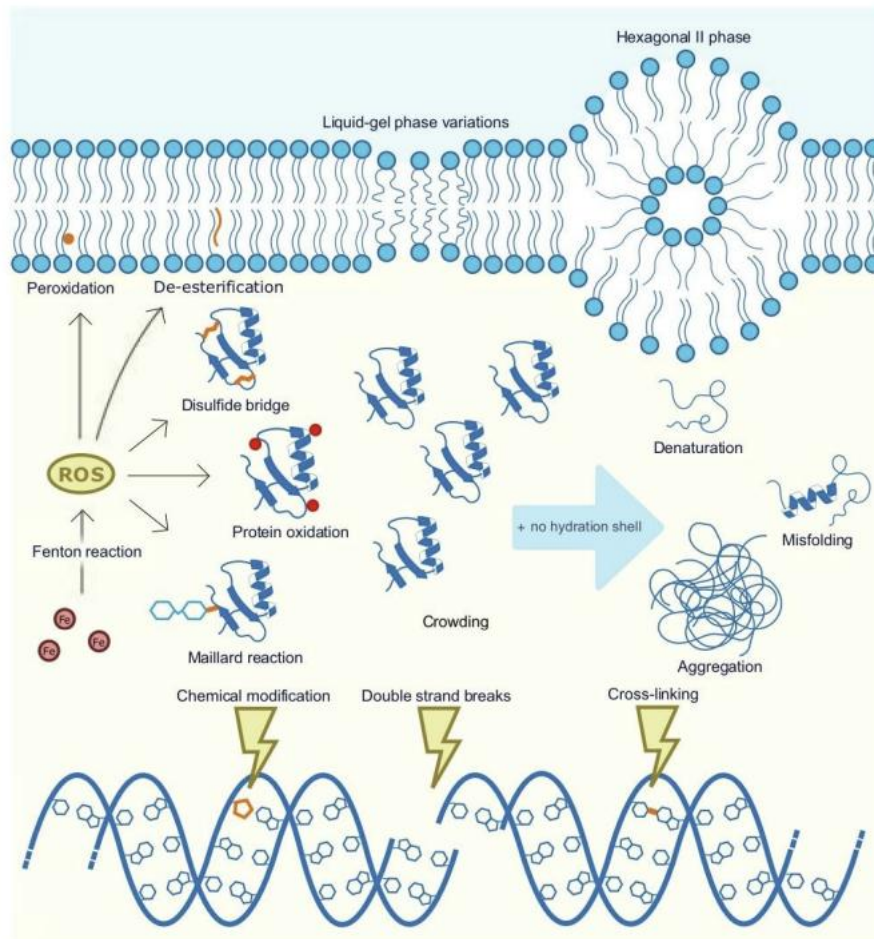


Figure 2 | Cellular damage affecting bacterial cells upon desiccation. During desiccation, bacterial membranes experience a transition from a liquid crystal to a gel phase. The transition can cause membrane breakages and leakage of cytoplasmic content. Moreover, dehydration triggers the production and accumulation of ROS, which can damage the membrane through lipid peroxidation and de-esterification. ROS can also induce the formation of disulfide bridges, resulting in the oxidation of proteins. The absence of a hydration shell can damage proteins, which can be misfolded and denatured, contributing to the generation of protein aggregates. DNA damage induced by desiccation occurs through covalent modifications, double-strand breaks, and cross-linking. Other details on molecular damage are described below (adapted from Greffe and Michiels, 2020).

Membrane damage

The cellular membrane constitutes the fundamental boundary delineating the intracellular compartment from the external milieu, and its integrity is profoundly influenced by alterations in external water availability (Lebre *et al.*, 2017). According to Singer and Nicolson's fluid-mosaic model, biological membranes consist of a phospholipid bilayer interspersed with transmembrane proteins (Singer and Nicolson, 1972). Within this bilayer, the phospholipid head groups are spaced apart by water molecules that establish hydrogen bonds, thereby maintaining the membrane in a fluid crystalline state (Rockinger *et al.*, 2021). During the initial stages of desiccation, water removal leads to a decrease in cell volume, while the surface area of the plasma membrane remains unchanged. This imbalance forces the plasma membrane to deform due to cellular shrinkage, while the absence of

hydration shells around phospholipid heads promotes their tighter packing (Grefe and Michiels, 2020). The resulting increase in packing density strengthens van der Waals interactions between adjacent hydrocarbon chains, elevates the melting temperature, and drives the transition of membranes from the liquid-crystalline to the gel phase (Billi and Potts, 2002; Grefe and Michiels, 2020; Fardelli *et al.*, 2022). This phase transition compromises membrane fluidity and generates discontinuities that promote fusion, disruption, and leakage upon rehydration (Lebre *et al.*, 2017; Grefe and Michiels, 2020). Under severe dehydration, additional lipid polymorphisms may arise, such as the hexagonal II phase, in which lipids reorganize with polar head groups oriented inward and hydrocarbon chains outward, destabilizing the lamellar architecture (Lebre *et al.*, 2017; Grefe and Michiels, 2020). When membrane integrity is endangered, the functioning of the respiratory chain is disrupted, and the accumulation of superoxide ions is triggered. The resulting ROS production sets off a series of biochemical reactions that amplify their harmful effects, including processes like lipid peroxidation and de-esterification, further compromising membrane integrity (França *et al.* 2007; Wang *et al.*, 2017; Grefe and Michiels 2020).

Protein damage

Protein activity is strictly dependent on the preservation of its native structure (Lebre *et al.*, 2017). During desiccation, the absence of water destabilizes proteins by eliminating their hydration shells, thereby promoting abnormal interactions with surrounding molecules. Moreover, the exposure of hydrophobic regions that are normally buried within the protein core results in protein denaturation and aggregation (França *et al.*, 2007; Grefe and Michiels, 2020). Conformational changes due to dehydration disrupt the electron transport chain and impair antioxidant enzymes, intensifying ROS accumulation (Franca *et al.*, 2007; Grefe and Michiels, 2020). The dysfunction of electron transport proteins in the presence of Fe^{2+} ions promotes the Fenton reaction, leading to the production of highly reactive hydroxyl and hydroperoxyl radicals (Grzyb and Skłodowska, 2022). Furthermore, the functional groups present in amino acids facilitate several possible harmful chemical reactions, like oxidation. In this scenario, cysteine-containing molecules are rapidly oxidized, as the thiol (-SH) group of the L-cysteine side chain possesses strong nucleophilic properties that render it highly reactive toward oxidizing agents (Ezraty *et al.*, 2017). ROS interact with protein thiols (R-SH), generating cysteine sulfenic acids (R-SOH), an unstable intermediate that can be further oxidized to cysteine sulfinic (R-SO₂H) and sulfonic acids (R-SO₃H) (Ezraty *et al.*, 2017). Such oxidative modifications irreversibly alter cysteine-containing proteins, ultimately leading to their functional inactivation. In parallel, oxidative stress promotes the formation of carbonyl groups and reactive aldehydes, which endanger proteins through Maillard reactions (Lebre *et al.* 2017), where reducing sugars covalently bind free amino groups, causing irreversible polymerization (Billi and Potts 2002;

Potts *et al.* 2005). Collectively, these processes (*i.e.*, denaturation, aggregation, oxidative modification, and polymerization) severely compromise protein integrity, leading to metabolic dysfunction and causing cell death.

Nucleic acid damage

The stability of genetic information within DNA molecules is essential for cell survival and multiplication. During dehydration, genome integrity becomes highly vulnerable, as its chemical stability relies on complete hydration. Consequently, such genomic instability may compromise replication, transcription, and protein synthesis (Billi and Potts 2002; Grzyb and Skłodowska, 2022). In particular, desiccation promotes harmful lesions such as cross-linking, double-strand breaks, base removal, and chemical modifications, including alkylation and oxidation (Billi and Potts, 2002; Lebre *et al.*, 2017; Greffe and Michiels, 2020). Moreover, the desiccation-induced oxidative stress further exacerbates DNA vulnerability. Although a comprehensive overview of oxidative stress-related DNA damage is still lacking, desiccation promotes the accumulation of ROS, which significantly impairs DNA integrity. ROS generated through metal-catalysed Haber-Weiss and Fenton reactions induce complex lesions that block replication (Figure 3) (Potts *et al.* 2005; Greffe and Michiels 2020).

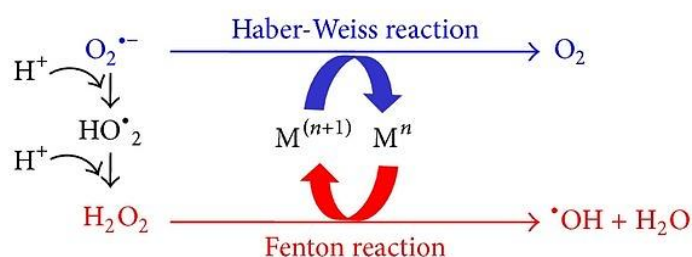


Figure 3 | Fenton and Haber-Weiss reaction. Reduced form of transition-metals (M^n) reacts through the Fenton reaction with hydrogen peroxide (H_2O_2), leading to the generation of hydroxyl radical ($\bullet OH$). Superoxide anion ($O_2^{\bullet-}$) can also react with the oxidized form of transition metals ($M^{(n+1)}$) in the Haber-Weiss reaction, leading to the production of M^n , which then again affects redox cycling (adapted from Ayala *et al.*, 2014).

In parallel, oxidative stress fosters Maillard reactions, leading to the production of melanoidins through the condensation of saccharide carbonyl groups with primary amines of proteins and nucleic acids. This process destabilizes DNA and impairs DNA and RNA polymerase activity, with damage progressively accumulating during desiccation and ultimately compromising genetic information (Potts *et al.* 2005; Lebre *et al.* 2017; Greffe and Michiels, 2020).

1.1.2. Molecular mechanisms of xerotolerance

The responses of xerotolerant bacteria to xeric stress implies a global reprogramming of transcriptional patterns. Omics approaches have provided valuable insights into both the spatial and temporal dynamics of microbial adaptation to desiccation stress (Lebre *et al.*, 2017). To counteract the negative morphological, physiological, and biochemical consequences of water loss, xerotolerant microorganisms have evolved a broad repertoire of adaptive strategies, summarized in Figure 4. Although the responses to hyperosmolarity and desiccation are distinct, they share common features, reflecting overlapping mechanisms that stabilize membranes and proteins (Potts, 1994).

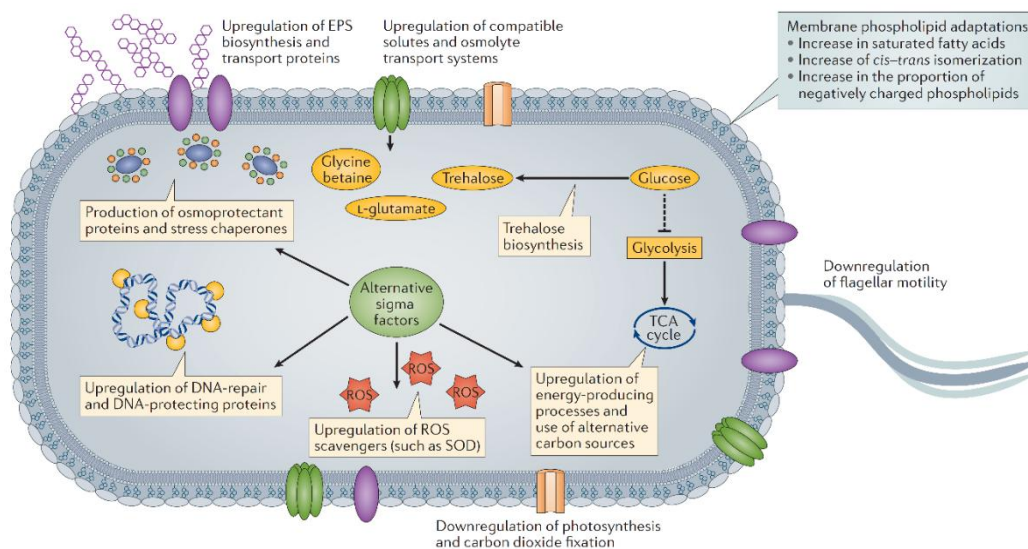


Figure 4 | Illustration of adaptive mechanisms of xerotolerant bacteria. Bacterial cells resist the xeric stress by preventing water loss and protein damage and by protecting DNA through the accumulation and expression of osmoprotectants. Moreover, alternative carbon sources are used to produce ATP, and energy-consuming processes, such as flagellar motility, are repressed. Reactive oxygen species scavengers, such as the superoxide dismutase (SOD) and catalase enzymes, are upregulated. Furthermore, bacteria accumulate solutes and modify membrane phospholipids (adapted from Lebre *et al.*, 2017).

Most adaptive strategies to low a_w are triggered by regulatory cascades which reprogram the expression of specific genes involved in stress responses. Although the detailed molecular switches remain unclear, alternative sigma factors, involved in regulation of the general stress response, are considered crucial for coordinating cellular adaptation to xeric stress (Lebre *et al.*, 2017). Central to this response in *Escherichia coli* is the activation of the alternative sigma factor RpoS (σ^S), which coordinates the expression of genes involved in DNA stabilization and oxidative stress response (Lebre *et al.*, 2017; Zeidler and Müller, 2019). Moreover, in *Salmonella enterica* serovar Typhimurium, an upregulation of the alternative sigma factor RpoE (σ^E) has been observed, which promotes the production of an external protective matrix composed of extracellular polymeric

substances (EPS) (Lebre *et al.*, 2017; Costa *et al.*, 2018). EPS constitutes the major component of biofilms, including polysaccharides, proteins, extracellular DNA, and lipids (Costa *et al.*, 2018; Di Martino, 2018). Due to their jelly nature and richness in water, biofilms represent the outer line of defence against xeric stress, as their physical and physiological characteristics confer protection. Their hygroscopic nature enables efficient absorption and retention of water, thereby enhancing the likelihood of prolonged survival under desiccation (Lebre *et al.*, 2017; Esbelin *et al.*, 2018). (Esbelin *et al.*, 2018).

In response to xeric stress, the cell membrane plays a crucial protective role by acting as a physical, electrical, and chemical barrier, serving as a shield that separates the cytoplasm from the external environment. Cell membranes are constantly exposed to environmental fluctuations, and reduced water availability represents one of the most critical stress factors (Lebre *et al.*, 2017). Under xeric conditions, dehydration disrupts phospholipid organization, compromising membrane integrity and fluidity (Greffé and Michiels, 2020). This process induces a phase transition that destabilizes membrane architecture (Ramos *et al.*, 2001). To counteract membrane collapse induced by cellular water leakage, bacteria, such as *Oceanomonas baumannii*, implement reversible modifications such as increasing the ratio of saturated to unsaturated fatty acids, shifting from *trans* to *cis* conformations, and synthesizing negatively charged phospholipids like cardiolipin. These adjustments help maintain membranes in the gel phase and preserve their structural stability under low a_w (Brown *et al.*, 2000; Ramos *et al.*, 2001).

To withstand dehydration, xerotolerant microorganisms increase the concentration of osmotically active substances in their cytoplasm to restore osmotic equilibrium and sustain protein function (Greffé and Michiels, 2020; Lebre *et al.*, 2017; Zeidler and Müller, 2019). This adaptive response typically follows a bi-phasic “salting-out” process. During the initial phase, cells rapidly accumulate charged solutes such as potassium and glutamate to compensate the osmotic stress. Subsequently, these are replaced by neutral organic compatible solutes either acquired from the environment or *de novo* biosynthesized that maintain long-term osmotic balance and stabilize proteins by preventing their unfolding, aggregation, and denaturation (Potts, 1994; Lebre *et al.*, 2017; Zeidler and Müller, 2019). Compatible solutes are osmotically active compounds that do not interfere with biological processes at high concentrations and so are compatible with cellular life. The classes of compatible solutes used by bacteria are limited (Figure 5). Among them, there are polyols (*e.g.*, glycerol), sugars and their derivatives (*e.g.*, trehalose, mannitol, and sulphotrehalose), amino acids and their derivatives (*e.g.*, proline and ectoine), and trimethylammonium derivatives such as glycine betaine (Grant, 2004; Zeidler and Müller, 2019). While they are compatible with all important cellular functions and do not interfere with metabolism, their hygroscopic properties inhibit membrane disruption and reduce intracellular fluidity during water loss (Grant, 2004; Greffé and Michiels, 2020; Lebre *et al.*, 2017).

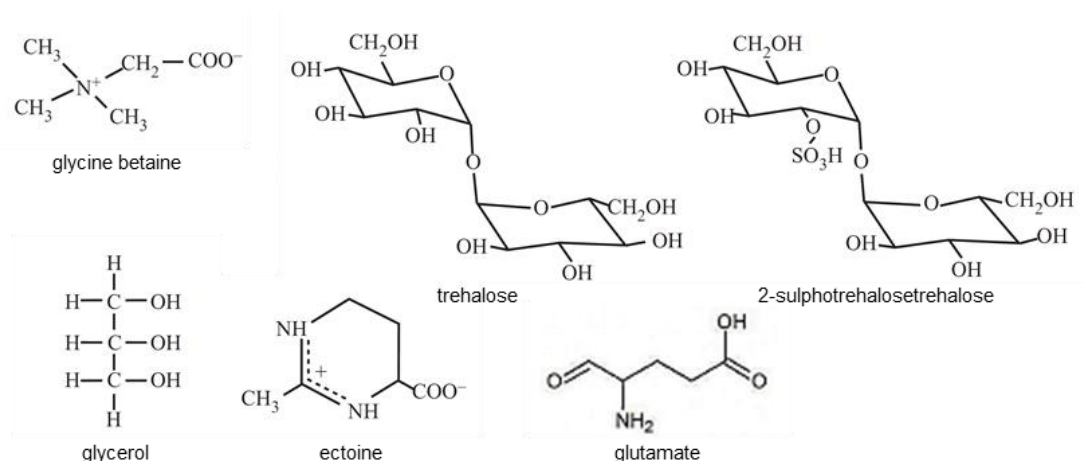


Figure 5 | Structure of compatible solutes. Compatible solutes are polar, normally uncharged or zwitterionic compounds accumulated by cells to avoid massive water loss under low a_w conditions (adapted from Grant *et al.*, 2004).

Among compatible solutes, trehalose has long attracted attention as it can restore osmotic balance and stabilize organic molecules through water replacement. When desiccation-tolerant cells experience the lack of a significant portion of internal water content, they can go through the so-called vitrification process (Grefe and Michiels, 2020). It consists of the synthesis and the substantial intracellular accumulation of the disaccharide trehalose. According to the water replacement hypothesis, trehalose substitutes water molecules in hydrogen-bond networks around membrane phospholipids and proteins, thereby halting chemical reactions and preserving membrane integrity (Lebre *et al.*, 2017; Grefe and Michiels, 2020). In addition to stabilizing macromolecules, trehalose may contribute to residual water retention and serve as an energy source during stress recovery (Bosch *et al.*, 2021). Importantly, the glassy state generated by trehalose is reversible, as rehydration restores water content and inverts the vitrification process (Crowe *et al.*, 1998; Ramos *et al.*, 2001; Billi and Potts, 2002). The accumulation of compatible solutes varies depending on the type of xeric stress: under osmotic stress, balance is achieved through water efflux or solute uptake and synthesis, whereas under matric stress, compatible solutes accumulate more gradually due to the slow drying process. Conversely, rapid air-drying induces cellular shrinkage that hampers their accumulation (Potts, 1994). Altogether, these strategies protect membranes and macromolecules from desiccation-induced damage, thereby increasing the probability of survival and preventing cell death (Lebre *et al.*, 2017; Zeidler and Müller, 2019).

Desiccation imposes a severe metabolic burden, forcing microorganisms to adjust their metabolism to meet changing energy demands (Lebre *et al.*, 2017). When suffering from xeric stress, bacteria markedly reduce metabolic activity by downregulating respiration-related pathways and entering a dormant, stress-resistant state. Central metabolic routes such as glycolysis, the tricarboxylic acid

(TCA) cycle, and oxidative phosphorylation are repressed to minimize energy expenditure. Under these conditions, the glyoxylate shunt (GS) in the yeast *Saccharomyces cerevisiae* remains active as a metabolic shortcut for energy production, supporting cellular survival under low a_w (Figure 6) (Erkut *et al.*, 2016). Unlike the TCA cycle, which fully oxidizes acetyl-CoA to CO_2 and generates NADH for oxidative phosphorylation, the GS pathway bypasses two CO_2 -generating steps, conserving carbon skeletons for biosynthesis. This adaptation allows cells to sustain anabolic processes and produce ATP more efficiently under nutrient-limited and xeric conditions. The GS serves as an anaplerotic route, replenishing TCA intermediates, particularly oxaloacetate, which can be consumed for gluconeogenesis (Dunn *et al.*, 2009; Erkut *et al.*, 2016). By bypassing decarboxylation steps, the GS prevents depletion of key intermediates that would otherwise be diverted to biosynthetic pathways, ensuring metabolic flexibility under stress (Erkut *et al.*, 2016).

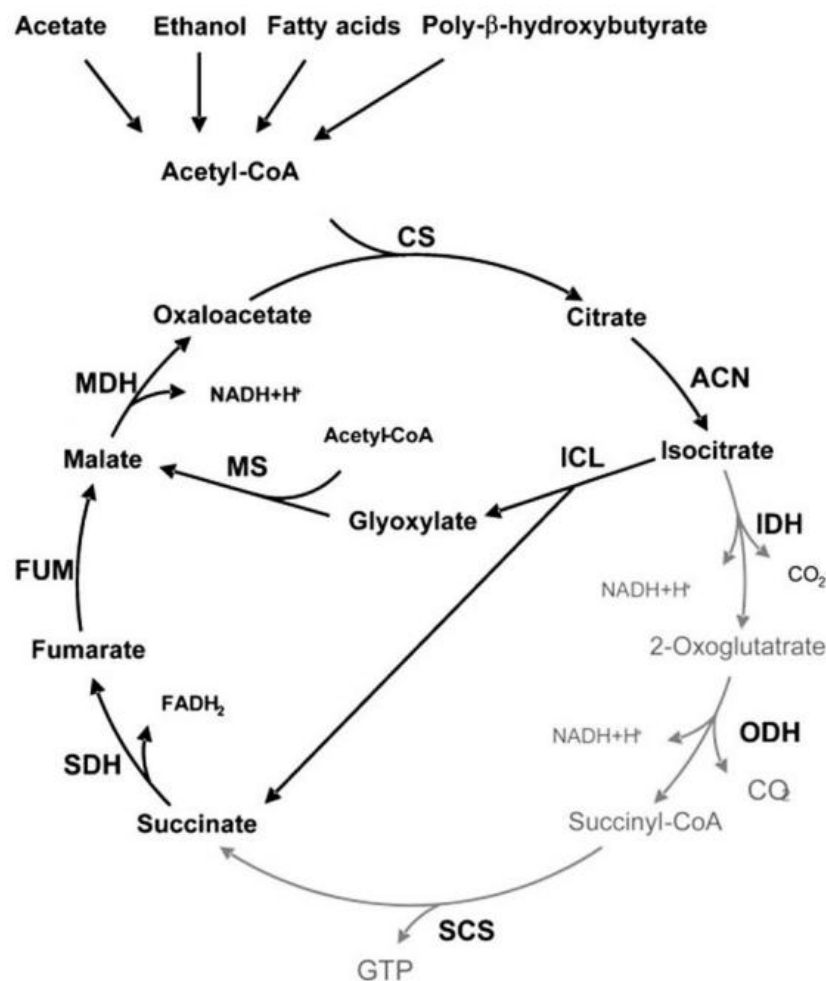


Figure 6 | Schematic representation of *E. coli* enzymatic reactions within the TCA and glyoxylate shunt pathways. Activation of the glyoxylate pathway allows cells to bypass the TCA cycle and its CO_2 -generating steps. Abbreviations: CS, citrate synthase; ACN, aconitase; IDH, isocitrate dehydrogenase; ODH, 2-oxoglutarate dehydrogenase; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; FUM, fumarase; MDH, malate dehydrogenase; MS, malate synthase; ICL, isocitrate lyase (adapted from Dunn *et al.*, 2009).

In response to xeric stress, bacteria differentially regulate metabolic pathways, leading to a general shift from anabolism to catabolism (Lebre *et al.*, 2017). The balance between these processes is complex, since metabolism can be diverted to alternative carbon sources. Indeed, fatty acid oxidation yields more ATP molecules per carbon atom than glucose, making fatty acids an efficient catabolic reservoir (Erkut *et al.*, 2016; Lebre *et al.*, 2017). Under desiccating conditions, glucose is often diverted to trehalose biosynthesis, while fatty acid oxidation becomes a highly effective alternative energy source that sustains cellular metabolism (Lebre *et al.*, 2017).

During xeric conditions, cellular ATP is redirected toward processes that are essential for bacterial survival. Among these, a central role is played by antioxidant defences, with the upregulation of genes encoding enzymes involved in ROS scavenging, including superoxide dismutase (SOD), catalases, peroxidases, and thioredoxins (Zeidler and Müller, 2019; Greffe and Michiels, 2020). Concomitantly, genes responsible for the synthesis of osmoprotectant proteins, molecular chaperones, and proteases, as well as DNA-protective and -binding proteins, are activated to preserve macromolecular integrity (Greffe and Michiels, 2020). This metabolic plasticity, ranging from enhanced activity to dormancy, highlights the diverse strategies bacteria employ to counteract desiccation stress and ensure persistence in xeric environments. Dormancy represents a widespread adaptive strategy observed across plants, animals, and microorganisms (Geiser *et al.*, 2013; Lennon *et al.*, 2021; McDonald *et al.*, 2024). Dormancy is defined as a temporary state of reduced metabolic activity and halted growth triggered by unfavourable conditions such as drought, extreme temperatures, or nutrient limitation (Lennon and Jones, 2011). By conserving energy and resources, dormancy increases the likelihood of survival until more favourable conditions are restored, playing a crucial role in the ecology and lifecycle of many bacterial species (Lennon *et al.*, 2021). In bacteria, multiple forms of dormancy have been documented (Rittershaus *et al.*, 2013), with sporulation representing the best-studied manifestation. Through an asymmetric cell division process, vegetative cells differentiate into metabolically inactive spores that are highly resistant to a wide range of biotic and abiotic stresses (Dworkin and Shah, 2010; Setlow, 2014; Huang and Hull, 2017).

In sporulating bacteria, dormancy has been considered a robust and definitive response to environmental stress. However, recent insights have blurred these boundaries in nonsporulating organisms, where dormancy is now recognized as a dynamic and progressive process (Ayrapetyan *et al.*, 2018). Beyond individual survival, dormancy also contributes to the long-term persistence of microbial communities by generating “seed banks,” reservoirs of dormant cells that preserve genetic diversity and can be revitalized under different environmental conditions (Lennon and Jones, 2011; Lebre *et al.*, 2017). This ability to withstand desiccation underscores the ecological importance of dormancy and its pivotal role in shaping microbial resilience in xeric environments.

1.2. The VBNC state as an adaptive strategy to counteract xeric stress

Bacteria inhabit environments characterized by constant fluctuations, where selective pressures have shaped their remarkable ability to adapt rapidly. One of the most effective adaptive strategies is dormancy, a state of reduced metabolic activity that enables the bacterial cells to withstand adverse conditions. To counteract xeric stress, non-sporulating bacteria can adopt a dormancy strategy, known as the viable but non-culturable (VBNC) state (Ayrapetyan *et al.*, 2018). The VBNC state is often confused with persistence, as both represent stress-induced survival subpopulations that remain viable and metabolically active in a nongrowing state. However, important distinctions exist between these two phenomena. In particular, culturability and viability serve as key criteria for differentiating VBNC cells from persisters, as well as from other related states such as dormant or uncultured cells (Figure 7). VBNC cells represent a subpopulation of bacteria that transiently loses the ability to grow on routine laboratory media, despite previously being culturable. This transition occurs progressively as cells initially grow on nutrient media but become nonculturable under stress conditions (Figure 7A). Conversely, persistence is a dormancy mechanism that enables a fraction of bacterial cells to survive diverse stresses (*e.g.*, antibiotic treatment, starvation, oxidative stress, DNA damage, and suboptimal pH) (Ayrapetyan *et al.*, 2018). Unlike resistant cells, which rely on heritable genetic determinants, persisters are genetically identical to the non-persister population and remain susceptible to antibiotics (Ayrapetyan *et al.*, 2015). Persistence is typically described by a biphasic killing curve (Figure 7B): an initial rapid decline reflects the death of sensitive cells, followed by a slower phase in which mortality decreases due to the survival of persisters, usually less than 1% of the population (Ayrapetyan *et al.*, 2018). A major difference between VBNC and persisters lies in their revitalization dynamics. Persisters typically resume growth on nutrient media shortly after stress removal, whereas VBNC cells remain unculturable even after stress alleviation (Figure 7). Their recovery requires a prolonged revitalization period or specific stimuli before culturability can be restored (Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). This process, known as resuscitation, involves physiological changes that ultimately enable VBNC cells to regain the ability to grow on nutrient media (Ramamurthy *et al.*, 2014).

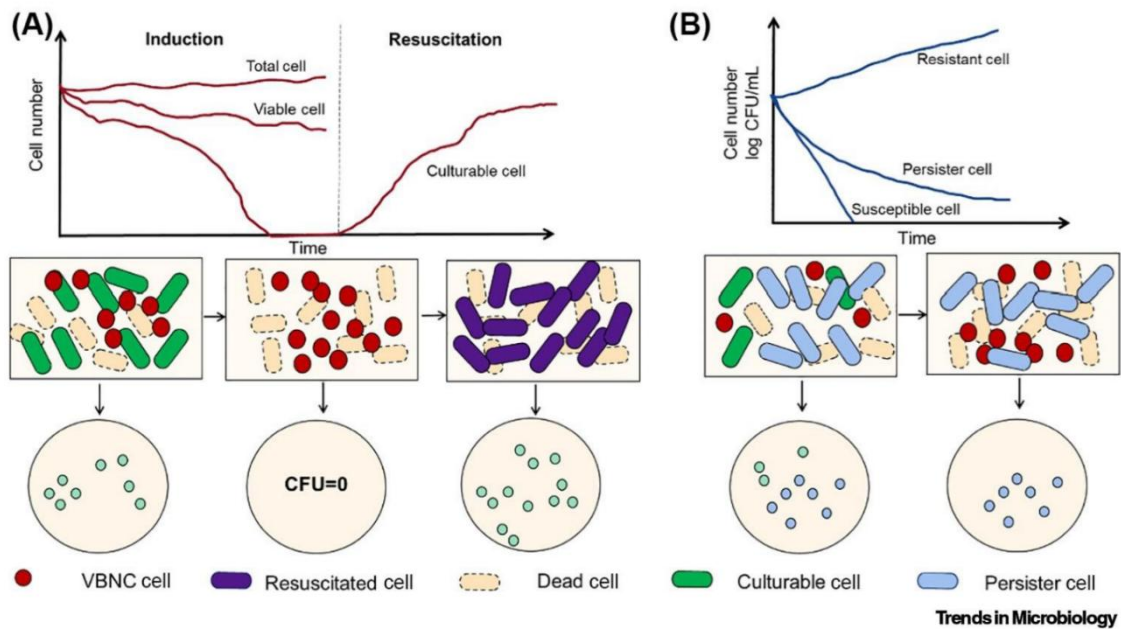


Figure 7 | Comparison between VBNC state and persistence. (A) The VBNC state is induced when cells are exposed to unfavorable conditions, leading to a progressive decline in culturability until it reaches zero, while the number of viable cells remains constant. Upon stress removal and provision of appropriate conditions or stimuli, VBNC cells can resuscitate and regain the ability to grow on nutrient media. (B) Persistence is induced when cells are challenged with stressful factors (*i.e.*, antimicrobial treatment). Unlike VBNC cells, persisters remain culturable and can be detected on microbiological media after stress removal (adapted from Liu *et al.*, 2023).

First introduced by Xu and colleagues in 1982 (Xu *et al.*, 1982), the VBNC concept raised questions about whether these cells were dying or instead adopting an adaptive survival strategy to withstand adverse conditions (Ramamurthy *et al.*, 2014). In the 1990s, the terms “dormancy” and “VBNC state” were often used interchangeably to describe cells that do not grow but can revert to a culturable state under favourable conditions (Liu *et al.*, 2023). More recent studies, however, highlight metabolic activity as a useful criterion for differentiating VBNC from dormant cells: while dormant cells exhibit undetectable metabolic activity, VBNC cells maintain measurable activity (Ramamurthy *et al.*, 2014; Liu *et al.*, 2023). The VBNC state can be triggered by diverse environmental and physiological factors, including nutrient deprivation, pH fluctuations, extreme temperatures, hypoxia, UV irradiation, osmotic stress, and exposure to heavy metals (Liu *et al.*, 2023; Balagurusamy *et al.*, 2024). Beyond these stress conditions, VBNC cells may also arise stochastically under apparently favourable conditions, such as routine laboratory growth (Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). This suggests a potential role of this physiological transformation in safeguarding a fraction of the bacterial population against unforeseen challenges.

1.2.1. Mechanisms underlying bacterial transition to the VBNC state

VBNC bacteria are widely distributed and display remarkable species diversity; however, the strategies by which they resist external stimuli and enter the VBNC state still require a deeper

understanding (Cai *et al.*, 2021). Upon transition into the VBNC state, bacterial cells preserve their structural integrity but undergo a marked size reduction, a feature that enhances protection against diverse environmental stresses (Liu *et al.*, 2017). Typically, entering the VBNC state, Gram-negative bacteria acquire a coccoidal morphology, whereas Gram-positive cells appear slightly elongated. These shape modifications are thought to reflect changes in the biochemical composition of the cell envelope (Signoretto *et al.*, 2000). Morphological transformations lead to an increase in cell wall thickness, enhancing bacterial tolerance to external stimuli and contributing to reduced metabolic activity and limited substance exchange across the cell surface (Cai *et al.*, 2021). Under adverse conditions, essential proteins may aggregate, impairing normal metabolic processes and facilitating entry into the VBNC state (Dong *et al.*, 2020). Collectively, these changes in morphology and chemical composition represent adaptive strategies that minimize cellular maintenance requirements and enhance survival under hostile environments.

Despite extensive characterization of the morphological and physiological features of VBNC cells, the genetic basis governing this state remains largely unclear. This uncertainty is compounded by the fact that numerous bacterial species can enter the VBNC state, denoting the existence of diverse, species-specific regulatory mechanisms.

A theory known as the gene regulatory theory has been proposed to explain VBNC formation, suggesting that the transition to the VBNC state results from a finely tuned process of gene regulation (Dong *et al.*, 2020).

In *Vibrio cholerae* VBNC cells, exposure to harsh environmental conditions, such as amino acid starvation, activates the stringent response mechanism (Cai *et al.*, 2021). This mechanism is mediated by RelA-dependent synthesis of the alarmones guanosine tetraphosphate and guanosine pentaphosphate, collectively referred to as (p)ppGpp (Ayrapetyan *et al.*, 2018; Cai *et al.*, 2021). Because (p)ppGpp inhibits exopolyphosphatase (PPX), the enzyme responsible for degrading polyphosphates (polyP), polyP accumulates through the constitutive activity of polyphosphate kinase (PPK) (Ayrapetyan *et al.*, 2015). Boaretti and colleagues demonstrated that *E. coli* mutants unable to produce (p)ppGpp are less likely to enter the VBNC state (Boaretti *et al.*, 2003). Conversely, the overexpression of (p)ppGpp enhances bacterial response to environmental stresses by negatively affecting the synthesis of DNA, RNA, and proteins, ultimately leading to growth arrest (Cai *et al.*, 2021).

Recent studies have implicated the toxin-antitoxin (TA) system of *Cronobacter sakazakii* in the regulation of bacterial entry into the VBNC state (Zhang *et al.*, 2020). The TA system consists of two co-expressed genes: one gene encodes a relatively stable toxin protein while the other encodes an unstable antitoxin. They are regulated by the stringent response, which promotes polyP accumulation and activates Lon and ClpP protease, thereby degrading antitoxins. This process alters the toxin-

antitoxin balance, allowing free toxins to exert downstream effects (*e.g.*, interfere with translation or DNA replication) (Ayrapetyan *et al.*, 2018; Cai *et al.*, 2021). In addition, general stress response factors play a crucial role in VBNC state entry. Specifically, the RNA polymerase alternative sigma factor RpoS (σ^S), regulated by the stringent response and ClpX protease, contributes to bacterial tolerance against diverse environmental stressors. Similarly, the LysR-type transcriptional regulator (OxyR) enables bacteria to cope with oxidative stress (Li *et al.*, 2014; Cai *et al.*, 2021). Finally, the degradation of the toxin transcriptional activator ToxR can promote the entry of *Vibrio cholerae* into the VBNC state, conferring an adaptive advantage under stressful conditions, as ToxR plays a central role in sensing and responding to environmental signals (Dong *et al.*, 2020). Figure 8 provides an overview of the molecular mechanisms that drive the induction of the VBNC state.

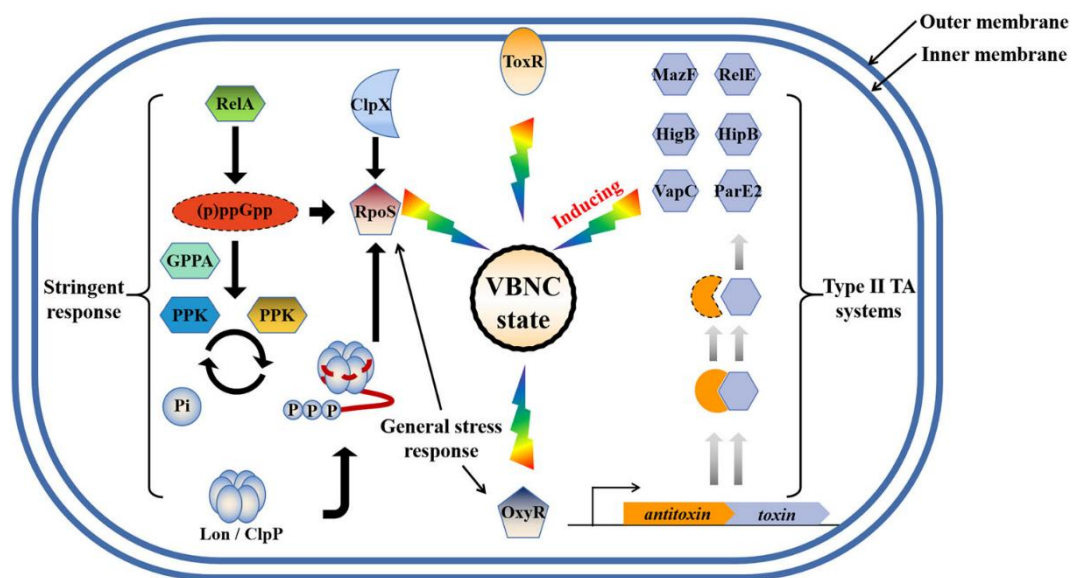


Figure 8 | The molecular mechanisms of VBNC bacteria formation. Molecular mechanisms of the VBNC cells formation mainly involve three pathways: the stringent response, the general stress response system, and the toxin-antitoxin systems (adapted from Cai *et al.*, 2021).

Although the VBNC state and antibiotic persistence (AP) were discovered independently and decades apart, these physiological states share striking phenotypic similarities that justify investigating their relatedness. Antibiotic persistence is defined as the ability of a small subpopulation of bacteria to survive exposure to an antibiotic without being genetically resistant to it (Niu *et al.*, 2024). Given the overlap in environmental stresses that induce VBNC and persister formation, as well as the comparable molecular mechanisms underlying their development, a new theory has recently emerged to explain VBNC formation. Specifically, it has been proposed that VBNC cells and persisters are not distinct phenomena but rather interconnected stages within a broader “continuum of dormancy” (Figure 9). This concept, referred to as the dormancy continuum hypothesis, suggests that both states

represent adaptive responses to unfavourable conditions and should be studied as part of a unified framework bridging VBNC and persister cells (Ayrapetyan *et al.*, 2015; Ayrapetyan *et al.*, 2018).

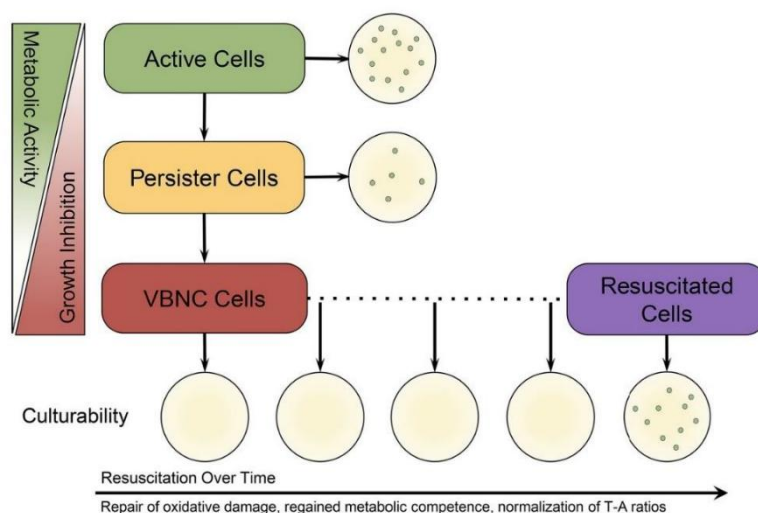


Figure 9 | Schematic dynamics of “dormancy continuum hypothesis”. When active cells are subjected to an environmental cue, they respond by inhibiting their growth and entering an early state of dormancy: persistence. If persister cells are removed from the stressful factor, they can grow on culture media. However, if the level of stress escalates, cells switch to a more profound dormancy phenotype: the VBNC state. VBNC cells, to return culturable, need to be resuscitated for extended periods and sometimes in the presence of external stimuli. Once resuscitation has occurred, bacteria become culturable again (adapted from Ayrapetyan *et al.*, 2018).

The hypothesis stems from evidence that active bacterial cells exposed to stochastic events and/or environmental signals can undergo reduced metabolic activity and growth arrest. It is plausible that in the very initial phase of stress exposure, persister cells are the first to be generated and are those capable of growing on nutrient media when the stress is relieved. However, prolonged exposure to stressful conditions drives persister cells into a deeper dormancy state, *i.e.*, the VBNC state. Two major hypotheses have been proposed to explain the transition between persister and VBNC cells. The first one, involving the TA system, hypothesizes that environmental stress triggers the degradation of antitoxins, thereby liberating toxins, which inhibit metabolism and growth. In the early stages of stress exposure, this process generates persister cells that can rapidly resume growth once the stress is relieved (Ayrapetyan *et al.*, 2015). However, prolonged stress leads to further toxin accumulation, deeper dormancy, and reduced metabolic activity, ultimately driving cells into the VBNC state. This framework posits that persisters and VBNC cells represent sequential stages along a continuum of dormancy, with resuscitation requiring the repair of oxidative damage and restoration of balanced toxin–antitoxin ratios (Ayrapetyan *et al.*, 2015; Ayrapetyan *et al.*, 2018).

A complementary hypothesis to the toxin–antitoxin model emphasizes the role of protein aggregation in driving bacterial dormancy (Figure 10).

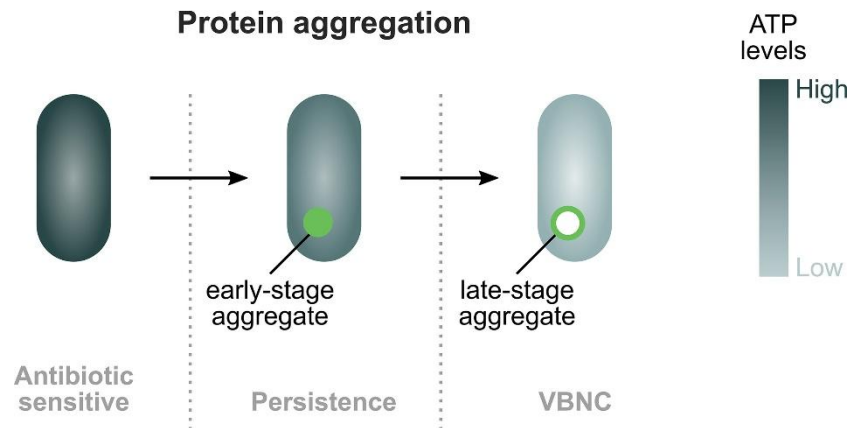


Figure 10 | Model for the development of bacterial dormancy through protein aggregation. Upon nutrient starvation in the stationary phase, cellular ATP concentrations decrease. As a consequence, proteostasis cannot be maintained, and protein aggregation occurs progressively. Persister cells are characterized by early-stage aggregates, while deeply dormant VBNC cells contain more developed aggregates (adapted from Dewachter *et al.*, 2021).

Experimental evidence indicates that, under stress conditions such as nutrient starvation, proteins involved in energy production are progressively sequestered into aggregates, leading to ATP depletion and the onset of dormancy (Dewachter *et al.*, 2021). Importantly, the structural features of these aggregates determine the ability of cells to exit dormancy. Persister cells typically harbor early-stage, liquid-like condensates that can be readily dissolved by the chaperone DnaK, enabling rapid regrowth. In contrast, VBNC cells often contain late-stage aggregates that have undergone a liquid-to-solid phase transition, characterized by intermolecular β -sheet structures that impede disaggregation. This solidification process hinders the recovery of cells from dormancy and pushes them into a deeper dormancy state. Thus, protein aggregation not only precedes dormancy development but also provides a mechanistic explanation for the transition from persister to VBNC cells, with aggregate structure serving as a critical determinant of dormancy depth and resuscitation potential (Dewachter *et al.*, 2021; Bollen *et al.*, 2025).

The key distinction between VBNC and persister cells lies in their resuscitation dynamics (Liu *et al.*, 2023). Persister cells can resume growth on nutrient media almost immediately or after a brief lag once the stressor, such as antibiotics, is removed. In contrast, VBNC cells require prolonged resuscitation periods outside of culture media following the elimination of the inducing stress, and in some bacterial species, specific treatments are necessary to trigger recovery (Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). This supports the view that VBNC cells represent a deeper dormancy state than persisters, necessitating additional time or external factors to restore metabolic activity, repair cellular damage, and regain culturability (Ayrapetyan *et al.*, 2015). Currently, over 100 species of bacteria have been shown to enter a nonculturable state, but only a subset of these species has been shown to

resuscitate (*i.e.*, confirmed to be VBNC *sensu stricto*). This is because (i) in some studies, nonculturable cells were detected but resuscitation was not attempted, (ii) the proper conditions to induce resuscitation are not yet known, or (iii) these cells are not in a true VBNC state (Ayrapetyan *et al.*, 2018). Indeed, further studies are needed to clarify mechanisms common to VBNC state entry and resuscitation processes.

1.2.2. Public health impact of VBNC cells

Bacterial cells in the VBNC state exhibit enhanced resistance to chemical stresses and antibiotics relative to culturable cells (Oliver, 2010). VBNC formation has been documented in more than 100 microbial species, many of which are pathogenic (Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). The presence of VBNC bacteria in food is well documented and represents a significant concern for public health. Food contaminants such as *E. coli* O157:H7, *Salmonella enterica* subsp. *enterica*, and *Pseudomonas putida* can enter the VBNC state under common food processing conditions, including refrigeration, pasteurization, cryopreservation, and desiccation (Fakruddin *et al.*, 2013). For instance, refrigerated pasteurized grapefruit juice has been shown to induce the VBNC state in *E. coli* O157:H7 and *S. typhimurium* within 24 hours of incubation, while pasteurized milk subjected to thermal treatment can harbour VBNC *E. coli* and *P. putida* (Fakruddin *et al.*, 2013). Moreover, foodborne outbreaks in Japan have been linked to *S. enterica* and *E. coli* O157 VBNC cells, highlighting their potential to persist undetected in processed products and subsequently cause disease. Apart from their pathogenicity, VBNC bacteria also influence the shelf life and microbial stability of food and beverages, posing a dual threat to both consumer health and product quality (Fakruddin *et al.*, 2013). VBNC cells are equally problematic in healthcare environments because of their ability to withstand environmental stress and persist in the inanimate environment. Hospital-acquired infections (HAIs) remain a major challenge, as pathogens can spread indirectly through contact with fomites. More than 40% of HAIs are attributed to cross-contamination by healthcare personnel, who may transfer pathogens via contaminated cloths, gloves, and frequently touched surfaces such as medical devices, tables, and beds (Sukhum *et al.*, 2022; Sandu *et al.*, 2025). Despite terminal disinfection, 5-30% of hospital surfaces remain contaminated, reflecting the limitations of current cleaning strategies (Weber *et al.*, 2016). Two main strategies are employed to reduce HAIs: touch methods, which rely on chemical disinfectants applied directly by healthcare personnel, and no-touch methods, such as vaporized hydrogen peroxide or germicidal ultraviolet (UV) systems, which aim to standardize and improve disinfection (Weber *et al.*, 2016; Strich and Palmore, 2017; Kelly *et al.*, 2022). However, recent evidence shows that these disinfection procedures can themselves induce the VBNC state in several pathogens. UV treatment has been reported to trigger VBNC formation in *E. coli* and

Pseudomonas aeruginosa (Zhang *et al.*, 2015), while hydrogen peroxide promotes VBNC cells in *Vibrio alginolyticus* (Zhou *et al.*, 2022). Importantly, in species such as *E. coli* O157:H7, *Listeria monocytogenes*, *Legionella pneumophila*, and various *Vibrio* spp., VBNC cells retain virulence and can cause severe infections once resuscitated in the host (Ramamurthy *et al.*, 2014).

The persistence and induction of VBNC cells in both the food industry and healthcare environments may therefore increase the incidence of infection outbreaks. This issue is compounded by the lack of internationally agreed guidelines for specific disinfection strategies. Although monitoring microbial contamination of hospital surfaces is crucial for containing outbreaks when an environmental reservoir is suspected, contamination of the hospital environment is rarely investigated, and harmonized methodological guidelines are unavailable (van der Schoor *et al.*, 2022). According to the standard procedure for *Cleanrooms and associated controlled environments - Biocontamination control* (BS EN 17141:2020), and *Microbiology of the food chain - Horizontal methods for surface sampling* (ISO 18593:2018), environmental sampling can be performed using different devices, including contact plates, sponges, and swabs. Among these, swabs are the most frequently employed in healthcare settings (Rawlinson *et al.*, 2019). After surface sampling with a swab moistened in a suitable transport buffer (TB), samples must be transported at 1-8 °C. An aliquot of the TB should then be directly plated on TSA or stored at 3 ± 2 °C for no longer than 48 hours before plating. Unfortunately, these standard culture-dependent methods may fail to detect bacteria in the VBNC state, leading to an underestimation of the actual microbial burden (Liu *et al.*, 2023). In the worst-case scenario, if all bacteria in a sample are VBNC, the sample may even be incorrectly regarded as germ-free (Li *et al.*, 2014). Therefore, the presence of VBNC cells in both food-related and hospital settings poses a serious risk to human health, since they can escape detection of environmental bacterial contamination where culture-based methods are employed to detect pathogens after decontamination interventions (Li *et al.*, 2014; Russotto *et al.*, 2015). Altogether, these factors underscore the critical role of the VBNC state in the persistence of pathogenic bacteria and highlight the limitations of conventional culture-based methods in successfully detecting them (Ramamurthy *et al.*, 2014).

1.3. *Acinetobacter baumannii*: a successful nosocomial pathogen

The genus *Acinetobacter*, from the Greek ακίνητος [akinetos], meaning motionless, belongs to the *Moraxellaceae* family, within the *Moraxellales* order and γ -*proteobacteria* class (Peleg *et al.*, 2008; Asif *et al.*, 2018). *Acinetobacter* spp. are Gram-negative, strictly aerobic, non-motile, non-fermenting, catalase-positive, oxidase-negative bacteria with a DNA G+C content of 39%-47% (Almasaudi, 2018). *Acinetobacter* cells are *ca.* 1.5 μm in length, with a shape varying from coccoid to coccobacillary, depending on the strain and growth stage (Figure 11) (Visca *et al.*, 2011).

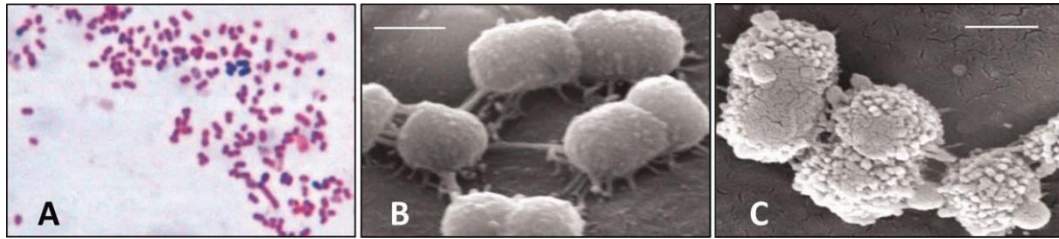


Figure 11 | Morphology and staining properties of *A. baumannii*. (A) Gram stain of stationary phase cells, some of which appear blue, due to retention of the crystal violet dye. (B) and (C) scanning electron micrograph of *A. baumannii* strains AYE and ACICU, respectively. White bar: 500 nm (adapted from Visca *et al.*, 2011).

Currently, the genus *Acinetobacter* includes 96 validly published species, most of which are environmental bacteria, while only a few are opportunistic human pathogens (<https://lpsn.dsmz.de/genus/acinetobacter>, consulted December 15th 2025). Among them, the most clinically significant members are grouped into the *Acinetobacter calcoaceticus-baumannii* complex (ACB) (Peleg *et al.*, 2008). The ACB complex consists of five pathogenic species: *A. baumannii*, *A. nosocomialis*, *A. pittii*, *A. seifertii*, and *A. lactucae* (the heterotypic synonym of *A. dijkshoorniae*), as well as one non-pathogenic species, *A. calcoaceticus* (Harding *et al.*, 2018). These pathogenic species are involved in ventilator-associated pneumonia (VAP), skin and soft-tissue infections, wound infections, urinary tract infections, secondary meningitis, and bloodstream infections, primarily affecting patients in Intensive Care Units (ICUs) (Harding *et al.*, 2018). Among the species in the ACB complex, *A. baumannii* is the most clinically relevant and well-characterized (Antunes *et al.*, 2014). *A. baumannii* infections impact critically ill patients with weakened immune defences and are typically linked to prolonged hospitalization (Visca *et al.*, 2011). The infection rates rise in the presence of mechanical ventilation, indwelling devices such as intravascular and urinary catheters, drainage tubes, invasive surgeries, and major trauma, especially burn or wound trauma (Dijkshoorn *et al.*, 2007; Peleg *et al.*, 2008). The main clinical manifestations caused by *A. baumannii* include ventilator-associated pneumonia and bloodstream infections, usually connected to extended hospital stays, especially in ICUs, where the microorganism is endemic. These infections account for approximately 19.2% of all healthcare-associated infections in the United States and Europe, with higher rates observed in Asia and the Middle East (Harding *et al.*, 2018; Sandu *et al.*, 2025). Additionally, it is noteworthy that *A. baumannii* has been among the most frequently isolated and deadly co-infecting agents in patients with COVID-19 (Clancy *et al.*, 2021; Russo *et al.*, 2023). Less commonly, *A. baumannii* can cause community-acquired infections; among these, community-acquired pneumonia tends to be more severe than nosocomial infections and can be fulminant, with mortality rates reaching up to 60% (Dijkshoorn *et al.*, 2007). Community-acquired infections are

more prevalent in males and are associated with predisposing factors such as advanced age, alcoholism, heavy smoking, diabetes mellitus, chronic obstructive pulmonary disease, and renal disease (Antunes *et al.*, 2014).

There is compelling evidence that the two most prominent features determining the success of *A. baumannii* as a nosocomial pathogen are drug resistance and environmental persistence (Harding *et al.*, 2018; Green *et al.*, 2022). Indeed, metabolic versatility together with resistance to disinfectants and antibiotics makes *A. baumannii* a serious challenge for clinicians and infection control specialists. In this regard, several studies have reported that *A. baumannii* strains responsible for epidemic outbreaks are resistant to the majority of commercially available antibiotics (Harding *et al.*, 2018). These antimicrobial-resistant strains, according to the international terminology established by the European Centre for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention (CDC), can be classified based on their resistance profile as: *i*) Multidrug-resistant (MDR), microorganisms non-susceptible to at least one agent in three or more antimicrobial categories. *ii*) Extremely drug-resistant (XDR), microorganisms non-susceptible to at least one agent in all but two or fewer antimicrobial categories. *iii*) Pan drug-resistant (PDR), microorganisms non-susceptible to all agents in all antimicrobial categories (Magiorakos *et al.*, 2012). Current therapeutic options for *A. baumannii* infections remain extremely limited, owing to its propensity to develop antimicrobial resistance even against last-resort agents such as carbapenems and, occasionally, colistin (Nguyen and Joshi, 2021). In this contest, the CDC has classified MDR *A. baumannii* strains as a significant threat, resulting in a compelling need for ongoing monitoring and prevention activities. Furthermore, the World Health Organization (WHO) has classified carbapenem-resistant *A. baumannii* in the critical group of top-priority pathogens for which research and development efforts for new antimicrobial treatment are needed (Tacconelli *et al.*, 2018; CDC, 2019).

1.3.1. Molecular and cellular mechanisms of *A. baumannii* xerotolerance

Besides drug resistance, another key factor in the success of *A. baumannii* is its remarkable adaptation to the hospital environment (Gayoso *et al.*, 2014; Zeidler and Mueller, 2019). This ability enables *A. baumannii* persistence on abiotic surfaces for extended periods, facilitating transmission, triggering ICU outbreaks, and complicating decontamination (Green *et al.*, 2022). Despite infection prevention and control measures, hospital surfaces, equipment, personnel, and indwelling devices remain major reservoirs of *A. baumannii* dissemination (Figure 12) (Dijkshoorn *et al.*, 2007; Chapartegui-González *et al.*, 2018).

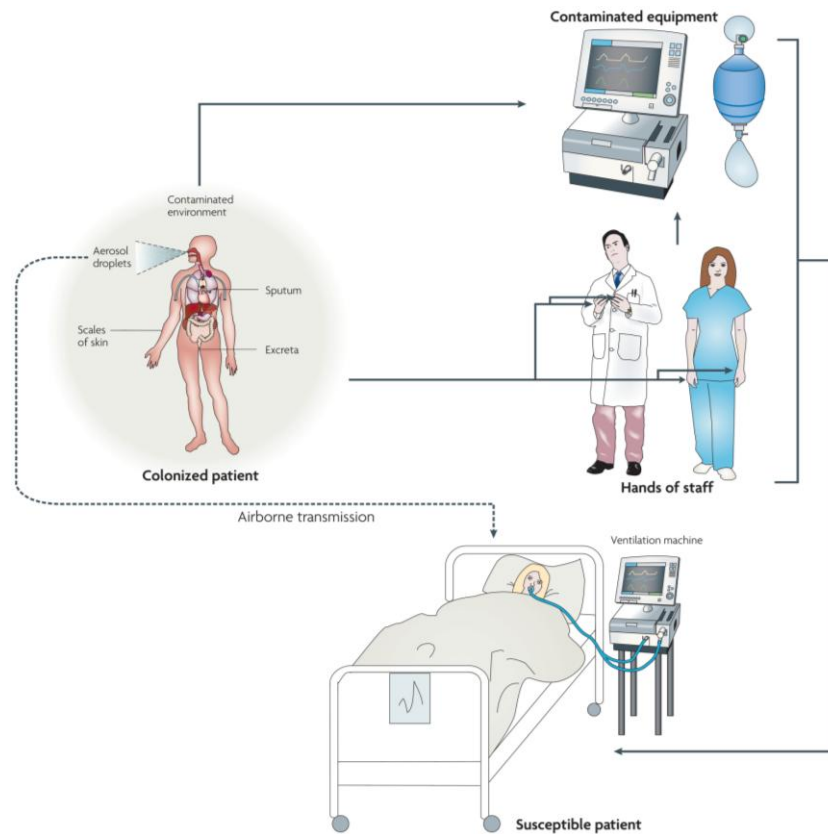


Figure 12 | Transmission routes of *A. baumannii* into a ward. The principal vehicles of infection are colonized patients, contaminated material or medical devices, and staff. Once on a ward, *A. baumannii* can spread from the colonized patient to the environment and other susceptible patients. Excreta, air droplets, and scales of skin can contaminate the direct environment of the patient. Due to the high capability of *A. baumannii* to survive and persist on dry abiotic surfaces, medical equipment also constitutes a source of transmission of this pathogen (adapted from Dijkshoorn *et al.*, 2007).

A. baumannii clinical isolates withstand prolonged desiccation more than laboratory strains, surviving from several weeks to months while maintaining culturability (Giannouli *et al.*, 2013; Zeidler and Mueller, 2019). This ability further sustains *A. baumannii* persistence on abiotic surfaces, increasing the risk of transmission by acting as a reservoir for future outbreaks (Campos *et al.*, 2019). *A. baumannii* desiccation resistance is a multifactorial and not completely understood phenomenon. It has been previously demonstrated that desiccation resistance is influenced by surface type, humidity, temperature, inoculum, and bacterial growth phase. In this regard, bacterial cultures harvested from the stationary phase and then desiccated show higher desiccation resistance compared to cells harvested from the logarithmic phase (Farrow *et al.*, 2018; Zeidler and Müller, 2019).

The molecular mechanisms at the basis of *A. baumannii* desiccation resistance remain elusive. Some regulatory pathways involved in desiccation resistance of *A. baumannii* cells are summarized hereafter.

Oxidative stress response induced by desiccation

During desiccation, the accumulation of oxidative stress and ROS activates the response regulator BfmR, which has been identified as a global regulator of the desiccation resistance phenotype (Zeidler and Müller, 2019). BfmR is part of a two-component regulatory system together with the sensor histidine kinase BfmS, which inactivates BfmR through dephosphorylation. BfmR phosphorylation determines the upregulation of several stress-related proteins, including KatE, a catalase HP11 (hydroperoxidase), and the *A. baumannii* stress-related protein A (AbsA), whose function remains undefined (Farrow *et al.*, 2018). Deletion of BfmR not only reduces resistance to nutrient starvation, oxidative stress, and osmotic stress but also results in a highly desiccation-susceptible phenotype (Farrow *et al.*, 2018; Zeidler and Müller, 2019). The importance of the BfmR/BfmS two-component system in enhancing desiccation resistance underscores the critical role of gene expression reshaping, enabling *A. baumannii* to mitigate the effects of water loss (Palethorpe *et al.*, 2022). Further studies on KatE confirmed its involvement in desiccation tolerance, as deletion of *katE* significantly reduced survival under desiccation (Farrow *et al.*, 2018). Moreover, BfmR was recently found to regulate two bacterial hydrophilins, DtpA and DtpB, which promote desiccation tolerance in *A. baumannii* by protecting DNA and proteins from desiccation-induced damage (Green *et al.*, 2022).

It has also been demonstrated that desiccation-stressed strains of *A. baumannii* significantly upregulate other proteins involved in ROS detoxification, such as [Cu²⁺-Zn²⁺]-SOD, peroxiredoxin (Gayoso *et al.*, 2014).

Furthermore, a transcriptomic analysis of the *A. baumannii* ATCC 17978 strain under desiccation conditions revealed the upregulation of genes related to oxidative stress response and proteostasis, including factors for ribosome assembly, chaperone activities, and protein translation and degradation (Wang *et al.*, 2020). Notably, this study identified increased protein aggregation as a dominant molecular signal associated with desiccation tolerance. Although protein aggregation is commonly considered detrimental to protein activity, it has been demonstrated that these aggregates retain functional proteins and act as a protective repository during water loss (Wang *et al.*, 2020). This reversible aggregation mechanism may shield the proteome from irreversible damage and provide functional proteins for recovery upon rehydration, suggesting that protein aggregation represents an adaptive strategy rather than a purely harmful process.

Compatible solutes accumulation

To withstand dehydration, xerotolerant microorganisms increase the intracellular concentration of compatible solutes to restore osmotic equilibrium and sustain protein function (Greffé and Michiels, 2020; Lebre *et al.*, 2017; Zeidler and Müller, 2019). These osmotically active compounds, either acquired from the environment or synthesized *de novo*, do not interfere with cellular processes and

are compatible with life. In response to salt stress, *A. baumannii* ATCC 19606^T accumulates glutamate, mannitol, and trehalose or, when available, takes up glycine betaine from the environment (Zeidler *et al.*, 2017). Trehalose, encoded by the *otsAB* operon (Figure 13), is considered one of the most important anhydrobiotic protectors against desiccation (Billi and Potts, 2002). However, compatible solutes involvement in *A. baumannii* desiccation tolerance remains unclear.

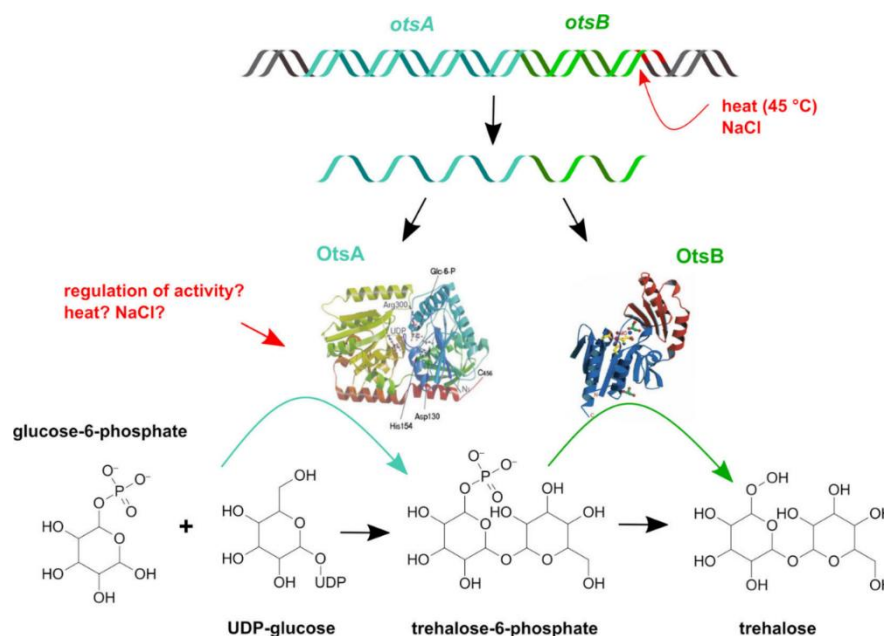


Figure 13 | Trehalose synthesis in *A. baumannii*. Trehalose is synthesized through the conserved *otsAB* pathway. The two genes are encoded by an operon controlled by a promoter inducible by heat-inactivation and osmolarity variations. OtsA catalyses the formation of the intermediate trehalose-6-phosphate. OtsB cleaves off the phosphate group and releases trehalose (adapted from Zeidler and Müller, 2019).

The prominent role of compatible solutes in *A. baumannii* ATCC 19606^T osmostress resistance is corroborated by the impaired growth of the $\Delta mtlD\Delta otsB$ double mutant under high NaCl concentrations, due to the defect in the biosynthesis of both mannitol and trehalose (Zeidler and Müller, 2018). However, the double mutant strain did not exhibit decreased survival on dry surfaces (Zeidler and Müller, 2018), suggesting that intracellular solute *de novo* synthesis is not essential for protection against desiccation. By contrast, *A. baumannii* cells suspended in trehalose-containing media before desiccation displayed a clear survival advantage compared to those exposed to water or other solutes (Zeidler and Müller, 2018). Notably, only exogenous trehalose, but not mannitol, glutamate, or glycine betaine, significantly increased survival times, confirming the prominent role of trehalose in desiccation resistance.

Outer membrane modification

The outermost structure of Gram-negative bacteria, called outer membrane, consists of a glycerophospholipid monolayer and an exposed monolayer of lipopolysaccharide or, in the case of *A. baumannii*, lipooligosaccharide (LOS) (Valcek *et al.*, 2025). Despite its permeability, the outer membrane is the most essential barrier separating the intracellular space from the surrounding environment and significantly contributes to survival under dehydration (Lopalco *et al.*, 2017; Wang *et al.*, 2017; Harding *et al.*, 2018). Desiccation resistance is closely linked to the composition of the outer membrane, and *A. baumannii* is known to modify the lipid A moiety of LOS. Unlike most Gram-negative bacteria, which synthesize hexa-acylated lipid A under standard growth conditions, *A. baumannii* predominantly produces hepta-acylated lipid A (Figure 14). This modification is catalysed by the two acyltransferases LpxL and LpxM, which reinforce the outer membrane and provide protection against desiccation and membrane-active antimicrobials, such as colistin (Boll *et al.*, 2015; Zeidler and Müller, 2019). Notably, the *A. baumannii* $\Delta lpxM$ mutant is more sensitive to desiccation (Boll *et al.*, 2015).

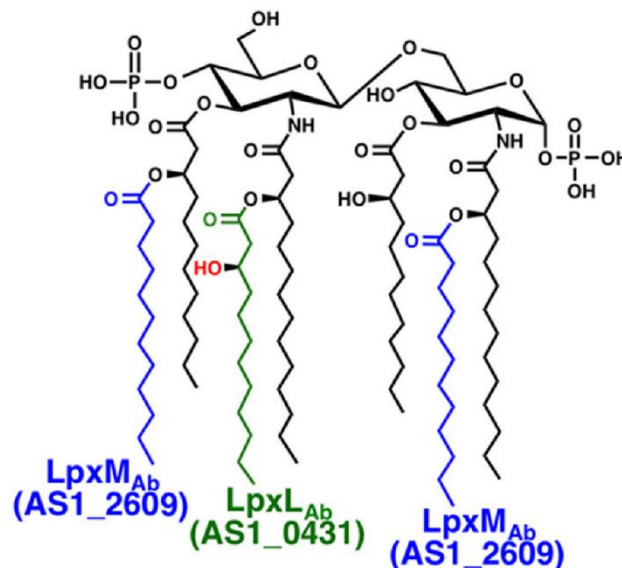


Figure 14 | *A. baumannii* lipid A. Acylation of *A. baumannii* lipid A is made by the acyltransferases LpxL_{Ab} and LpxM_{Ab}. LpxL_{Ab} catalyzes the addition of a secondary lauroyl group to position 2 (dark green), which can be hydroxylated (red). LpxM_{Ab} catalyzes the transfer of a secondary acyl chain at both positions 2 and 3 (blue) (adapted from Boll *et al.*, 2015).

In this regard, many desiccation stress responses rely on biochemical modifications of membrane phospholipids. The higher packing density of phospholipids enhances van der Waals interactions between hydrocarbon chains, thereby raising the melting temperature (Greffé and Michiels, 2020). Consequently, membrane fluidity is compromised, and the bilayer undergoes a transition from the liquid-crystalline to the gel phase. Chemical analyses revealed that, under desiccation, *A. baumannii*

ATCC 19606^T alters its membrane composition by increasing the proportion of saturated fatty acids relative to unsaturated ones (Fardelli *et al.*, 2022). This adjustment reduces membrane fluidity and confirms the phase transition from the liquid-crystalline to the gel phase (Fardelli *et al.*, 2022).

EPS and biofilm production

Several Gram-negative bacteria carry an additional EPS layer, constituting the so-called capsule, composed of repeated carbohydrate units, which works as a glycan shield. The capsular layer is an important virulence factor in *A. baumannii*, which enables its survival in human serum and increases its antibiotic resistance (Chin *et al.*, 2018; Lucidi *et al.*, 2024). EPS production was identified as a water-retaining shell in the local microenvironment to protect *A. baumannii* during dehydration (Farrow *et al.*, 2018). Despite the capsular layer protecting against water loss, huge differences in desiccation resistance were observed among capsule-producing strains (Zeidler and Müller, 2019). Recent studies showed the existence of two distinct subpopulations of colonies in the *A. baumannii* AB5075 strain: the virulent opaque (VIR-O) and the avirulent translucent (AV-T) phenotype. The differences are due to the production of distinct capsules between the two types of colonies. VIR-O cells produce a capsule with two-fold increased thickness compared with AV-T cells (Chin *et al.*, 2018). Therefore, the VIR-O subpopulation is more resistant to desiccation, host antimicrobial compounds, such as CRAMP and lysozyme, and disinfectants, whereas the AV-T type is advantageous in the environment outside the host. Indeed, AV-T cells show increased growth rates in nutrient-poor media and are more efficient biofilm formers than VIR-O cells (Chin *et al.*, 2018). Biofilms are matrices produced by bacteria to facilitate colonization of natural environments, and *A. baumannii* can form biofilm on biotic and abiotic surfaces (Chapartegui-Gonzales *et al.*, 2018). Being composed of extracellular carbohydrates, DNA, and other hydrophilic macromolecules, biofilms enable the retention of water, thereby increasing survivability on dry surfaces and mediating a higher rate of medical device-associated infections (Zeidler and Müller, 2019). However, not all *A. baumannii* strains can form biofilms, and non-biofilm-forming strains are more sensitive to desiccation than biofilm-producing strains. This could explain the relatively poor desiccation tolerance of the *A. baumannii* ATCC 19606^T and ATCC 17978 strains, which are known not to be good biofilm producers compared with more recent clinical *A. baumannii* isolates (Giannouli *et al.*, 2013).

Chapter 2. Aim of the thesis

Water is the cornerstone of life, and its scarcity imposes severe physiological and biochemical challenges on microorganisms. A distinctive feature of bacteria is their extraordinary ability to survive and adapt to fluctuations in the surrounding environment. Among the multitude of stresses faced by microorganisms, xeric stress, caused by water loss under hyperosmotic and desiccation conditions, constitutes one of the most widespread and alarming threats. The capacity of bacteria to withstand desiccation is not only an ecological adaptation but also a major concern for food safety and hospital hygiene. Pathogens that survive drying can persist on abiotic surfaces, thereby enhancing their capability of being transmitted to vulnerable individuals and, ultimately, facilitating their spread in both nosocomial and community settings. This phenomenon is particularly critical in healthcare environments, where bacterial resistance to desiccation poses a significant challenge in the management of nosocomial pathogens. Indeed, many infection outbreaks originate from bacteria capable of surviving for extended periods on inanimate surfaces. Upon contamination, such surfaces act as reservoirs that enable transmission through hospital fomites and personnel.

Among Gram-negative bacteria, *Acinetobacter baumannii* stands out for its extraordinary ability to withstand prolonged desiccation, a trait that has established it as a model organism of environmental persistence in healthcare settings. This remarkable resilience renders *A. baumannii* a critical pathogen, particularly in hospitals and Intensive Care Units, where its presence contributes to numerous hospital-acquired infections and their associated mortality. The challenge of eradicating this pathogen is further compounded by its resistance to commonly used antibiotics and disinfectants.

The environmental persistence of *A. baumannii* is strongly supported by its ability to enter the Viable But Non-Culturable (VBNC) state, which enables survival under extreme temperatures, pH fluctuations, and hypoxic conditions (König *et al.*, 2023). In this dormant condition, cells remain metabolically active but lose the capacity to grow on conventional media. Importantly, VBNC cells can regain culturability once favourable conditions are restored, making this survival strategy a hidden threat to infection control and environmental monitoring.

This thesis aims to elucidate the molecular mechanisms enabling *A. baumannii* to enter the VBNC state following desiccation stress (Chapter 3). Furthermore, the thesis focuses on the analysis of gene expression during transition to the VBNC state (Chapter 3) and the resuscitation process (Chapter 4), aiming to identify potential key genes involved in the reversible processes of VBNC cell formation and recovery.

VBNC cells have important clinical and public health implications. Indeed, previous studies have demonstrated that Gram-negative pathogens commonly associated with HAIs, such as *A. baumannii*,

P. aeruginosa, and *Klebsiella pneumoniae*, can enter a VBNC state under environmental stresses like desiccation on abiotic surfaces (König *et al.*, 2023; Qi *et al.*, 2023; Zhao *et al.*, 2024). Building on this evidence, this thesis investigates whether other ESKAPE bacterial pathogens, namely *Enterococcus* spp., *Staphylococcus aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp., all recognized as major threats due to their antibiotic resistance, can adopt the VBNC survival strategy to withstand the desiccation stress. In addition, the thesis assesses the limitations of conventional culture-based methods in detecting VBNC cells and introduces an innovative resuscitation-based protocol to enhance their recovery (Chapter 5). Finally, the thesis illustrates a patented method called MORECOVERY developed on the work described above, which increases the detection of some ESKAPE bacteria in various settings, and underscores its potential impact on microbiological surveillance and infection control (Chapter 6).

Chapter 3. The response to desiccation in *Acinetobacter baumannii*

Massimiliano Lucidi^{a,b*}, Giulia Capecchi^{a*}, Cinzia Spagnoli^a, Arianna Basile^a, Irene Artuso^a, Luca Persichetti^a, Elisa Fardelli^a, Giovanni Capellini^a, Daniela Visaggio^{a,b,c}, Francesco Imperi^{a,b,c}, Giordano Rampioni^{a,c}, Livia Leoni^a, and Paolo Visca^{a,b,c}

^aDepartment of Science, Roma Tre University, Rome, Italy

^bNBFC, National Biodiversity Future Center, Palermo, Italy

^cSanta Lucia Foundation IRCCS, Rome, Italy

*These authors contributed equally to this work.

Virulence, 2025, VOL. 16, NO. 1, 2490209 <https://doi.org/10.1080/21505594.2025.2490209>

Publication synopsis

A. baumannii exhibits an extraordinary capacity to withstand prolonged desiccation, a trait that has established it as a model organism of environmental persistence in healthcare settings. This pathogen can maintain its culturability for weeks or even months on abiotic surfaces, thereby contributing to long-term environmental contamination and efficient spread in hospitals (Giannouli *et al.*, 2013). A key factor supporting the environmental persistence of *A. baumannii* is its capacity to enter the Viable But Non-Culturable (VBNC) state, a dormant condition that enables survival under extreme stresses such as temperature fluctuations, osmotic imbalance, and hypoxia (König *et al.*, 2023). In this state, cells remain metabolically active but lose culturability on standard media, regaining growth only when favourable conditions are restored. *A. baumannii* cells in VBNC state represent a hidden reservoir for infections since they escape conventional culture-based surveillance. Despite its clinical relevance, the cellular and molecular mechanisms that sustain survival under prolonged desiccation remain poorly understood.

In this study, the mechanisms underlying desiccation resistance and VBNC entry in the *A. baumannii* ATCC 19606^T and ACICU strains were investigated under conditions mimicking airborne drying on hospital surfaces. A multimodal experimental approach was employed, combining microbiological assays, viability staining, culturability measurements, and transcriptomic analyses to monitor survival dynamics and physiological transitions during prolonged desiccation. Comparative analyses between the reference strain ATCC 19606^T and the clinical isolates ACICU revealed marked strain-dependent differences in desiccation tolerance and kinetics of transition to the VBNC state. Transcriptomic profiling confirmed VBNC entry in both strains, highlighting a shift toward alternative metabolic pathways associated with quiescence.

Overall, these findings demonstrate that entry into the VBNC state is a major strategy enabling *A. baumannii* to withstand desiccation and persist in hospital environments. Understanding this dormant phenotype is crucial for enhancing infection control measures and for developing surveillance strategies that can detect VBNC cells.

RESEARCH ARTICLE

OPEN ACCESS

The response to desiccation in *Acinetobacter baumannii*

Massimiliano Lucidi ^{a,b,*}, Giulia Capecchi ^{a,*}, Cinzia Spagnoli^a, Arianna Basile^a, Irene Artuso^a, Luca Persichetti ^a, Elisa Fardelli ^a, Giovanni Capellini ^a, Daniela Visaggio ^{a,b,c}, Francesco Imperi^{a,b,c}, Giordano Rampioni ^{a,c}, Livia Leoni ^a, and Paolo Visca ^{a,b,c}

^aDepartment of Science, Roma Tre University, Rome, Italy; ^bNBFC, National Biodiversity Future Center, Palermo, Italy; ^cSanta Lucia Foundation IRCCS, Rome, Italy

ABSTRACT

The long-term resistance to desiccation on abiotic surfaces is a key determinant of the adaptive success of *Acinetobacter baumannii* as a healthcare-associated bacterial pathogen. Here, the cellular and molecular mechanisms enabling *A. baumannii* to resist desiccation and persist on abiotic surfaces were investigated. Experiments were set up to mimic the *A. baumannii* response to air-drying that would occur when bacterial cells contaminate fomites in hospitals. Resistance to desiccation and transition to the “viable but nonculturable” (VBNC) state were determined in the laboratory-adapted strain ATCC 19606^T and the epidemic strain ACICU. Culturability, membrane integrity, metabolic activity, virulence, and gene expression profile were compared between the two strains at different stages of desiccation. Upon desiccation, ATCC 19606^T and ACICU cells lose culturability and membrane integrity, lower their metabolism, and enter the VBNC state. However, desiccated *A. baumannii* cells fully recover culturability and virulence in an insect infection model following rehydration in physiological buffers or human biological fluids. Transcriptome and chemical analyses of *A. baumannii* cells during desiccation unveiled the production of protective metabolites (L-cysteine and L-glutamate) and decreased energetic metabolism consequent to activation of the glyoxylate shunt (GS) pathway, as confirmed by reduced resuscitation efficiency of *aceA* mutants, lacking the key enzyme of the GS pathway. VBNC cell formation and extensive metabolic reprogramming provide a biological basis for the response of *A. baumannii* to desiccation, with implications on environmental control measures aimed at preventing the transmission of *A. baumannii* infection in hospitals.

ARTICLE HISTORY

Received 26 September 2024
Revised 10 February 2025
Accepted 2 April 2025

KEYWORDS

AceA; glyoxylate shunt;
membrane permeability;
resuscitation; VBNC

Introduction

Acinetobacter baumannii is a frequent colonizer of hospitalized patients and causes severe disease, including ventilator-associated pneumonia, urinary tract infection, bacteraemia, meningitis, and skin or soft tissue infections, particularly in intensive care unit (ICU) patients [1–3]. Recently, the incidence of *A. baumannii* infections increased by more than 110% due to the SARS-CoV-2 pandemic, often resulting in lethal co-infections [4,5]. Therefore, *A. baumannii* infections are now considered a worrisome problem on a global scale due to the healthcare burden, increasing trend of antibiotic resistance, and high transmissibility among patients [2]. Contamination of the environment has been documented in outbreaks for which information about environmental sampling was reported [6]. Asymptomatic *A. baumannii* carriage on the skin, in the pharynx, and the gastrointestinal tract has frequently been linked to significant contamination

in the near surroundings of patients (reviewed by Dijkshoorn *et al.*, 2007 [1]). Transmission of this pathogen is facilitated by hospital personnel, airflow, shared equipment, plumbing systems, and other fomites that constitute an *A. baumannii* reservoir of infections [7]. Managing outbreaks of *A. baumannii* sometimes necessitate ward closure and impose decontamination interventions or modifications to infrastructure which cause clinical, financial, and logistical burdens [1,2].

The striking ability to endure long-term desiccation is a major obstacle to *A. baumannii* eradication from the hospital environment, making this pathogen a model organism to investigate the effects of air-desiccation and osmoprotection on Gram-negative bacteria [8]. The ability of *A. baumannii* to survive water loss has so far been ascribed to the biosynthesis of compatible solutes, namely L-glutamate, D-mannitol, and trehalose, which act as osmoprotectants to prevent

CONTACT Massimiliano Lucidi  massimiliano.lucidi@uniroma3.it; Paolo Visca  paolo.visca@uniroma3.it

*These authors contributed equally to this work: Massimiliano Lucidi, Giulia Capecchi.

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macromolecular damages caused by dehydration (reviewed by Zeidler and Müller [8]), and the expression of the DtpA and DtpB hydrophilins, which act as molecular chaperones to prevent desiccation-induced protein denaturation [9]. It has also been demonstrated that *A. baumannii* enters a state of metabolic quiescence induced by dehydration, known as the “viable but not culturable” (VBNC) state [10]. Since culture-based procedures for assessing bacterial contamination in the hospital environment rely on the assumption that one viable cell gives rise to one colony on a solid medium [11], transition to the VBNC state would reduce bacterial detection given that VBNC cells, by definition, do not grow on solid media normally used for environmental control [12]. Indeed, the notion that the most successful *A. baumannii* clinical strains are endowed with higher desiccation resistance compared to laboratory-adapted strains is based on their ability to form colonies after desiccation [13,14]. However, the existence of a VBNC state challenges the paradigm “one viable cell=one colony” in assessing bacterial viability, posing the need to consider VBNC cells as an integral, still-alive component of the entire population challenged with the desiccation stress. Here, we elucidate the cellular and molecular mechanisms governing the transition to the desiccation-induced VBNC state in two *A. baumannii* prototypic strains endowed with different levels of desiccation resistance and discuss the impact of VBNC cell formation on environmental surveillance and hospital infection control.

Results

A. baumannii culturability during desiccation

Preliminarily, we set the culture conditions ensuring 100% culturability of *A. baumannii* cells for desiccation experiments. To this aim, culturability [expressed as colony-forming unit (CFU) values], cell density (OD_{600}), and membrane integrity (the principal viability marker) were compared between the type strain ATCC 19606^T and the epidemic strain ACICU taken at different times during growth in Luria Bertani (LB) medium (Figure S1). Although the CFU/ OD_{600} values did not change during 24-h growth, membrane damage became detectable after 14 h (Figure S1). Hence, to evaluate the clonogenic potential of *A. baumannii* undergoing desiccation, 6-h cultures of ATCC 19606^T and ACICU, showing maximum culturability and membrane integrity, were desiccated on a glass surface for up to 8 weeks or maintained in water as control (Figure 1a). Desiccation caused

a progressive reduction of culturability of both strains, with ACICU retaining its colony-forming ability 6 weeks longer than ATCC 19606^T. Storage in water for up to 4 weeks did not affect *A. baumannii* culturability, whereas a moderate (<1 Log) decrease in CFU counts was observed after 8 weeks (Figure 1b).

A. baumannii transition to the VBNC state during desiccation

To study the clonogenic potential of desiccated cells at the single-cell level, the pVRL1gfp plasmid directing constitutive expression of the green fluorescent protein (GFP [15]) was introduced in both *A. baumannii* ATCC 19606^T and ACICU strains. GFP-expressing cells desiccated for 0, 6, and 24 h were overlaid with a pad of LB soft (0.5% agarose) medium supplemented with a subinhibitory concentration of propidium iodide (PI, 60 μ M; Figure S2) to label membrane-damaged cells, and bacterial replication was continuously monitored for 4 h by time-lapse confocal microscopy. Four distinct *A. baumannii* sub-populations were found to co-exist after desiccation, hereafter classified as type I to type IV cells (Figure 1c): type I, clonogenic green-fluorescent cells; type II, non-clonogenic green-fluorescent cells; type III, non-clonogenic cells, which lose green fluorescence and adsorb PI over time; type IV, non-clonogenic red-fluorescent cells (Figure 1d and Supplementary videos 1–2). The inability to replicate despite membrane integrity, as proven by the cytoplasmic retention of GFP (27-kDa protein; *ca.* 4 $\times$ 3 nm size [16]) and PI exclusion, conforms type II cells to typical VBNC cells [12]. Conversely, type III and IV cells are endowed with typical features of dead cells due to PI intake, GFP release, and loss of clonogenicity. Quantification of these subpopulations revealed that the number of type II cells and, to a lesser extent, type IV cells increased during desiccation for both strains, denoting a progressive switch to the VBNC and dead states, respectively (Figure 1e). Moreover, the percentage of type II (typical VBNC) cells after 6- and 24-h desiccation was higher in ATCC 19606^T than in ACICU (Figure 1e). The decrease of green fluorescence emission by type III and IV cells should be attributed to GFP leakage from damaged cells, since GFP levels in the supernatants increased proportionally to the desiccation time and loss of cell-associated fluorescence (Figure S3). Plasmid loss was excluded since nearly 100% of the population of both strains maintained the pVRL1gfp plasmid (Figure S4).

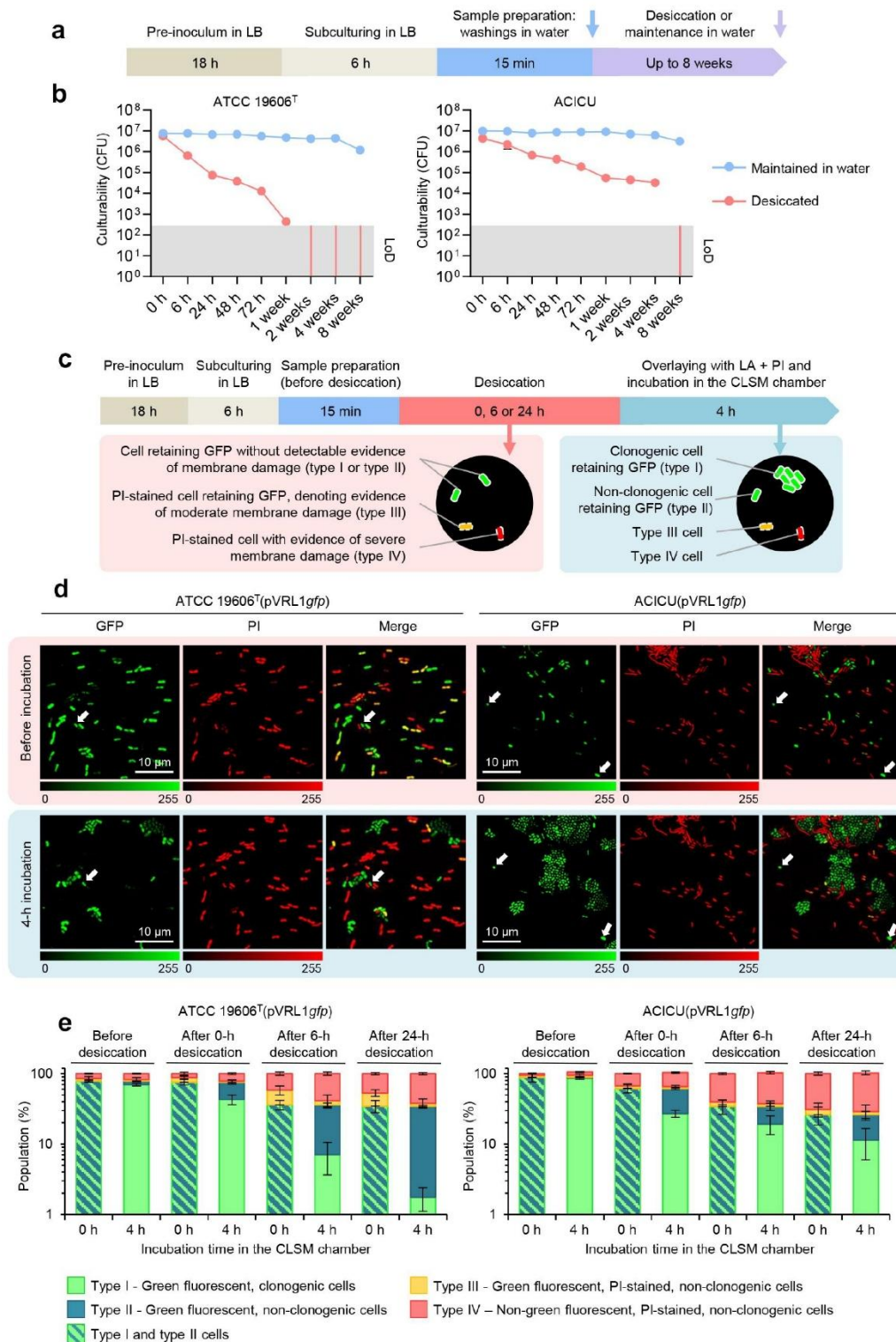


Figure 1. Resistance to desiccation and entry of *A. baumannii* cells in the non-clonogenic state after desiccation. (a) Experimental timeline of the desiccation resistance assay. Colored arrows indicate the sampling times for CFU counts; cyan, before desiccation; purple, after desiccation. (b) Clonogenic ability (CFU counts) of ATCC 19606^T and ACICU cells kept in water or desiccated for up to 8 weeks. The grey area indicates the lower detection limit (LoD) of CFU counts, corresponding to 4×10^2 CFU, with red segments

Factors affecting the resuscitation of VBNC *A. baumannii* cells

Resuscitation is defined as the regain of clonogenicity (*i.e.*, the ability to generate a colony from a single cell on a solid medium) by stress-induced VBNC cells, and it can occur by removing the stressing factor [12]. As previously demonstrated, ATCC 19606^T cells can be resuscitated after osmotic stress and dehydration, although with a low efficiency [10]. Therefore, the resuscitation of desiccated *A. baumannii* cells was investigated by measuring their culturability and membrane integrity after 24-h incubation at 37°C in M9 basal salt solution (M9SS Mg²⁺Ca²⁺), a mineral medium containing Mg²⁺ and Ca²⁺ ions but devoid of any carbon source (Figure 2a). Interestingly, a complete regain of clonogenicity was observed for both ATCC 19606^T and ACICU strains after 24-h incubation in M9SS Mg²⁺Ca²⁺, irrespective of the initial composition of the population (Figure 2b). Resuscitation was associated with complete recovery of membrane integrity, as inferred from PI exclusion (Figure 2c). This indicates that not only the typical VBNC population (type II) but also the populations composed of apparently dead cells, characterized by severely damaged membranes and leakage of intracellular GFP (type III and IV; Figure 1c), can be resuscitated, and therefore should be considered in a VBNC-like state. Low temperature and absence of membrane-stabilizing cations dramatically affected resuscitation, since culturability and regain of membrane integrity were reduced by incubation at 4°C and depletion of Mg²⁺ and Ca²⁺ ions [M9SS, phosphate-buffered saline (PBS)] (Figure S5). Under these sub-optimal conditions, the resuscitation efficiency greatly differed between ATCC 19606^T and ACICU, the latter being less sensitive to Mg²⁺ and Ca²⁺ depletion and low temperature (Figure S5). Thus, the epidemic strain ACICU remains culturable during longer desiccation periods than ATCC 19606^T (Figure 1b) and regains culturability and membrane integrity under sub-optimal resuscitation conditions (Figure S5). Intriguingly, resuscitation kinetic assays

revealed that 12-h incubation in M9SS Mg²⁺Ca²⁺ at 37°C was sufficient to ensure complete recovery of culturability and membrane integrity of both the *A. baumannii* strains (Figure S6).

We also used vancomycin (Van), a glycopeptide antibiotic excluded by the *A. baumannii* outer membrane but capable of penetrating it when the membrane is damaged [17], to probe membrane permeability of cells before and after desiccation, and after resuscitation. The minimal inhibitory concentration (MIC) of Van was similarly high (256 mg/L) for ATCC 19606^T and ACICU in all the test conditions (Figure S7). However, time-killing assays revealed that desiccated cells were more rapidly killed by inhibitory Van concentrations (512 mg/L; 2 × MIC) than cells before desiccation or after resuscitation, confirming that desiccation perturbs the outer membrane and facilitates Van penetration (Figure S7).

Since both *A. baumannii* strains were able to resuscitate after desiccation, we wondered if VBNC formation and resuscitation are common features of other members of the *Acinetobacter* genus. Therefore, four additional *A. baumannii* strains (ATCC 17978, AB5075, RUH 5875, and AYE) and representative strains belonging to other species of the genus (*Acinetobacter nosocomialis*, *Acinetobacter pittii*, *Acinetobacter baylyi*, *Acinetobacter seifertii*, and *Acinetobacter lactucae*) were desiccated, and their ability to resuscitate was assessed. Albeit with different resuscitation efficiencies, all *A. baumannii* strains and *Acinetobacter* species entered the VBNC state after desiccation and recovered culturability upon incubation in M9SS Mg²⁺Ca²⁺. Notably, *A. baumannii* AB5075 and *A. pittii* showed the highest resistance to desiccation (Figure S8).

Structural and functional modifications of desiccated *A. baumannii* cells

To better characterize the transitions in and out of the VBNC state, desiccated and resuscitated cells of

representing all CFU values below the LoD. Data are the mean ± standard deviation (SD; error bars) of three independent experiments. (c) Graphical representation of the confocal laser-scanning microscopy (CLSM) imaging experiment. ATCC 19606^T (pVRL1gfp) and ACICU(pVRL1gfp) cells were desiccated on a glass coverslip, covered with a pad of LB 0.5% agarose supplemented with 60 µM PI, and observed in a CLSM incubation chamber. Bacterial subpopulations (type I-IV) were quantified before and after 4-h incubation at 37°C. (d) Representative time-lapse CLSM micrographs of cells before and after 4-h incubation at 37°C. White arrows indicate type II cells. Green and red scales denote pixel intensity. (e) Percentages of cells belonging to type I, II, III, and IV subpopulations, calculated before (0 h) and after (4 h) incubation in the CLSM chamber (*n* > 6,500 for each sample). The distinction between type I and II cells is based on bacterial replication after 4-h incubation at 37°C.

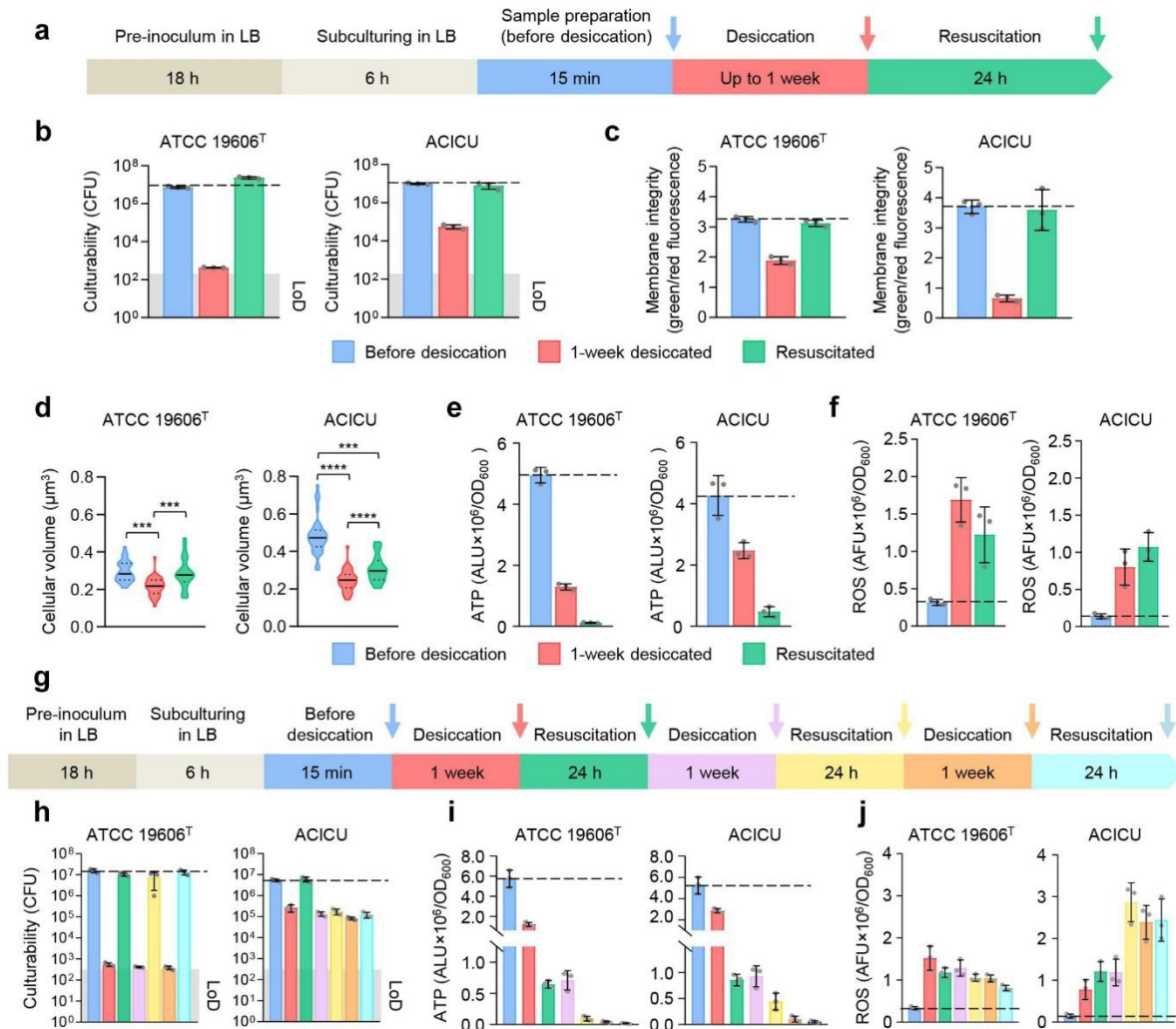


Figure 2. Formation of VBNC cells upon desiccation and analysis of structural and functional alterations occurring during *A. baumannii* desiccation and resuscitation. (a) Timeline of the resuscitation assay. ATCC 19606^T and ACICU cells were air-dried for 1 week. After desiccation, cells were suspended in M9SS Mg²⁺Ca²⁺ and incubated at 37°C for 24 h under shaking (resuscitation). Colored arrows indicate the sampling times for CFU counts; cyan, before desiccation; red, after desiccation; green, after resuscitation. (b) Culturability (expressed as CFU) of cells before desiccation, after desiccation, and after resuscitation. (c) Membrane integrity expressed as the ratio between green (SYTO 9) and red (PI) fluorescence emissions. (d) Cell volume before desiccation, after 1-week desiccation, and after resuscitation, as determined by AFM imaging of 30 cells of ATCC 19606^T and ACICU. The median (filled lines) and interquartile ranges (dashed lines) are shown. ATP (e) and ROS (f) levels in ATCC 19606^T and ACICU cells before desiccation, after desiccation, and after resuscitation. (g) Timeline of the resuscitation assays performed after three rounds of desiccation. Colored arrows indicate the sampling times for CFU counts (h), ATP levels (i), and ROS content (j). Color codes of histograms in (h,i,j) are those used in the experimental timeline (g). The grey area in (b) and (h) indicates the LoD of CFU counts (4 × 10² CFU). Dashed lines in histograms (b,c,e,f,h,i,j) indicate the pre-desiccation values. Data are the mean ± SD (error bars) of three independent experiments. Statistical significance was determined by the unpaired *t*-test (***) *p* < 0.001; **** *p* < 0.0001).

ATCC 19606^T and ACICU were analysed at both morphological and metabolic levels. Atomic force microscopy (AFM) measurements revealed that desiccation results in a significant loss of cellular volume, consequent to water evaporation, more evident in ACICU than in ATCC 19606^T. Upon

resuscitation, desiccated ATCC 19606^T cells fully recovered their pre-desiccation volume, whereas ACICU did not. However, ATCC 19606^T and ACICU showed nearly identical cellular volumes after desiccation and after resuscitation (Figure 2d, Figure S9).

Dehydration causes energy consumption and the generation of reactive oxygen species (ROS) due to membrane damage and respiratory chain malfunction [18]. Quantitative ATP and ROS content estimation demonstrated that ATCC 19606^T and ACICU suffer from severe ATP depletion and ROS production during desiccation (Figure 2e-f). During resuscitation, intracellular ATP was further consumed (Figure 2e), while ROS production continued (Figure 2f), denoting that resuscitation is an energy-demanding process laden with oxidative stress.

Since both desiccation and resuscitation are energy-demanding processes, we wondered whether *A. baumannii* could endure multiple rounds of desiccation and resuscitation in the absence of carbon and energy source supply (Figure 2g). ATCC 19606^T recovered full culturability across 3 rounds of desiccation and resuscitation, while ACICU failed to resuscitate after the first round, despite its higher tolerance to multiple rounds of desiccation (Figure 2h). Both strains experienced a progressive decline in ATP content during three rounds of desiccation and resuscitation (Figure 2i). Different from ATCC 19606^T, ROS levels progressively increased in ACICU during multiple rounds of desiccation and resuscitation (Figure 2j), in line with reduced culturability.

Resuscitation in biological fluids and virulence of desiccated *A. baumannii* cells

Given that the vast majority of *A. baumannii* cells are in the VBNC state after 1-week desiccation (Figure 2b), we wondered if resuscitation also occurs in biological fluids, as this would be a pre-requisite for ensuing *in vivo* proliferation. Since biological fluids contain carbon sources that would sustain bacterial replication during 24-h incubation at 37°C, we tested *A. baumannii* resuscitation in heat-inactivated human serum (HIS), urine, and saliva supplemented with bacteriostatic concentrations of ciprofloxacin (Cip) to prevent bacterial growth (Figure 3a). After desiccation, only ATCC 19606^T was resuscitated in saliva, whereas both ATCC 19606^T and ACICU were resuscitated in HIS and urine (Figure 3b), raising the possibility that desiccated *A. baumannii* cells which contaminate the hospital environment can resuscitate in human biological fluids to cause infection. Consequently, the virulence potential of both *A. baumannii* strains before and after desiccation, and after resuscitation in M9SS Mg²⁺Ca²⁺ or biological fluids, was tested in the *Galleria mellonella* insect model of infection. Although desiccated cells of both strains were significantly less virulent than the non-desiccated counterparts, virulence was

fully recovered after resuscitation in biological fluids (Figure 3c). The reduced virulence of desiccated cells suggests that part of the bacterial inoculum is unable to replicate *in vivo* and/or is cleared by the insect immune system before resuscitation. Overall, these data demonstrate that the VBNC *A. baumannii* population retains infectivity and lethality in the insect model of infection after resuscitation in biological fluids.

Transcriptional reprogramming in desiccated *A. baumannii* cells

To gain insights into the regulatory mechanisms implicated in the transition to the VBNC state, the transcriptional profiles of ATCC 19606^T and ACICU were compared before and after 1-week desiccation by RNA-sequencing (Figure 4a). Desiccation caused an extensive transcriptional reprogramming, involving *ca.* one-third of *A. baumannii* genes (Table S1). ACICU showed more differentially expressed genes (DEGs) than ATCC 19606^T (1,101 vs 975, respectively). Of the 539 DEGs shared by the two strains, only 9 showed opposite regulation (Figure 4b). To shed light on the functional categories of shared DEGs, each DEG was assigned to a cluster of orthologous groups of protein (COG) category (Table S2). COGs within the P (inorganic ion transport and metabolism) and C (energy production and conversion) categories were enriched with upregulated DEGs (Figure 4c; Table S3). The downregulated DEGs were enriched in COGs related to the J (translation, including ribosome structure and biogenesis) and O (molecular chaperones and related functions) categories (Figure 4c; Table S4). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that the upregulated DEGs common to both strains were enriched in pathways involving ATP-binding cassette (ABC) transporters (ko02010), sulphur and biotin metabolism (ko00920 and ko00780, respectively), and fatty acid biosynthesis (ko00061) (Figure 4d; Table S5). The shared downregulated DEGs enriched the pathways related to ribosome biogenesis (ko03010), valine, leucine, and isoleucine degradation (ko00280), benzoate degradation (ko00362), and geraniol degradation (ko00281) (Figure 4e; Table S6).

Involvement of L-cysteine, L-glutamate, and glyoxylate shunt pathway in *A. baumannii* desiccation resistance and resuscitation

Analysis of individual DEGs belonging to upregulated COG categories (P and C) and KEGG pathways (ABC transporters and sulphur metabolism) shared by ATCC

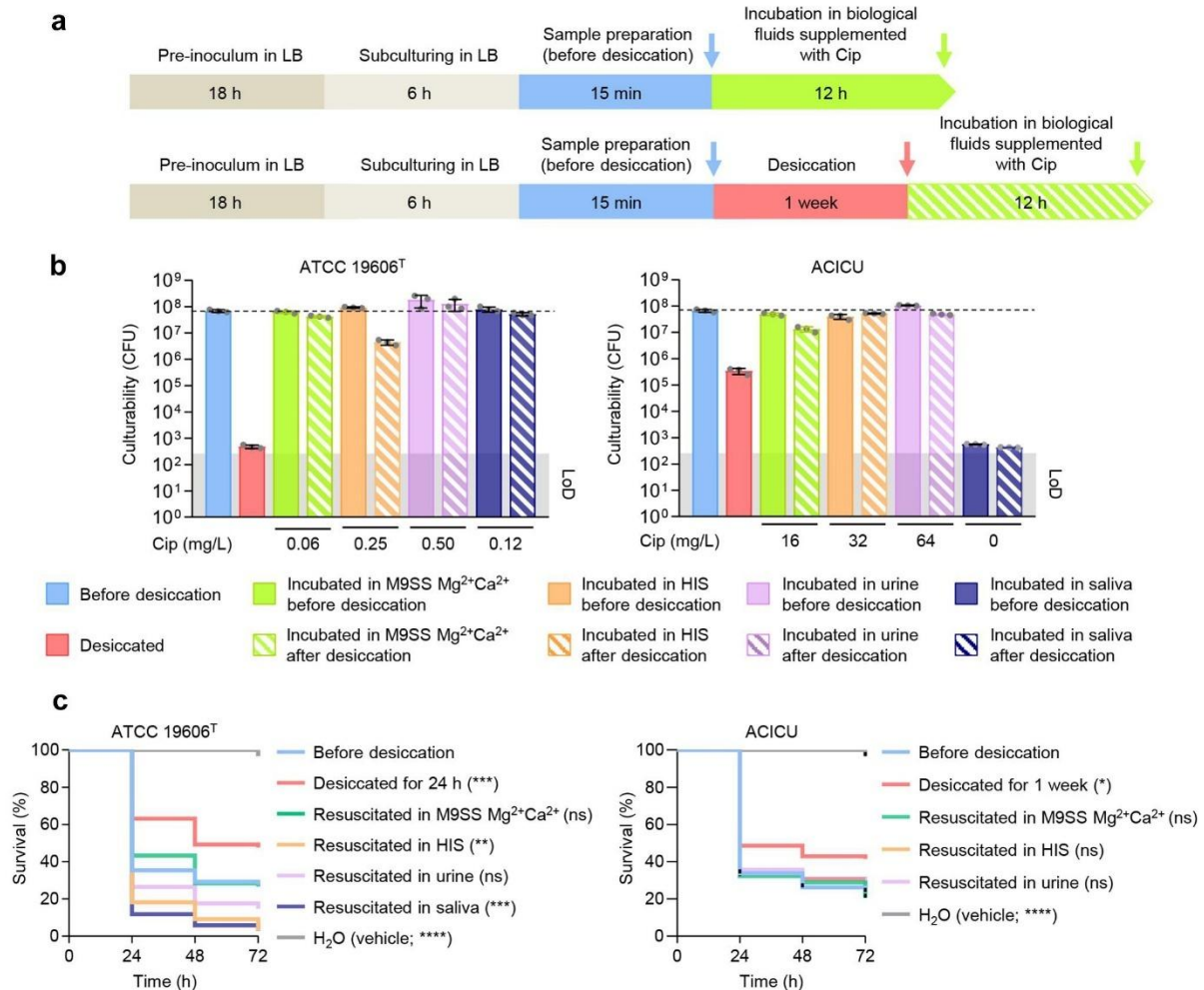


Figure 3. Resuscitation of desiccated *A. baumannii* cells in different biological fluids, and lethality in an insect infection model. (a) Experimental timeline of the resuscitation assay in different biological fluids. Colored arrows indicate the sampling times for CFU counts; cyan, before desiccation; red, after desiccation; fluo-green, after resuscitation. (b) Suspensions of ATCC 19606^T and ACICU cells before and after 1-week desiccation were incubated at 37°C for 12 h in HIS, urine, or saliva supplemented with Cip. The concentrations shown beneath the bars indicate the bacteriostatic concentration of Cip for each resuscitation medium. M9SS Mg²⁺Ca²⁺ was used as the resuscitation control medium. After incubation, clonogenicity was evaluated by CFU counts. Color codes of histograms are those used in the experimental timeline. The grey area indicates the LoD of CFU counts (4×10^2 CFU). Dashed lines indicate the pre-desiccation values. Data are the mean $\pm$ SD (error bars) of three independent experiments. (c) Lethality of ATCC 19606^T and ACICU cells in *G. mellonella*. The larvae ($n = 120$ per group) were injected with *A. baumannii* cells taken before desiccation, after desiccation, and after resuscitation in M9SS Mg²⁺Ca²⁺, HIS, urine, or saliva. Ten μ L of bacterial suspensions at OD₆₀₀ = 0.1 ($ca. 5 \times 10^6$) and OD₆₀₀ = 0.01 ($ca. 5 \times 10^5$) for ATCC 19606^T and ACICU, respectively, corresponding to the LD₅₀ of each strain, were inoculated into each caterpillar. After injection, larvae were kept at 37°C, and their survival was monitored every 24 h for 3 days. p-values were determined by the log-rank test (ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Asterisks indicate statistically significant differences between the survival plots of larvae infected with ATCC 19606^T and ACICU cells before desiccation and the test condition.

19606^T and ACICU, unveiled a metabolic flux of sulphur- and carbon-containing compounds towards the biosynthesis of L-cysteine, L-glutamate, and glyoxylate (Figure 5a). The upregulation of genes encoding ABC transporters of sulphur compounds (*cysPUWA*, *tauABC*, and *ssuABC* for sulphate, taurine, and

alkanesulfonate uptake, respectively) and enzymes implicated in the conversion of dimethylsulfoxide to sulphite (*ssuD*, *ssuE*, and *sfnG*) direct the metabolism towards the generation of sulphite, a central precursor of L-cysteine. Furthermore, the upregulation of both *cysJ* and *cysE* would increase the production of sulphide

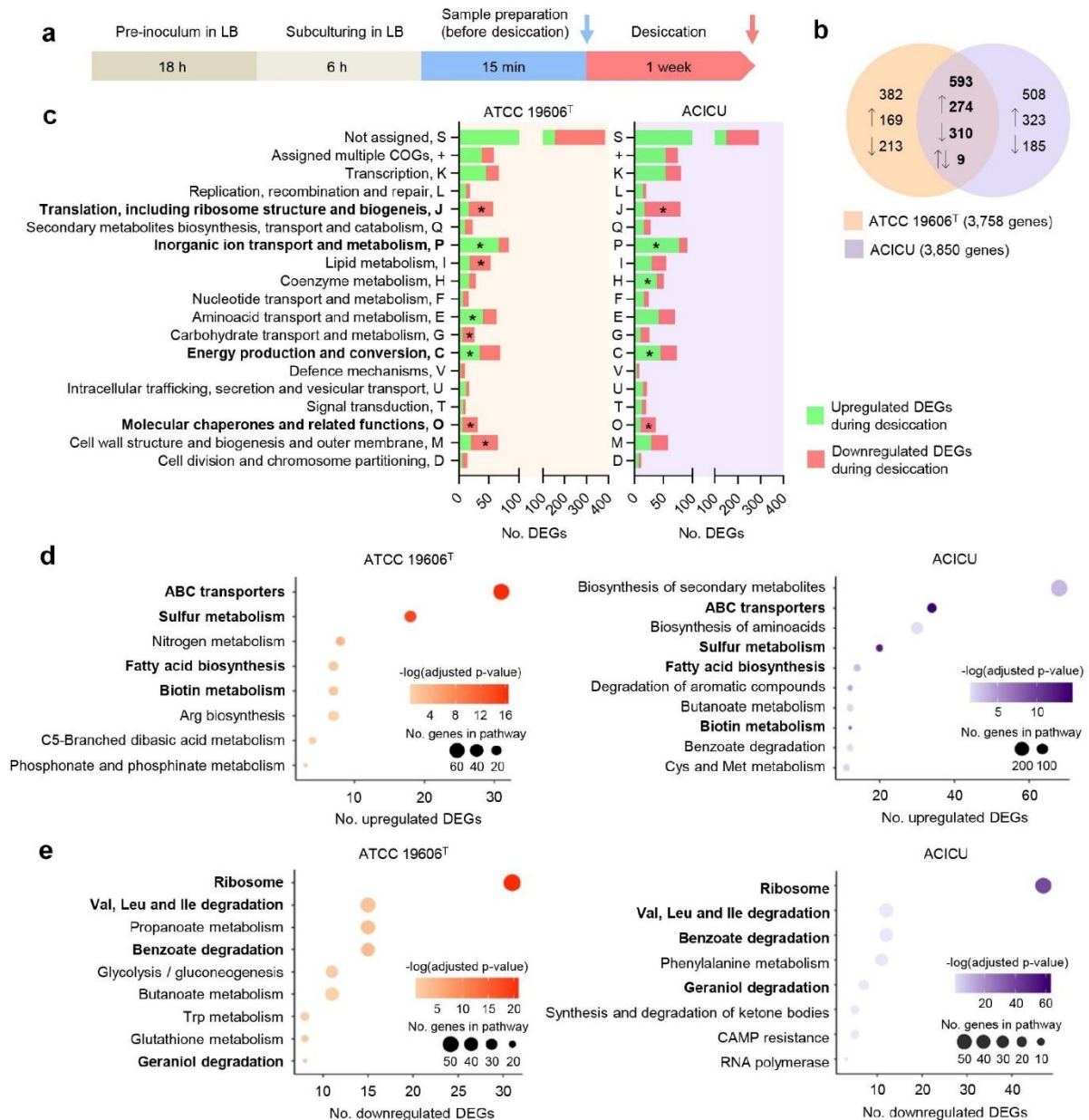


Figure 4. Analysis of the *A. baumannii* transcriptome before and after desiccation. (a) Timeline of sample collection for transcriptome analysis. Arrows indicate the sampling times of cells for RNA-seq before (cyan) and after (red) desiccation. (b) Venn diagram showing the number of DEGs in ATCC 19606^T and ACICU after 1-week desiccation. The number of shared DEGs is shown in bold. Upwards and downwards arrows indicate up- and down-regulated DEGs, respectively. (c) COG functional categories of ATCC 19606^T and ACICU DEGs in desiccated cells. Asterisks in green and red bars denote COGs enriched with up- and down-regulated DEGs, respectively. Shared categories are highlighted in bold. (d-e) KEGG pathway-based enrichment analysis of upregulated (d) and downregulated (e) DEGs of ATCC 19606^T and ACICU during desiccation. Shared pathways are highlighted in bold. Abbreviations: ABC, ATP-binding cassette; CAMP, cationic antimicrobial peptide.

(resulting from sulphite oxidation catalysed by CysJ) and O-acetylserine (generated from L-serine by CysE), respectively, both serving as substrates for the biosynthesis of L-cysteine and acetate (Figure 5a). Upregulation

of genes involved in L-cysteine biosynthesis was confirmed by reverse transcription-quantitative PCR (RT-qPCR) analysis of *cysU*, *sfnG*, *ssuE*, *cysJ*, and *cysE* transcripts. All five genes were upregulated in both ATCC

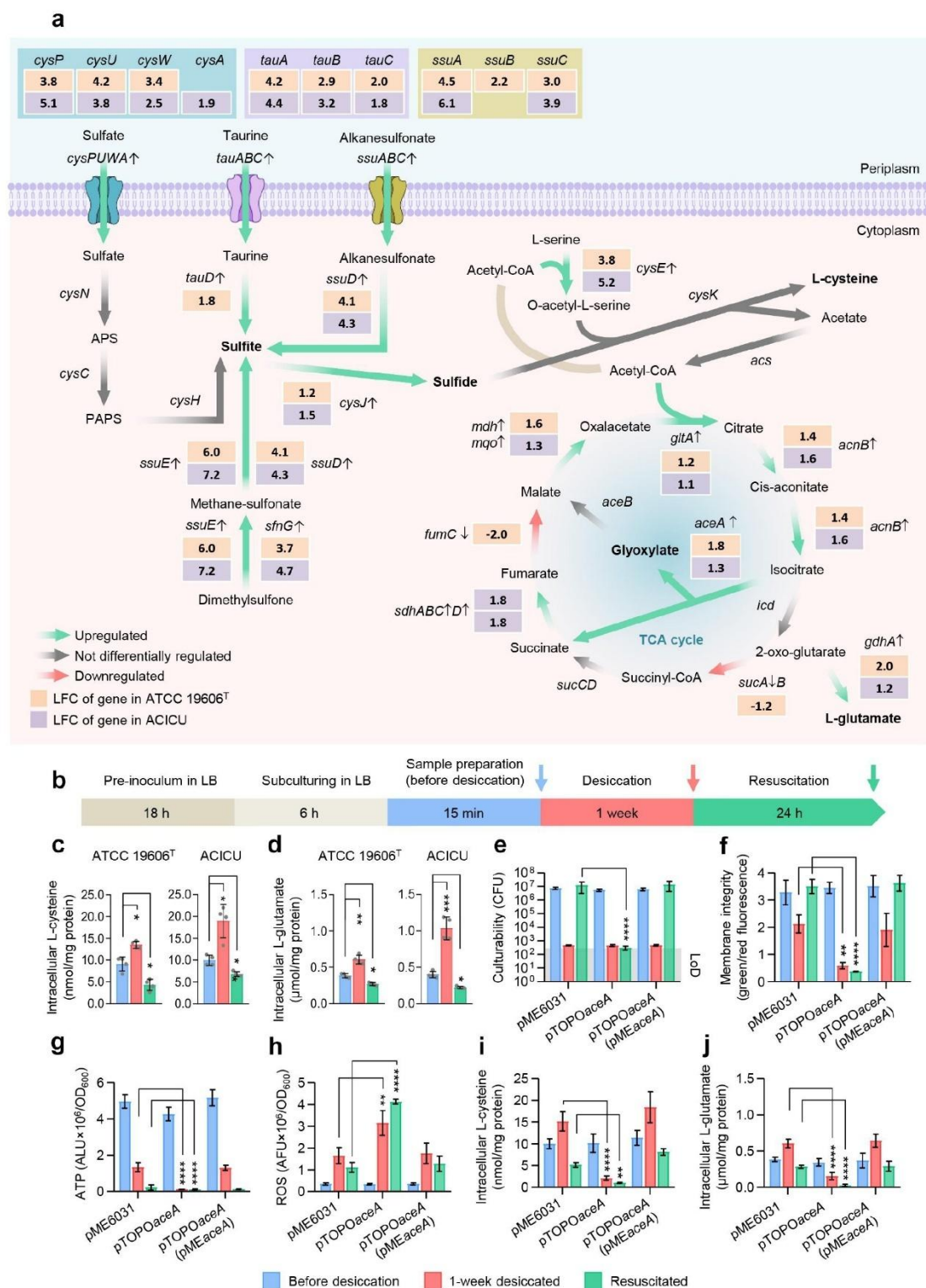


Figure 5. Role of L-cysteine, L-glutamate, and GS pathway in *A. baumannii* desiccation resistance. (a) Schematic representation of the sulfur metabolism, TCA, and GS pathway, with relevant metabolites in bold. Values in orange and purple boxes indicate the log₂ fold-change (LFC) of ATCC 19606^T and ACICU DEGs, respectively. The LFC value of non-DEGs is not shown. Gene, locus tag, and enzyme designations are provided in Figure S10. (b) Experimental timeline. Colored arrows indicate the sampling times for cells

19606^T and ACICU during desiccation, and downregulated or not differentially expressed during resuscitation (Figure S10), paralleling the increase of intracellular L-cysteine levels occurring during desiccation and its consumption during resuscitation (Figure 5b-c). Acetate is converted into acetyl-CoA by acetyl-CoA synthetase (Acs), serving as substrate for CysE or entering the tricarboxylic acid (TCA) cycle. Notably, DEGs involved in the glyoxylate shunt (GS) pathway, namely *gltA* (citrate synthase), *acnB* (aconitase), and *aceA* (isocitrate lyase), were upregulated during desiccation in both strains (Table S7). Moreover, the repression of *sucA* (α -ketoglutarate dehydrogenase) concomitant with the activation of *gdhA* (glutamate dehydrogenase) suggests a metabolic flux towards L-glutamate production from 2-oxo-glutarate (Table S7; Figure 5a). RT-qPCR analysis of *gltA*, *acnB*, *aceA*, and *gdhA* transcripts confirmed the upregulation of glutamate synthesis and GS pathway genes in desiccated cells of both ATCC 19606^T and ACICU and showed that these genes were expressed below the basal level in resuscitated cells (Figure S10). Accordingly, intracellular L-glutamate levels significantly increased during desiccation and decreased during resuscitation in both strains (Figure 5d), while the levels of trehalose and D-mannitol, two compatible solutes that accumulate during salt-induced stress [19], remained unchanged (Figure S11). Overall, gene expression analysis and chemical assays suggest that desiccated *A. baumannii* cells activate the GS pathway and overproduce L-cysteine and L-glutamate, whereas resuscitated cells shut off the GS pathway and consume both amino acids.

To substantiate the importance of the GS pathway in the *A. baumannii* response to desiccation, the *aceA* gene, encoding the key enzyme that deviates the TCA cycle to the GS pathway by converting isocitrate into glyoxylate plus succinate, was inactivated in ATCC 19606^T. Knock-out of *aceA* severely impaired the resuscitation efficiency without affecting post-desiccation culturability (Figure 5e). This result indicates that

activation of the GS pathway during desiccation ensures the recovery of the clonogenic ability upon resuscitation. Compared with the wild type, desiccated cells of the *aceA* mutant did not recover from membrane damage appearing extremely permeable to PI even after resuscitation (Figure 5f). The *aceA* knock-out also endured severe ATP, L-cysteine, and L-glutamate depletion, and increased ROS production (Figure 5g-j). Complementation *in trans* of the *aceA* mutation rescued the defect in resuscitation, improved membrane integrity, and restored wild-type levels of ATP, ROS, L-cysteine, and L-glutamate (Figure 5e-j).

Discussion

Microorganisms have the exceptional ability to endure harsh environments and adapt to changes in external conditions. Dehydration, induced by either water evaporation (desiccation or matric stress) or exposure to high solute concentrations (hyperosmotic stress), poses a serious challenge to microorganisms. Although both matric and hyperosmotic stresses result in water subtraction from the cell, these two stresses are mechanistically distinct. While dehydration can be obtained by exposing cells to a hypertonic solution, it should be noted that water is still physically present around the cell during hyperosmotic stress. Conversely, air-desiccated cells are not suspended in a water solution, implying that several vital functions are impaired as the result of water evaporation, particularly nutrient diffusion and solute transport, which are indeed essential for the hyperosmotic stress response [3,20]. These differences require distinct adaptive responses as evidenced by the accumulation of different metabolites: while D-mannitol and trehalose protect *A. baumannii* cells during hyperosmotic stress [19], we identified L-cysteine and L-glutamate as possible protectants during desiccation. Based on these differences, our experimental set-up was intended to mimic the *A. baumannii* response to air-drying that would occur when bacterial cells are shed in the hospital environment. To account,

before desiccation (cyan), after desiccation (red), and after resuscitation in M9SS $Mg^{2+}Ca^{2+}$ for 24 h at 37°C (green). (c) L-cysteine concentration in ATCC 19606^T and ACICU cells. (d) L-glutamate concentration in ATCC 19606^T and ACICU cells. (e) Culturability, (f) membrane integrity, (g) ATP content, (h) ROS levels, (i) intracellular L-cysteine, and (j) L-glutamate concentration in wild type and *aceA*-defective ATCC 19606^T strains before desiccation, after desiccation, and after resuscitation. The grey area in (e) indicates the LoD of CFU counts (4×10^2 CFU). Data in (c-j) are the mean $\pm$ SD (error bars) of at least three independent experiments. Statistical significance was determined by the unpaired *t*-test (**p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001). Asterisks in (c-d) indicate statistically significant differences relative to cells before desiccation. Asterisks in (e-j) indicate statistically significant differences between the test strain and ATCC 19606^T(pME6031).

at least in part, for the intra-species *A. baumannii* variability [3], experiments were conducted using the laboratory-adapted type strain ATCC 19606^T [21] and the epidemic isolate ACICU [22], which belongs to International Clonal Lineage II and represents the most widespread *A. baumannii* lineage [23].

While the drying process is a complex phenomenon impacting all macromolecular components of the cell [18], recent findings have linked the gradual loss of *A. baumannii* culturability occurring during desiccation to membrane gelification, a physical process in which phospholipids of both inner and outer membranes become densely packed, stiffening the membranes and leading to membrane ruptures [24,25]. At the functional level, we observed that desiccation causes progressive loss of membrane integrity with volume reduction, ATP depletion, and oxidative stress, ultimately resulting in a dramatic loss of clonogenicity in both ATCC 19606^T and ACICU strains. While the two *A. baumannii* model strains used in this study showed remarkable differences in the timing of their response to the desiccation, it is worth noting that >90% of the population became unculturable after 24-h desiccation on an inert glass substrate, disclosing structural and functional features suggestive of cell death. However, the whole non-culturable *A. baumannii* population, including apparently dead cells characterized by extensively compromised membrane integrity, was able to recover clonogenicity when suspended in a carbon-free physiological buffer, a typical feature of VBNC cells [12]. Resuscitation was optimal under conditions favouring membrane fluidity and stability, *i.e.* optimal temperature (37°C) and availability of membrane-stabilizing divalent cations (Ca²⁺ and Mg²⁺). Following desiccation, the reversible transition to the VBNC state was demonstrated for several *A. baumannii* clinical strains, as well as strains belonging to non-*A. baumannii* pathogenic species. One can speculate that resistance to desiccation and entrance in the VBNC state are effective survival strategies for species endowed with broad ecological fitness, allowing members of the *Acinetobacter* genus to face harsh conditions and thrive in diverse environments [1,26].

The consequences of the above findings on the transmissibility of *A. baumannii* infection in hospitals can be inferred by the ability of desiccated VBNC cells to resuscitate in some biological fluids and recover their virulence in the *G. mellonella* insect model of infection. The desiccation-induced transition to the VBNC state observed in pathogenic *Acinetobacter* species imposes a paradigm shift in interpreting

environmental surveillance results in healthcare settings. Infection control specialists should know that VBNC *Acinetobacter* cells constitute a reservoir of potentially infectious bacteria that cannot be detected in routine monitoring of hospital surfaces through direct culture-based methods.

Although the VBNC state has previously been associated with *A. baumannii* survival to hyperosmotic stress, the underlying molecular mechanisms have been elucidated only in part [10]. Our results suggest that *A. baumannii* extensively remodels its transcriptome when exposed to air-desiccation for one week, *i.e.* when the population consists of >99% VBNC cells. In this condition, comparative transcriptome analysis showed that *ca.* one-third of *A. baumannii* genes are differentially expressed with remarkable differences between ATCC 19606^T and ACICU, suggesting that *A. baumannii* adopts multiple strategies to face desiccation and enter the VBNC state. In search of common response mechanisms to desiccation, we focused on DEGs shared by the two strains, observing that both ATCC 19606^T and ACICU activate genes for the synthesis of L-cysteine and L-glutamate and shift their energetic metabolism towards the GS pathway. The metabolic overview provided by KEGG and COG analysis also denotes a reduction of protein synthesis (ko03010, ribosome) and expression of molecular chaperones (O category, molecular chaperones and related functions), consistent with prior findings [27,28]. Intriguingly, although DtpA and DtpB molecular chaperones appeared essential for resistance to desiccation in a Lon protease-deficient mutant of *A. baumannii* ATCC 17978 [9], in our experimental conditions *dtpA* and *dtpB* were not differentially expressed (Table S1). It is plausible that the expression of these two chaperones occurs at different stages of desiccation, is strain-specific, and/or restricted to the Δlon mutant. Even more intriguing is the observation that the catalase gene *katE*, previously hypothesized to play a role in the detoxification of ROS produced during desiccation [9], was downregulated in ATCC 19606^T and not differentially expressed in ACICU (Table S1), raising the possibility that *A. baumannii* employs alternative strategies to withstand the desiccation-induced oxidative stress. Production of antioxidants, like L-cysteine, is a viable defence strategy from oxidative stress, and we provide evidence that L-cysteine is accumulated during desiccation and is consumed during resuscitation. L-cysteine is known to protect bacteria from ROS through S-thiolation, a reversible post-translational modification that links low molecular weight thiols (*e.g.*, L-cysteine and glutathione) to protein sulfhydryl groups via disulfide bonds, safeguarding thiol-

containing proteins from irreversible oxidative damage [29,30].

Transcriptomic and chemical analyses revealed that *A. baumannii* cells overproduce the osmolyte L-glutamate during desiccation, consistent with the upregulation of the L-glutamate dehydrogenase (*gdhA*) gene. Notably, increased L-glutamate synthesis has previously been documented in VBNC cells of ATCC 19606^T induced by hyperosmolarity (Table S8; ref. [10]) and in nutrient-starved VBNC cells of *Vibrio parahaemolyticus* [31], suggesting that overproduction of L-glutamate is a general stress-response mechanism associated with the transition to a quiescence state. Among the *de novo* synthesized compatible solutes, L-glutamate has a broader function in bacterial stress response than trehalose and mannitol, whose function is limited to osmoprotection. Indeed, induction of L-glutamate synthesis has been associated with acid tolerance, growth under oxygen limitation, resistance to lantibiotics, and protection against oxidative stress [32].

A prominent role of the GS pathway in resuscitation from the VBNC state can be inferred from the expression analysis and mutagenesis of *aceA*. By shortcutting the TCA cycle into the GS pathway, desiccating *A. baumannii* cells would slow down their energetic metabolism, consistent with the established role of the GS pathway in the maintenance of the stress-induced latency and VBNC state in other bacterial pathogens [33–35].

It is interesting to notice that in *A. baumannii* the mutational inactivation of the GS pathway has no effect on clonogenicity after desiccation but impairs the resuscitation of desiccated (VBNC) cells. Wild type and *aceA*-mutant cells show a similar decay in clonogenicity after 1-week desiccation, but only the *aceA*-mutant does not resuscitate from the VBNC state. During desiccation, ATP consumption and ROS production increase in the *aceA* mutant compared with the wild type. Concomitantly, the synthesis of protective metabolites (L-cysteine and L-glutamate) is strongly reduced. These metabolic alterations are likely to impair the ability of the *aceA* mutant to relieve the desiccation stress, as shown by a further increase in ROS production and consumption of residual L-cysteine and L-glutamate levels during resuscitation. In addition, *aceA* mutant cells disclose more severe membrane damage during desiccation and are impaired in membrane repair during the resuscitation step. All these features suggest that, following desiccation, the *aceA* mutant cells are dead or enter a deeper state of dormancy that cannot be reversed under the resuscitation conditions of this study.

Master regulators controlling the expression of *A. baumannii* desiccation- and stress-responsive proteins are promising targets for the development of

alternative strategies against *A. baumannii* [36–39]. Since resuscitation from the VBNC state is essential for the virulence of desiccated *A. baumannii* cells, inhibition of AceA would represent a novel approach to impede *A. baumannii* resuscitation, “freezing” the cells in the less virulent VBNC state. Notably, itaconate, a lysosomal inducer produced by macrophages in response to bacterial infection [40] acts as a potent AceA inhibitor [41], showcasing how innate immunity has evolved AceA inhibitory strategies to combat bacterial infection.

Materials and methods

Bacterial strains and culture media

The bacterial strains used in this study are listed in Table S9. The *A. baumannii* type strain ATCC 19606 [21] and the *A. baumannii* strain ACICU, a prototype of the epidemic International Clonal Lineage II [22] were used for comparison. *A. baumannii* ATCC 19606^T and ACICU had formerly been categorized as “desiccation-sensitive” and “desiccation-resistant,” respectively [13,14]. Bacteria were routinely grown in Luria Bertani broth (LB; 42) or LB supplemented with 1.5% (w/v) agar (LA) at 37°C. Antibiotics were used at the following concentrations for *Escherichia coli* DH5α and *A. baumannii*, respectively: gentamicin (Gm) 10 mg/L and 100 mg/L; tetracycline (Tc) 12.5 mg/L and 100 mg/L; kanamycin (Km) 25 mg/L and 50 mg/L.

Preparation of competent cells

Competent *E. coli* cells were prepared by the rubidium-calcium chloride method and transformed according to the heat shock protocol [42]. Electrocompetent cells of *A. baumannii* were prepared as previously described [43], and plasmids were introduced in ATCC 19606^T and ACICU by electroporation, as reported in ref [43].

Bacterial growth measurements, culturability, and SYTO 9 and PI staining

ATCC 19606^T and ACICU were grown in LB and incubated at 37°C under shaking for 18 h. Bacterial cultures were sub-cultured (1:100 dilution) in a flask and incubated at 37°C under shaking. Bacterial growth was monitored for up to 24 h, measuring the optical density at 600 nm (OD_{600}) in a BioSpectrometer Basic spectrophotometer (Eppendorf). Aliquots of the cultures were collected after 6, 14, and 24 h for colony-forming units (CFU) counts on LA and LIVE/DEAD staining using the BacLight kit, containing SYTO 9 and PI dyes (Thermo Fisher),

according to the manufacturer's instructions. Fluorescence emissions of SYTO 9- and PI-stained samples were quantified using a Spark 10 M microplate reader (Tecan) at the excitation/emission wavelengths of 480/500 nm and 515/635 nm, respectively. Stained samples were observed by using a Leica SP5 inverted confocal laser-scanning microscope (CLSM) equipped with a 63× oil immersion objective.

Preparation of bacterial inocula and desiccation conditions

ATCC 19606^T and ACICU were grown in LB at 37°C for 18 h, diluted 1:100 in the same medium, and incubated at 37°C under shaking for 6 h. Then, bacteria were harvested by centrifugation (3,000 $g \times 5$ min), washed twice, and suspended in ultrapure distilled water at the desired OD₆₀₀. Aliquots of bacterial suspensions were air-dried under the laminar flow hood at $25.19 \pm 1.49^\circ\text{C}$ and stored in a 16 L-vacuum bell containing 50 g of silica gel at an average temperature of $20.88 \pm 0.59^\circ\text{C}$ and an average relative humidity (RH) of $13.00 \pm 5.58\%$. Bacterial suspensions were desiccated on the surface of glass coverslips (Corning cover glasses, Sigma-Aldrich), glass Petri dishes, and glass chamber slides (4-well on lumox detachable, Sarstedt).

Bacterial culturability during maintenance in water and after desiccation

ATCC 19606^T and ACICU inocula were diluted in ultrapure distilled water to OD₆₀₀ = 1.0 (corresponding to $ca. 5 \times 10^8$ CFU/mL for both strains). The bacterial suspensions (20 μL , $ca. 10^7$ CFU) were poured onto a glass coverslip and air-dried or maintained in ultrapure distilled water (15 mL) in 50 mL-polystyrene tubes (Falcon) and incubated at $20.88 \pm 0.59^\circ\text{C}$ for up to 8 weeks. Culturability of desiccated and water-maintained cells was evaluated by CFU counts on LA plates at 0 h, 6 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 4 weeks, and 8 weeks. Air-dried samples on glass coverslips were rehydrated in 2 mL of ultrapure distilled water, incubated for 15 min at room temperature, mixed by vortexing for 30 s, and 10-fold serially diluted in sterile saline for CFU counts, as previously described [13,14]. To evaluate bacterial culturability in water, 20- μL samples were appropriately diluted for CFU counts on LA plates.

PI toxicity and time-lapse microscopy assessment of bacterial clonogenicity

ATCC 19606^T(pVRL1gfp) and ACICU(pVRL1gfp) were suspended in ultrapure distilled water and air-dried. Culturability before and after desiccation was assessed

by CFU counts on LA. The OD₆₀₀ of samples before and after desiccation was adjusted to inoculate the same number of clonogenic cells ($ca. 10^5$ CFU/mL) in LB containing serial twofold PI dilutions (ranging from 120 μM to 30 μM). Bacterial growth (OD₆₀₀) at 37°C was monitored over time using the Spark 10 M (Tecan) microplate reader. The subinhibitory PI concentration (60 μM) was determined by comparison of growth profiles of desiccated and non-desiccated cells treated with increasing PI concentrations (30–120 μM).

Time-lapse fluorescence microscopy experiments were performed with a Leica SP5 CLSM equipped with a 63× oil immersion objective. ATCC 19606^T(pVRL1gfp) and ACICU(pVRL1gfp) were suspended in water, diluted to OD₆₀₀ = 0.5, and air-dried on glass coverslips for 0 h, 6 h, and 24 h. Desiccated bacteria were covered with a 0.1 mm thick layer of LB supplemented with 0.5% (w/v) agarose and 60 μM propidium iodide (PI), and placed in a CLSM incubation chamber at 37°C, 80% RH, and 20% P(O₂). Twenty μL of samples before air-drying were directly deposited on LB 0.5% (w/v) agarose supplemented with 60 μM PI and placed in the CLSM incubation chamber under the same conditions. Time-lapse microscopy images of at least 20 different fields of view (400 $\mu\text{m} \times 400 \mu\text{m}$) were acquired every 30 min for 4 h, since thereafter cells stopped dividing, probably due to lack of nutrients and/or phototoxicity. Due to the low probability of spotting clonogenic cells after long-term desiccation (see Results), 24 h was designated as the desiccation endpoint for clonogenicity assessment.

Automatic cell- and microcolony-counting was performed using CellProfiler [44]. Cells organized in clusters before incubation were manually identified and excluded from the analysis.

Quantification of GFP release during desiccation

ATCC 19606^T(pVRL1gfp) and ACICU(pVRL1gfp) were suspended in ultrapure distilled water and air-dried on glass Petri dishes for 6 h and 24 h. Cells before and after desiccation were diluted to OD₆₀₀ = 0.5 in ultrapure distilled water and centrifuged (3,000 $g \times 5$ min). Supernatants were collected to determine the emission spectra ranging from 500 to 530 nm with a resolution of 0.5 nm employing the LS-50B fluorimeter (PerkinElmer).

Membrane integrity assessment and resuscitation of desiccated cells in different media

Bacterial cells were diluted in ultrapure distilled water up to OD₆₀₀ = 1.0. Twenty μL of the bacterial suspensions were poured onto a glass coverslip and air-dried

for 0 h, 24 h, and 1 week. After desiccation, CFU counts were performed. Alternatively, air-dried bacteria were incubated at 37°C or 4°C for 24 h under shaking in 2 mL of the following resuscitation media: *i*) PBS without calcium chloride and magnesium chloride (Sigma-Aldrich); *ii*) M9 basal salt solution (M9SS) without carbon source (7 g/L Na₂HPO₄, 3 g/L KH₂PO₄, 0.5 g/L NaCl, 1 g/L NH₄Cl; 42); *iii*) PBS supplemented with 1 mM MgSO₄ and 0.2 mM CaCl₂ (PBS Mg²⁺Ca²⁺); *iv*) M9SS supplemented with 1 mM MgSO₄ and 0.2 mM CaCl₂ (M9SS Mg²⁺Ca²⁺; ref. [42]). After resuscitation, bacterial culturability was evaluated by CFU counts, and bacterial membrane integrity was assessed by SYTO 9/PI staining by using the LIVE/DEAD BacLight kit (Thermo Fisher). Similarly, bacterial culturability and membrane integrity were evaluated during resuscitation, harvesting cells after 6-, 12-, 24-, and 48-h incubation in M9SS Mg²⁺Ca²⁺ at 37°C.

Membrane integrity was also assessed by the Van susceptibility test. Briefly, ATCC 19606^T and ACICU were washed twice, suspended in ultrapure distilled water, and air-dried on glass Petri dishes for 24 h. An aliquot of desiccated cells was resuscitated for 24 h at 37°C in M9SS Mg²⁺Ca²⁺. Bacterial samples were resuspended at OD₆₀₀ = 0.001 (corresponding to ca. 5×10^5 CFU/mL) in 100 µL of cation-adjusted Mueller Hinton broth (CAMHB; Becton Dickinson) supplemented with increasing concentrations of vancomycin (Van; 512–0.5 mg/L). MIC was defined as the lowest concentration that completely inhibited bacterial growth as detected by the unaided eye, according to Clinical Laboratory Standards Institute recommendations (CLSI, 2024). For Van time-killing assay, bacterial samples were resuspended at OD₆₀₀ = 0.1 in saline and saline supplemented with 512 mg/L Van, incubated at 37°C, and plated on LA after 0, 3, 6, 9, and 12 h for CFU counts. For each time, the survival percentages were calculated by dividing the CFU obtained from the Van-treated sample by the CFU obtained from saline.

Plasmid stability

Bacterial cells before, after 1-week desiccation on glass Petri dishes, and after resuscitation at 37°C for 24 h in M9SS Mg²⁺Ca²⁺ were washed in distilled water and diluted at OD₆₀₀ = 1. CFU were determined on LA (N₀) and LA supplemented with the appropriate antibiotic (N_{Ant}). The plasmid stability percentage was expressed as (N_{Ant}/N₀)×100, as previously reported [43,45].

AFM analysis of bacterial cells and image processing

ATCC 19606^T and ACICU were diluted in ultrapure distilled water to OD₆₀₀ = 0.5. Twenty µL of bacterial suspensions were poured onto a microscope glass (Thermo Scientific SuperFrost Microscope slides 76×26 mm with 1 mm thickness) and imaged by AFM before and after desiccation, and after resuscitation. AFM measurements were carried out at 24°C using a Dimension ICON AFM (Bruker, Santa Barbara, USA) operating in the PeakForce mode, as previously described with minor modifications [46]. Briefly, the AFM cantilever (Bruker Scan-Asyst-Air) was set for “soft” nominal spring constant of 0.4 N/m and had a sharpened silicon tip with a nominal radius of 2 nm. The cantilever oscillation frequency and oscillation amplitude were set to 1 kHz and 150 nm, respectively. The force set point was optimized in the 15–25 nN range. The image size was 512 pixels/line/row, and the minimum lateral resolution was determined by the scan size. AFM images were analysed with Gwyddion Software v.248 [47]. All images were first-order flattened and set to the same range of height (0–900 nm) to facilitate comparison between conditions. After levelling, a metrological analysis was performed on at least 30 different randomly selected cells to measure their biophysical parameters. Briefly, cellular length and width were referred to as elongated and short horizontal axes, respectively. Cell height was determined based on the maximum height measured along the cell cross-section. Cell volume and surface area were obtained by the Gwyddion grain-analysis algorithm after individual cell masking.

Evaluation of ATP levels and ROS production in cells before desiccation, after desiccation, and after resuscitation

Bacterial cells were washed twice, suspended in ultrapure distilled water, and air-dried for 1 week. After desiccation, the samples were diluted and resuscitated in M9SS Mg²⁺Ca²⁺. The cellular ATP content was quantified by the BacTiter-Glo Microbial Cell Viability Assay (Promega) according to the manufacturer's instructions. Briefly, the cells taken before and after desiccation or after resuscitation were suspended in 50 µL of ultrapure distilled water, mixed with an equal volume of the BacTiter-Glo reagent, and incubated in the dark for 5 min. Then, luminescence was measured employing the Spark 10 M (Tecan)

microplate reader. The ATP content was expressed as the ratio between arbitrary luminescence units (ALU) and the OD₆₀₀ of the bacterial suspension. ROS levels were quantified by employing the 2,7-dichlorofluorescein diacetate (DCFDA)-based assay [48]. Cells taken before and after desiccation or after resuscitation were suspended in 100 µL of ultrapure distilled water containing 10 µM DCFDA. The fluorescence excitation/emission at 485/535 nm of DCFHDA and the OD₆₀₀ of samples were measured in a Spark 10 M (Tecan) microplate reader. ROS production was expressed as the ratio between arbitrary fluorescence unit (AFU) emission and OD₆₀₀.

Resuscitation in biological fluids

The biological fluids used in this study are residual samples from previous works [49,50]. An existing stock of heat-inactivated human serum (HIS) pooled from 125 healthy donors was used [49]. Urine and saliva specimens were previously collected from 10 healthy donors, pooled, and filter-sterilized [50]. ATCC 19606^T and ACICU cells were air-dried for 1 week on glass Petri dishes. Cells before and after desiccation were diluted at OD₆₀₀ = 0.1 in HIS, urine, saliva, or M9SS Mg²⁺Ca²⁺ supplemented with different two-fold serially diluted ciprofloxacin (Cip) concentrations ranging from 128 to 0.03 µg/mL. CFU counts were performed after 12-h incubation at 37°C with shaking.

Galleria mellonella infection assay

The LD₅₀ of ATCC 19606^T and ACICU, corresponding to ca. 5×10^6 and 5×10^5 CFU, respectively, was preliminarily determined [51]. Since ATCC 19606^T is more susceptible to desiccation than ACICU (see Results), to appreciate any difference between different resuscitation conditions, the *G. mellonella* killing assay was set up to inject the larvae with bacterial inocula causing ca. 50% lethality. To this purpose, ATCC 19606^T and ACICU were washed twice with ultrapure distilled water, air-dried for 24 h and 1 week, respectively, and then incubated in M9SS Mg²⁺Ca²⁺, HIS, urine, or saliva for 12 h at 37°C. After 24-h and 1-week desiccation, ATCC 19606^T and ACICU showed comparable levels of culturability (see Results). Fifth-instar *G. mellonella* larvae (0.406 ± 0.054 g) were injected with 10 µL of bacterial suspensions before air-drying, after desiccation, or after incubation in M9SS Mg²⁺Ca²⁺ or biological fluids at OD₆₀₀ = 0.1 (ca. 5×10^6 CFU) and 0.01 (ca. 5×10^5 CFU) for ATCC 19606^T and ACICU, respectively, corresponding to the LD₅₀ of the

two strains. The *G. mellonella* infection assay was performed as previously described [52], using sterile water as vehicle and control. A cohort of 120 larvae was injected for each experimental group. Larvae unresponsive to multiple tactile stimulations were considered dead.

Isolation and purification of bacterial RNA

ATCC 19606^T and ACICU cells were washed in ultrapure distilled water, suspended in ultrapure distilled water at OD₆₀₀ = 1.0, and 4 mL of suspension were uniformly spread on the surface of 78.5 cm² glass Petri dishes for one-week desiccation. Before and after desiccation, bacteria were directly suspended in 2 mL of RNA Protect Bacteria Reagent (Qiagen). Total RNA isolation was performed by using miRNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. RNA was purified with RNeasy MiniElute Cleanup Kit (Qiagen), and RNA samples were treated with TURBO DNase (Invitrogen) to remove genomic DNA. PCR reactions were performed to confirm the absence of DNA contamination by using the oligonucleotides 16S_FW and 16S_RV (Table S10). RNA concentration and purity were measured with a NanoDropTM spectrophotometer (Thermo Fisher Scientific). Three independent RNA isolation and purification experiments were performed for each condition.

Genome-wide expression analyses and functional annotations

The RNA-seq analysis, RNA quality assessment, library preparation, sequencing, and statistical analysis of the data were performed at the GENEWIZ Biotechnology Facility (GENEWIZ, Azenta Life Sciences Company, Leipzig, Germany), as previously described [53]. In particular, RNA samples were quantified using the Qubit 4.0 Fluorometer (Life Technologies), and RNA integrity was checked with an RNA kit on an Agilent 5300 Fragment Analyzer (Agilent Technologies). The average RNA integrity numbers $\pm$ SD, calculated on three biological replicates for each sample, were as follows: 9.15 ± 0.23 , ATCC 19606^T cells before desiccation; 9.13 ± 0.41 , ATCC 19606^T cells after 1-week desiccation; 8.95 ± 0.12 , ACICU cells before desiccation; 9.01 ± 0.21 , ACICU cells after 1-week desiccation. rRNA depletion was performed using the NEB Next rRNA Depletion kit (New England Biolabs). RNA sequencing library preparation was performed using the NEB Next Ultra II RNA Library Prep kit for Illumina, following the manufacturer's recommendations. The library

preparation was not directional. Briefly, enriched RNAs were fragmented according to the manufacturer's instructions. First-strand and second-strand cDNA were subsequently synthesized. cDNA fragments were end-repaired and adenylated at the 3' ends, and a universal adapter was ligated to cDNA fragments, followed by index addition and library enrichment with limited-cycle PCR. Sequencing libraries were validated using the NGS kit on the Agilent 5300 Fragment Analyzer (Agilent Technologies) and quantified using the Qubit 4.0 Fluorometer. The sequencing libraries were multiplexed and loaded onto the flow cell on the Illumina NovaSeq 6000 instrument, according to the manufacturer's instructions. The samples were sequenced using a 2 × 150 Pair-End configuration v1.5. Image analysis and base calling were conducted by NovaSeq Control Software v1.7 on the NovaSeq instrument. Raw sequence data (.bcl files) generated from Illumina NovaSeq were converted into fastq files and demultiplexed using the Illumina bcl2fastq program v2.20. One mismatch was allowed for index sequence identification. After investigating the quality of the raw data, sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v0.36. The trimmed reads were mapped to the reference genome using the Bowtie2 aligner v2.2.6. BAM files were generated as a result of this step. Unique gene hit counts were calculated using feature counts from the Subread package v1.5.2. Only unique reads that fell within gene regions were counted. After gene hit counts extraction, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the customer-defined groups of samples was performed. All RNA-seq reads were aligned to the genome of ATCC 19606^T (NZ_CP058289.1) and ACICU (NZ_CP031380.1) for data analysis. The Wald test was used to generate p-values and log₂ fold-change (LFC). Only genes with an adjusted p-value (p adj) < 0.05 were considered differentially expressed genes (DEGs). Plasmid genes were excluded for the comparative gene expression analysis between ATCC 19606^T and ACICU.

The EggNOG-mapper v5.0 [54] was used to determine the COG categories [55] and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways [56]. All selected DEGs were visualized within their relative metabolic pathway using the KEGG Mapper-Reconstruct tool, in the KEGG pathway database [56]. Enrichment analysis of DEGs in COG

categories and KEGG pathways was performed with the "enrichr" function from the clusterProfiler package v4.0 [57]. Annotations of EggNOG-mapper v5.0 (.emapper.annotations) were used to develop the corresponding reference databases. p-values were adjusted for multiple comparisons using the false discovery rate algorithm. The bubble plots illustrating significantly enriched pathways (p-value < 0.05) were drawn with the R package ggplot2.

Reverse-transcriptase quantitative PCR analysis

To determine RNA expression levels of the *cysU*, *sfnG*, *ssuE*, *cysJ*, *cysE*, *gltA*, *acnB*, *aceA*, and *gdhA* genes, cDNA synthesis was performed using the iScript Reverse Transcription Supermix Kit (Bio-Rad). RT-qPCR reactions were performed using the iTaq Universal SYBR Green Supermix (Bio-Rad). Gene-specific primers used for the analysis are listed in Table S10. The 16S rRNA gene was used as an internal control to normalize the RT-qPCR data and to calculate the relative fold change in gene expression by using the $2^{-\Delta\Delta CT}$ method, as previously described [52]. RT-qPCR analysis was performed on three different RNA pools for each condition (biological replicates).

Disruption of the *aceA* gene and genetic complementation of the *aceA* mutation

A 517-bp DNA fragment internal to the ATCC 19606^T *aceA* gene (HTZ92_RS12445) was generated by PCR with *aceATOPO_FW* and *aceATOPO_RV* primers (Table S10) for direct cloning in pCR-BluntII-TOPO [58], yielding pTOPO*aceA* (Table S9). pTOPO*aceA* was used as an integrative suicide vector in ATCC 19606^T, and the *aceA* knock-out mutants were selected on LA plates containing Km. Disruption of *aceA* was verified by PCR using the M13_FW and *aceATOPO_RV* primers (Table S10) and amplicon sequencing. The *aceA* knock-out mutant, designated ATCC 19606^T pTOPO*aceA*, was grown in the presence of Km to stabilize the *aceA* mutation [58]. To complement the *aceA* mutation, the entire *aceA* gene with its endogenous promoter was amplified from ATCC 19606^T genomic DNA with primers listed in Table S10. The 2107-bp amplicon was digested using BamHI and EcoRI and cloned into the BglII and EcoRI sites of pME6031 [59], yielding the pME*aceA* plasmid (Table S9). pME*aceA* was introduced in ATCC 19606^T(pTOPO*aceA*) by electrotransformation.

Quantification of compatible solutes and L-cysteine

Bacterial cells before, after 1-week desiccation on glass Petri dishes, and after resuscitation at 37°C for 24 h in M9SS $Mg^{2+}Ca^{2+}$ were diluted at $OD_{600} = 2.0$ in 1 mL of distilled water. Bacterial suspensions were centrifuged ($3,000\text{ g} \times 5\text{ min}$), and pellets were stored at -80°C until used. An aliquot of the bacterial pellet was used to quantify the total protein content with the Coomassie protein assay reagent (Sigma Aldrich) [52]. Another aliquot was frozen at -80°C and thawed at room temperature three times to facilitate cell lysis. For quantification of compatible solutes, the intracellular concentrations of L-glutamate, trehalose, and D-mannitol were determined using L-Glutamic Acid Assay Kit (Megazyme, Neogene), Trehalose Assay Kit (Megazyme, Neogene), and D-Mannitol Assay Kit (Megazyme, Neogene), respectively, following the manufacturer's instructions. Intracellular L-cysteine was quantified using Cysteine Assay Kit (Fluorometric) (Sigma-Aldrich), according to the manufacturer's instructions. The levels of L-glutamate, trehalose, D-mannitol, and L-cysteine were normalized to the total protein content of each sample.

Statistical analysis

Statistical analysis was performed with the GraphPad InStat software v8.0. Survival curves were generated by the Kaplan–Meier method, and the significance of survival differences was estimated by the log rank test. Differences having a p -value ≤ 0.05 were considered statistically significant. All *in vitro* experiments were analysed using a two-tailed unpaired student's t -test.

Ethical statement

The use of human-derived samples was conducted according to the Helsinki Declaration statement of ethical principles. In particular, the biological fluids used in this study are by-products from previous works, upon approval by the review board of Policlinico Umberto I, Sapienza University, Rome [49,50]. Human serum, urine, and saliva samples were obtained from healthy donors, following illustration, approval, and subscription of informed consent. Then, samples were anonymously submitted to the laboratory, pooled, and used for experimental purposes.

Acknowledgements

A preliminary version of this manuscript has been published as a pre-print on SSRN (doi: 10.2139/ssrn.4731192; licenced under CC BY 4.0). Data from this paper were also presented as a conference talk with interim findings at the “13th Symposium on the Biology of Acinetobacter,” and the corresponding abstract was published at <https://13acinetobacter.org/nideia.pt/wp/book-of-abstracts-2/>.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was partly supported by the Excellence Department grant [art. 1, comma 314–337 Legge 232/2016] to the Department of Science, Roma Tre University from Ministero dell'Università e della Ricerca and by grant A0375-2020-36558 GAVAP from Regione Lazio to PV. PV also acknowledges the financial support of Rome Technopole [grant F83B22000040006] for transcriptomic analyses. ML was supported by a NBFC fellowship [grant CN00000033] funded by Ministero dell'Università e della Ricerca. The funders had no role in study design, data collection, interpretation, or the decision to submit the work for publication.

CRedit authorship contribution statement

Lucidi Massimiliano: Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing.

Capecchi Giulia: Data curation, Investigation, Methodology, Writing – original draft.

Spagnoli Cinzia: Data curation, Investigation, Methodology.

Basile Arianna: Data curation, Investigation.

Artuso Irene: Data curation, Investigation.

Persichetti Luca: Data curation, Investigation.

Fardelli Elisa: Data curation, Investigation.

Capellini Giovanni: Supervision, Validation, Writing – review and editing.

Visaggio Daniela: Conceptualization, Project administration, Supervision, Writing – original draft.

Imperi Francesco: Conceptualization, Validation, Writing – review and editing.

Rampioni Giordano: Conceptualization, Validation, Writing – review and editing.

Leoni Livia: Conceptualization, Validation, Writing – review and editing.

Visca Paolo: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – review and editing.

Lucidi Massimiliano and Capecchi Giulia contributed equally to this study as co-first authors.

All authors have read and approved the manuscript.

Data availability statement

Raw data that support the findings of this study, the Supplementary Tables S1–S10, and the Supplementary Video S1 and S2 are openly available in the Open Science Framework (OSF) repository at <https://osf.io/g9rf4/> (DOI: 10.17605/OSF.IO/G9RF4). Transcriptomic data have been deposited in the Gene Expression Omnibus database under the accession number GSE249021.

ORCID

Massimiliano Lucidi  <http://orcid.org/0000-0003-3238-9164>
 Giulia Capecci  <http://orcid.org/0000-0002-0304-6712>
 Luca Persichetti  <http://orcid.org/0000-0001-6578-254X>
 Elisa Fardelli  <http://orcid.org/0000-0002-4181-1841>
 Giovanni Capellini  <http://orcid.org/0000-0002-5169-2823>
 Daniela Visaggio  <http://orcid.org/0000-0002-3192-8974>
 Giordano Rampioni  <http://orcid.org/0000-0002-1735-8565>
 Livia Leoni  <http://orcid.org/0000-0002-2046-4297>
 Paolo Visca  <http://orcid.org/0000-0002-6128-7039>

References

- [1] Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol*. 2007;5(12):939–951. doi: 10.1038/nrmicro1789
- [2] Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. 2018;18:318–327. doi: 10.1016/S1473-3099(17)30753-3
- [3] Lucidi M, Visaggio D, Migliaccio A, et al. Pathogenicity and virulence of *Acinetobacter baumannii*: factors contributing to the fitness in healthcare settings and the infected host. *Virulence*. 2024;15(1):2289769. doi: 10.1080/21505594.2023.2289769
- [4] Zhang H, Zhang Y, Wu J, et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. *Emerg Microbes Infect*. 2020;9(1):1958–1964. doi: 10.1080/22221751.2020.1812437
- [5] Ellis RC, Roberts EK, Grier JT, et al. *Acinetobacter baumannii* infections that are resistant to treatment: warning signs from the COVID-19 pandemic. *Future Microbiol*. 2022;17(17):1345–1347. doi: 10.2217/fmb-2022-0153
- [6] van den Broek PJ, Arends J, Bernards AT, et al. Epidemiology of multiple *Acinetobacter* outbreaks in the Netherlands during the period 1999–2001. *Clin Microbiol Infect*. 2006;12:837–843. doi: 10.1111/j.1469-0691.2006.01510.x
- [7] Doughty EL, Liu H, Moran RA, et al. Endemicity and diversification of carbapenem-resistant *Acinetobacter baumannii* in an intensive care unit. *Lancet Reg Health West Pac*. 2023;37:100780. doi: 10.1016/j.lanwpc.2023.100780
- [8] Zeidler S, Müller V. Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environ Microbiol*. 2019;21(7):2212–2230. doi: 10.1111/1462-2920.14565
- [9] Green ER, Fakhoury JN, Monteith AJ, et al. Bacterial hydrophilins promote pathogen desiccation tolerance. *Cell Host Microbe*. 2022;30(7):975–987.e7. doi: 10.1016/j.chom.2022.03.019
- [10] König P, Wilhelm A, Schaudinn C, et al. The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *MBio*. 2023;14:e0213923. doi: 10.1128/mbio.02139-23
- [11] Rawlinson S, Ciric L, Cloutman-Green E. How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. *J Hosp Infect*. 2019;103:363–374. doi: 10.1016/j.jhin.2019.07.015
- [12] Liu J, Yang L, Kjellerup BV, et al. Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. *Trends Microbiol*. 2023;31(10):1013–1023. doi: 10.1016/j.tim.2023.04.009
- [13] Jawad A, Seifert H, Snelling AM, et al. Survival of *Acinetobacter baumannii* on dry surfaces: comparison of outbreak and sporadic isolates. *J Clin Microbiol*. 1998;36(7):1938–1941. doi: 10.1128/JCM.36.7.1938-1941.1998
- [14] Giannouli M, Antunes LCS, Marchetti V, et al. Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I–III and to the emerging genotypes ST25 and ST78. *BMC Infect Dis*. 2013;13(1):282. doi: 10.1186/1471-2334-13-282
- [15] Sabatini A, Lucidi M, Ciolli S, et al. Innate immune mechanisms promote human response to *Acinetobacter baumannii* infection. *Eur J Immunol*. 2024;54(11):e2451170. doi: 10.1002/eji.202451170
- [16] Yang F, Moss LG, Phillips GN. The molecular structure of green fluorescent protein. *Nat Biotechnol*. 1996;14(10):1246–1251. doi: 10.1038/nbt1096-1246
- [17] Geisinger E, Mortman NJ, Dai Y, et al. Antibiotic susceptibility signatures identify potential antimicrobial targets in the *Acinetobacter baumannii* cell envelope. *Nat Commun*. 2020;11(1):4522. doi: 10.1038/s41467-020-18301-2
- [18] Lebre PH, De Maayer P, Cowan DA. Xerotolerant bacteria: surviving through a dry spell. *Nat Rev Microbiol*. 2017;15:285–296. doi: 10.1038/nrmicro.2017.16
- [19] Zeidler S, Müller V. Unusual deprivation of compatible solutes in *Acinetobacter baumannii*. *Environ Microbiol*. 2020;22:1370–1380. doi: 10.1111/1462-2920.14951
- [20] Bosch J, Varliero G, Hallsworth JE, et al. Microbial anhydrobiosis. *Environ Microbiol*. 2021;23(11):6377–6390. doi: 10.1111/1462-2920.15699
- [21] Artuso I, Lucidi M, Visaggio D, et al. Genome diversity of domesticated *Acinetobacter baumannii* ATCC 19606^T strains. *Microb Genom*. 2022;8. doi: 10.1099/mgen.0.000749
- [22] Iacono M, Villa L, Fortini D, et al. Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. *Antimicrob Agents Chemother*. 2008;52(7):2616–2625. doi: 10.1128/AAC.01643-07

- [23] Roberts LW, Hoi LT, Khokhar FA, et al. Genomic characterisation of multidrug-resistant *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in two intensive care units in Hanoi, Vietnam: a prospective observational cohort study. *Lancet Microbe*. 2022;3:e857–66. doi: 10.1016/S2666-5247(22)00181-1
- [24] Fardelli E, Lucidi M, Di Gioacchino M, et al. Biophysical mechanisms of dehydrating membranes of *Acinetobacter baumannii* linked to drought-resistance. *Biochim et Biophys Acta (BBA) - Biomembr*. 2022;1864(12):184045. doi: 10.1016/j.bbamem.2022.184045
- [25] Fardelli E, Di Gioacchino M, Lucidi M, et al. Evidence of correlation between membrane phase transition and clonogenicity in dehydrating *Acinetobacter baumannii*: a combined micro-Raman and AFM study. *J Phys Chem B*. 2024;128(28):6806–6815. doi: 10.1021/acs.jpcc.4c01246
- [26] Pulami D, Kämpfer P, Glaeser SP. High diversity of the emerging pathogen *Acinetobacter baumannii* and other *Acinetobacter* spp. in raw manure, biogas plants digestates, and rural and urban wastewater treatment plants with system specific antimicrobial resistance profiles. *Sci Total Environ*. 2023;859:160182. doi: 10.1016/j.scitotenv.2022.160182
- [27] Gayoso CM, Mateos J, Méndez JA, et al. Molecular mechanisms involved in the response to desiccation stress and persistence in *Acinetobacter baumannii*. *J Proteome Res*. 2014;13:460–476. doi: 10.1021/pr400603f
- [28] Wang X, Cole CG, Cd D, et al. Protein aggregation is associated with *Acinetobacter baumannii* desiccation tolerance. *Microorganisms*. 2020;8:343. doi: 10.3390/microorganisms8030343
- [29] Ansong C, Wu S, Meng D, et al. Top-down proteomics reveals a unique protein S-thiolation switch in *Salmonella* Typhimurium in response to infection-like conditions. *Proc Natl Acad Sci USA*. 2013;110:10153–10158. doi: 10.1073/pnas.1221210110
- [30] Loi VV, Rossius M, Antelmann H. Redox regulation by reversible protein S-thiolation in bacteria. *Front Microbiol*. 2015;6:187. doi: 10.3389/fmicb.2015.00187
- [31] Wagley S, Morcrette H, Kovacs-Simon A, et al. Bacterial dormancy: a subpopulation of viable but non-culturable cells demonstrates better fitness for revival. *PloS Pathog*. 2021;17(1):e1009194. doi: 10.1371/journal.ppat.1009194
- [32] Feehily C, Karatzas KAG. Role of glutamate metabolism in bacterial responses towards acid and other stresses. *J Appl Microbiol*. 2013;114(1):11–24. doi: 10.1111/j.1365-2672.2012.05434.x
- [33] Nandakumar M, Nathan C, Rhee KY. Isocitrate lyase mediates broad antibiotic tolerance in *Mycobacterium tuberculosis*. *Nat Commun*. 2014;5:4306. doi: 10.1038/ncomms5306
- [34] Bhusal RP, Barr JJ, Subedi D. A metabolic perspective into antimicrobial tolerance and resistance. *Lancet Microbe*. 2022;3:e160–1. doi: 10.1016/S2666-5247(22)00006-4
- [35] Qi Z, Sun N, Liu C. Glyoxylate cycle maintains the metabolic homeostasis of *Pseudomonas aeruginosa* in viable but nonculturable state induced by chlorine stress. *Microbiol Res*. 2023;270:127341. doi: 10.1016/j.micres.2023.127341
- [36] Farrow JM, Wells G, Pesci EC. Desiccation tolerance in *Acinetobacter baumannii* is mediated by the two-component response regulator BfmR. *PLOS ONE*. 2018;13:e0205638. doi: 10.1371/journal.pone.0205638
- [37] Perez Mora B, Giordano R, Permingeat V, et al. BfmRS encodes a regulatory system involved in light signal transduction modulating motility and desiccation tolerance in the human pathogen *Acinetobacter baumannii*. *Sci Rep*. 2023;13:175. doi: 10.1038/s41598-022-26314-8
- [38] Oda Y, Shapiro MM, Lewis NM, et al. CsrA-controlled proteins reveal new dimensions of *Acinetobacter baumannii* desiccation tolerance. *J Bacteriol*. 2022;204(4):e0047921. doi: 10.1128/jb.00479-21
- [39] Maharjan RP, Sullivan GJ, Adams FG, et al. DksA is a conserved master regulator of stress response in *Acinetobacter baumannii*. *Nucleic Acids Res*. 2023;51(12):6101–6119. doi: 10.1093/nar/gkad341
- [40] Zhang Z, Chen C, Yang F, et al. Itaconate is a lysosomal inducer that promotes antibacterial innate immunity. *Mol Cell*. 2022;82(15):2844–2857.e10. doi: 10.1016/j.molcel.2022.05.009
- [41] Chen M, Sun H, Boot M, et al. Itaconate is an effector of a Rab GTPase cell-autonomous host defense pathway against *Salmonella*. *Science*. 2020;369:450–455. doi: 10.1126/science.aaz1333
- [42] Sambrook EF, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 1989.
- [43] Lucidi M, Visaggio D, Prencipe E, et al. New shuttle vectors for real-time gene expression analysis in multidrug-resistant *Acinetobacter* species: *in vitro* and *in vivo* responses to environmental stressors. *Appl Environ Microbiol*. 2019;85(18):e01334–19. doi: 10.1128/AEM.01334-19
- [44] Carpenter AE, Jones TR, Lamprecht MR, et al. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biol*. 2006;7(10):R100. doi: 10.1186/gb-2006-7-10-r100
- [45] Lucidi M, Runci F, Rampioni G, et al. New shuttle vectors for gene cloning and expression in multidrug-resistant *Acinetobacter* species. *Antimicrob Agents Chemother*. 2018;62:e02480–17. doi: 10.1128/AAC.02480-17
- [46] Bashiri S, Lucidi M, Visaggio D, et al. Growth phase- and desiccation-dependent *Acinetobacter baumannii* morphology: an atomic force microscopy investigation. *Langmuir*. 2021;37:1110–1119. doi: 10.1021/acs.langmuir.0c02980
- [47] Nečas D, Klapetek P. Gwyddion: an open-source software for SPM data analysis. *Open Phys*. 2012;10:181–188. doi: 10.2478/s11534-011-0096-2
- [48] Eruslanov E, Kusmartsev S. Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods Mol Biol*. 2010;594:57–72. doi: 10.1007/978-1-60761-411-1_4
- [49] Runci F, Bonchi C, Frangipani E, et al. *Acinetobacter baumannii* biofilm formation in human serum and disruption by gallium. *Antimicrob Agents Chemother*. 2017;61(1):e01563–16. doi: 10.1128/AAC.01563-16

- [50] Visaggio D, Pirolo M, Frangipani E, et al. A highly sensitive luminescent biosensor for the microvolumetric detection of the *Pseudomonas aeruginosa* siderophore pyochelin. *ACS Sens.* 2021;6(9):3273–3283. doi: 10.1021/acssensors.1c01023
- [51] Antunes LCS, Imperi F, Carattoli A, et al. Deciphering the multifactorial nature of *Acinetobacter baumannii* pathogenicity. *PLOS ONE.* 2011;6:e22674. doi: 10.1371/journal.pone.0022674
- [52] Runci F, Gentile V, Frangipani E, et al. Contribution of active iron uptake to *Acinetobacter baumannii* pathogenicity. *Infect Immun.* 2019;87:e00755–18. doi: 10.1128/IAI.00755-18
- [53] Letizia M, Mellini M, Fortuna A, et al. PqsE expands and differentially modulates the RhlR quorum sensing regulon in *Pseudomonas aeruginosa*. *Microbiol Spectr.* 2022;10(3):e0096122. doi: 10.1128/spectrum.00961-22
- [54] Huerta-Cepas J, Szklarczyk D, Heller D, et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res.* 2019;47:D309–14. doi: 10.1093/nar/gky1085
- [55] Galperin MY, Kristensen DM, Makarova KS, et al. Microbial genome analysis: the COG approach. *Brief Bioinform.* 2019;20:1063–1070. doi: 10.1093/bib/bbx117
- [56] Kanehisa M, Sato Y, Kawashima M. KEGG mapping tools for uncovering hidden features in biological data. *Protein Sci.* 2022;31(1):47–53. doi: 10.1002/pro.4172
- [57] Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb).* 2021;2:100141. doi: 10.1016/j.xinn.2021.100141
- [58] Aranda J, Poza M, Pardo BG, et al. A rapid and simple method for constructing stable mutants of *Acinetobacter baumannii*. *BMC Microbiol.* 2010;10(1):279. doi: 10.1186/1471-2180-10-279
- [59] Heeb S, Itoh Y, Nishijyo T, et al. Small, stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. *Mol Plant Microbe Interact.* 2000;13:232–237. doi: 10.1094/MPMI.2000.13.2.232

Raw data that support the findings of this study, the Supplementary Tables S1-S10, and the Supplementary Videos S1 and S2 are openly available in the Open Science Framework (OSF) repository at <https://osf.io/g9rf4/> (DOI: 10.17605/OSF.IO/G9RF4).

Chapter 4. Transcriptomic landscape associated with the resuscitation of viable but not culturable *Acinetobacter baumannii* cells

Cinzia Spagnoli¹, Dillon E. Kunkle^{2,3}, Daniela Visaggio^{1,4,5}, Paolo Visca^{1,4,5}, Eric P. Skaar^{2,3,6}

¹Department of Science, Roma Tre University, Rome, Italy

²Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

³Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University Medical Center, Nashville, TN, USA

⁴NBFC, National Biodiversity Future Center, Palermo, Italy

⁵Santa Lucia Foundation IRCCS, Rome, Italy

⁶Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

Manuscript in preparation

Publication synopsis

A. baumannii has emerged as one of the most resilient pathogens in healthcare settings, combining multidrug resistance with an extraordinary ability to survive under harsh conditions. In the previous chapter (Chapter 3, Publication I), it was demonstrated that desiccation, a stress commonly encountered by bacteria colonizing hospital surfaces, drives *A. baumannii* into a VBNC state. This dormant phenotype allows cells to remain metabolically active while eluding detection by conventional culture-based methods (Li *et al.*, 2014), creating a hidden reservoir of bacteria with a potential impact on infection control practices.

The VBNC state is reversible, as dormant cells can regain culturability after a recovery period or upon exposure to specific stimuli (Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). This process, known as “resuscitation”, involves physiological changes that ultimately restore the ability to grow (form colonies) on nutrient media (Ramamurthy *et al.*, 2014). Importantly, VBNC cells retain virulence and can cause severe infections once resuscitated in a suitable host, underscoring the clinical relevance of this phenomenon (Ramamurthy *et al.*, 2014; Lucidi *et al.*, 2025). Despite its relevance, the molecular basis enabling the transition of *A. baumannii* from the dormant VBNC state to active growth is still unexplored.

This chapter aims to bridge this gap by investigating the global transcriptional changes that occur during resuscitation of desiccation-induced VBNC cells of *A. baumannii* ATCC 19606^T. To this purpose, a genome-wide transcriptional approach was applied to identify functional pathways supporting the transition from dormancy to active growth. Comparative analysis of transcriptomic profiles between resuscitated and desiccated cells revealed a distinct set of genes specifically associated with the recovery process. Functional analysis indicated that DNA repair pathways were predominant among the resuscitation-specific genes, indicating that maintenance of genome integrity is a critical requirement for successful recovery of growth capabilities. In addition, functional assays demonstrated that a component of DNA repair systems plays a pivotal role in restoring culturability, linking genome integrity to the resuscitation of VBNC cells. To assess the contribution of the DNA repair system to bacterial virulence and infection, its impact was evaluated using an *in vivo* murine pulmonary infection model.

By integrating transcriptomic data with phenotypic assays, this study points to the DNA repair system as a key determinant of successful resuscitation and highlights its role in restoring virulence after desiccation.

ABSTRACT

Acinetobacter baumannii is a leading cause of hospital-acquired infections, where outbreaks are fuelled by its ability to persist on dry surfaces in a viable but non-culturable (VBNC) state. While the molecular basis of entry in the VBNC state has been explored, the molecular and cellular mechanisms enabling recovery from the VBNC state remain unknown. Here, we show that resuscitation of desiccation-induced VBNC cells is an active process requiring extensive transcriptional reprogramming. Genome-wide analysis revealed strong induction of genes involved in DNA repair, replication, and transcription, with *uvrA*, encoding the sensor of the nucleotide excision repair system, emerging as one of the most highly upregulated and resuscitation-specific genes. Knock-out of *uvrA* abolished resuscitation of VBNC cells and sensitized cells to genotoxic stress. Promoter activity assays confirmed progressive *uvrA* activation during resuscitation, underscoring the role of UvrA in repairing desiccation-induced DNA lesions. Using a murine pneumonia model, we found that resuscitation restores, and even enhances virulence compared to pre-desiccation conditions in the wild type, while the *uvrA* mutant remains attenuated. These findings reveal that recovery from dormancy is DNA repair-dependent and suggest that resuscitation may transiently potentiate pathogenicity. By identifying UvrA as a player in both stress adaptation and infection, this work uncovers a critical determinant of *A. baumannii* persistence and pathogenesis and highlights DNA repair as a potential target to limit both environmental survival and pathogenicity.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen and a leading cause of severe nosocomial infections (Morris *et al.*, 2019; Lucidi *et al.*, 2024; Antochévis *et al.*, 2025). *A. baumannii* is responsible for ventilator-associated pneumonia, urinary tract infection, bacteremia, meningitis, and skin or soft tissue infections (Peleg *et al.*, 2008; Wong *et al.*, 2017). Over the last decades, *A. baumannii* has consolidated its role as a major pathogen in Intensive Care Units (ICUs), frequently affecting critically ill patients and causing infections associated with high mortality rates (Jiang *et al.*, 2022; Antochévis *et al.*, 2025). *A. baumannii* infections are complicated by intrinsic antibiotic resistance, with many clinical isolates exhibiting acquired multidrug resistance (Cavallo *et al.*, 2023). Carbapenem-resistant *A. baumannii* has been designated by the World Health Organization (WHO) as a critical priority pathogen and an urgent public health threat (WHO, 2024). The widespread incidence of multidrug-resistant strains underscores the urgent need for innovative therapeutics and new antimicrobial strategies (Antochévis *et al.*, 2025).

Managing *A. baumannii* infections is further challenged by its persistence in the hospital environment and resilience against disinfection (Sunenshine *et al.*, 2007; Tipton *et al.*, 2018). Despite infection control efforts, hospital surfaces remain a critical source of pathogen transmission (Jiang *et al.*, 2022). Indeed, the bacterium has been isolated from diverse hospital equipment and surfaces, including invasive medical devices such as endotracheal tubes and central venous catheters, as well as bed rails, door handles, furniture, and even the clothing of healthcare personnel (Dijkshoorn *et al.*, 2007; Peleg *et al.*, 2008; Chapartegui-González *et al.*, 2018; Ng *et al.*, 2018). During the COVID-19 pandemic, routine infection control practices were disrupted (Gottesman *et al.*, 2021), and prioritization of SARS-CoV-2 prevention often led to lapses in routine infection control activities, such as hand hygiene or room cleaning, facilitating the spread of multidrug-resistant organisms (Gottesman *et al.*, 2021). Notably, *A. baumannii* emerged as the most prevalent co-infecting agent with SARS-CoV-2 in ICU patients (Clancy *et al.*, 2021; Ellis *et al.*, 2022). The persistence of *A. baumannii* on abiotic surfaces is potentiated by its capacity to compensate for water loss during desiccation (Zeidler and Müller, 2019). Clinical isolates remain culturable for weeks to months, increasing the risk of future outbreaks (Giannouli *et al.*, 2013; Campos *et al.*, 2019). This capacity to survive has been associated with the expression of hydrophilins DtpA and DtpB, which protect against desiccation-induced protein damage (Green *et al.*, 2022).

For years, loss of culturability was equated with bacterial death. However, recent evidence has shown that *A. baumannii* can enter a viable but non-culturable (VBNC) state following loss of intracellular water triggered by different stresses, including desiccation and hyperosmotic conditions (König *et al.*, 2023; Lucidi *et al.*, 2025). VBNC cells remain viable but metabolically quiescent, temporarily unable to grow on routine laboratory media despite retaining basal metabolic activity. Transcriptomic

analyses performed under desiccation and hyperosmotic stress have shown that entry of *A. baumannii* into the VBNC state is tightly linked to intracellular water loss and is accompanied by a marked downregulation of tricarboxylic acid (TCA) cycle genes, indicating a global suppression of central carbon metabolism (König *et al.*, 2023; Lucidi *et al.*, 2025). In desiccation-induced VBNC cells, the metabolic slowdown is reinforced by the concomitant activation of the glyoxylate shunt (GS) pathway, which bypasses the decarboxylating steps of the TCA cycle and supports a low-energy, metabolic quiescence state (Lucidi *et al.*, 2025). The VBNC state is reversible, as bacterial cells can restore metabolic activity through a process known as “resuscitation”, which requires stress removal and favourable conditions that enable the recovery of colony growth on solid nutrient media (Ramamurthy *et al.*, 2014; Ayrapetyan *et al.*, 2018; Liu *et al.*, 2023). Since culture-based procedures for the assessment of bacterial contamination assume that one viable cell produces one colony (Rawlinson *et al.*, 2019), the VBNC state reduces bacterial detection, as these cells do not grow on standard media used for environmental monitoring (Liu *et al.*, 2023). Bacterial transition to the VBNC state not only obscures the assessment of bacterial contamination but also enhances long-term survival, reinforcing the *A. baumannii* reputation as a formidable nosocomial threat (Rawlinson *et al.*, 2019; Liu *et al.*, 2023).

While recent studies have identified molecular mechanisms governing desiccation resistance and entry into the VBNC state (Zeidler and Müller, 2019; König *et al.*, 2023; Lucidi *et al.*, 2025), the processes underlying the resuscitation of *A. baumannii* VBNC cells remain largely unexplored. In this work, we employed a transcriptomic approach to investigate the physiological changes that enable *A. baumannii* to resuscitate from the VBNC state and evaluated the impact of these changes on infectivity and pathogenicity.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table S1. Bacterial cells were grown in Luria-Bertani (LB) broth or on LB agar plates (LB supplemented with 1.5% w/v agar, LA). Carbenicillin (carb) was added at a final concentration of 75 µg/mL for plasmid maintenance, when required.

Bacterial inocula and desiccation conditions. Bacterial overnight cultures in LB were initiated from single *A. baumannii* ATCC 19606^T colonies on LA plates and incubated at 37 °C for 18 h. Cultures were then diluted 1:100 in fresh LB and grown for an additional 6 h at 37 °C with shaking. Bacterial cells were harvested by centrifugation (3,000×g, 5 min), washed twice with double-distilled water, and resuspended to an optical density of 600 nm (OD₆₀₀) of 1.0 (*ca.* 5×10^8 CFU/mL). Aliquots of the bacterial suspensions were spotted on glass surfaces and air-dried under a laminar flow hood at 25.2 ± 1.5 °C. Samples were stored for 1 week at an average temperature of 20.9 ± 0.6 °C and relative humidity of $13.00 \pm 5.6\%$. Bacterial cells were desiccated either on glass coverslips (Fisherbrand™ Cover Glasses, Fisher Scientific) or glass Petri dishes (Sigma-Aldrich).

Bacterial culturability after desiccation and resuscitation. The bacterial suspensions were prepared using the procedure described above, and 20 µL (*ca.* 10^7 CFU) were pipetted onto a glass coverslip and air-dried. After 1 week, samples were rehydrated in 2 mL of resuscitation buffer (RB, 6.8 g/L Na₂HPO₄, 3 g/L KH₂PO₄, 0.5 g/L NaCl, 1 g/L NH₄Cl; 0.02 g/L CaCl₂ and 0.12 g/L MgSO₄; Lucidi *et al.*, 2025) and incubated at room temperature for 15 min (post-desiccation bacterial counts) or incubated in RB at 37 °C for 24 h with shaking (post-resuscitation bacterial counts). Samples were vortexed for 30 s and serially diluted 10-fold in sterile saline for CFU enumeration on LA plates, as previously described (Giannouli *et al.*, 2013; Jawad *et al.*, 1998).

Isolation and purification of bacterial RNA. *A. baumannii* ATCC 19606^T cells were washed twice with bidistilled water and resuspended in bidistilled water at an OD₆₀₀ of 1.0. Four mL of suspension were uniformly spread onto the surface of sterile glass Petri dishes and air-dried as described above. After 1 week, samples were rehydrated in 4 mL of RB and incubated at 37 °C for 24 h under shaking conditions to allow bacterial resuscitation. Before and after resuscitation, bacterial cells were immediately stabilized by resuspension in 2 mL of RNA Protect Bacteria Reagent (Qiagen). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen), following the manufacturer's instructions. RNA samples were further purified using the RNeasy MiniElute Cleanup Kit (Qiagen) and treated with TURBO DNase (Invitrogen) to remove residual genomic DNA. The absence of DNA contamination was verified by PCR using the oligonucleotides 16S_FW and 16S_RV (Table S2).

RNA concentration and purity were assessed using a NanoDropTM spectrophotometer (Thermo Fisher Scientific). All procedures were performed in biological triplicate for each experimental condition.

Genome-wide expression analyses and functional annotations. The RNA-seq analysis, RNA quality assessment, library preparation, sequencing, and statistical analysis of the data were performed at the GENEWIZ Biotechnology Facility (GENEWIZ, Azenta Life Sciences Company, Leipzig, Germany), as previously described (Lucidi *et al.*, 2025). RNA samples were quantified using the Qubit 4.0 Fluorometer (Life Technologies), and RNA integrity was checked with an RNA kit on an Agilent 5300 Fragment Analyzer (Agilent Technologies). rRNA depletion was performed using the NEB Next rRNA Depletion kit (New England Biolabs). The RNA sequencing library preparation was performed using the NEB Next Ultra II RNA Library Prep kit for Illumina, following the manufacturer's recommendations. Briefly, enriched RNAs were fragmented according to the manufacturer's instructions. First-strand and second-strand cDNA were subsequently synthesized. cDNA fragments were end-repaired and adenylated at the 3' ends, and a universal adapter was ligated to cDNA fragments, followed by index addition and library enrichment with limited-cycle PCR. Sequencing libraries were validated using the NGS kit on the Agilent 5300 Fragment Analyzer (Agilent Technologies) and quantified using the Qubit 4.0 Fluorometer. The sequencing libraries were multiplexed and loaded onto the flow cell on an Illumina NovaSeq 6000 sequencer, according to the manufacturer's instructions. The samples were sequenced using a 2 × 150 Pair-End configuration v1.5. Image analysis and base calling were conducted by NovaSeq Control Software v1.7 on the NovaSeq instrument. Raw sequence data (.bcl files) generated from Illumina NovaSeq were converted into fastq files and demultiplexed using the Illumina bcl2fastq program v2.20. One mismatch was allowed for index sequence identification. After investigating the quality of the raw data, sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v0.36. The trimmed reads were mapped to the *A. baumannii* ATCC 19606^T reference genome using the Bowtie2 aligner v2.2.6. BAM files were generated as a result of this step. Unique gene hit counts were calculated using feature counts from the Subread package v1.5.2. Only unique reads falling within coding gene regions were counted. After gene hit counts extraction, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the customer-defined groups of samples was performed. All RNA-seq reads were aligned to the genome of ATCC 19606^T (NZ_CP058289.1) for data analysis. The Wald test was used to generate p-values and Log₂Fold-Change (LFC). Only genes with an adjusted p-value ($p_{adj} < 0.05$) were considered differentially expressed genes (DEGs). The EggNOG-mapper v5.0 (Huerta-Cepas *et al.*, 2019) was used to determine the COG categories (Galperin *et al.*, 2019).

Reverse-transcriptase quantitative PCR analysis. To determine RNA expression levels of the *uvrA* gene, RNA was extracted as described above, and cDNA was synthesized using the iScript Reverse Transcription Supermix Kit (Bio-Rad). RT-qPCR reactions were performed using the iTaq Universal SYBR Green Supermix (Bio-Rad). Gene-specific primers used for the analysis are listed in Table S2. The 16S rRNA gene was used as an internal control to normalize the RT-qPCR data and to calculate the relative fold change in gene expression by using the $2^{-\Delta\Delta CT}$ method, as previously described (Runci *et al.*, 2019). RT-qPCR analysis was performed on three different RNA pools for each condition (biological replicates).

Gene cloning and genetic manipulations. All plasmids and primers used in this study are listed in Table S2. Prior to use, all cloned vectors were confirmed by Oxford Nanopore Technologies (ONT) (Plasmidsaurus) sequencing. *PuvrA* m-Scarlet-I transcriptional reporter plasmids were generated by amplifying genomic regions upstream of the gene using *A. baumannii* ATCC 19606^T genomic DNA as a PCR template and amplicon cloning into a HindIII-HF and BamHI-HF digested pWH1266-CG-VmS vector (Kunkle *et al.*, manuscript in preparation) using HiFi Assembly (NEB) following the manufacturer's protocol. For the generation of the $\Delta uv rA$ construct 1,000 bp of DNA in both the 5' and 3' flanking regions surrounding the *uvrA* gene were amplified using *A. baumannii* genomic DNA as a PCR template. These products were cloned into a BamHI-HF and XbaI digested pFLP2 vector using HiFi Assembly. The $\Delta uv rA$ mutant was created by allelic exchange, using the resulting construct, as follows. The construct was introduced by electro-transformation into WT *A. baumannii* ATCC 19606^T electro-competent cells as previously described (Stahl *et al.*, 2015). After electroporation, cells were incubated in 1 ml pre-warmed SOC medium at 37 °C. After 1 h of incubation, transformants were selected on LA plates containing 75 µg/mL carbenicillin and incubated for 24 h at 37 °C. Integration of the plasmid was verified by PCR using appropriate primer pairs (Table S2). Strains were then plated onto LA containing 10% sucrose and incubated at 30 °C for 24 h to select for clones that had resolved the integrated plasmid. Resulting sucrose-resistant colonies were tested for carbenicillin sensitivity by replica plating onto LA supplemented with 75 µg/mL carbenicillin. Carbenicillin-sensitive colonies were subjected to PCR analysis using appropriate primer pairs (Table S2). Deletion of *uvrA* was confirmed for carbenicillin-sensitive colonies first by PCR with the appropriate primers (Table S2), followed by whole-genome sequencing (ONT).

For genetic complementation of the $\Delta uv rA$ mutant, a fragment of 2865 bp containing the full *uvrA* coding sequence and its native promoter region (324 bp upstream of the start codon) was PCR-amplified from *A. baumannii* ATCC 19606^T genomic DNA. The fragment was cloned into the

pWH1266 vector, introducing a 3×FLAG tag at the 5' of the *uvrA* coding sequence. The construct was introduced by electro-transformation into the $\Delta uv rA$ *A. baumannii* electro-competent cells as previously described (Stahl *et al.*, 2015). After electroporation, cells were incubated for 1 h in 1 ml pre-warmed SOC medium at 37 °C, then selected on LA plates containing 75 µg/mL carbenicillin.

***PuvrA*::mScarlet-I transcriptional reporter assays.** Overnight cultures of the indicated *A. baumannii* ATCC 19606^T WT strain harboring *PuvrA*::mScarlet-I transcriptional reporter plasmid were started in LB plus 75 µg/mL carbenicillin. The day after, overnight cultures were diluted 1:100 in fresh LB and grown for 6 h at 37 °C with shaking. Bacterial cultures were desiccated for 1 week as previously described, then desiccated bacteria were rehydrated and resuspended to an OD₆₀₀ of 0.25 in 2 ml of RB and resuscitated by 24-h incubation at 37 °C with shaking. Aliquots of bacterial suspension (100 µl) were used to determine mScarlet-I fluorescence (excitation λ =575 nm, emission λ =635 nm) normalized to OD₆₀₀ (mScarlet/OD₆₀₀) at 0, 2, 4, 6, and 24 h during resuscitation.

UV-C light exposure of *A. baumannii*. Overnight cultures of *A. baumannii* ATCC 19606^T were initiated from single colonies in LB, with antibiotics added when appropriate. The following day, overnight cultures were diluted 1:50 into fresh LB and incubated at 37 °C for 1 h. Cultures were adjusted to an OD₆₀₀ of 0.5 in double-distilled water, and serially 10-fold diluted. Aliquots (5 µL) from each dilution were spotted onto LBA plates, with antibiotics added when necessary, and air-dried under a laminar flow hood until the water had completely evaporated, forming a monolayer of bacterial cells (Dai *et al.*, 2012). Plates were exposed to UV-C light at 254 nm (2 mJ/cm²) using a Stratalinker UV 2400 Crosslinker (Stratagene, La Jolla, CA). After UV-C light exposure, plates were incubated at 37 °C for 18 h, and survivors were quantified by CFU counts.

Analysis of the bacterial growth. Overnight cultures of WT and $\Delta uv rA$ *A. baumannii* ATCC 19606^T strains harboring either empty vector pWH1266, and the complemented strain ($\Delta uv rA$ + *uvrA*) were started from single colonies in LB, with antibiotics added when appropriate. The day after, overnight cultures were diluted 1:50 into fresh LB and incubated at 37 °C for 1 h with shaking. These cultures were then diluted 1:100 into the indicated media and inoculated into 96-well microtiter plates. Plates were incubated at 37 °C with continuous shaking for 24 h, and the OD₆₀₀ was measured every 60 min. Bacterial growth was monitored both in the absence and presence of mechlorethamine (30 µM) or paraquat (75 µM).

Mouse pneumonia model of *A. baumannii* infection. C57BL/6 female mice were purchased from Jackson Laboratories and infected at 9-10 weeks of age. For the before desiccation condition, two

days before infection, *A. baumannii* ATCC 19606^T and $\Delta uvrA$ derivative were streaked onto LA plates for single colony isolation, followed by overnight growth at 37°C with shaking at 180 rpm. On the day of infection, bacteria were subcultured into 5 mL at a 1:100 dilution and grown for 6 hours at 37°C with shaking at 180 rpm. Bacterial cells were harvested by centrifugation ($3,000 \times g$, 5 min), washed twice with double-distilled water, and adjusted to yield a final inoculum of 3×10^8 CFU in 40 μ L of bidistilled water. For the after-desiccation condition, one week before infection, cells of *A. baumannii* ATCC 19606^T and the $\Delta uvrA$ derivative were prepared using the procedure mentioned above, and adjusted to yield a final concentration of 5×10^8 CFU/mL in 10 mL of double-distilled water, as confirmed CFU counts. Aliquots of bacterial suspensions were spotted on glass coverslips and air-dried under a laminar flow hood at 25.2 ± 1.5 °C. Samples were stored for 1 week at an average temperature of 20.9 ± 0.6 °C and relative humidity of $13.00 \pm 5.6\%$. After 1 week, samples were rehydrated with double-distilled water, collected through a cell scraper and yield a final inoculum of 3×10^8 CFU in 40 μ L of bidistilled water. For the resuscitation condition, desiccated cells were rehydrated to yield a final inoculum of 3×10^8 CFU in 1 mL of RB and incubated at 37 °C for 24 h with shaking 180 rpm. Bacterial inocula were serially diluted and plated on LA to confirm equivalent bacterial concentrations between groups.

Prior to infections, mice were anesthetized by intraperitoneal injection of 2,2,2 tribromoethanol diluted in sterile PBS. Anesthetized mice were infected intranasally with a 40 μ L volume of the bacterial inoculum (*ca.* 3×10^8 CFU). During infection, mouse weight and survival were monitored. At 36 hours post-infection, mice were humanely euthanized by forced CO₂ inhalation followed by cervical dislocation, and lungs, hearts, livers, spleens, and kidneys were aseptically harvested and stored on ice. Organs were homogenized and serial dilutions of homogenized tissues were plated onto LA for CFU counts. Organ weights were measured before and after sample collection for accurate CFU quantification of per gram of tissue.

Quantification, statistical analysis, and software. Raw data were recorded in Microsoft Excel and imported into GraphPad Prism 8 for figures generation and statistical analysis. Data were analyzed as indicated in figure legends. Asterisks denote statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Details regarding specific statistical tests, significance levels, data dispersion, group sizes, and measurement precision are provided in the figure legends.

RESULTS

***A. baumannii* undergoes transcriptional reprogramming during resuscitation of VBNC cells.**

To investigate the molecular mechanisms that restore essential cellular functions and metabolic activity during recovery from the VBNC state, RNA sequencing was performed, and the transcriptional profile of resuscitated (RES) *A. baumannii* ATCC 19606^T cells was compared with that of VBNC cells generated after 1-week desiccation (AD). Resuscitation triggered genome-wide transcriptional reprogramming, with a total of 975 genes differentially expressed between RES and AD cells, corresponding to 27.8% of *A. baumannii* genes (DEGs; $|\text{LFC}| > 1$; $\text{padj} < 0.05$) (Figure 1A, Table S3). In particular, 425 and 550 genes were significantly upregulated and downregulated, respectively. To identify biological processes associated with resuscitation, DEGs were functionally annotated using the Cluster of Orthologous Groups (COG) classification (Table S4). Downregulated DEGs were assigned to categories related to core cellular functions, including translation, ribosome structure and biogenesis (J), inorganic ion transport and metabolism (P), energy production and conversion (C), and cell wall/membrane biogenesis (M) (Figure 1B, Table S5). Upregulated DEGs were predominantly assigned to categories replication, recombination, and repair (L), transcription (K), secondary metabolites biosynthesis, transport, and catabolism (Q), and defence mechanisms (V) (Figure 1B, Table S5). Category L genes, including replication, recombination, and repair genes, showed strong transcriptional activation during resuscitation; of 35 DEGs assigned to category L, 26 (74.3%) were upregulated (Figure S1). The abundance of upregulated genes in the COG category L suggests that DNA repair and recombination are key processes enabling the recovery of *A. baumannii* from the VBNC state. Significant DEGs from this study (AD vs RES) were compared with DEGs previously reported for the transition from vegetative cells to desiccated VBNC cells (BD vs AD) (Lucidi *et al.*, 2025). Venn diagram analysis revealed a subset of 75 DEGs specific to the resuscitation process since they were upregulated during resuscitation (AD vs RES) and downregulated during desiccation (BD vs AD) (Figure 1C, Table S6). The COG analysis of these 75 DEGs revealed a predominance of unassigned genes (NA, ~38), and a secondary distribution assigned to multiple COGs (X, ~6), transcription (K, ~4), replication, recombination and repair (L, ~4), and translation, including ribosome structure and biogenesis (J, ~4). This result indicates that still unannotated genes together with core processes in transcription, replication, and translation contribute to the transition from desiccation to resuscitation (Figure S2, Table S7).

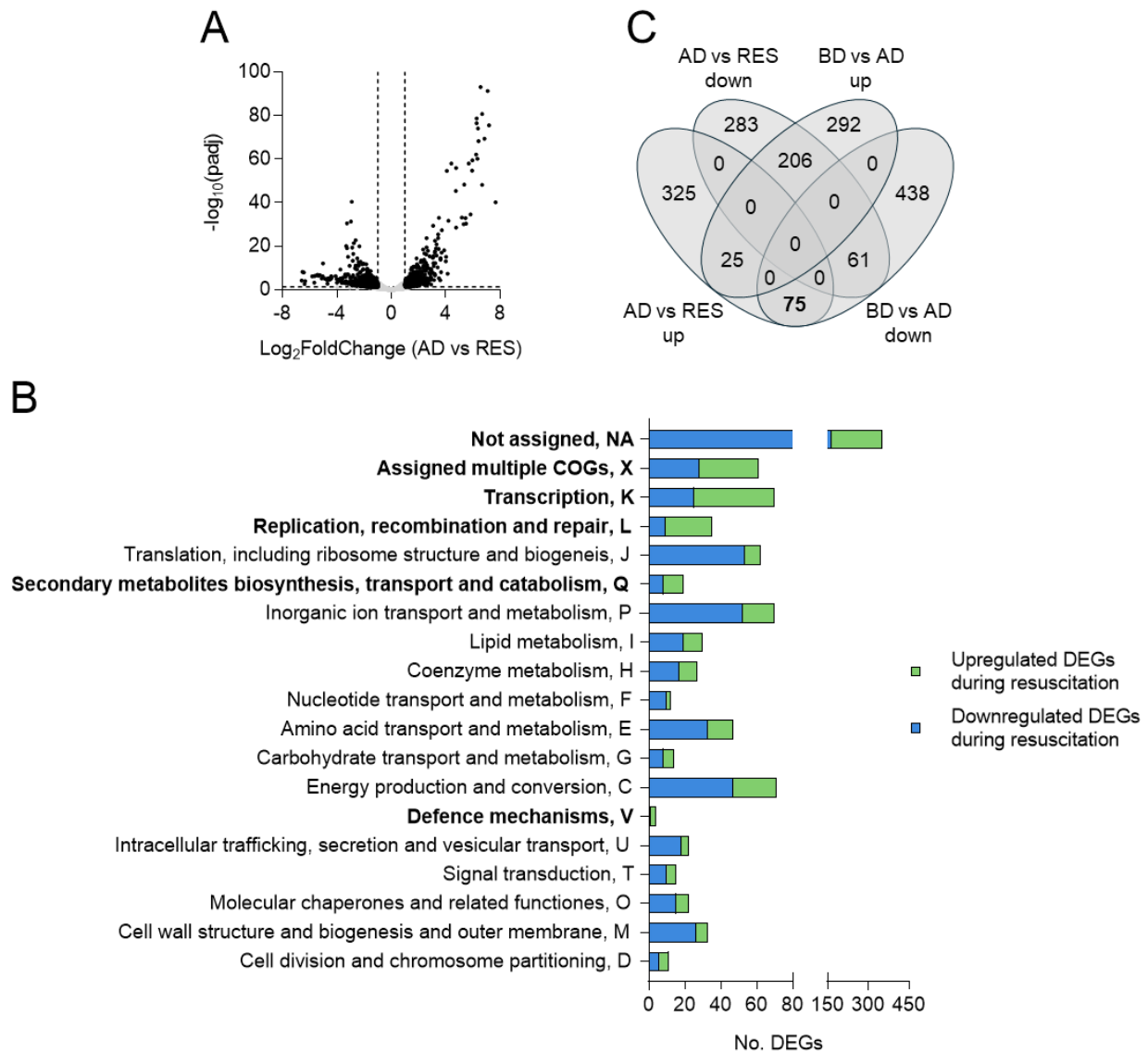


Figure 1 | Transcriptional analysis of *A. baumannii* ATCC 19606^T VBNC cells after resuscitation. (A) Volcano plot depicting the Log₂FoldChange (LFC) of differentially expressed genes (DEGs) of the resuscitated cells (RES) compared to the VBNC cells generated after 1-week desiccation (AD). (B) COG functional categories of *A. baumannii* DEGs in resuscitated cells. The green and blue bars indicate the upregulated and downregulated DEGs, respectively. COGs categories with >50% upregulated DEGs than downregulated DEGs are highlighted in bold type. (C) Venn diagram showing the numbers of significantly upregulated and downregulated DEGs after 1-week desiccation (BD vs AD) and resuscitation (AD vs RES). The subset of genes upregulated during resuscitation (AD vs RES up) and concurrently downregulated during desiccation (BD vs AD down) is highlighted in bold. Data are the mean ± SD (error bars) of three independent experiments.

Loss of UvrA impairs DNA damage tolerance in *A. baumannii*.

Desiccation promotes the intracellular accumulation of reactive oxygen species (ROS), which damage macromolecules, including DNA, via oxidation, crosslinking, and covalent modifications (Lebre *et al.*, 2017; Greffe & Michiels, 2020). Notably, ROS increase has previously been documented during desiccation of *A. baumannii* ATCC 19606^T cells (Lucidi *et al.*, 2025). Consistent

with this, transcriptomic profiling of *A. baumannii* ATCC 19606^T VBNC cells during resuscitation revealed a marked induction of DNA repair-related genes. Among these, *uvrA*, encoding a component of the UvrABC nucleotide excision repair system (COG category L, replication, recombination and repair), emerged as one of the most strongly upregulated genes during resuscitation and was downregulated during desiccation, denoting its specific role during the resuscitation process. This transcriptional pattern was validated by reverse transcription-quantitative PCR (RT-qPCR), confirming *uvrA* repression in desiccated cells and reactivation upon resuscitation (Figure 2A). To further investigate *uvrA* regulation during resuscitation, the *uvrA* promoter activity directed by the *PuvrA::mScarlet-I* transcriptional reporter was monitored over a 24-h resuscitation time course experiment (Figure S3). Fluorescence values, normalized to OD₆₀₀ and expressed relative to the empty vector (EV) control, revealed a time-dependent increase in *uvrA* promoter activation. A modest signal was observed immediately after bacterial suspension in RB (0 h), followed by a significant induction at 4 h ($***P < 0.001$), which further increased by 24 h ($****P < 0.0001$). These results indicate a progressive and sustained reactivation of *uvrA* transcription during resuscitation (Figure 2B).

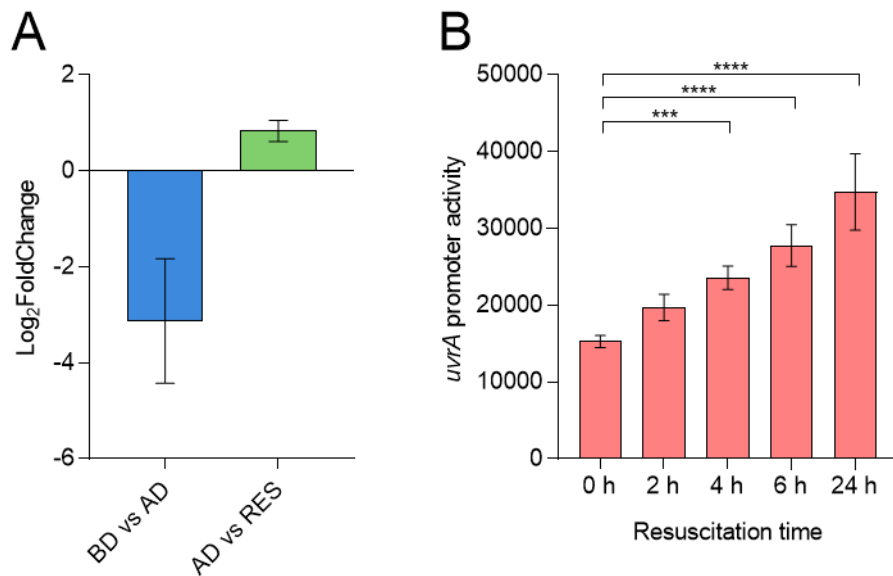


Figure 2 | Transcriptional activation of the *uvrA* gene during resuscitation of *A. baumannii* ATCC 19606^T. (A) Transcription (mRNA) levels of *uvrA* quantified by RT-qPCR in dells desiccated for 1 week relative to the pre-desiccation levels (BD vs AD), and in resuscitated cells relative to 1-week desiccated cells (AD vs RES). Data are the mean $\pm$ SD (error bars) of three independent experiments ($n = 3$). (B) Fluorescence quantification of *PuvrA::mScarlet-I* reporter normalized to OD₆₀₀ (mScarlet/OD₆₀₀) at different times (0, 2, 4, 6, and 24 h) during resuscitation in RB. Background correction was performed using the mean signal from the empty vector (EV) used as a control. Data are mean $\pm$ SD of three biological replicates, each performed in technical triplicate. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test; $***P < 0.001$, $****P < 0.0001$.

The *uvrA* gene has previously been linked with DNA damage repair in *A. baumannii* cells exposed to genotoxic stress (Norton *et al.*, 2013). Consistent with these observations, the protective role of UvrA was functionally validated by assessing the consequences of *uvrA* deletion on DNA repair phenotypes of *A. baumannii* ATCC 19606^T. Bacterial survival following UV-C irradiation was first evaluated. Wild-type (WT) cells carrying the empty vector (EV) tolerated UV-C exposure and remained viable, whereas the $\Delta uv r A$ EV mutant showed significantly reduced colony-forming unit (CFU) counts following UV-C irradiation (Figure 3A). To further evaluate genotoxic stress sensitivity, *A. baumannii* WT and $\Delta uv r A$ EV mutant strains were exposed to mechlorethamine and paraquat, which induce DNA crosslinking and oxidative stress-mediated DNA damage, respectively (Kunkle *et al.*, 2024; Machigov *et al.*, 2022). For both DNA-damaging treatments, exposed cultures displayed pronounced growth delay compared with the untreated controls, with the $\Delta uv r A$ EV strain showing the most severe impairment (Figures 3B, C). Notably, stress sensitivity of the $\Delta uv r A$ EV strain was fully restored upon expression of *uvrA* *in trans*, with the complemented strain ($\Delta uv r A + uv r A$) exhibiting enhanced resistance relative to the WT EV (Figure 3), likely due to the multicopy nature of the pWH1266 plasmid. Collectively, these data demonstrate that *uvrA* is transcriptionally induced during *A. baumannii* resuscitation, and that UvrA-mediated repair contributes to bacterial tolerance against both direct and oxidative stress-induced genotoxicity.

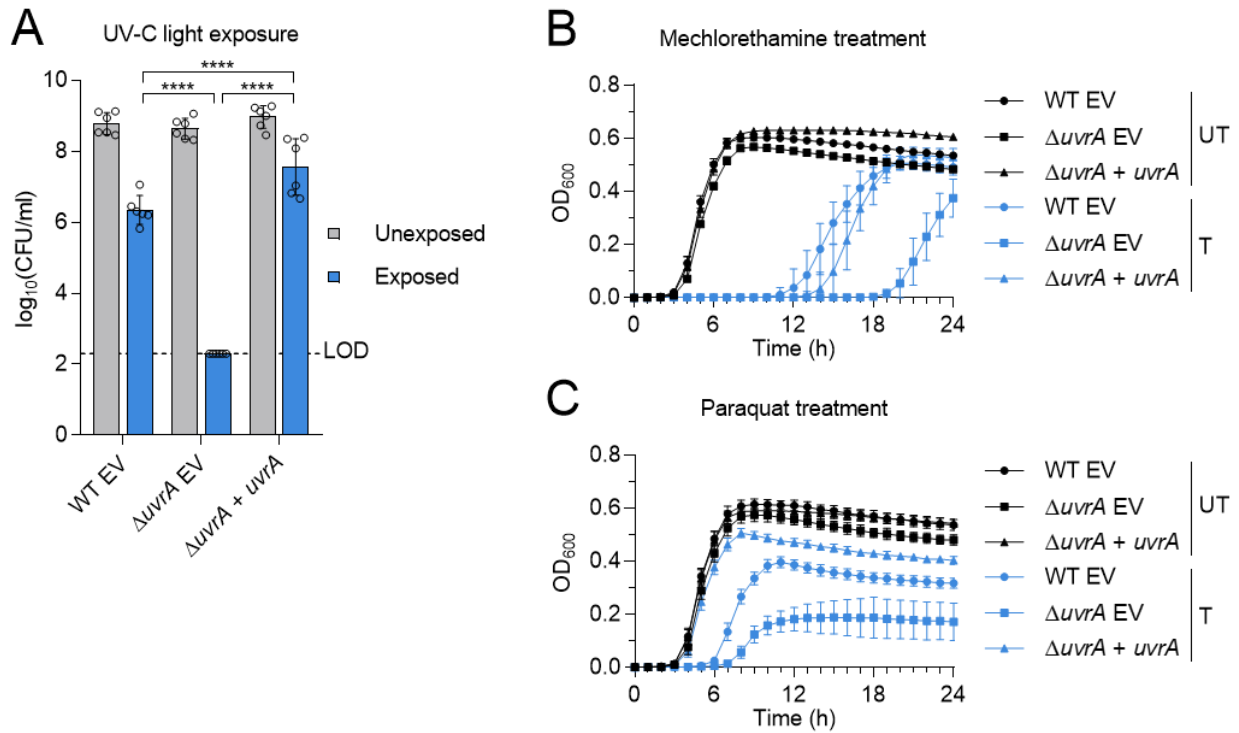


Figure 3 | Mutation of the *uvrA* gene increases the susceptibility of *A. baumannii* ATCC 19606^T to DNA damaging agents. (A) Culturability of WT and Δ*uvrA* *A. baumannii* ATCC 19606^T strains harboring either empty vector pWH1266 (EV) or the complementing plasmid pWH1266-5'*FuvrA*, unexposed (grey) or exposed (blue) to UV-C light (2 mJ/cm²). The dashed line represents the log₁₀ of the limit of detection (LOD) of CFU counts (10² CFU/ml). Data represent mean ± SD of six biological replicates, each depicted as a dot, with each replicate performed in technical triplicate. Statistical significance was determined by Tukey's multiple comparison test, *****P* < 0.0001. (B-C) WT and Δ*uvrA* *A. baumannii* ATCC 19606^T strains carrying either pWH1266 EV or pWH1266-5'*FuvrA* were cultured in LB medium, and untreated (UT, black) or treated (T, blue) with (B) mechlorethamine (30 μM) or (C) paraquat (75 μM). OD₆₀₀ was recorded every 60 minutes to monitor bacterial growth. Data represent mean ± SD of three biological replicates, each performed in technical triplicate.

The *A. baumannii* Δ*uvrA* mutant does not resuscitate from the desiccation-induced VBNC state

To assess the contribution of *uvrA* to desiccation tolerance and recovery from the desiccation-induced VBNC state, the culturability of WT EV, Δ*uvrA* EV, and complemented strains was assessed before desiccation, after 1-week desiccation, and after 24-h resuscitation in RB. Prior to desiccation, all the strains displayed comparable CFU levels, indicating similar baseline viability. After 1 week of desiccation, CFU counts for all strains dropped below the limit of detection, confirming the strong impact of prolonged dehydration on *A. baumannii* culturability. Upon resuscitation, WT and complemented strains regained culturability, with CFU levels increasing relative to the desiccated state. In contrast, the Δ*uvrA* strain failed to recover, with CFU counts remaining below the detection threshold (Figure 4A). Collectively, these data underscore the essential contribution of *uvrA* to resuscitation and its pivotal role in desiccation stress resilience.

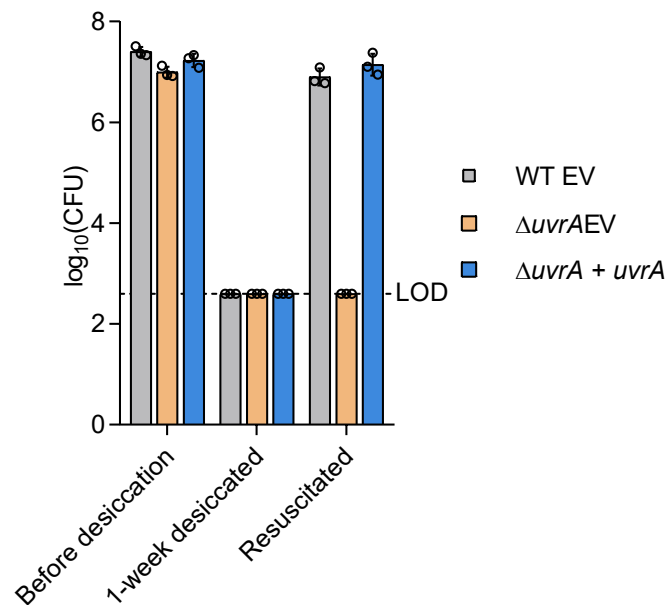


Figure 4 | The *A. baumannii* $\Delta uvrA$ mutant does not resuscitate from the desiccation-induced VBNC state. Culturability of WT and $\Delta uvrA$ *A. baumannii* ATCC 19606^T strains harboring the pWH1266 empty vector (EV) or the pWH1266-5'*FuvrA* complementing plasmid was assessed before desiccation, after 1-week desiccation, and after 24-h resuscitation in RB. The dashed line represents the log₁₀ of the limit of detection (LOD) of CFU counts (4×10^2 CFU). Data are the mean $\pm$ SD; each dot represents an individual biological replicate, each performed in technical triplicate.

Lung colonization by WT and $\Delta uvrA$ *A. baumannii* strains before and after resuscitation

Resuscitation restores the culturability and metabolic activity of *A. baumannii* VBNC cells. Furthermore, *uvrA* plays a critical role in this process, likely because DNA lesions accumulated during desiccation cannot be efficiently repaired in its absence. During infection, bacterial pathogens are continuously exposed to host-derived stresses that generate DNA damage (van der Veen & Tang, 2015), suggesting that the ability to recover genome integrity may directly influence the *in vivo* fitness. Therefore, to determine how resuscitation and *uvrA* contribute to *A. baumannii* *in vivo* fitness and pathogenicity, pulmonary colonization by WT and $\Delta uvrA$ strains before desiccation, after 1-week desiccation, and after resuscitation, were compared in mouse model of bacterial pneumonia. Female C57BL/6J mice (9-10 weeks old) were intranasally infected with either the WT strain or the $\Delta uvrA$ mutant. At 36 hours post-infection (hpi), mice were humanely euthanized, and the bacterial burden in the lungs and other organs was quantified to evaluate colonization and dissemination. Both WT and $\Delta uvrA$ strains exhibited a comparable pulmonary colonization before desiccation, and a modest, non-significant reduction after 1 week of desiccation (Figure S4). Bacterial dissemination to peripheral organs (liver, heart, kidneys, and spleen) was generally low across all conditions, with CFU counts markedly lower than those detected in the lungs (Figure S4). Moreover, no substantial differences in dissemination to peripheral organs were observed between the WT and the $\Delta uvrA$

mutant or among the different physiological states, indicating that desiccation and resuscitation primarily influence pulmonary colonization rather than systemic spread. Following resuscitation, both strains regained their ability to colonize the lungs. However, the WT strain displayed an even higher pulmonary burden than in the pre-desiccation condition (Figure 5 and S4). Importantly, resuscitated WT bacteria achieved significantly greater lung colonization than the $\Delta uvrA$ mutant, demonstrating that *uvrA* is required not only for efficient recovery from the VBNC state but also for maintaining full virulence in animal infection. Together, these findings suggest that recovery from desiccation may transiently potentiate WT virulence in the lung, whereas the absence of *uvrA* compromises the ability of *A. baumannii* to fully recover its pathogenicity in the mouse model of infection.

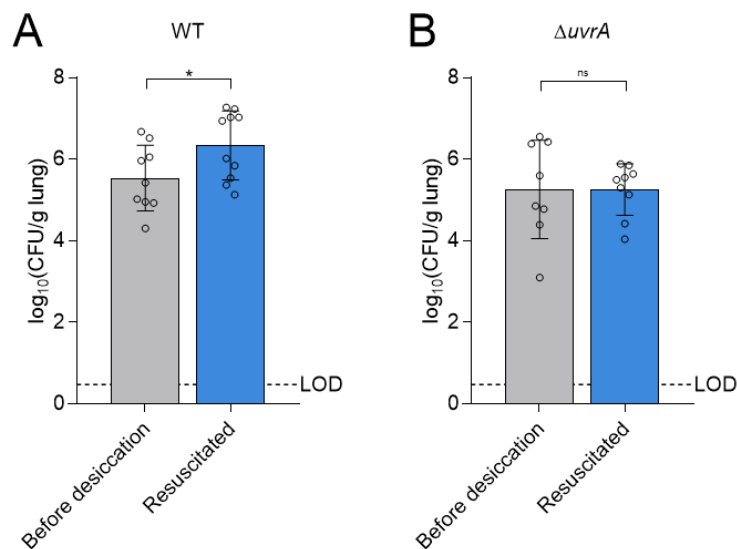


Figure 5 | Lung colonization of mice by *A. baumannii* ATCC 19606^T WT and the $\Delta uvrA$ mutant before and after resuscitation. Female C57BL/6J mice (9-10 weeks old) were intranasally infected with *A. baumannii* WT (A) or $\Delta uvrA$ (B) strains, either before desiccation or after resuscitation. At 36 hpi, mice were euthanized, and bacterial burdens in the lungs were enumerated. The dashed line represents the log₁₀ of the limit of detection (LOD) of CFU counts (4 CFU/g lungs). Data represent the mean $\pm$ SEM; each data point represents one mouse. * $P < 0.05$ as determined by unpaired t-test.

DISCUSSION

Bacteria are endowed with remarkable resilience to environmental stresses, and desiccation represents one of the most challenging conditions encountered in both natural environments and clinical settings. For healthcare-associated pathogens, such as *Acinetobacter baumannii*, the ability to withstand water loss is not only an ecologically relevant trait but a critical determinant of the bacterial persistence on hospital surfaces and medical devices, thereby facilitating cross-transmission and outbreaks (Giannouli *et al.*, 2013). Previous studies have characterized the molecular mechanisms underlying desiccation tolerance and transition to the VBNC state in *A. baumannii*, including transcriptomic analyses that revealed global metabolic downregulation and stress adaptation in VBNC cells (Lucidi *et al.*, 2025). However, the processes that govern the reversal of this state, *i.e.* the recovery of cultivability (resuscitation) of VBNC cells, remain poorly understood, despite empiric evidence that both infectivity and pathogenicity are restored after prolonged environmental persistence. To date, few, if any, studies have addressed the molecular basis of resuscitation in *A. baumannii* VBNC cells.

Evidence from other bacteria suggests that recovery from dormancy is an active process requiring the repair of cellular damage that has caused the dormant state. For instance, resuscitation of *Pseudomonas putida* VBNC cells induced by desiccation relies on metabolic reprogramming for energy production and respiration (López-Lara *et al.*, 2020). Similarly, work on desert microbial communities demonstrated that rapid bacterial rehydration triggers a strong transcriptional response involving DNA repair and energy generation (Imminger *et al.*, 2024). Genes associated with the repair of severe DNA lesions, such as double-stranded breaks, are prominently induced across multiple taxa, highlighting conserved strategies to restore genome integrity and resume metabolism during resuscitation (Imminger *et al.*, 2024). Building on these observations, our study provides a genome-wide analysis of the *A. baumannii* transcriptome during resuscitation from desiccation-induced VBNC status. Functional annotation revealed that, during recovery from dormancy, genes belonging to the functional categories replication, recombination, DNA repair, transcription, and energy metabolism are strongly expressed, consistent with the need to rapidly restore genome integrity and metabolic capacity before full growth resumes. Comparative analysis with the VBNC transcriptome previously reported for *A. baumannii* (Lucidi *et al.*, 2025) enabled the identification of a subset of genes specifically associated with resuscitation. This was achieved by contrasting the resuscitation gene dataset with the previously published dataset describing gene expression during entry into the desiccation-induced VBNC state (Lucidi *et al.*, 2025). Through this comparison, genes that were upregulated during resuscitation but downregulated during transition to the VBNC state were identified, plausibly due to their specific role in recovery. Among these, *uvrA* emerged as a key determinant in the resuscitation process, suggesting that nucleotide excision repair (NER) is a pivotal

process during exit from VBNC. The *uvrA* gene encodes the damage sensor of the UvrABC NER system, which removes bulky DNA lesions and cross-links to restore genomic integrity (Genta *et al.*, 2025). Previous studies on *A. baumannii* have linked the induction of *uvrA* to genotoxic stress, showing that *uvrA* expression is RecA-dependent and triggered by DNA-damaging conditions such as UV irradiation, antibiotic exposure, and acidic pH (Norton *et al.*, 2013; Kunkle *et al.*, 2024). Despite this information on *uvrA* regulation, no genetic evidence of its functional role in *A. baumannii* response to the genotoxic stress was available until now. To address this knowledge gap, we generated a $\Delta uv rA$ mutant in *A. baumannii* ATCC 19606^T and performed survival assays exposing the wild-type and the $\Delta uv rA$ mutant to different DNA-damaging conditions, including UV-C irradiation, DNA crosslinking by mechlorethamine, and ROS-mediated damage induced through paraquat. Our results provide evidence that deletion of *uvrA* severely compromises survival under all the tested genotoxic conditions, underscoring the essential contribution of the NER system to genome maintenance in *A. baumannii* ATCC 19606^T exposed to genotoxic stress.

Transcriptional fusion assays revealed a clear, time-dependent activation of the *uvrA* promoter during resuscitation. Based on this observation, we hypothesized that *uvrA* is essential for successful recovery from dormancy of VBNC cells. To test this, we compared the ability to withstand desiccation and regain culturability between the wild type and the $\Delta uv rA$ mutant, obtaining evidence that *uvrA* is indispensable for resuscitation from the VBNC state. Desiccation imposes severe stress on *A. baumannii* cells, primarily by increasing intracellular levels of reactive oxygen species (ROS) (Lucidi *et al.*, 2025). Elevated ROS levels are deleterious because they induce DNA strand breaks and nucleotide alterations, leading to the accumulation of lesions that compromise genome integrity (Poetsch *et al.*, 2020). Therefore, repairing such lesions during resuscitation is essential to restore cellular functions. In this context, we hypothesize that UvrA-mediated NER plays a pivotal role in *A. baumannii* recovery from the VBNC state, as suggested by its strong overexpression during resuscitation.

Interestingly, oxidative stress encountered by *A. baumannii* ATCC 19606^T during desiccation (Lucidi *et al.*, 2025) somehow resembles the conditions that *A. baumannii* faces within the host. During lung infection, neutrophils are recruited to the site of infection and produce hydrogen peroxide (H₂O₂) as part of the oxidative burst, generating high levels of ROS that act as antimicrobial agents (Juttukonda *et al.*, 2019; Lee *et al.*, 2020). c. Therefore, we hypothesized that nucleotide excision repair plays a critical role not only in recovery from dormancy but also in *A. baumannii* ATCC 19606^T pathogenesis. To investigate this aspect, we conducted intrapulmonary murine infection experiments to evaluate both the contribution of UvrA to virulence and the effect of resuscitation on the ability of *A. baumannii* to establish infection *in vivo*. To our knowledge, this is the first study aimed at assessing the pathogenic potential of VBNC and resuscitated cells in a mammalian infection model. Previous

studies using strain ATCC 17978 reported that desiccation does not significantly impair virulence (Green *et al.*, 2022). In contrast, our data show that both the wild-type strain ATCC 19606^T and the *uvrA* mutant exhibit a markedly reduced ability to cause infection following desiccation. This discrepancy may reflect strain-specific differences in stress tolerance or regulatory networks. Consistent with previous observations in the *Galleria mellonella* infection model (Lucidi *et al.*, 2025), we found that resuscitation restores and even enhances virulence compared to the pre-desiccation condition. This suggests that the transition from dormancy to active growth may prime *A. baumannii* for effective host colonization, possibly through the activation of stress response and repair pathways. Importantly, the *uvrA* mutant did not display increased virulence after resuscitation, as opposed to the parent strain. This finding supports our hypothesis that UvrA-mediated nucleotide excision repair is critical for recovery from oxidative damage and for virulence expression *in vivo*. Together, these results indicate that both resuscitation and UvrA function are key pathogenicity determinants.

One limitation of this study is that all experiments were performed using a single reference strain, namely *A. baumannii* ATCC 19606^T. While this approach provided original insights into the complex process of resuscitation from the VBNC state, it does not capture the potential variability among other strains. Intraspecific diversity in *A. baumannii*, including differences in stress tolerance and regulatory networks, has been documented (Valcek *et al.*, 2025), and it is plausible that other strains may exhibit distinct transcriptional responses or repair strategies during recovery from the VBNC state.

Despite this limitation, our findings provide a solid framework for understanding the molecular basis of resuscitation in *A. baumannii* and underscore the central role of DNA repair in this process.

In conclusion, this study provides a genome-wide picture of *A. baumannii* resuscitation from the VBNC state and identifies *uvrA* as a key determinant of recovery and virulence. UvrA-mediated nucleotide excision DNA repair emerges as essential for survival under genotoxic stress and for regaining culturability after desiccation. Importantly, our *in vivo* experiments demonstrate that both resuscitation and UvrA function are critical for pathogenicity. These findings highlight resuscitation as a pivotal event shaping bacterial virulence.

Future research should expand this analysis to a broader range of clinical isolates to determine whether the mechanisms identified here are conserved or strain-specific, and to clarify their implications for persistence and pathogenicity. In perspective, studies aimed at targeting DNA repair pathways may offer innovative approaches to limit both environmental survival and infection, ultimately providing new strategies to combat *A. baumannii*.

REFERENCES

- Antochevis, L. C., Wilhelm, C. M., Arns, B., Sganzerla, D., Sudbrack, L. O., Nogueira, T. C. R. L., Guzman, R. D., Martins, A. S., Cappa, D. S., dos Santos, Â. C., Pascual, J. C., Perugini, V. H., Vespero, E. C., Rigatto, M. H. P., Pereira, D. C., Lutz, L., Leão, R. S., Marques, E. A., Henrique, D. M., ... Zavascki, A. P. (2025). World Health Organization priority antimicrobial resistance in *Enterobacterales*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecium* healthcare-associated bloodstream infections in Brazil (ASCENSION): a prospective, multicentre, observational study. *Lancet Regional Health - Americas*, 43, 101004. <https://doi.org/10.1016/j.lana.2025.101004>
- Ayrapetyan, M., Williams, T., & Oliver, J. D. (2018). Relationship between the Viable but Nonculturable State and antibiotic persister cells. *Journal of Bacteriology*, 200(20), e00249-18. <https://doi.org/10.1128/JB.00249-18>
- Campos, A., Lopes, M. S., Carvalheira, A., Barbosa, J., & Teixeira, P. (2019). Survival of clinical and food *Acinetobacter* spp. isolates exposed to different stress conditions. *Food Microbiology*, 77, 202-207. <https://doi.org/10.1016/j.fm.2018.09.009>
- Cavallo, I., Oliva, A., Pages, R., Sivori, F., Truglio, M., Fabrizio, G., Pasqua, M., Pimpinelli, F., & Di Domenico, E. G. (2023). *Acinetobacter baumannii* in the critically ill: complex infections get complicated. *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1196774>
- Chapartegui-González, I., Lázaro-Díez, M., Bravo, Z., Navas, J., Icardo, J. M., & Ramos-Vivas, J. (2018). *Acinetobacter baumannii* maintains its virulence after long-time starvation. *PLoS ONE*, 13(8), e0201961. <https://doi.org/10.1371/journal.pone.0201961>
- Clancy, C. J., Schwartz, I. S., Kula, B., & Nguyen, M. H. (2021). Bacterial superinfections among persons with Coronavirus disease 2019: a comprehensive review of data from postmortem studies. *Open Forum Infectious Diseases*, 8(3), ofab065. <https://doi.org/10.1093/ofid/ofab065>
- Dai, T., Murray, C. K., Vrahas, M. S., Baer, D. G., Tegos, G. P., & Hamblin, M. R. (2012). Ultraviolet C light for *Acinetobacter baumannii* wound infections in mice: potential use for battlefield wound decontamination? *The Journal of Trauma and Acute Care Surgery*, 73(3), 661-667. <https://doi.org/10.1097/TA.0b013e31825c149c>
- Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nature Reviews Microbiology*, 5(12), 939-951. <https://doi.org/10.1038/nrmicro1789>

- Ellis, R. C., Roberts, E. K., Grier, J. T., & Fiester, S. E. (2022). *Acinetobacter baumannii* Infections that are resistant to treatment: warning signs from the COVID-19 pandemic. *Future Microbiology*, 17(17), 1345-1347. <https://doi.org/10.2217/fmb-2022-0153>
- Galperin, M. Y., Makarova, K. S., Wolf, Y. I., & Koonin, E. V. (2015). Expanded microbial genome coverage and improved protein family annotation in the COG database. *Nucleic Acids Research*, 43(D1), D261-D269. <https://doi.org/10.1093/nar/gku1223>
- Giannouli, M., Antunes, L. C. S., Marchetti, V., Triassi, M., Visca, P., & Zarrilli, R. (2013). Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78. *BMC Infectious Diseases*, 13, 282. <https://doi.org/10.1186/1471-2334-13-282>
- Gottesman, T., Fedorowsky, R., Yerushalmi, R., Lellouche, J., & Nutman, A. (2021). An outbreak of carbapenem-resistant *Acinetobacter baumannii* in a COVID-19 dedicated hospital. *Infection Prevention in Practice*, 3(1), 100113. <https://doi.org/10.1016/j.infpip.2021.100113>
- Green, E. R., Fakhoury, J. N., Monteith, A. J., Pi, H., Giedroc, D. P., & Skaar, E. P. (2022). Bacterial hydrophilins promote pathogen desiccation tolerance. *Cell Host & Microbe*, 30(7), 975-987.e7. <https://doi.org/10.1016/j.chom.2022.03.019>
- Grefe, V. R. G., & Michiels, J. (2020). Desiccation-induced cell damage in bacteria and the relevance for inoculant production. *Applied Microbiology and Biotechnology*, 104(9), 3757-3770. <https://doi.org/10.1007/s00253-020-10501-6>
- Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J., von Mering, C., & Bork, P. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, 47(D1), D309-D314. <https://doi.org/10.1093/nar/gky1085>
- Imminger, S., Meier, D. V., Schintlmeister, A., Legin, A., Schnecker, J., Richter, A., Gillor, O., Eichorst, S. A., & Wobken, D. (2024). Survival and rapid resuscitation permit limited productivity in desert microbial communities. *Nature Communications*, 15(1), 3056. <https://doi.org/10.1038/s41467-024-46920-6>
- Jawad, A., Seifert, H., Snelling, A. M., Heritage, J., & Hawkey, P. M. (1998). Survival of *Acinetobacter baumannii* on dry surfaces: Comparison of outbreak and sporadic isolates. *Journal of Clinical Microbiology*, 36(7), 1938-1941. <https://doi.org/10.1128/JCM.36.7.1938-1941.1998>

- Jiang, Y., Ding, Y., Wei, Y., Jian, C., Liu, J., & Zeng, Z. (2022). Carbapenem-resistant *Acinetobacter baumannii*: a challenge in the intensive care unit. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1045206>
- Juttukonda, L. J., Green, E. R., Lonergan, Z. R., Heffern, M. C., Chang, C. J., & Skaar, E. P. (2018). *Acinetobacter baumannii* OxyR regulates the transcriptional response to hydrogen peroxide. *Infection and Immunity*, 87(1), e00413-18. <https://doi.org/10.1128/IAI.00413-18>
- König, P., Wilhelm, A., Schaudinn, C., Poehlein, A., Daniel, R., Widera, M., Averhoff, B., & Müller, V. (2023). The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *mBio*, 14(5), e0213923. <https://doi.org/10.1128/mbio.02139-23>
- Kunkle, D. E., Cai, Y., Eichman, B. F., & Skaar, E. P. (2024). An interstrand DNA crosslink glycosylase aids *Acinetobacter baumannii* pathogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 121(27), e2402422121. <https://doi.org/10.1073/pnas.2402422121>
- Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, 15(5), 285-296. <https://doi.org/10.1038/nrmicro.2017.16>
- Lee, H. H., Aslanyan, L., Vidyasagar, A., Brennan, M. B., Tauber, M. S., Carrillo-Sepulveda, M. A., Dore, M. R., Rigel, N. W., & Martinez, L. R. (2020). Depletion of alveolar macrophages increases pulmonary neutrophil infiltration, tissue damage, and sepsis in a murine model of *Acinetobacter baumannii* pneumonia. *Infection and Immunity*, 88(7), e00128-20. <https://doi.org/10.1128/IAI.00128-20>
- Liu, J., Yang, L., Kjellerup, B. V., & Xu, Z. (2023). Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. *Trends in Microbiology*, 31(10), 1013-1023. <https://doi.org/10.1016/j.tim.2023.04.009>
- López-Lara, L. I., Pazos-Rojas, L. A., López-Cruz, L. E., Morales-García, Y. E., Quintero-Hernández, V., de la Torre, J., van Dillewijn, P., Muñoz-Rojas, J., & Baez, A. (2020). Influence of rehydration on transcriptome during resuscitation of desiccated *Pseudomonas putida* KT2440. *Annals of Microbiology*, 70(1), 54. <https://doi.org/10.1186/s13213-020-01596-3>
- Lucidi, M., Capecchi, G., Spagnoli, C., Basile, A., Artuso, I., Persichetti, L., Fardelli, E., Capellini, G., Visaggio, D., Imperi, F., Rampioni, G., Leoni, L., & Visca, P. (2025). The response to desiccation in *Acinetobacter baumannii*. *Virulence*, 16(1), 2490209. <https://doi.org/10.1080/21505594.2025.2490209>

- Lucidi, M., Visaggio, D., Migliaccio, A., Capecchi, G., Visca, P., Imperi, F., & Zarrilli, R. (2024). Pathogenicity and virulence of *Acinetobacter baumannii*: factors contributing to the fitness in healthcare settings and the infected host. *Virulence*, 15(1), 2289769. <https://doi.org/10.1080/21505594.2023.2289769>
- Machigov, E. A., Igonina, E. V., Sviridova, D. A., Rubanovich, A. V., & Abilev, S. K. (2022). The genotoxic effect of the paraquat radiomimetic on *Escherichia coli* bacteria. *Biology Bulletin*, 49(12), 2486-2494. <https://doi.org/10.1134/S106235902212010X>
- Morris, F. C., Dexter, C., Kostoulas, X., Uddin, M. I., & Peleg, A. Y. (2019). The mechanisms of disease caused by *Acinetobacter baumannii*. *Frontiers in Microbiology*, 10, 1601. <https://doi.org/10.3389/fmicb.2019.01601>
- Ng, D. H. L., Marimuthu, K., Lee, J. J., Khong, W. X., Ng, O. T., Zhang, W., Poh, B. F., Rao, P., Raj, M. D. R., Ang, B., & De, P. P. (2018). Environmental colonization and onward clonal transmission of carbapenem-resistant *Acinetobacter baumannii* (CRAB) in a medical intensive care unit: the case for environmental hygiene. *Antimicrobial Resistance & Infection Control*, 7(1), 51. <https://doi.org/10.1186/s13756-018-0343-z>
- Norton, M. D., Spilkia, A. J., & Godoy, V. G. (2013). Antibiotic resistance acquired through a DNA damage-inducible response in *Acinetobacter baumannii*. *Journal of Bacteriology*, 195(6), 1335-1345. <https://doi.org/10.1128/JB.02176-12>
- Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews*, 21(3), 538-582. <https://doi.org/10.1128/CMR.00058-07>
- Poetsch, A. R. (2020). The genomics of oxidative DNA damage, repair, and resulting mutagenesis. *Computational and Structural Biotechnology Journal*, 18, 207-219. <https://doi.org/10.1016/j.csbj.2019.12.013>
- Ramamurthy, T., Ghosh, A., Pazhani, G. P., & Shinoda, S. (2014). Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Frontiers in Public Health*, 2, 103. <https://doi.org/10.3389/fpubh.2014.00103>
- Rawlinson, S., Ciric, L., & Cloutman-Green, E. (2019). How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. *The Journal of Hospital Infection*, 103(4), 363-374. <https://doi.org/10.1016/j.jhin.2019.07.015>

- Runci, F., Gentile, V., Frangipani, E., Rampioni, G., Leoni, L., Lucidi, M., Visaggio, D., Harris, G., Chen, W., Stahl, J., Averhoff, B., & Visca, P. (2019). Contribution of active iron uptake to *Acinetobacter baumannii* pathogenicity. *Infection and Immunity*, 87(4), e00755-18. <https://doi.org/10.1128/IAI.00755-18>
- Stahl, J., Bergmann, H., Göttig, S., Ebersberger, I., & Averhoff, B. (2015). *Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases D. *PloS One*, 10(9), e0138360. <https://doi.org/10.1371/journal.pone.0138360>
- Sunenshine, R. H., Wright, M.-O., Maragakis, L. L., Harris, A. D., Song, X., Hebden, J., Cosgrove, S. E., Anderson, A., Carnell, J., Jernigan, D. B., Kleinbaum, D. G., Perl, T. M., Standiford, H. C., & Srinivasan, A. (2007). Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization-Volume 13, Number 1-January 2007-Emerging Infectious Diseases journal-CDC. <https://doi.org/10.3201/eid1301.060716>
- Tipton, K. A., Chin, C.-Y., Farokhyfar, M., Weiss, D. S., & Rather, P. N. (2018). Role of capsule in resistance to disinfectants, host antimicrobials, and desiccation in *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*, 62(12), e01188-18. <https://doi.org/10.1128/AAC.01188-18>
- van der Veen, S., & Tang, C. M. (2015). The BER necessities: the repair of DNA damage in human-adapted bacterial pathogens. *Nature Reviews Microbiology*, 13(2), 83-94. <https://doi.org/10.1038/nrmicro3391>
- WHO bacterial priority pathogens list 2024: bacterial pathogens of public health importance, to guide research, development, and strategies to prevent and control antimicrobial resistance (1st ed). (2024). World Health Organization. <https://www.who.int/publications/i/item/9789240093461>
- Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., & Spellberg, B. (2017). Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clinical Microbiology Reviews*, 30(1), 409-447. <https://doi.org/10.1128/CMR.00058-16>
- Zeidler, S., & Müller, V. (2019a). Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environmental Microbiology*, 21(7), 2212-2230. <https://doi.org/10.1111/1462-2920.14565>
- Zeidler, S., & Müller, V. (2019b). The role of compatible solutes in desiccation resistance of *Acinetobacter baumannii*. *MicrobiologyOpen*, 8(5), e00740. <https://doi.org/10.1002/mbo3.740>

Supplemental Material

Transcriptomic landscape associated with the resuscitation of viable but not culturable *Acinetobacter baumannii* cells

Cinzia Spagnoli¹, Dillon E. Kunkle^{2,3}, Daniela Visaggio^{1,4,5}, Paolo Visca^{1,4,5}, Eric P. Skaar^{2,3,6}

¹Department of Science, Roma Tre University, Rome, Italy

²Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

³Vanderbilt Institute for Infection, Immunology, and Inflammation, Vanderbilt University Medical Center, Nashville, TN, USA

⁴NBFC, National Biodiversity Future Center, Palermo, Italy

⁵Santa Lucia Foundation IRCCS, Rome, Italy

⁶Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

Manuscript in preparation

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Table S1. Bacterial strains used in this study

Strain	Relevant characteristics	Source/Reference
<i>Acinetobacter baumannii</i> ATCC 19606 ^T	Wild-type strain	Artuso <i>et al.</i> , 2022
<i>A. baumannii</i> ATCC 19606 ^T Δ <i>uvrA</i>	Clear <i>uvrA</i> deletion mutant	This study
<i>Acinetobacter baumannii</i> ATCC 19606 ^T pWH1266-CG-VmS	Empty vector control strain	This study
<i>Acinetobacter baumannii</i> ATCC 19606 ^T pWH1266-CG-VmS_ P_{uvrA}	m-Scarlet-I reporter strain for P_{uvrA}	This study
<i>Acinetobacter baumannii</i> ATCC 19606 ^T pWH1266	Empty vector control strain	This study
<i>A. baumannii</i> ATCC 19606 ^T Δ <i>uvrA</i> pWH1266	Empty vector control strain	This study
<i>A. baumannii</i> ATCC 19606 ^T Δ <i>uvrA</i> pWH1266-5' <i>FuvrA</i>	Complemented strain carrying <i>uvrA</i> fused to a 3×FLAG tag at the 5' end	This study
<i>Escherichia coli</i> DH5a	Plasmid maintenance <i>E. coli</i> strain used for gene cloning	Skaar lab stock
<i>Escherichia coli</i> DH5a pFLp2	Cloning strain carrying the <i>A. baumannii</i> suicide vector with sucrose resistance (pFLp2)	Herbert Schweizer (Hoang <i>et al.</i> , 1998)
<i>Escherichia coli</i> DH5a pFLp2- <i>DuvrA</i>	Strain carrying the <i>uvrA</i> knockout vector	This study
<i>Escherichia coli</i> DH5a pWH1266	Strain carrying the <i>A. baumannii</i> shuttle vector (pWH1266)	(Hunger <i>et al.</i> , 1990)
<i>Escherichia coli</i> DH5a pWH1266-5' <i>FuvrA</i>	Strain carrying the vector for <i>uvrA</i> complementation	This study
<i>Escherichia coli</i> DH5a pWH1266-CG-VmS	Strain carrying the <i>A. baumannii</i> vector for m-Scarlet-I expression (pWH1266-CG-VmS)	(Kunkle <i>et al.</i> , manuscript in preparation)
<i>Escherichia coli</i> DH5a pWH1266-CG-VmS_ P_{uvrA}	Strain carrying the vector with a transcriptional fusion between <i>PuvrA</i> and mScarlet-I	This study

Table S2. Primer used in this study

Primer	Primer sequence	Primer description
pFLP2_XbaI_fwd	AATAATAGTGAACGGCAGGTATAT GTGATGGGT	5' primer for amplifying to control pFLp2 cloning
pFLP2_BamHI_rev	TGCGACGTGAACAGTGAGCTGTAT GTGC	3' primer for amplifying to control pFLp2 cloning
pWH1266_BamHI_fwd	AGGCCAGTCTTTCGACTGAGCC	5' primer for amplifying to control pWH1266 cloning
pWH1266_HindIII_rev	TGTCCCTTCGTAAGGACGGCCTTCC	3' primer for amplifying to control pWH1266 cloning
uvrA_up_fwd	GGTAAAAAAGGATCGATCCTACTTC GCCTGCTTTTGGTTC	5' primer for amplifying upstream flanking region for <i>uvrA</i> knockout construct
<i>uvrA</i> _up_rev	TGTTTTATTGGCTCATAAAACATCT CAATTGTGTATTG	3' primer for amplifying upstream flanking region for <i>uvrA</i> knockout construct
uvrA_down_fwd	TTTATGAGCCAATAAAACAAAAA GCCGG	5' primer for amplifying downstream flanking region for <i>uvrA</i> knockout construct
uvrA_down_rev	AAGTTCCTATTCTCTAGGGGTCATC GCCTTTACTCAAG	3' primer for amplifying downstream flanking region for <i>uvrA</i> knockout construct
uvrA_inCTRL_fwd	CTGGTATCTAGAATGTGTTGTC	5' primer for checking merodiploid and WT/KO after sucrose selection; anneals upstream of <i>uvrA</i>
uvrA_inCTRL_rev	GGTGTTTGAGCCTCTTATTC	3' primer for checking merodiploid and WT/KO after sucrose selection; anneals downstream of <i>uvrA</i>
P _{uvrA} _comp-5'FLAG_fwd	TTGACAGCTTATCATCGATAATTCA TCATAAACAACTATTAGACC	5' primer for amplifying <i>uvrA</i> promoter region for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
P _{uvrA} _comp-5'FLAG_rev	TATAATCCATAAAACATCTCAATTG TGTATTG	3' primer for amplifying <i>uvrA</i> promoter region for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
3X FLAG_uvrA_comp5'_fwd	GAGATGTTTTATGGATTATAAGGAT CATGATGGTGATTATAAGGATC	5' primer for amplifying 3xFLAG for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
3X FLAG_uvrA_comp5'_rev	TTTGGCTCATACCACCACCACCACC CTTG	3' primer for amplifying 3xFLAG for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
uvrA_comp-5'FLAG_fwd	TGGTGGTGGTATGAGCCAAAGTCA TATC	5' primer for amplifying <i>uvrA</i> for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
uvrA_comp-5'FLAG_rev	ACGATGCGTCCGGCGTAGAGTTATT GTTTCAACATCGGTTTC	3' primer for amplifying <i>uvrA</i> for the complementation construct with <i>uvrA</i> fused to a 3×FLAG tag at the 5' end
P _{uvrA} _fwd	CGGCCGCTCTAGAACTAGTGTTC TGCATTCATCATAAAC	5' primer for amplifying <i>uvrA</i> promoter region for the pWH1266-CG-VmS cloning
P _{uvrA} _rev	TGGTATATCTCCTTCGCTAAAAAAC ATCTCAATTGTGTATTG	3' primer for amplifying <i>uvrA</i> promoter region for the pWH1266-CG-VmS cloning
uvrA_RT_fwd	TGAGCGCTTACACTTCTTGG	5' primer for amplifying an internal region of <i>uvrA</i> for the RT-qPCR
uvrA_RT_rev	CACCCATCAAACCTGCACC	3' primer for amplifying an internal region of <i>uvrA</i> for the RT-qPCR
16S_FW	TGTGAAGAAGGCCATTATGGTT	5' primer for amplifying of 16S rRNA gene by RT-qPCR
16S_RV	CTGTATTACCGCGGCTGCT	3' primer for amplifying of 16S rRNA gene by RT-qPCR

Table S3. List of DEGs during resuscitation

Gene name	Locus Tag	LFC	pvalue	padj	Gene name	Locus Tag	LFC	pvalue	padj
	HTZ92_RS00110	2.3291	2.87E-07	4.07E-06		HTZ92_RS09940	1.6344	4.96E-08	8.69E-07
	HTZ92_RS00125	3.3285	3.43E-18	2.13E-16		HTZ92_RS09980	1.9912	2.19E-10	6.16E-09
	HTZ92_RS00130	2.4109	8.36E-08	1.37E-06		HTZ92_RS10005	1.0419	1.43E-02	4.24E-02
	HTZ92_RS00135	2.4361	4.72E-11	1.43E-09		HTZ92_RS10030	1.3737	8.07E-05	6.01E-04
	HTZ92_RS00145	2.0714	2.45E-04	1.52E-03		HTZ92_RS10065	-1.1060	6.84E-03	2.34E-02
	HTZ92_RS00170	1.5847	2.47E-06	2.78E-05		HTZ92_RS10070	1.1901	1.09E-03	5.18E-03
	HTZ92_RS00220	1.4381	1.34E-08	2.68E-07		HTZ92_RS10095	1.4675	1.45E-02	4.28E-02
<i>hisH</i>	HTZ92_RS00245	1.2232	5.47E-04	2.97E-03	<i>paaG</i>	HTZ92_RS10100	1.9715	2.67E-03	1.08E-02
	HTZ92_RS00275	-2.0998	1.72E-07	2.59E-06		HTZ92_RS10105	2.0545	1.99E-03	8.40E-03
	HTZ92_RS00295	-1.2257	5.36E-03	1.91E-02	<i>paaK</i>	HTZ92_RS10110	2.7200	8.06E-05	6.01E-04
	HTZ92_RS00305	-1.6254	3.69E-03	1.41E-02	<i>paaJ</i>	HTZ92_RS10115	2.8478	3.23E-06	3.52E-05
	HTZ92_RS00310	-2.0034	2.67E-03	1.08E-02	<i>paaC</i>	HTZ92_RS10120	2.6918	5.45E-05	4.36E-04
	HTZ92_RS00330	-5.0228	1.82E-14	8.28E-13	<i>paaB</i>	HTZ92_RS10125	3.1240	3.34E-06	3.62E-05
	HTZ92_RS00335	-4.0949	3.08E-08	5.71E-07	<i>paaA</i>	HTZ92_RS10130	3.5937	5.53E-06	5.71E-05
	HTZ92_RS00340	-3.6137	3.70E-07	5.10E-06	<i>paaZ</i>	HTZ92_RS10135	1.8751	7.52E-03	2.53E-02
	HTZ92_RS00345	-2.8560	2.84E-05	2.45E-04		HTZ92_RS10145	2.1628	5.16E-15	2.47E-13
	HTZ92_RS00350	-1.7806	7.65E-05	5.78E-04		HTZ92_RS10150	1.2461	1.30E-07	2.02E-06
	HTZ92_RS00385	-1.0642	8.29E-03	2.73E-02		HTZ92_RS10175	1.8195	2.45E-07	3.56E-06
	HTZ92_RS00405	-3.2533	4.74E-05	3.84E-04		HTZ92_RS10180	1.8518	1.01E-04	7.21E-04
	HTZ92_RS00470	1.5465	1.04E-05	1.00E-04		HTZ92_RS10195	1.7070	5.06E-06	5.27E-05
	HTZ92_RS00475	1.2346	1.44E-04	9.75E-04		HTZ92_RS10225	1.0958	5.62E-05	4.47E-04
<i>gltB</i>	HTZ92_RS00495	-2.3600	1.15E-04	8.04E-04		HTZ92_RS10280	-1.7160	1.71E-02	4.87E-02
	HTZ92_RS00500	-1.5001	1.25E-02	3.80E-02		HTZ92_RS10285	-1.0074	9.20E-03	2.97E-02
	HTZ92_RS00580	2.5100	1.38E-06	1.65E-05	<i>tssF</i>	HTZ92_RS10310	-1.6974	9.15E-04	4.52E-03
	HTZ92_RS00605	-1.7737	2.32E-04	1.46E-03	<i>tssE</i>	HTZ92_RS10315	-2.3318	5.02E-04	2.76E-03
<i>rpsP</i>	HTZ92_RS00615	-1.8017	2.86E-11	8.79E-10	<i>tssC</i>	HTZ92_RS10325	-1.5033	4.69E-03	1.71E-02
<i>rimM</i>	HTZ92_RS00620	-1.6315	5.34E-07	7.06E-06	<i>tssB</i>	HTZ92_RS10330	-1.2850	7.96E-03	2.65E-02
<i>trmD</i>	HTZ92_RS00625	-1.7790	2.47E-10	6.82E-09		HTZ92_RS10335	-1.4691	4.77E-03	1.73E-02
<i>rplS</i>	HTZ92_RS00630	-1.6878	1.37E-09	3.34E-08		HTZ92_RS10340	-1.8462	7.49E-06	7.46E-05
	HTZ92_RS00655	-2.7615	2.76E-05	2.39E-04		HTZ92_RS10355	-1.5504	1.44E-02	4.26E-02
	HTZ92_RS00720	1.2280	6.54E-04	3.43E-03		HTZ92_RS10380	2.1847	1.12E-07	1.78E-06
	HTZ92_RS00730	1.5664	6.35E-04	3.35E-03	<i>uca</i>	HTZ92_RS10390	-1.6768	1.33E-02	4.01E-02
	HTZ92_RS00750	-1.0254	2.30E-04	1.46E-03		HTZ92_RS10395	-2.2425	4.22E-03	1.57E-02
<i>astA</i>	HTZ92_RS00765	-1.9650	3.86E-07	5.28E-06		HTZ92_RS10400	-2.5299	3.29E-03	1.28E-02
<i>astD</i>	HTZ92_RS00770	-1.6993	2.38E-04	1.48E-03		HTZ92_RS10415	1.0498	7.20E-03	2.44E-02
<i>astB</i>	HTZ92_RS00775	-1.7548	3.11E-05	2.67E-04		HTZ92_RS10435	-1.8302	1.40E-02	4.15E-02
	HTZ92_RS00790	-3.9985	9.77E-05	7.01E-04		HTZ92_RS10455	-1.2053	3.06E-03	1.21E-02
	HTZ92_RS00805	-1.7424	7.01E-05	5.36E-04		HTZ92_RS10465	-1.1056	4.52E-03	1.66E-02
	HTZ92_RS00820	-1.3965	6.00E-05	4.69E-04	<i>mumR</i>	HTZ92_RS10470	2.0713	1.70E-08	3.30E-07
<i>glyQ</i>	HTZ92_RS00830	-1.5082	1.04E-03	4.99E-03		HTZ92_RS10500	-2.8729	3.64E-03	1.39E-02
	HTZ92_RS00855	2.9128	8.05E-04	4.06E-03		HTZ92_RS10530	1.8786	2.40E-04	1.49E-03
<i>ribA</i>	HTZ92_RS00885	-1.0896	2.04E-05	1.82E-04		HTZ92_RS10615	-2.4558	1.81E-05	1.64E-04
	HTZ92_RS00900	-1.7404	8.34E-04	4.20E-03		HTZ92_RS10620	-1.4897	7.06E-04	3.65E-03
	HTZ92_RS00910	1.1054	8.62E-05	6.32E-04	<i>cysS</i>	HTZ92_RS10675	-1.2256	4.05E-04	2.31E-03
	HTZ92_RS00920	2.5894	2.25E-26	2.19E-24		HTZ92_RS10695	1.3361	5.98E-05	4.68E-04
	HTZ92_RS00925	2.5669	1.19E-18	7.49E-17		HTZ92_RS10725	-1.7565	3.44E-03	1.33E-02
	HTZ92_RS00930	2.8092	8.09E-18	4.85E-16		HTZ92_RS10745	1.2493	6.65E-05	5.14E-04

	HTZ92_RS00935	-1.2963	8.85E-05	6.45E-04		HTZ92_RS10750	1.4028	1.04E-02	3.28E-02
	HTZ92_RS00940	-1.5067	7.22E-03	2.44E-02		HTZ92_RS10755	3.0419	4.92E-17	2.81E-15
	HTZ92_RS00995	-1.0869	5.41E-03	1.92E-02		HTZ92_RS10760	2.2063	1.01E-10	2.97E-09
	HTZ92_RS01005	-1.0600	2.30E-03	9.54E-03		HTZ92_RS10765	3.1996	3.41E-20	2.36E-18
<i>rpsJ</i>	HTZ92_RS01020	-3.2696	1.14E-21	8.88E-20		HTZ92_RS10770	3.4353	4.16E-28	4.28E-26
<i>rplC</i>	HTZ92_RS01025	-2.8020	7.04E-19	4.52E-17		HTZ92_RS10775	3.7048	4.94E-30	5.22E-28
<i>rplD</i>	HTZ92_RS01030	-3.2736	2.69E-33	3.34E-31		HTZ92_RS10780	4.1996	1.47E-34	1.95E-32
<i>rplW</i>	HTZ92_RS01035	-3.3153	9.71E-23	8.31E-21		HTZ92_RS10785	5.3079	7.09E-33	8.22E-31
<i>rplB</i>	HTZ92_RS01040	-2.9136	2.21E-43	3.78E-41		HTZ92_RS10790	6.2623	2.84E-82	2.55E-79
<i>rpsS</i>	HTZ92_RS01045	-2.7678	5.43E-24	4.88E-22		HTZ92_RS10795	7.0809	3.78E-95	6.79E-92
<i>rplV</i>	HTZ92_RS01050	-2.9693	4.47E-34	5.74E-32		HTZ92_RS10800	7.6570	4.55E-43	7.44E-41
<i>rplP</i>	HTZ92_RS01060	-1.5204	1.64E-08	3.22E-07		HTZ92_RS10805	6.3318	2.53E-63	8.26E-61
<i>rpsN</i>	HTZ92_RS01090	-1.1074	9.25E-05	6.68E-04		HTZ92_RS10810	5.3540	3.22E-51	6.44E-49
<i>rpsH</i>	HTZ92_RS01095	-1.3773	1.39E-06	1.66E-05		HTZ92_RS10815	4.4162	4.18E-61	1.07E-58
<i>rplF</i>	HTZ92_RS01100	-1.4844	1.02E-08	2.07E-07		HTZ92_RS10820	6.2663	5.06E-80	3.64E-77
<i>rplR</i>	HTZ92_RS01105	-1.7060	3.82E-11	1.17E-09		HTZ92_RS10825	5.9636	1.52E-62	4.55E-60
<i>rpsE</i>	HTZ92_RS01110	-1.5204	1.07E-10	3.13E-09		HTZ92_RS10830	6.2331	2.84E-65	1.02E-62
<i>rpmD</i>	HTZ92_RS01115	-1.5517	6.14E-08	1.04E-06		HTZ92_RS10835	6.4050	1.48E-71	5.92E-69
<i>rpmJ</i>	HTZ92_RS01130	-1.3167	2.65E-06	2.97E-05		HTZ92_RS10840	5.4999	3.78E-33	4.53E-31
<i>rpsM</i>	HTZ92_RS01135	-2.1110	2.43E-11	7.54E-10		HTZ92_RS10845	5.1901	5.83E-36	8.73E-34
<i>rpsK</i>	HTZ92_RS01140	-1.7761	6.18E-12	2.08E-10		HTZ92_RS10850	6.6736	3.74E-51	7.07E-49
<i>rpsD</i>	HTZ92_RS01145	-1.6933	5.11E-10	1.35E-08		HTZ92_RS10855	1.0905	5.87E-05	4.62E-04
<i>rpoA</i>	HTZ92_RS01150	-1.7051	3.64E-09	8.24E-08		HTZ92_RS10860	5.8242	1.62E-37	2.52E-35
<i>rplQ</i>	HTZ92_RS01155	-1.1367	1.62E-03	7.12E-03		HTZ92_RS10865	6.3640	1.88E-77	9.63E-75
	HTZ92_RS01200	1.1813	6.89E-05	5.28E-04		HTZ92_RS10870	7.1777	5.00E-79	3.00E-76
	HTZ92_RS01235	1.1414	6.57E-04	3.43E-03		HTZ92_RS10875	6.5483	2.70E-97	9.70E-94
	HTZ92_RS01290	-1.9212	3.23E-06	3.52E-05		HTZ92_RS10880	6.6779	1.84E-84	2.20E-81
<i>ybeY</i>	HTZ92_RS01300	-1.0459	3.53E-04	2.06E-03		HTZ92_RS10885	5.6867	3.70E-61	1.02E-58
	HTZ92_RS01325	-1.5576	1.24E-04	8.65E-04		HTZ92_RS10890	5.9451	1.09E-57	2.45E-55
	HTZ92_RS01335	1.7010	3.56E-08	6.49E-07		HTZ92_RS10895	6.8506	1.15E-72	5.18E-70
	HTZ92_RS01360	2.6039	1.29E-03	5.94E-03		HTZ92_RS10905	5.4520	9.21E-36	1.27E-33
	HTZ92_RS01370	1.2137	9.12E-03	2.95E-02		HTZ92_RS10910	3.8816	1.10E-20	7.94E-19
	HTZ92_RS01375	2.5448	1.65E-03	7.25E-03		HTZ92_RS10945	1.8756	8.05E-07	1.00E-05
<i>rpsI</i>	HTZ92_RS01450	-1.2050	8.74E-06	8.54E-05		HTZ92_RS10960	1.6050	2.68E-05	2.34E-04
<i>rplM</i>	HTZ92_RS01455	-1.8169	1.85E-08	3.56E-07		HTZ92_RS10965	1.5091	1.10E-06	1.34E-05
<i>pdxA</i>	HTZ92_RS01460	1.4579	1.80E-05	1.63E-04		HTZ92_RS10995	-1.3594	7.31E-03	2.46E-02
	HTZ92_RS01475	-2.1255	3.04E-07	4.28E-06		HTZ92_RS11000	-2.5868	1.85E-06	2.14E-05
	HTZ92_RS01510	-1.4575	2.29E-03	9.51E-03		HTZ92_RS11020	1.5267	5.60E-09	1.19E-07
	HTZ92_RS01515	-1.2383	9.68E-04	4.71E-03		HTZ92_RS11025	-1.0901	1.63E-03	7.15E-03
	HTZ92_RS01520	-1.7757	9.03E-08	1.46E-06		HTZ92_RS11030	-1.6947	7.05E-07	8.92E-06
	HTZ92_RS01525	2.6174	1.17E-03	5.50E-03		HTZ92_RS11045	-1.5408	9.95E-04	4.82E-03
	HTZ92_RS01535	1.4047	7.61E-03	2.55E-02		HTZ92_RS11055	1.5887	7.20E-09	1.49E-07
	HTZ92_RS01540	2.5377	1.69E-03	7.37E-03		HTZ92_RS11060	1.3122	3.04E-04	1.83E-03
	HTZ92_RS01575	1.1265	3.18E-03	1.25E-02		HTZ92_RS11120	-1.7995	4.16E-04	2.37E-03
	HTZ92_RS01585	-1.8820	4.34E-04	2.43E-03	<i>ahpC</i>	HTZ92_RS11135	-1.0414	8.94E-03	2.91E-02
	HTZ92_RS01600	-1.5743	1.54E-03	6.89E-03		HTZ92_RS11185	2.6542	1.15E-06	1.40E-05
	HTZ92_RS01610	-1.4869	1.75E-03	7.59E-03		HTZ92_RS11200	1.2578	6.72E-05	5.18E-04
<i>secD</i>	HTZ92_RS01640	-1.4263	3.22E-04	1.91E-03		HTZ92_RS11215	-1.5925	1.16E-03	5.44E-03
<i>yajC</i>	HTZ92_RS01645	-2.2702	2.91E-06	3.19E-05		HTZ92_RS11235	-1.7385	6.94E-06	6.97E-05
<i>tgt</i>	HTZ92_RS01650	-1.5367	3.13E-04	1.88E-03	<i>clpB</i>	HTZ92_RS11240	1.4950	8.59E-05	6.32E-04

	HTZ92_RS01735	-1.7212	8.71E-06	8.53E-05		HTZ92_RS11250	-1.2869	1.35E-02	4.05E-02
<i>aspS</i>	HTZ92_RS01745	-1.0928	2.52E-04	1.54E-03		HTZ92_RS11275	1.0359	5.07E-04	2.78E-03
	HTZ92_RS01760	1.1863	3.75E-05	3.09E-04		HTZ92_RS11280	-2.8176	2.35E-04	1.47E-03
	HTZ92_RS01770	1.8846	2.48E-09	5.79E-08		HTZ92_RS11320	1.3238	1.24E-02	3.79E-02
	HTZ92_RS01775	-1.3161	2.52E-03	1.03E-02		HTZ92_RS11325	1.3727	5.07E-05	4.09E-04
	HTZ92_RS01825	-1.5530	5.74E-05	4.54E-04		HTZ92_RS11365	1.1825	1.41E-02	4.17E-02
<i>folC</i>	HTZ92_RS01885	-1.1511	7.97E-04	4.04E-03		HTZ92_RS11370	1.1445	1.11E-04	7.81E-04
	HTZ92_RS01905	-4.4333	5.58E-08	9.51E-07		HTZ92_RS11380	1.2948	7.60E-03	2.55E-02
	HTZ92_RS01920	-1.1502	1.53E-02	4.46E-02		HTZ92_RS11390	1.2444	1.01E-03	4.90E-03
	HTZ92_RS01925	-1.4286	8.92E-04	4.45E-03		HTZ92_RS11420	1.2507	2.26E-04	1.44E-03
	HTZ92_RS01945	-1.5891	1.07E-03	5.12E-03		HTZ92_RS11425	1.4476	1.05E-04	7.50E-04
	HTZ92_RS01955	-1.2239	5.76E-04	3.09E-03		HTZ92_RS11445	1.7205	2.18E-08	4.13E-07
	HTZ92_RS01980	-1.3435	5.68E-05	4.51E-04		HTZ92_RS11530	-1.6598	2.04E-07	3.02E-06
	HTZ92_RS01985	-1.1571	3.29E-03	1.28E-02		HTZ92_RS11610	-1.0611	7.21E-04	3.71E-03
	HTZ92_RS02005	-2.6270	1.96E-13	8.10E-12		HTZ92_RS11635	1.0867	1.25E-05	1.17E-04
	HTZ92_RS02015	-1.9665	1.18E-04	8.27E-04		HTZ92_RS11640	1.0555	5.61E-03	1.98E-02
	HTZ92_RS02020	-1.8694	1.77E-08	3.43E-07		HTZ92_RS11655	1.3590	3.26E-05	2.77E-04
<i>mscL</i>	HTZ92_RS02045	-1.1039	1.61E-02	4.63E-02		HTZ92_RS11730	-1.3772	3.78E-03	1.44E-02
	HTZ92_RS02085	-1.7401	1.04E-04	7.40E-04	<i>mhpT</i>	HTZ92_RS11735	1.1304	2.02E-03	8.52E-03
	HTZ92_RS02090	-1.4594	1.68E-03	7.33E-03		HTZ92_RS11740	2.0457	1.26E-09	3.12E-08
	HTZ92_RS02100	-2.2589	2.73E-10	7.49E-09	<i>gudD</i>	HTZ92_RS11795	1.1849	6.11E-06	6.24E-05
	HTZ92_RS02115	-1.8853	8.84E-03	2.89E-02		HTZ92_RS11820	1.1697	7.33E-03	2.47E-02
	HTZ92_RS02135	1.4620	6.78E-03	2.32E-02		HTZ92_RS11860	1.0190	4.15E-03	1.55E-02
<i>dapE</i>	HTZ92_RS02170	-1.0575	1.84E-05	1.66E-04		HTZ92_RS11865	-1.0686	6.80E-05	5.24E-04
	HTZ92_RS02195	-1.3280	4.20E-04	2.38E-03		HTZ92_RS11885	-2.1194	1.68E-08	3.28E-07
	HTZ92_RS02255	-1.6096	8.20E-03	2.70E-02		HTZ92_RS11905	-2.0611	7.39E-06	7.38E-05
	HTZ92_RS02260	-1.2513	1.36E-02	4.06E-02		HTZ92_RS11910	-1.2221	4.86E-05	3.93E-04
<i>sbmA</i>	HTZ92_RS02280	-1.1316	1.47E-04	9.92E-04		HTZ92_RS11935	-2.3929	1.02E-05	9.82E-05
	HTZ92_RS02300	-1.3854	1.68E-06	1.95E-05		HTZ92_RS11965	1.3681	1.17E-05	1.10E-04
<i>gatA</i>	HTZ92_RS02310	-1.4916	3.49E-04	2.05E-03		HTZ92_RS11970	1.8555	8.99E-17	4.89E-15
<i>gatC</i>	HTZ92_RS02315	-1.5417	1.31E-04	9.01E-04		HTZ92_RS11975	1.0383	1.36E-04	9.26E-04
	HTZ92_RS02345	-2.0887	4.24E-03	1.58E-02		HTZ92_RS11980	1.8291	5.19E-08	8.98E-07
<i>hchA</i>	HTZ92_RS02405	-1.0321	5.06E-03	1.82E-02		HTZ92_RS11990	1.5065	7.03E-07	8.92E-06
	HTZ92_RS02415	-2.0722	1.20E-11	3.94E-10	<i>merD</i>	HTZ92_RS12005	3.9932	2.07E-15	1.05E-13
	HTZ92_RS02455	1.7751	1.17E-07	1.85E-06	<i>merA</i>	HTZ92_RS12010	3.3915	1.69E-12	6.09E-11
	HTZ92_RS02460	-1.2116	3.98E-04	2.28E-03	<i>merC</i>	HTZ92_RS12015	3.1849	2.45E-09	5.76E-08
	HTZ92_RS02465	-2.8255	2.08E-05	1.85E-04	<i>merP</i>	HTZ92_RS12020	3.2139	1.17E-08	2.35E-07
	HTZ92_RS02470	-2.4301	8.24E-05	6.12E-04	<i>merT</i>	HTZ92_RS12025	2.9168	3.40E-07	4.74E-06
	HTZ92_RS02475	-2.1344	1.61E-03	7.10E-03	<i>merR</i>	HTZ92_RS12030	1.5803	2.37E-05	2.09E-04
	HTZ92_RS02480	-2.6909	1.15E-05	1.09E-04	<i>lspA</i>	HTZ92_RS12040	-1.1527	1.25E-02	3.81E-02
	HTZ92_RS02485	-2.0440	9.09E-03	2.94E-02		HTZ92_RS12045	-1.8477	7.39E-04	3.80E-03
	HTZ92_RS02490	-2.7227	7.32E-05	5.59E-04	<i>cadR</i>	HTZ92_RS12050	2.6022	3.21E-05	2.74E-04
<i>adeJ</i>	HTZ92_RS02550	-1.2840	1.15E-03	5.40E-03		HTZ92_RS12060	1.3037	1.86E-03	7.97E-03
<i>adeI</i>	HTZ92_RS02555	-1.3762	5.72E-03	2.02E-02	<i>rnt</i>	HTZ92_RS12155	-1.0172	8.82E-05	6.44E-04
<i>lolA</i>	HTZ92_RS02590	1.8136	5.16E-10	1.35E-08		HTZ92_RS12165	-1.1170	3.52E-05	2.95E-04
	HTZ92_RS02610	-1.0867	8.90E-03	2.90E-02		HTZ92_RS12225	-2.1176	5.27E-03	1.88E-02
	HTZ92_RS02615	2.3290	1.89E-05	1.70E-04		HTZ92_RS12245	1.2808	2.09E-03	8.73E-03
	HTZ92_RS02635	-1.4433	6.27E-08	1.06E-06		HTZ92_RS12250	-3.7764	6.94E-06	6.97E-05
	HTZ92_RS02655	-1.6537	3.43E-04	2.02E-03		HTZ92_RS12255	1.9055	6.08E-13	2.33E-11
	HTZ92_RS02660	-2.3959	2.11E-13	8.60E-12		HTZ92_RS12285	-1.2627	2.99E-03	1.18E-02

<i>gltA</i>	HTZ92_RS02675	-2.3658	1.79E-07	2.67E-06	<i>ata</i>	HTZ92_RS12325	1.0177	1.99E-03	8.38E-03
<i>sdhC</i>	HTZ92_RS02680	-1.4592	5.77E-06	5.93E-05	<i>lon</i>	HTZ92_RS12330	1.2449	5.37E-03	1.91E-02
<i>sdhD</i>	HTZ92_RS02685	-1.3664	3.47E-05	2.92E-04	<i>gdhA</i>	HTZ92_RS12350	-1.5695	3.64E-04	2.12E-03
	HTZ92_RS02695	-1.0917	3.67E-05	3.05E-04	<i>ureG</i>	HTZ92_RS12400	-2.2562	1.45E-03	6.56E-03
<i>sucC</i>	HTZ92_RS02725	-1.0036	1.96E-04	1.26E-03		HTZ92_RS12405	-3.3100	2.74E-05	2.38E-04
	HTZ92_RS02775	-1.4574	2.78E-03	1.11E-02	<i>ureE</i>	HTZ92_RS12410	-2.0022	7.99E-03	2.66E-02
<i>yhbY</i>	HTZ92_RS02815	-1.3256	4.36E-04	2.44E-03		HTZ92_RS12420	-2.7949	1.60E-03	7.08E-03
<i>ftsH</i>	HTZ92_RS02825	1.6274	6.64E-13	2.51E-11	<i>ureA</i>	HTZ92_RS12425	-2.5543	2.30E-03	9.54E-03
	HTZ92_RS02835	-1.5077	1.55E-02	4.51E-02		HTZ92_RS12430	-1.9817	1.03E-03	4.97E-03
	HTZ92_RS02855	-1.7549	1.68E-03	7.35E-03		HTZ92_RS12445	-2.4315	1.08E-05	1.04E-04
	HTZ92_RS02880	-1.1131	5.57E-03	1.97E-02	<i>cysD</i>	HTZ92_RS12480	-1.3795	5.75E-03	2.02E-02
	HTZ92_RS02895	-1.9823	1.31E-06	1.59E-05		HTZ92_RS12485	-2.4553	2.64E-04	1.62E-03
	HTZ92_RS02925	-3.7507	6.40E-06	6.48E-05		HTZ92_RS12500	-1.0087	1.35E-03	6.16E-03
<i>htpX</i>	HTZ92_RS02950	1.2322	7.21E-07	9.06E-06		HTZ92_RS12505	-1.5140	1.69E-03	7.37E-03
	HTZ92_RS02955	-1.1089	4.57E-03	1.68E-02		HTZ92_RS12510	-1.3853	2.76E-07	3.94E-06
	HTZ92_RS02965	-1.8102	3.32E-05	2.81E-04		HTZ92_RS12515	-1.0165	2.49E-03	1.02E-02
	HTZ92_RS02990	-1.8600	6.99E-03	2.38E-02		HTZ92_RS12585	1.2543	7.70E-04	3.94E-03
	HTZ92_RS03025	2.4618	5.54E-09	1.19E-07		HTZ92_RS12600	-1.5870	4.78E-03	1.74E-02
	HTZ92_RS03035	1.1597	9.19E-03	2.97E-02		HTZ92_RS12610	1.3894	6.89E-03	2.35E-02
	HTZ92_RS03060	1.4857	5.49E-08	9.44E-07		HTZ92_RS12625	-1.1832	6.01E-03	2.09E-02
	HTZ92_RS03070	-1.4394	8.32E-05	6.15E-04		HTZ92_RS12650	1.0056	1.07E-03	5.12E-03
<i>lysA</i>	HTZ92_RS03080	-1.0769	9.41E-03	3.02E-02		HTZ92_RS12705	1.0756	5.68E-04	3.06E-03
	HTZ92_RS03095	-1.0504	7.25E-03	2.45E-02		HTZ92_RS12745	1.1148	2.88E-04	1.75E-03
	HTZ92_RS03125	-2.0891	5.79E-03	2.03E-02		HTZ92_RS12750	1.3906	3.43E-04	2.02E-03
	HTZ92_RS03130	-3.4791	5.24E-05	4.20E-04	<i>betA</i>	HTZ92_RS12855	1.1887	6.97E-03	2.38E-02
	HTZ92_RS03135	-3.1317	1.06E-04	7.53E-04		HTZ92_RS12870	-1.8500	4.61E-03	1.69E-02
	HTZ92_RS03140	-4.3971	3.48E-07	4.81E-06		HTZ92_RS12880	1.6580	1.53E-07	2.33E-06
<i>tolR</i>	HTZ92_RS03290	-1.9897	6.27E-04	3.32E-03		HTZ92_RS12885	1.0649	1.50E-04	1.01E-03
<i>tolQ</i>	HTZ92_RS03295	-1.5060	9.30E-03	2.99E-02		HTZ92_RS12895	-1.2232	1.47E-03	6.62E-03
	HTZ92_RS03305	-1.0235	1.40E-02	4.15E-02		HTZ92_RS12915	1.4963	3.64E-08	6.58E-07
	HTZ92_RS03330	1.1820	5.91E-04	3.15E-03		HTZ92_RS12930	1.3534	1.28E-04	8.82E-04
	HTZ92_RS03365	1.4090	4.33E-04	2.43E-03		HTZ92_RS12935	2.4568	1.44E-05	1.34E-04
	HTZ92_RS03390	-4.4028	4.27E-08	7.63E-07		HTZ92_RS12940	1.4693	6.34E-04	3.35E-03
	HTZ92_RS03395	-3.7540	2.89E-06	3.19E-05		HTZ92_RS12965	1.1609	2.08E-03	8.72E-03
<i>cysW</i>	HTZ92_RS03400	-5.0533	4.31E-08	7.68E-07		HTZ92_RS12970	2.3729	1.39E-13	5.88E-12
<i>cysT</i>	HTZ92_RS03405	-4.5342	1.47E-06	1.74E-05	<i>cas3f</i>	HTZ92_RS12985	1.8716	1.39E-10	4.00E-09
	HTZ92_RS03410	-5.6717	8.27E-05	6.13E-04		HTZ92_RS12990	2.5322	8.19E-11	2.45E-09
	HTZ92_RS03415	-6.4433	3.90E-10	1.05E-08		HTZ92_RS13060	1.4215	4.83E-08	8.52E-07
<i>nadB</i>	HTZ92_RS03440	-1.4091	6.68E-04	3.47E-03		HTZ92_RS13170	1.3562	3.15E-04	1.88E-03
<i>lepB</i>	HTZ92_RS03460	-3.1558	1.18E-04	8.28E-04		HTZ92_RS13175	1.0684	1.76E-04	1.15E-03
	HTZ92_RS03465	-1.9652	1.40E-04	9.48E-04		HTZ92_RS13195	1.7525	1.52E-06	1.79E-05
<i>era</i>	HTZ92_RS03475	1.5184	6.67E-04	3.47E-03		HTZ92_RS13200	1.3960	2.53E-07	3.66E-06
	HTZ92_RS03480	1.3410	5.32E-04	2.91E-03		HTZ92_RS13215	-3.3495	1.35E-05	1.25E-04
	HTZ92_RS03545	-1.5451	3.68E-05	3.05E-04		HTZ92_RS13225	-1.6854	3.66E-04	2.13E-03
	HTZ92_RS03555	1.3929	7.50E-07	9.36E-06		HTZ92_RS13245	-1.5123	3.20E-03	1.25E-02
<i>uvrB</i>	HTZ92_RS03560	1.3786	1.31E-09	3.22E-08	<i>minC</i>	HTZ92_RS13255	-1.0868	1.09E-05	1.04E-04
	HTZ92_RS03640	-1.0017	4.86E-03	1.75E-02	<i>minD</i>	HTZ92_RS13260	-1.2364	5.81E-04	3.12E-03
	HTZ92_RS03670	-1.0459	1.54E-03	6.88E-03		HTZ92_RS13280	1.0580	9.40E-06	9.16E-05
	HTZ92_RS03685	1.6364	3.40E-07	4.74E-06	<i>rimI</i>	HTZ92_RS13300	-1.1584	1.29E-02	3.92E-02
	HTZ92_RS03750	1.4521	5.00E-07	6.69E-06	<i>fusA</i>	HTZ92_RS13320	-1.7645	2.97E-09	6.84E-08

	HTZ92_RS03770	1.3651	1.58E-04	1.05E-03	<i>rpsG</i>	HTZ92_RS13325	-1.1866	1.57E-03	6.97E-03
	HTZ92_RS03785	-1.1952	1.18E-03	5.50E-03		HTZ92_RS13335	-1.0858	4.43E-04	2.47E-03
<i>fabR</i>	HTZ92_RS03790	-1.4654	4.47E-04	2.49E-03		HTZ92_RS13405	-1.0543	3.54E-03	1.36E-02
	HTZ92_RS03795	-2.4068	4.71E-15	2.29E-13		HTZ92_RS13440	1.2056	2.68E-03	1.08E-02
	HTZ92_RS03800	-1.9456	9.80E-09	2.00E-07		HTZ92_RS13455	-1.2902	1.01E-02	3.21E-02
	HTZ92_RS03835	1.0318	1.39E-02	4.15E-02		HTZ92_RS13495	-2.0735	5.55E-08	9.50E-07
	HTZ92_RS03850	-5.2645	9.26E-09	1.90E-07	<i>rplY</i>	HTZ92_RS13500	1.6095	2.20E-06	2.52E-05
<i>pstC</i>	HTZ92_RS03855	-3.9038	2.46E-06	2.78E-05		HTZ92_RS13510	-1.3873	3.52E-03	1.36E-02
<i>pstA</i>	HTZ92_RS03860	-4.0174	1.03E-06	1.27E-05	<i>ribB</i>	HTZ92_RS13525	-1.0854	1.05E-02	3.30E-02
<i>pstB</i>	HTZ92_RS03865	-2.1197	7.36E-05	5.61E-04	<i>lysM</i>	HTZ92_RS13540	-1.4967	2.38E-04	1.48E-03
<i>sohB</i>	HTZ92_RS03870	1.0833	4.43E-06	4.68E-05	<i>acpP</i>	HTZ92_RS13545	-1.8428	1.62E-03	7.14E-03
<i>dacC</i>	HTZ92_RS03910	-2.0352	2.60E-14	1.17E-12	<i>fabG</i>	HTZ92_RS13550	-1.0918	2.55E-05	2.23E-04
	HTZ92_RS03915	-1.8663	2.08E-04	1.33E-03		HTZ92_RS13575	1.5536	1.14E-04	8.03E-04
	HTZ92_RS03920	-2.3180	1.78E-03	7.71E-03		HTZ92_RS13580	-1.9163	7.01E-08	1.17E-06
<i>surE</i>	HTZ92_RS03925	-1.7164	8.05E-06	7.93E-05		HTZ92_RS13605	1.8748	1.49E-07	2.28E-06
<i>gloA</i>	HTZ92_RS03950	1.6494	1.76E-04	1.15E-03		HTZ92_RS13725	-2.7804	2.25E-04	1.44E-03
	HTZ92_RS04005	1.3886	1.89E-03	8.04E-03		HTZ92_RS13795	2.9051	7.08E-08	1.18E-06
	HTZ92_RS04010	1.6408	4.91E-09	1.08E-07	<i>nuoM</i>	HTZ92_RS13830	-1.4484	1.73E-07	2.60E-06
<i>bauD</i>	HTZ92_RS04050	2.0669	6.30E-08	1.06E-06	<i>nuoL</i>	HTZ92_RS13835	-1.6095	1.36E-07	2.10E-06
<i>bauE</i>	HTZ92_RS04060	1.2710	4.88E-03	1.76E-02	<i>nuoI</i>	HTZ92_RS13850	-1.0141	4.67E-03	1.70E-02
<i>barA</i>	HTZ92_RS04100	1.1965	6.64E-04	3.46E-03	<i>nuoH</i>	HTZ92_RS13855	-1.5542	5.98E-06	6.13E-05
<i>basI</i>	HTZ92_RS04115	1.3748	1.09E-03	5.18E-03	<i>nuoG</i>	HTZ92_RS13860	-1.4471	2.27E-04	1.44E-03
<i>basJ</i>	HTZ92_RS04120	1.4499	9.19E-05	6.68E-04	<i>nuoF</i>	HTZ92_RS13865	-1.5915	1.62E-04	1.07E-03
<i>folE</i>	HTZ92_RS04155	-1.7268	1.42E-06	1.69E-05	<i>nuoE</i>	HTZ92_RS13870	-1.9065	4.94E-06	5.16E-05
<i>glnA</i>	HTZ92_RS04215	-2.2136	9.23E-05	6.68E-04	<i>nuoC</i>	HTZ92_RS13875	-1.2601	1.87E-03	7.98E-03
	HTZ92_RS04230	-1.6439	8.78E-11	2.61E-09		HTZ92_RS13880	-1.1400	8.57E-04	4.29E-03
	HTZ92_RS04265	-1.1006	5.50E-03	1.95E-02		HTZ92_RS13935	2.3496	2.89E-15	1.44E-13
	HTZ92_RS04275	1.5919	5.45E-04	2.96E-03		HTZ92_RS13940	1.9191	2.07E-09	4.90E-08
<i>ahcY</i>	HTZ92_RS04300	-1.2922	9.82E-04	4.76E-03		HTZ92_RS13965	2.2376	2.53E-06	2.85E-05
	HTZ92_RS04330	1.1878	5.60E-05	4.46E-04		HTZ92_RS13975	1.3719	8.76E-03	2.87E-02
	HTZ92_RS04335	-2.1133	1.82E-03	7.84E-03		HTZ92_RS13995	2.5298	8.25E-15	3.90E-13
<i>tsf</i>	HTZ92_RS04355	-1.8650	4.92E-09	1.08E-07	<i>tcuA</i>	HTZ92_RS14025	1.3513	2.64E-07	3.79E-06
	HTZ92_RS04385	-2.2435	1.78E-04	1.16E-03		HTZ92_RS14040	1.1362	3.33E-05	2.82E-04
	HTZ92_RS04400	-1.6588	4.10E-05	3.36E-04		HTZ92_RS14045	1.0834	4.24E-04	2.40E-03
<i>queF</i>	HTZ92_RS04405	-1.5678	1.62E-04	1.07E-03		HTZ92_RS14050	1.5487	3.46E-04	2.04E-03
	HTZ92_RS04430	1.8906	2.22E-06	2.52E-05		HTZ92_RS14055	2.2015	2.21E-06	2.52E-05
	HTZ92_RS04435	2.3905	4.18E-07	5.69E-06		HTZ92_RS14120	-3.0776	2.52E-13	1.02E-11
	HTZ92_RS04440	1.5140	1.18E-03	5.50E-03		HTZ92_RS14125	-2.8790	5.45E-22	4.35E-20
	HTZ92_RS04445	2.7712	8.22E-10	2.07E-08		HTZ92_RS14130	-2.4300	1.80E-13	7.52E-12
	HTZ92_RS04450	2.4178	3.27E-16	1.76E-14		HTZ92_RS14175	1.1584	2.43E-04	1.51E-03
	HTZ92_RS04455	1.9580	1.32E-06	1.59E-05	<i>hisC</i>	HTZ92_RS14220	-1.0248	1.36E-02	4.06E-02
	HTZ92_RS04470	2.0521	5.31E-06	5.50E-05		HTZ92_RS14260	2.5526	1.63E-03	7.15E-03
	HTZ92_RS04475	2.3482	1.59E-06	1.86E-05		HTZ92_RS14265	1.5729	1.32E-03	6.09E-03
	HTZ92_RS04485	2.1788	3.76E-04	2.17E-03		HTZ92_RS14275	2.5998	1.25E-03	5.83E-03
	HTZ92_RS04495	1.2080	1.25E-02	3.81E-02	<i>gigA</i>	HTZ92_RS14285	-1.1922	2.31E-04	1.46E-03
	HTZ92_RS04510	2.4366	3.63E-05	3.02E-04		HTZ92_RS14310	-1.3613	1.88E-03	8.00E-03
	HTZ92_RS04520	1.9577	3.05E-04	1.84E-03		HTZ92_RS14320	1.0558	1.56E-04	1.05E-03
	HTZ92_RS04525	1.8764	4.16E-05	3.39E-04	<i>trxA</i>	HTZ92_RS14365	1.5757	1.84E-04	1.19E-03
	HTZ92_RS04530	1.5534	6.93E-07	8.83E-06	<i>rho</i>	HTZ92_RS14370	-1.1538	1.19E-04	8.28E-04
	HTZ92_RS04535	2.2925	3.36E-05	2.84E-04		HTZ92_RS14375	-3.3728	9.21E-08	1.49E-06

	HTZ92_RS04540	1.8451	1.38E-06	1.65E-05		HTZ92_RS14380	-2.4978	5.41E-09	1.17E-07
	HTZ92_RS04550	1.9660	3.02E-04	1.82E-03		HTZ92_RS14385	1.2315	1.10E-04	7.79E-04
	HTZ92_RS04555	1.4559	1.18E-03	5.50E-03		HTZ92_RS14405	-1.1013	1.43E-02	4.22E-02
	HTZ92_RS04560	1.4406	3.48E-04	2.05E-03		HTZ92_RS14410	-2.3804	7.88E-06	7.80E-05
	HTZ92_RS04580	-2.1644	3.38E-06	3.65E-05		HTZ92_RS14435	-1.2030	2.99E-04	1.81E-03
	HTZ92_RS04585	-1.2136	1.08E-02	3.36E-02	<i>infC</i>	HTZ92_RS14440	2.1110	1.50E-05	1.38E-04
	HTZ92_RS04600	-1.5954	1.47E-02	4.31E-02		HTZ92_RS14450	-1.7110	2.13E-08	4.05E-07
	HTZ92_RS04605	1.0101	5.29E-03	1.89E-02	<i>pcnB</i>	HTZ92_RS14485	-1.2145	3.72E-03	1.42E-02
	HTZ92_RS04740	-1.9753	7.39E-05	5.62E-04		HTZ92_RS14530	-1.2618	2.86E-06	3.17E-05
	HTZ92_RS04755	1.4200	8.97E-03	2.92E-02		HTZ92_RS14555	-1.3072	4.59E-04	2.55E-03
	HTZ92_RS04805	-1.1792	9.85E-06	9.55E-05		HTZ92_RS14560	-1.2007	5.37E-04	2.93E-03
	HTZ92_RS04830	-5.4297	6.69E-09	1.41E-07		HTZ92_RS14565	-1.3810	1.33E-04	9.07E-04
<i>hisQ</i>	HTZ92_RS04835	-2.9075	6.19E-04	3.29E-03		HTZ92_RS14610	-1.0726	2.77E-03	1.11E-02
	HTZ92_RS04840	-2.8700	5.80E-04	3.11E-03	<i>acnA</i>	HTZ92_RS14615	1.2785	1.95E-03	8.24E-03
<i>yegQ</i>	HTZ92_RS04860	1.1911	4.71E-04	2.61E-03		HTZ92_RS14645	-2.2658	4.72E-04	2.61E-03
	HTZ92_RS04875	-1.9534	1.26E-04	8.74E-04	<i>ihvC</i>	HTZ92_RS14660	-1.5954	2.52E-03	1.03E-02
	HTZ92_RS04885	2.1868	3.88E-08	6.97E-07	<i>ihvN</i>	HTZ92_RS14665	-1.6404	2.63E-03	1.07E-02
	HTZ92_RS04900	1.6477	3.36E-06	3.64E-05		HTZ92_RS14670	-1.3809	1.64E-02	4.71E-02
	HTZ92_RS04910	-2.6618	4.75E-12	1.61E-10		HTZ92_RS14675	-1.5106	1.50E-02	4.37E-02
	HTZ92_RS04945	-1.1672	1.44E-03	6.53E-03	<i>leuS</i>	HTZ92_RS14680	-1.5644	4.22E-06	4.47E-05
	HTZ92_RS05000	-1.1429	8.71E-03	2.86E-02		HTZ92_RS14710	-1.0212	3.22E-04	1.91E-03
	HTZ92_RS05010	-1.2827	1.55E-03	6.91E-03	<i>rsgA</i>	HTZ92_RS14725	-1.2163	6.10E-03	2.12E-02
	HTZ92_RS05045	-1.0843	2.31E-03	9.57E-03	<i>pilW</i>	HTZ92_RS14780	1.7456	8.24E-10	2.07E-08
	HTZ92_RS05050	-1.1538	1.58E-04	1.05E-03	<i>rlmN</i>	HTZ92_RS14785	1.4317	3.38E-03	1.31E-02
	HTZ92_RS05085	1.1170	3.08E-06	3.37E-05	<i>ndk</i>	HTZ92_RS14790	-1.0028	1.76E-02	4.99E-02
	HTZ92_RS05160	-1.1177	8.04E-04	4.06E-03		HTZ92_RS14805	-1.1717	6.67E-04	3.47E-03
	HTZ92_RS05170	-1.2170	6.94E-04	3.60E-03		HTZ92_RS14820	-1.4845	2.51E-04	1.54E-03
<i>csuB</i>	HTZ92_RS05270	-1.5882	7.68E-03	2.56E-02	<i>eda</i>	HTZ92_RS14850	-1.2428	7.53E-04	3.87E-03
<i>csuC</i>	HTZ92_RS05275	-3.1705	4.81E-09	1.07E-07		HTZ92_RS14860	-1.2404	6.96E-03	2.37E-02
<i>csuD</i>	HTZ92_RS05280	-1.9772	1.30E-07	2.02E-06	<i>pta</i>	HTZ92_RS14865	-1.6962	1.85E-03	7.91E-03
<i>uraH</i>	HTZ92_RS05345	-1.5499	1.57E-05	1.44E-04		HTZ92_RS14875	-1.0079	4.08E-04	2.33E-03
	HTZ92_RS05350	-2.5399	4.09E-03	1.54E-02		HTZ92_RS14880	1.0537	1.16E-05	1.10E-04
	HTZ92_RS05370	-2.2477	8.99E-03	2.92E-02	<i>leuA</i>	HTZ92_RS14910	1.0430	2.68E-06	3.00E-05
	HTZ92_RS05410	-2.3654	3.75E-03	1.43E-02		HTZ92_RS14935	-1.1390	1.33E-02	4.01E-02
	HTZ92_RS05415	-2.2489	4.57E-03	1.68E-02		HTZ92_RS14940	-4.7104	4.84E-06	5.09E-05
	HTZ92_RS05445	1.2007	1.34E-04	9.14E-04	<i>tatB</i>	HTZ92_RS14945	-5.8164	5.01E-08	8.75E-07
	HTZ92_RS05460	1.8579	3.03E-08	5.64E-07	<i>tatC</i>	HTZ92_RS14950	-2.9847	6.84E-05	5.25E-04
	HTZ92_RS05475	-1.1008	2.24E-05	1.99E-04		HTZ92_RS14955	-1.9424	3.64E-03	1.39E-02
	HTZ92_RS05520	-1.0591	9.62E-03	3.07E-02		HTZ92_RS14995	-1.6252	3.65E-03	1.40E-02
<i>alr</i>	HTZ92_RS05545	1.1017	1.02E-02	3.24E-02		HTZ92_RS15055	-1.0769	1.36E-02	4.07E-02
<i>rpsR</i>	HTZ92_RS05560	-1.7781	8.00E-06	7.90E-05		HTZ92_RS15065	-1.2863	1.05E-03	5.03E-03
<i>rpsF</i>	HTZ92_RS05565	-1.5097	2.46E-07	3.57E-06		HTZ92_RS15080	1.7844	1.48E-08	2.93E-07
<i>cyoB</i>	HTZ92_RS05585	-1.0256	7.98E-04	4.04E-03		HTZ92_RS15100	2.4736	1.58E-06	1.85E-05
<i>pgaB</i>	HTZ92_RS05615	-2.5038	5.11E-05	4.11E-04		HTZ92_RS15125	3.2176	1.66E-14	7.67E-13
<i>pgaD</i>	HTZ92_RS05625	-2.4687	6.51E-04	3.42E-03		HTZ92_RS15140	1.1478	1.24E-05	1.16E-04
	HTZ92_RS05630	-1.5969	8.03E-05	6.01E-04	<i>gltP</i>	HTZ92_RS15180	-1.1266	8.51E-04	4.27E-03
	HTZ92_RS05640	3.4436	2.21E-26	2.19E-24	<i>truA</i>	HTZ92_RS15210	1.3785	3.58E-08	6.51E-07
	HTZ92_RS05645	2.3104	2.41E-12	8.32E-11	<i>infA</i>	HTZ92_RS15220	-1.6579	4.11E-06	4.39E-05
	HTZ92_RS05670	-1.3869	6.79E-07	8.68E-06	<i>leuD</i>	HTZ92_RS15245	-2.6591	2.80E-07	3.97E-06
<i>kdpC</i>	HTZ92_RS05725	1.4674	3.21E-09	7.31E-08	<i>leuC</i>	HTZ92_RS15250	-2.3322	7.67E-08	1.27E-06

	HTZ92_RS05775	-1.4664	2.23E-04	1.42E-03		HTZ92_RS15265	1.5744	8.47E-04	4.26E-03
	HTZ92_RS05780	1.4542	2.22E-03	9.28E-03		HTZ92_RS15335	-1.1888	1.09E-03	5.18E-03
	HTZ92_RS05795	-2.2350	1.80E-03	7.76E-03		HTZ92_RS15340	-1.4594	1.02E-02	3.24E-02
	HTZ92_RS05825	-1.4262	2.08E-03	8.72E-03		HTZ92_RS15350	-1.0646	7.60E-03	2.55E-02
	HTZ92_RS05830	-1.9884	9.76E-04	4.75E-03		HTZ92_RS15375	-3.6050	1.77E-05	1.61E-04
	HTZ92_RS05860	1.0922	1.35E-03	6.16E-03		HTZ92_RS15385	-1.2874	7.96E-04	4.04E-03
	HTZ92_RS05875	-1.9153	2.43E-04	1.51E-03		HTZ92_RS15395	-1.6785	3.23E-07	4.54E-06
	HTZ92_RS05890	-1.5790	1.71E-05	1.56E-04		HTZ92_RS15500	1.6594	4.91E-04	2.70E-03
	HTZ92_RS05910	-1.6855	4.75E-04	2.62E-03	<i>pnp</i>	HTZ92_RS15525	-1.6535	2.73E-05	2.38E-04
	HTZ92_RS05920	-1.4780	4.12E-03	1.54E-02	<i>rpsO</i>	HTZ92_RS15530	1.2309	4.11E-05	3.36E-04
	HTZ92_RS05935	1.7584	6.77E-13	2.54E-11		HTZ92_RS15565	-1.0697	6.09E-03	2.11E-02
	HTZ92_RS05975	-1.9978	5.11E-03	1.84E-02	<i>ubiE</i>	HTZ92_RS15570	-1.0921	2.02E-07	3.00E-06
	HTZ92_RS06020	1.1309	1.53E-04	1.03E-03		HTZ92_RS15585	-1.2768	3.75E-05	3.09E-04
	HTZ92_RS06080	-6.3371	1.21E-05	1.13E-04	<i>rimP</i>	HTZ92_RS15650	-2.1563	1.25E-07	1.96E-06
	HTZ92_RS06185	-1.6676	2.44E-04	1.51E-03		HTZ92_RS15655	-1.3335	6.30E-04	3.34E-03
	HTZ92_RS06195	-1.4873	4.00E-04	2.30E-03		HTZ92_RS15705	-1.3677	1.08E-05	1.03E-04
	HTZ92_RS06200	-1.5337	7.77E-06	7.71E-05	<i>fadB</i>	HTZ92_RS15790	-1.3358	1.28E-04	8.83E-04
	HTZ92_RS06215	-1.1706	3.27E-03	1.28E-02		HTZ92_RS15800	1.6799	1.08E-02	3.36E-02
	HTZ92_RS06230	-2.3172	5.92E-10	1.54E-08		HTZ92_RS15815	1.2541	2.64E-03	1.07E-02
<i>accC</i>	HTZ92_RS06250	-1.6393	3.84E-07	5.27E-06		HTZ92_RS15845	1.8512	1.28E-03	5.93E-03
<i>accB</i>	HTZ92_RS06255	-2.2327	8.88E-08	1.44E-06		HTZ92_RS15865	3.6456	3.34E-10	9.10E-09
<i>aroQ</i>	HTZ92_RS06260	-1.2882	1.01E-02	3.20E-02		HTZ92_RS15870	1.7469	7.25E-07	9.09E-06
	HTZ92_RS06280	-2.4638	3.37E-04	2.00E-03	<i>rpoB</i>	HTZ92_RS15880	-1.3045	6.97E-06	6.98E-05
	HTZ92_RS06285	-6.3916	3.83E-04	2.20E-03	<i>rplJ</i>	HTZ92_RS15890	-1.3307	9.58E-08	1.53E-06
<i>nirB</i>	HTZ92_RS06290	-1.9005	1.34E-02	4.03E-02	<i>rplA</i>	HTZ92_RS15895	-1.8431	5.29E-14	2.29E-12
<i>nirD</i>	HTZ92_RS06295	-2.6080	1.28E-03	5.93E-03	<i>rplK</i>	HTZ92_RS15900	-2.2470	3.69E-15	1.82E-13
	HTZ92_RS06300	-1.3816	1.57E-02	4.55E-02	<i>nusG</i>	HTZ92_RS15905	-1.0820	3.32E-03	1.29E-02
<i>moaA</i>	HTZ92_RS06315	-1.2371	5.99E-07	7.75E-06		HTZ92_RS15915	-1.3035	2.39E-03	9.83E-03
	HTZ92_RS06345	1.2863	7.74E-05	5.84E-04		HTZ92_RS15940	-1.0408	1.04E-02	3.28E-02
<i>tusD</i>	HTZ92_RS06370	1.4178	1.93E-03	8.18E-03		HTZ92_RS15975	-2.1591	4.70E-04	2.60E-03
	HTZ92_RS06415	-1.2001	2.69E-03	1.08E-02		HTZ92_RS16020	-1.9850	4.25E-04	2.40E-03
<i>argF</i>	HTZ92_RS06420	-1.0516	1.56E-03	6.95E-03	<i>phoU</i>	HTZ92_RS16025	-3.5520	2.13E-07	3.12E-06
<i>glnG</i>	HTZ92_RS06430	-2.5257	5.77E-05	4.55E-04		HTZ92_RS16040	1.4809	9.46E-08	1.52E-06
	HTZ92_RS06435	-3.2378	5.84E-09	1.24E-07		HTZ92_RS16085	1.9860	2.74E-06	3.05E-05
<i>rimO</i>	HTZ92_RS06445	-1.1240	1.59E-03	7.02E-03		HTZ92_RS16090	1.4160	1.89E-03	8.04E-03
<i>pyrH</i>	HTZ92_RS06450	-1.4062	1.12E-03	5.30E-03		HTZ92_RS16105	-1.0481	1.94E-04	1.25E-03
<i>frr</i>	HTZ92_RS06455	-1.4541	1.07E-05	1.03E-04	<i>thrC</i>	HTZ92_RS16115	-1.0015	8.82E-04	4.40E-03
<i>bamA</i>	HTZ92_RS06480	-1.4451	2.77E-05	2.39E-04		HTZ92_RS16135	1.5728	5.42E-09	1.17E-07
	HTZ92_RS06485	-1.6437	6.49E-04	3.42E-03		HTZ92_RS16175	-2.3022	2.49E-04	1.53E-03
<i>recA</i>	HTZ92_RS06520	2.2780	2.15E-10	6.07E-09	<i>ribD</i>	HTZ92_RS16190	1.1324	8.46E-06	8.31E-05
	HTZ92_RS06540	-1.0270	5.84E-03	2.04E-02	<i>nrdR</i>	HTZ92_RS16195	1.2987	1.17E-02	3.60E-02
	HTZ92_RS06545	-2.9221	1.64E-07	2.49E-06		HTZ92_RS16200	-1.8413	5.96E-03	2.08E-02
	HTZ92_RS06600	1.0871	9.57E-04	4.68E-03		HTZ92_RS16235	-4.7092	1.13E-06	1.38E-05
	HTZ92_RS06605	-2.8660	1.32E-04	9.03E-04		HTZ92_RS16240	-1.4215	5.68E-06	5.85E-05
	HTZ92_RS06640	-2.2491	2.03E-04	1.30E-03		HTZ92_RS16250	-1.2021	5.96E-04	3.18E-03
	HTZ92_RS06645	-3.7389	1.73E-11	5.47E-10	<i>dinB</i>	HTZ92_RS16305	1.2526	1.40E-03	6.35E-03
	HTZ92_RS06685	-2.0417	1.03E-07	1.64E-06		HTZ92_RS16320	-1.4517	2.28E-04	1.44E-03
	HTZ92_RS06695	-3.0595	1.34E-07	2.06E-06		HTZ92_RS16330	1.0539	1.02E-03	4.91E-03
	HTZ92_RS06720	-1.1149	7.85E-05	5.90E-04		HTZ92_RS16335	1.1279	4.32E-03	1.60E-02
	HTZ92_RS06755	-1.0118	3.86E-03	1.46E-02		HTZ92_RS16355	-1.2918	1.25E-02	3.81E-02

<i>kdsA</i>	HTZ92_RS06885	-2.0835	3.09E-05	2.66E-04		HTZ92_RS16365	2.5431	1.68E-03	7.35E-03
<i>eno</i>	HTZ92_RS06890	-1.4356	2.26E-05	1.99E-04		HTZ92_RS16380	2.5921	1.40E-03	6.34E-03
	HTZ92_RS07005	1.5582	5.06E-07	6.74E-06		HTZ92_RS16390	2.0572	3.00E-12	1.03E-10
	HTZ92_RS07020	1.5028	2.29E-12	7.99E-11		HTZ92_RS16415	1.0466	7.86E-04	4.00E-03
<i>cata</i>	HTZ92_RS07130	-1.6000	1.82E-03	7.84E-03		HTZ92_RS16430	-1.0288	1.85E-04	1.19E-03
<i>catC</i>	HTZ92_RS07135	-2.7011	4.44E-06	4.68E-05		HTZ92_RS16450	-1.8844	6.51E-05	5.06E-04
	HTZ92_RS07140	-1.2326	4.04E-03	1.52E-02	<i>atpD</i>	HTZ92_RS16465	-1.1291	3.93E-06	4.21E-05
	HTZ92_RS07160	1.9452	5.27E-13	2.04E-11	<i>atpG</i>	HTZ92_RS16470	-2.1461	4.58E-14	2.03E-12
<i>abaQ</i>	HTZ92_RS07165	1.7970	1.19E-09	2.98E-08	<i>atpA</i>	HTZ92_RS16475	-1.7037	7.22E-10	1.85E-08
	HTZ92_RS07170	1.0572	1.54E-03	6.89E-03		HTZ92_RS16480	-3.1897	1.60E-17	9.30E-16
	HTZ92_RS07175	1.2527	7.58E-05	5.75E-04		HTZ92_RS16485	-2.8454	1.10E-15	5.66E-14
	HTZ92_RS07210	1.0133	5.86E-03	2.04E-02	<i>atpE</i>	HTZ92_RS16490	-2.6438	1.26E-12	4.66E-11
	HTZ92_RS07300	-1.2836	5.19E-03	1.86E-02		HTZ92_RS16550	-1.3683	1.33E-03	6.11E-03
	HTZ92_RS07305	-1.5274	1.06E-04	7.55E-04		HTZ92_RS16640	1.0695	8.17E-03	2.70E-02
	HTZ92_RS07340	1.5841	3.50E-06	3.77E-05		HTZ92_RS16650	1.4903	9.26E-07	1.15E-05
	HTZ92_RS07350	2.0926	9.44E-06	9.17E-05		HTZ92_RS16660	1.6883	5.12E-06	5.32E-05
	HTZ92_RS07375	3.3275	2.12E-23	1.85E-21		HTZ92_RS16665	1.5997	1.91E-08	3.65E-07
	HTZ92_RS07380	3.4703	2.49E-21	1.87E-19		HTZ92_RS16720	1.0044	1.81E-04	1.18E-03
	HTZ92_RS07385	3.6062	3.87E-16	2.05E-14		HTZ92_RS16735	1.4046	5.64E-03	2.00E-02
	HTZ92_RS07395	4.7376	2.67E-48	4.80E-46		HTZ92_RS16745	3.5058	4.23E-07	5.74E-06
	HTZ92_RS07400	4.7527	5.84E-59	1.40E-56		HTZ92_RS16750	1.7537	8.68E-05	6.35E-04
	HTZ92_RS07405	4.0865	1.29E-57	2.74E-55		HTZ92_RS16790	1.2006	4.28E-03	1.59E-02
	HTZ92_RS07430	1.2562	6.40E-05	4.99E-04		HTZ92_RS16805	1.7729	1.58E-09	3.84E-08
	HTZ92_RS07435	1.2691	3.07E-03	1.21E-02		HTZ92_RS16815	2.4260	3.51E-19	2.29E-17
	HTZ92_RS07460	3.6836	1.62E-19	1.08E-17		HTZ92_RS16835	2.9395	1.84E-11	5.76E-10
	HTZ92_RS07465	2.6169	1.04E-13	4.45E-12		HTZ92_RS16855	-1.0414	2.48E-03	1.01E-02
	HTZ92_RS07470	1.0574	1.02E-03	4.91E-03		HTZ92_RS16940	-1.3847	2.32E-04	1.46E-03
<i>dcm</i>	HTZ92_RS07510	1.9438	2.62E-13	1.04E-11		HTZ92_RS16955	1.5933	1.51E-05	1.39E-04
	HTZ92_RS07560	1.0689	1.48E-02	4.34E-02	<i>tviB</i>	HTZ92_RS16985	-1.0943	1.37E-02	4.08E-02
	HTZ92_RS07625	1.3582	1.98E-03	8.38E-03		HTZ92_RS16990	-1.1272	1.47E-02	4.31E-02
	HTZ92_RS07645	3.4910	6.33E-36	9.11E-34		HTZ92_RS17000	-1.2448	2.24E-05	1.99E-04
<i>adeR</i>	HTZ92_RS07670	1.2125	7.19E-04	3.71E-03	<i>nadC</i>	HTZ92_RS17025	1.5506	6.99E-09	1.45E-07
	HTZ92_RS07685	1.4118	2.13E-07	3.12E-06		HTZ92_RS17030	2.0198	1.52E-10	4.33E-09
	HTZ92_RS07700	-3.3929	4.89E-09	1.08E-07		HTZ92_RS17065	1.4622	2.58E-04	1.58E-03
	HTZ92_RS07740	-1.5515	5.93E-03	2.07E-02		HTZ92_RS17100	-1.5514	1.38E-03	6.28E-03
<i>pqqB</i>	HTZ92_RS07780	-1.4749	2.25E-03	9.36E-03		HTZ92_RS17110	2.6310	1.80E-12	6.40E-11
	HTZ92_RS07805	1.0563	5.57E-04	3.01E-03		HTZ92_RS17115	2.2770	3.14E-09	7.18E-08
	HTZ92_RS07810	3.2738	1.51E-21	1.16E-19		HTZ92_RS17135	-1.1841	1.57E-03	6.96E-03
<i>modA</i>	HTZ92_RS07825	1.1260	1.62E-05	1.48E-04	<i>sul2</i>	HTZ92_RS17140	1.6061	1.28E-10	3.72E-09
	HTZ92_RS07830	2.1341	5.81E-07	7.54E-06		HTZ92_RS17145	1.3391	9.85E-07	1.22E-05
<i>antA</i>	HTZ92_RS07835	2.9856	3.63E-10	9.80E-09		HTZ92_RS17150	2.3310	4.60E-21	3.38E-19
<i>antB</i>	HTZ92_RS07840	2.1639	3.64E-05	3.03E-04		HTZ92_RS17155	1.8506	1.94E-12	6.83E-11
	HTZ92_RS07850	1.3299	8.99E-03	2.92E-02		HTZ92_RS17160	2.6918	1.74E-20	1.23E-18
	HTZ92_RS07870	1.2640	5.73E-04	3.08E-03		HTZ92_RS17165	2.6128	1.69E-05	1.55E-04
	HTZ92_RS07875	1.4681	3.58E-05	3.00E-04	<i>arsH</i>	HTZ92_RS17170	3.1314	1.91E-09	4.57E-08
	HTZ92_RS07960	-4.7052	3.15E-04	1.88E-03		HTZ92_RS17175	3.6140	5.50E-17	3.09E-15
	HTZ92_RS07965	-3.3403	2.36E-04	1.48E-03		HTZ92_RS17185	2.9036	1.32E-11	4.27E-10
	HTZ92_RS07985	-1.4048	1.07E-02	3.34E-02		HTZ92_RS17190	2.6073	2.70E-07	3.87E-06
	HTZ92_RS08020	-2.8331	1.07E-03	5.12E-03		HTZ92_RS17195	1.7779	2.42E-05	2.12E-04
	HTZ92_RS08095	-1.8033	4.07E-03	1.53E-02	<i>trbL</i>	HTZ92_RS17200	1.8402	7.82E-10	1.99E-08

	HTZ92_RS08100	-1.9602	3.33E-03	1.30E-02	<i>trbK</i>	HTZ92_RS17205	1.9835	1.59E-09	3.84E-08
	HTZ92_RS08105	-1.8974	2.59E-03	1.05E-02	<i>trbJ</i>	HTZ92_RS17210	1.6514	1.31E-07	2.04E-06
	HTZ92_RS08150	-1.4703	9.19E-04	4.53E-03		HTZ92_RS17220	2.5087	4.69E-13	1.83E-11
	HTZ92_RS08170	-1.2177	1.08E-03	5.15E-03		HTZ92_RS17225	3.0889	5.54E-18	3.38E-16
	HTZ92_RS08205	-3.8919	2.23E-06	2.53E-05		HTZ92_RS17230	4.1444	1.99E-09	4.74E-08
	HTZ92_RS08245	1.2965	8.14E-03	2.69E-02		HTZ92_RS17235	1.9159	9.26E-05	6.68E-04
	HTZ92_RS08275	-1.5309	4.15E-04	2.36E-03		HTZ92_RS17270	-1.5004	1.31E-04	9.01E-04
	HTZ92_RS08290	-1.4561	2.77E-03	1.11E-02		HTZ92_RS17290	1.3093	2.69E-05	2.34E-04
	HTZ92_RS08340	-1.0904	9.78E-03	3.11E-02		HTZ92_RS17300	2.0132	7.61E-05	5.76E-04
	HTZ92_RS08345	3.0948	1.38E-14	6.42E-13		HTZ92_RS17325	3.1916	1.02E-19	6.91E-18
	HTZ92_RS08420	-1.5237	5.51E-07	7.17E-06		HTZ92_RS17340	-6.5372	2.36E-10	6.58E-09
	HTZ92_RS08425	-2.5237	1.70E-11	5.45E-10		HTZ92_RS17345	-4.8846	7.12E-07	8.98E-06
	HTZ92_RS08430	-1.2152	5.89E-04	3.15E-03	<i>ssuD</i>	HTZ92_RS17350	-5.6101	2.40E-08	4.53E-07
<i>iscU</i>	HTZ92_RS08445	1.7448	5.43E-04	2.95E-03	<i>ssuC</i>	HTZ92_RS17355	-5.3537	2.69E-08	5.03E-07
<i>iscA</i>	HTZ92_RS08450	1.1181	8.06E-03	2.67E-02		HTZ92_RS17360	-4.6618	5.44E-07	7.11E-06
	HTZ92_RS08505	-1.4128	2.85E-04	1.73E-03		HTZ92_RS17405	-1.1105	4.73E-03	1.72E-02
<i>rpsT</i>	HTZ92_RS08510	-1.7745	5.18E-08	8.98E-07		HTZ92_RS17415	2.5312	1.84E-03	7.90E-03
	HTZ92_RS08520	1.1165	1.03E-04	7.33E-04		HTZ92_RS17420	1.3653	1.26E-02	3.82E-02
	HTZ92_RS08525	-2.0714	1.66E-06	1.93E-05		HTZ92_RS17430	2.6076	1.38E-03	6.28E-03
	HTZ92_RS08530	3.0772	3.90E-32	4.38E-30		HTZ92_RS17445	1.8901	4.71E-14	2.06E-12
	HTZ92_RS08545	-1.4644	6.35E-03	2.19E-02		HTZ92_RS17555	1.2357	1.88E-03	8.00E-03
	HTZ92_RS08555	-1.7874	9.62E-04	4.69E-03	<i>dmeF</i>	HTZ92_RS17565	3.2042	4.27E-25	3.94E-23
	HTZ92_RS08560	-1.0739	8.78E-03	2.87E-02	<i>gidC</i>	HTZ92_RS17580	-1.0134	1.66E-03	7.28E-03
	HTZ92_RS08570	-1.4454	2.92E-03	1.15E-02	<i>gidD</i>	HTZ92_RS17585	-1.0484	7.16E-03	2.43E-02
	HTZ92_RS08575	-1.2515	7.30E-03	2.46E-02	<i>rnpA</i>	HTZ92_RS17590	-1.6613	9.29E-05	6.69E-04
	HTZ92_RS08580	-1.5426	4.32E-03	1.60E-02	<i>rpmH</i>	HTZ92_RS17595	-2.8108	1.40E-08	2.78E-07
	HTZ92_RS08585	-2.9789	3.11E-04	1.87E-03	<i>cybC</i>	HTZ92_RS17620	1.0524	1.52E-02	4.44E-02
	HTZ92_RS08600	-1.4973	4.57E-08	8.09E-07		HTZ92_RS17625	-1.1937	9.72E-03	3.10E-02
<i>pyrF</i>	HTZ92_RS08625	-1.2202	1.35E-03	6.16E-03		HTZ92_RS17670	2.6140	1.14E-03	5.37E-03
	HTZ92_RS08650	-1.5915	6.13E-04	3.26E-03		HTZ92_RS17680	1.5325	8.95E-04	4.46E-03
	HTZ92_RS08690	-1.6810	1.23E-02	3.78E-02		HTZ92_RS17685	2.5457	1.70E-03	7.38E-03
	HTZ92_RS08770	-1.0498	6.57E-03	2.26E-02		HTZ92_RS17700	-1.4201	1.14E-05	1.09E-04
<i>fba</i>	HTZ92_RS08775	-1.0707	9.15E-04	4.52E-03		HTZ92_RS17770	-1.5957	3.63E-04	2.12E-03
	HTZ92_RS08780	-2.5072	7.44E-17	4.12E-15		HTZ92_RS17780	-2.2563	5.82E-04	3.12E-03
	HTZ92_RS08795	-1.0026	6.41E-03	2.21E-02	<i>dapB</i>	HTZ92_RS17785	1.0884	3.77E-05	3.09E-04
	HTZ92_RS08800	-1.4037	4.37E-03	1.61E-02		HTZ92_RS17795	1.8413	3.88E-06	4.16E-05
	HTZ92_RS08805	-1.3148	1.30E-02	3.92E-02		HTZ92_RS17805	1.0388	4.36E-03	1.61E-02
<i>putP</i>	HTZ92_RS08855	-2.0008	1.51E-06	1.78E-05		HTZ92_RS17810	1.5674	2.77E-03	1.11E-02
	HTZ92_RS08860	1.3198	5.98E-05	4.68E-04		HTZ92_RS17820	1.0827	2.34E-04	1.47E-03
<i>trmA</i>	HTZ92_RS08875	-1.8088	4.63E-05	3.76E-04		HTZ92_RS17830	-1.3094	2.89E-04	1.75E-03
	HTZ92_RS08915	1.9875	2.89E-06	3.19E-05		HTZ92_RS17865	1.5515	6.30E-06	6.42E-05
	HTZ92_RS08925	2.8549	2.57E-09	5.96E-08		HTZ92_RS17875	1.1522	6.55E-04	3.43E-03
	HTZ92_RS08930	-1.2324	1.32E-02	3.98E-02		HTZ92_RS17890	-1.0651	8.32E-04	4.20E-03
	HTZ92_RS08965	-2.1608	9.89E-03	3.15E-02		HTZ92_RS17955	1.7484	1.67E-07	2.52E-06
	HTZ92_RS08970	-3.8504	4.86E-06	5.10E-05	<i>zigA</i>	HTZ92_RS17960	1.6148	3.46E-07	4.81E-06
	HTZ92_RS09015	-1.4616	5.63E-04	3.04E-03	<i>hutH</i>	HTZ92_RS17985	-1.4972	4.34E-07	5.87E-06
	HTZ92_RS09100	1.6862	1.25E-03	5.82E-03		HTZ92_RS17990	-1.5636	5.21E-07	6.91E-06
	HTZ92_RS09125	-1.4550	6.60E-06	6.67E-05	<i>hutI</i>	HTZ92_RS17995	-1.1954	1.35E-04	9.21E-04
	HTZ92_RS09130	-1.6934	4.33E-10	1.15E-08		HTZ92_RS18010	2.5483	1.25E-11	4.07E-10
	HTZ92_RS09145	-5.1148	6.28E-07	8.06E-06	<i>phoR</i>	HTZ92_RS18110	-2.0980	1.57E-03	6.97E-03

	HTZ92_RS09150	-4.5851	4.40E-07	5.92E-06	<i>phoB</i>	HTZ92_RS18115	-2.1149	2.55E-03	1.04E-02
	HTZ92_RS09155	-3.6845	1.12E-05	1.07E-04		HTZ92_RS18125	1.7381	6.23E-10	1.61E-08
	HTZ92_RS09160	-3.5192	5.19E-05	4.17E-04		HTZ92_RS18185	1.0550	4.64E-04	2.58E-03
	HTZ92_RS09170	-1.7197	5.66E-03	2.00E-02	<i>parE</i>	HTZ92_RS18190	1.1329	1.58E-03	7.00E-03
	HTZ92_RS09185	1.0178	9.00E-04	4.48E-03	<i>hpxO</i>	HTZ92_RS18210	-2.4850	1.71E-03	7.42E-03
	HTZ92_RS09265	-1.7942	1.86E-04	1.20E-03		HTZ92_RS18215	-3.7444	4.64E-07	6.23E-06
<i>arsB</i>	HTZ92_RS09315	3.1054	3.49E-13	1.38E-11	<i>uraD</i>	HTZ92_RS18230	-3.0693	7.95E-05	5.97E-04
<i>arsC</i>	HTZ92_RS09325	-1.5008	1.44E-02	4.25E-02		HTZ92_RS18240	-2.0890	3.63E-03	1.39E-02
	HTZ92_RS09340	1.5207	1.96E-05	1.76E-04		HTZ92_RS18275	-1.3292	5.22E-04	2.86E-03
	HTZ92_RS09350	1.2274	1.45E-04	9.81E-04	<i>ftsZ</i>	HTZ92_RS18330	1.1121	5.43E-07	7.11E-06
<i>tauD</i>	HTZ92_RS09365	-2.3636	4.81E-04	2.65E-03		HTZ92_RS18355	1.5164	5.24E-09	1.14E-07
<i>tauC</i>	HTZ92_RS09370	-2.9962	4.31E-04	2.42E-03		HTZ92_RS18375	1.4493	1.76E-06	2.04E-05
	HTZ92_RS09375	-3.6460	9.49E-05	6.83E-04	<i>pdxH</i>	HTZ92_RS18395	1.4363	1.74E-11	5.47E-10
<i>tauA</i>	HTZ92_RS09380	-4.4793	1.52E-06	1.79E-05		HTZ92_RS18405	1.7276	2.42E-07	3.53E-06
	HTZ92_RS09385	1.0069	1.34E-03	6.14E-03	<i>sfhG</i>	HTZ92_RS18460	-5.6565	3.53E-08	6.48E-07
	HTZ92_RS09395	-1.8145	1.14E-02	3.53E-02	<i>msuE</i>	HTZ92_RS18465	-6.5929	6.33E-06	6.43E-05
	HTZ92_RS09400	-3.1108	6.56E-05	5.08E-04		HTZ92_RS18485	-2.6173	2.05E-25	1.94E-23
	HTZ92_RS09450	2.4063	9.33E-04	4.59E-03		HTZ92_RS18490	-2.3530	1.44E-22	1.20E-20
	HTZ92_RS09520	1.5106	6.08E-07	7.84E-06	<i>uvrA</i>	HTZ92_RS18515	2.5920	3.96E-22	3.23E-20
<i>yfcF</i>	HTZ92_RS09525	-1.5439	1.27E-03	5.86E-03		HTZ92_RS18520	1.7405	8.87E-08	1.44E-06
	HTZ92_RS09545	-3.4576	8.41E-05	6.19E-04		HTZ92_RS18525	1.4516	6.79E-09	1.42E-07
	HTZ92_RS09550	-4.5651	1.92E-06	2.21E-05	<i>ssb</i>	HTZ92_RS18555	1.8290	3.24E-05	2.76E-04
	HTZ92_RS09555	-4.5339	1.20E-06	1.46E-05		HTZ92_RS18580	-1.3635	1.09E-03	5.18E-03
	HTZ92_RS09560	-2.8410	2.37E-04	1.48E-03		HTZ92_RS18585	-1.7062	2.50E-03	1.02E-02
	HTZ92_RS09575	-2.1005	7.06E-03	2.40E-02		HTZ92_RS18610	1.0379	3.08E-03	1.21E-02
	HTZ92_RS09585	-3.6652	1.45E-05	1.35E-04		HTZ92_RS18615	-2.9894	4.20E-06	4.47E-05
	HTZ92_RS09590	-2.7389	6.52E-04	3.42E-03		HTZ92_RS18620	2.6677	1.45E-12	5.25E-11
	HTZ92_RS09595	-3.6578	8.37E-05	6.18E-04		HTZ92_RS18630	1.6484	3.17E-05	2.71E-04
	HTZ92_RS09600	-3.1831	3.63E-04	2.12E-03		HTZ92_RS18635	1.8304	5.43E-07	7.11E-06
	HTZ92_RS09625	-2.0826	4.55E-03	1.67E-02		HTZ92_RS18645	1.1798	3.76E-04	2.17E-03
	HTZ92_RS09635	1.2295	9.02E-03	2.92E-02		HTZ92_RS18650	1.8558	1.08E-08	2.19E-07
<i>umuD</i>	HTZ92_RS09640	2.8031	9.95E-12	3.31E-10		HTZ92_RS18675	1.3438	3.47E-08	6.40E-07
	HTZ92_RS09645	4.0251	1.46E-17	8.59E-16		HTZ92_RS18680	2.3438	1.96E-05	1.76E-04
	HTZ92_RS09660	1.8593	7.86E-08	1.30E-06		HTZ92_RS18690	2.2013	8.20E-16	4.27E-14
	HTZ92_RS09735	2.4305	9.41E-04	4.62E-03		HTZ92_RS18695	2.3428	2.25E-05	1.99E-04
	HTZ92_RS09825	1.1788	1.89E-03	8.04E-03		HTZ92_RS18700	1.5997	1.42E-12	5.22E-11
	HTZ92_RS09845	1.6539	4.74E-09	1.06E-07		HTZ92_RS18780	4.7498	2.67E-31	2.91E-29
	HTZ92_RS09865	1.9938	1.97E-04	1.27E-03	<i>rnpB</i>		2.5103	1.25E-04	8.66E-04
	HTZ92_RS09895	-1.4617	1.59E-02	4.58E-02	<i>ssrA</i>		2.4562	1.78E-04	1.16E-03
	HTZ92_RS09900	-2.7273	3.57E-04	2.09E-03	<i>ssrS</i>		2.0440	2.84E-03	1.13E-02
	HTZ92_RS09905	-3.0481	1.62E-04	1.07E-03					

Abbreviation: LFC, Log₂FoldChange

Table S4. Distribution of DEGs across COG categories

Gene name	Locus Tag	LFC	COG category	Gene name	Locus Tag	LFC	COG category
	HTZ92_RS00110	2.3291	K		HTZ92_RS09940	1.6344	C
	HTZ92_RS00125	3.3285	-		HTZ92_RS09980	1.9912	-
	HTZ92_RS00130	2.4109	K		HTZ92_RS10005	1.0419	G
	HTZ92_RS00135	2.4361	-		HTZ92_RS10030	1.3737	K
	HTZ92_RS00145	2.0714	-		HTZ92_RS10065	-1.1060	-
	HTZ92_RS00170	1.5847	F		HTZ92_RS10070	1.1901	Q
	HTZ92_RS00220	1.4381	EG		HTZ92_RS10095	1.4675	I
<i>hisH</i>	HTZ92_RS00245	1.2232	E	<i>paaG</i>	HTZ92_RS10100	1.9715	I
	HTZ92_RS00275	-2.0998	C		HTZ92_RS10105	2.0545	I
	HTZ92_RS00295	-1.2257	K	<i>paaK</i>	HTZ92_RS10110	2.7200	C
	HTZ92_RS00305	-1.6254	-	<i>paaJ</i>	HTZ92_RS10115	2.8478	-
	HTZ92_RS00310	-2.0034	E	<i>paaC</i>	HTZ92_RS10120	2.6918	-
	HTZ92_RS00330	-5.0228	-	<i>paaB</i>	HTZ92_RS10125	3.1240	Q
	HTZ92_RS00335	-4.0949	MU	<i>paaA</i>	HTZ92_RS10130	3.5937	-
	HTZ92_RS00340	-3.6137	M	<i>paaZ</i>	HTZ92_RS10135	1.8751	CI
	HTZ92_RS00345	-2.8560	P		HTZ92_RS10145	2.1628	E
	HTZ92_RS00350	-1.7806	P		HTZ92_RS10150	1.2461	C
	HTZ92_RS00385	-1.0642	-		HTZ92_RS10175	1.8195	E
	HTZ92_RS00405	-3.2533	P		HTZ92_RS10180	1.8518	E
	HTZ92_RS00470	1.5465	NU		HTZ92_RS10195	1.7070	EM
	HTZ92_RS00475	1.2346	U		HTZ92_RS10225	1.0958	-
<i>gltB</i>	HTZ92_RS00495	-2.3600	E		HTZ92_RS10280	-1.7160	-
	HTZ92_RS00500	-1.5001	E		HTZ92_RS10285	-1.0074	M
	HTZ92_RS00580	2.5100	NU	<i>tssF</i>	HTZ92_RS10310	-1.6974	-
	HTZ92_RS00605	-1.7737	NU	<i>tssE</i>	HTZ92_RS10315	-2.3318	-
<i>rpsP</i>	HTZ92_RS00615	-1.8017	J	<i>tssC</i>	HTZ92_RS10325	-1.5033	-
<i>rimM</i>	HTZ92_RS00620	-1.6315	J	<i>tssB</i>	HTZ92_RS10330	-1.2850	-
<i>trmD</i>	HTZ92_RS00625	-1.7790	J		HTZ92_RS10335	-1.4691	-
<i>rplS</i>	HTZ92_RS00630	-1.6878	J		HTZ92_RS10340	-1.8462	-
	HTZ92_RS00655	-2.7615	-		HTZ92_RS10355	-1.5504	P
	HTZ92_RS00720	1.2280	D		HTZ92_RS10380	2.1847	K
	HTZ92_RS00730	1.5664	-	<i>uca</i>	HTZ92_RS10390	-1.6768	EI
	HTZ92_RS00750	-1.0254	E		HTZ92_RS10395	-2.2425	-
<i>astA</i>	HTZ92_RS00765	-1.9650	E		HTZ92_RS10400	-2.5299	-
<i>astD</i>	HTZ92_RS00770	-1.6993	C		HTZ92_RS10415	1.0498	K
<i>astB</i>	HTZ92_RS00775	-1.7548	E		HTZ92_RS10435	-1.8302	U
	HTZ92_RS00790	-3.9985	-		HTZ92_RS10455	-1.2053	-
	HTZ92_RS00805	-1.7424	-		HTZ92_RS10465	-1.1056	P
	HTZ92_RS00820	-1.3965	-	<i>mumR</i>	HTZ92_RS10470	2.0713	K
<i>glyQ</i>	HTZ92_RS00830	-1.5082	J		HTZ92_RS10500	-2.8729	-
	HTZ92_RS00855	2.9128	KT		HTZ92_RS10530	1.8786	K
<i>ribA</i>	HTZ92_RS00885	-1.0896	H		HTZ92_RS10615	-2.4558	-
	HTZ92_RS00900	-1.7404	L		HTZ92_RS10620	-1.4897	L
	HTZ92_RS00910	1.1054	Q	<i>cysS</i>	HTZ92_RS10675	-1.2256	J
	HTZ92_RS00920	2.5894	Q		HTZ92_RS10695	1.3361	-
	HTZ92_RS00925	2.5669	Q		HTZ92_RS10725	-1.7565	-
	HTZ92_RS00930	2.8092	-		HTZ92_RS10745	1.2493	-

	HTZ92_RS00935	-1.2963	P		HTZ92_RS10750	1.4028	-
	HTZ92_RS00940	-1.5067	-		HTZ92_RS10755	3.0419	-
	HTZ92_RS00995	-1.0869	K		HTZ92_RS10760	2.2063	-
	HTZ92_RS01005	-1.0600	-		HTZ92_RS10765	3.1996	-
<i>rpsJ</i>	HTZ92_RS01020	-3.2696	J		HTZ92_RS10770	3.4353	-
<i>rplC</i>	HTZ92_RS01025	-2.8020	J		HTZ92_RS10775	3.7048	-
<i>rplD</i>	HTZ92_RS01030	-3.2736	J		HTZ92_RS10780	4.1996	-
<i>rplW</i>	HTZ92_RS01035	-3.3153	J		HTZ92_RS10785	5.3079	-
<i>rplB</i>	HTZ92_RS01040	-2.9136	J		HTZ92_RS10790	6.2623	L
<i>rpsS</i>	HTZ92_RS01045	-2.7678	J		HTZ92_RS10795	7.0809	L
<i>rplV</i>	HTZ92_RS01050	-2.9693	J		HTZ92_RS10800	7.6570	-
<i>rplP</i>	HTZ92_RS01060	-1.5204	J		HTZ92_RS10805	6.3318	-
<i>rpsN</i>	HTZ92_RS01090	-1.1074	J		HTZ92_RS10810	5.3540	L
<i>rpsH</i>	HTZ92_RS01095	-1.3773	J		HTZ92_RS10815	4.4162	-
<i>rplF</i>	HTZ92_RS01100	-1.4844	J		HTZ92_RS10820	6.2663	L
<i>rplR</i>	HTZ92_RS01105	-1.7060	J		HTZ92_RS10825	5.9636	-
<i>rpsE</i>	HTZ92_RS01110	-1.5204	J		HTZ92_RS10830	6.2331	-
<i>rpmD</i>	HTZ92_RS01115	-1.5517	J		HTZ92_RS10835	6.4050	-
<i>rpmJ</i>	HTZ92_RS01130	-1.3167	J		HTZ92_RS10840	5.4999	-
<i>rpsM</i>	HTZ92_RS01135	-2.1110	J		HTZ92_RS10845	5.1901	-
<i>rpsK</i>	HTZ92_RS01140	-1.7761	J		HTZ92_RS10850	6.6736	-
<i>rpsD</i>	HTZ92_RS01145	-1.6933	J		HTZ92_RS10855	1.0905	K
<i>rpoA</i>	HTZ92_RS01150	-1.7051	K		HTZ92_RS10860	5.8242	-
<i>rplQ</i>	HTZ92_RS01155	-1.1367	J		HTZ92_RS10865	6.3640	-
	HTZ92_RS01200	1.1813	-		HTZ92_RS10870	7.1777	-
	HTZ92_RS01235	1.1414	UW		HTZ92_RS10875	6.5483	L
	HTZ92_RS01290	-1.9212	-		HTZ92_RS10880	6.6779	-
<i>ybeY</i>	HTZ92_RS01300	-1.0459	J		HTZ92_RS10885	5.6867	L
	HTZ92_RS01325	-1.5576	C		HTZ92_RS10890	5.9451	K
	HTZ92_RS01335	1.7010	-		HTZ92_RS10895	6.8506	-
	HTZ92_RS01360	2.6039	-		HTZ92_RS10905	5.4520	-
	HTZ92_RS01370	1.2137	-		HTZ92_RS10910	3.8816	-
	HTZ92_RS01375	2.5448	-		HTZ92_RS10945	1.8756	-
<i>rpsI</i>	HTZ92_RS01450	-1.2050	J		HTZ92_RS10960	1.6050	K
<i>rplM</i>	HTZ92_RS01455	-1.8169	J		HTZ92_RS10965	1.5091	EGP
<i>pdxA</i>	HTZ92_RS01460	1.4579	H		HTZ92_RS10995	-1.3594	-
	HTZ92_RS01475	-2.1255	-		HTZ92_RS11000	-2.5868	K
	HTZ92_RS01510	-1.4575	M		HTZ92_RS11020	1.5267	K
	HTZ92_RS01515	-1.2383	T		HTZ92_RS11025	-1.0901	-
	HTZ92_RS01520	-1.7757	-		HTZ92_RS11030	-1.6947	-
	HTZ92_RS01525	2.6174	-		HTZ92_RS11045	-1.5408	-
	HTZ92_RS01535	1.4047	-		HTZ92_RS11055	1.5887	-
	HTZ92_RS01540	2.5377	-		HTZ92_RS11060	1.3122	-
	HTZ92_RS01575	1.1265	I		HTZ92_RS11120	-1.7995	-
	HTZ92_RS01585	-1.8820	-	<i>ahpC</i>	HTZ92_RS11135	-1.0414	O
	HTZ92_RS01600	-1.5743	-		HTZ92_RS11185	2.6542	-
	HTZ92_RS01610	-1.4869	D		HTZ92_RS11200	1.2578	-
<i>secD</i>	HTZ92_RS01640	-1.4263	U		HTZ92_RS11215	-1.5925	F
<i>yajC</i>	HTZ92_RS01645	-2.2702	U		HTZ92_RS11235	-1.7385	-
<i>tgt</i>	HTZ92_RS01650	-1.5367	F	<i>clpB</i>	HTZ92_RS11240	1.4950	O

	HTZ92_RS01735	-1.7212	-		HTZ92_RS11250	-1.2869	K
<i>aspS</i>	HTZ92_RS01745	-1.0928	J		HTZ92_RS11275	1.0359	M
	HTZ92_RS01760	1.1863	I		HTZ92_RS11280	-2.8176	-
	HTZ92_RS01770	1.8846	-		HTZ92_RS11320	1.3238	-
	HTZ92_RS01775	-1.3161	-		HTZ92_RS11325	1.3727	-
	HTZ92_RS01825	-1.5530	O		HTZ92_RS11365	1.1825	-
<i>folC</i>	HTZ92_RS01885	-1.1511	H		HTZ92_RS11370	1.1445	D
	HTZ92_RS01905	-4.4333	O		HTZ92_RS11380	1.2948	-
	HTZ92_RS01920	-1.1502	EGP		HTZ92_RS11390	1.2444	-
	HTZ92_RS01925	-1.4286	-		HTZ92_RS11420	1.2507	-
	HTZ92_RS01945	-1.5891	-		HTZ92_RS11425	1.4476	-
	HTZ92_RS01955	-1.2239	I		HTZ92_RS11445	1.7205	-
	HTZ92_RS01980	-1.3435	C		HTZ92_RS11530	-1.6598	-
	HTZ92_RS01985	-1.1571	-		HTZ92_RS11610	-1.0611	-
	HTZ92_RS02005	-2.6270	NU		HTZ92_RS11635	1.0867	P
	HTZ92_RS02015	-1.9665	-		HTZ92_RS11640	1.0555	-
	HTZ92_RS02020	-1.8694	-		HTZ92_RS11655	1.3590	K
<i>mscL</i>	HTZ92_RS02045	-1.1039	M		HTZ92_RS11730	-1.3772	K
	HTZ92_RS02085	-1.7401	-	<i>mhpT</i>	HTZ92_RS11735	1.1304	EGP
	HTZ92_RS02090	-1.4594	O		HTZ92_RS11740	2.0457	I
	HTZ92_RS02100	-2.2589	-	<i>gudD</i>	HTZ92_RS11795	1.1849	H
	HTZ92_RS02115	-1.8853	-		HTZ92_RS11820	1.1697	C
	HTZ92_RS02135	1.4620	-		HTZ92_RS11860	1.0190	E
<i>dapE</i>	HTZ92_RS02170	-1.0575	E		HTZ92_RS11865	-1.0686	K
	HTZ92_RS02195	-1.3280	-		HTZ92_RS11885	-2.1194	K
	HTZ92_RS02255	-1.6096	-		HTZ92_RS11905	-2.0611	-
	HTZ92_RS02260	-1.2513	-		HTZ92_RS11910	-1.2221	-
<i>sbmA</i>	HTZ92_RS02280	-1.1316	I		HTZ92_RS11935	-2.3929	P
	HTZ92_RS02300	-1.3854	-		HTZ92_RS11965	1.3681	L
<i>gatA</i>	HTZ92_RS02310	-1.4916	J		HTZ92_RS11970	1.8555	L
<i>gatC</i>	HTZ92_RS02315	-1.5417	H		HTZ92_RS11975	1.0383	V
	HTZ92_RS02345	-2.0887	-		HTZ92_RS11980	1.8291	V
<i>hchA</i>	HTZ92_RS02405	-1.0321	-		HTZ92_RS11990	1.5065	D
	HTZ92_RS02415	-2.0722	E	<i>merD</i>	HTZ92_RS12005	3.9932	K
	HTZ92_RS02455	1.7751	EGP	<i>merA</i>	HTZ92_RS12010	3.3915	C
	HTZ92_RS02460	-1.2116	MU	<i>merC</i>	HTZ92_RS12015	3.1849	-
	HTZ92_RS02465	-2.8255	-	<i>merP</i>	HTZ92_RS12020	3.2139	P
	HTZ92_RS02470	-2.4301	KT	<i>merT</i>	HTZ92_RS12025	2.9168	P
	HTZ92_RS02475	-2.1344	T	<i>merR</i>	HTZ92_RS12030	1.5803	K
	HTZ92_RS02480	-2.6909	-	<i>lspA</i>	HTZ92_RS12040	-1.1527	IMU
	HTZ92_RS02485	-2.0440	-		HTZ92_RS12045	-1.8477	P
	HTZ92_RS02490	-2.7227	P	<i>cadR</i>	HTZ92_RS12050	2.6022	K
<i>adeJ</i>	HTZ92_RS02550	-1.2840	V		HTZ92_RS12060	1.3037	L
<i>adeI</i>	HTZ92_RS02555	-1.3762	M	<i>rnt</i>	HTZ92_RS12155	-1.0172	L
<i>lolA</i>	HTZ92_RS02590	1.8136	M		HTZ92_RS12165	-1.1170	P
	HTZ92_RS02610	-1.0867	J		HTZ92_RS12225	-2.1176	L
	HTZ92_RS02615	2.3290	K		HTZ92_RS12245	1.2808	-
	HTZ92_RS02635	-1.4433	-		HTZ92_RS12250	-3.7764	P
	HTZ92_RS02655	-1.6537	-		HTZ92_RS12255	1.9055	K
	HTZ92_RS02660	-2.3959	-		HTZ92_RS12285	-1.2627	-

<i>gltA</i>	HTZ92_RS02675	-2.3658	C	<i>ata</i>	HTZ92_RS12325	1.0177	UW
<i>sdhC</i>	HTZ92_RS02680	-1.4592	C	<i>lon</i>	HTZ92_RS12330	1.2449	O
<i>sdhD</i>	HTZ92_RS02685	-1.3664	C	<i>gdhA</i>	HTZ92_RS12350	-1.5695	E
	HTZ92_RS02695	-1.0917	C	<i>ureG</i>	HTZ92_RS12400	-2.2562	KO
<i>sucC</i>	HTZ92_RS02725	-1.0036	F		HTZ92_RS12405	-3.3100	O
	HTZ92_RS02775	-1.4574	L	<i>ureE</i>	HTZ92_RS12410	-2.0022	O
<i>yhbY</i>	HTZ92_RS02815	-1.3256	J		HTZ92_RS12420	-2.7949	E
<i>ftsH</i>	HTZ92_RS02825	1.6274	O	<i>ureA</i>	HTZ92_RS12425	-2.5543	E
	HTZ92_RS02835	-1.5077	-		HTZ92_RS12430	-1.9817	O
	HTZ92_RS02855	-1.7549	-		HTZ92_RS12445	-2.4315	C
	HTZ92_RS02880	-1.1131	EGP	<i>cysD</i>	HTZ92_RS12480	-1.3795	H
	HTZ92_RS02895	-1.9823	H		HTZ92_RS12485	-2.4553	-
	HTZ92_RS02925	-3.7507	M		HTZ92_RS12500	-1.0087	-
<i>htpX</i>	HTZ92_RS02950	1.2322	O		HTZ92_RS12505	-1.5140	-
	HTZ92_RS02955	-1.1089	M		HTZ92_RS12510	-1.3853	C
	HTZ92_RS02965	-1.8102	I		HTZ92_RS12515	-1.0165	C
	HTZ92_RS02990	-1.8600	Q		HTZ92_RS12585	1.2543	P
	HTZ92_RS03025	2.4618	K		HTZ92_RS12600	-1.5870	P
	HTZ92_RS03035	1.1597	C		HTZ92_RS12610	1.3894	-
	HTZ92_RS03060	1.4857	-		HTZ92_RS12625	-1.1832	P
	HTZ92_RS03070	-1.4394	E		HTZ92_RS12650	1.0056	IQ
<i>lysA</i>	HTZ92_RS03080	-1.0769	E		HTZ92_RS12705	1.0756	C
	HTZ92_RS03095	-1.0504	-		HTZ92_RS12745	1.1148	IQ
	HTZ92_RS03125	-2.0891	M		HTZ92_RS12750	1.3906	P
	HTZ92_RS03130	-3.4791	-	<i>betA</i>	HTZ92_RS12855	1.1887	E
	HTZ92_RS03135	-3.1317	M		HTZ92_RS12870	-1.8500	H
	HTZ92_RS03140	-4.3971	G		HTZ92_RS12880	1.6580	-
<i>tolR</i>	HTZ92_RS03290	-1.9897	U		HTZ92_RS12885	1.0649	-
<i>tolQ</i>	HTZ92_RS03295	-1.5060	U		HTZ92_RS12895	-1.2232	-
	HTZ92_RS03305	-1.0235	-		HTZ92_RS12915	1.4963	K
	HTZ92_RS03330	1.1820	EGP		HTZ92_RS12930	1.3534	-
	HTZ92_RS03365	1.4090	-		HTZ92_RS12935	2.4568	-
	HTZ92_RS03390	-4.4028	K		HTZ92_RS12940	1.4693	-
	HTZ92_RS03395	-3.7540	P		HTZ92_RS12965	1.1609	-
<i>cysW</i>	HTZ92_RS03400	-5.0533	P		HTZ92_RS12970	2.3729	-
<i>cysT</i>	HTZ92_RS03405	-4.5342	O	<i>cas3f</i>	HTZ92_RS12985	1.8716	L
	HTZ92_RS03410	-5.6717	-		HTZ92_RS12990	2.5322	-
	HTZ92_RS03415	-6.4433	P		HTZ92_RS13060	1.4215	L
<i>nadB</i>	HTZ92_RS03440	-1.4091	H		HTZ92_RS13170	1.3562	-
<i>lepB</i>	HTZ92_RS03460	-3.1558	U		HTZ92_RS13175	1.0684	NU
	HTZ92_RS03465	-1.9652	NU		HTZ92_RS13195	1.7525	-
<i>era</i>	HTZ92_RS03475	1.5184	-		HTZ92_RS13200	1.3960	-
	HTZ92_RS03480	1.3410	-		HTZ92_RS13215	-3.3495	-
	HTZ92_RS03545	-1.5451	-		HTZ92_RS13225	-1.6854	G
	HTZ92_RS03555	1.3929	K		HTZ92_RS13245	-1.5123	I
<i>uvrB</i>	HTZ92_RS03560	1.3786	L	<i>minC</i>	HTZ92_RS13255	-1.0868	D
	HTZ92_RS03640	-1.0017	-	<i>minD</i>	HTZ92_RS13260	-1.2364	D
	HTZ92_RS03670	-1.0459	C		HTZ92_RS13280	1.0580	D
	HTZ92_RS03685	1.6364	C	<i>rimI</i>	HTZ92_RS13300	-1.1584	K
	HTZ92_RS03750	1.4521	-	<i>fusA</i>	HTZ92_RS13320	-1.7645	J

	HTZ92_RS03770	1.3651	-	<i>rpsG</i>	HTZ92_RS13325	-1.1866	J
	HTZ92_RS03785	-1.1952	-		HTZ92_RS13335	-1.0858	C
<i>fabR</i>	HTZ92_RS03790	-1.4654	K		HTZ92_RS13405	-1.0543	K
	HTZ92_RS03795	-2.4068	C		HTZ92_RS13440	1.2056	-
	HTZ92_RS03800	-1.9456	I		HTZ92_RS13455	-1.2902	M
	HTZ92_RS03835	1.0318	K		HTZ92_RS13495	-2.0735	F
	HTZ92_RS03850	-5.2645	P	<i>rplY</i>	HTZ92_RS13500	1.6095	J
<i>pstC</i>	HTZ92_RS03855	-3.9038	P		HTZ92_RS13510	-1.3873	H
<i>pstA</i>	HTZ92_RS03860	-4.0174	P	<i>ribB</i>	HTZ92_RS13525	-1.0854	H
<i>pstB</i>	HTZ92_RS03865	-2.1197	P	<i>lysM</i>	HTZ92_RS13540	-1.4967	-
<i>sohB</i>	HTZ92_RS03870	1.0833	OU	<i>acpP</i>	HTZ92_RS13545	-1.8428	IQ
<i>dacC</i>	HTZ92_RS03910	-2.0352	M	<i>fabG</i>	HTZ92_RS13550	-1.0918	IQ
	HTZ92_RS03915	-1.8663	-		HTZ92_RS13575	1.5536	J
	HTZ92_RS03920	-2.3180	-		HTZ92_RS13580	-1.9163	D
<i>surE</i>	HTZ92_RS03925	-1.7164	-		HTZ92_RS13605	1.8748	E
<i>gloA</i>	HTZ92_RS03950	1.6494	E		HTZ92_RS13725	-2.7804	-
	HTZ92_RS04005	1.3886	-		HTZ92_RS13795	2.9051	-
	HTZ92_RS04010	1.6408	-	<i>nuoM</i>	HTZ92_RS13830	-1.4484	C
<i>bauD</i>	HTZ92_RS04050	2.0669	P	<i>nuoL</i>	HTZ92_RS13835	-1.6095	CP
<i>bauE</i>	HTZ92_RS04060	1.2710	P	<i>nuoI</i>	HTZ92_RS13850	-1.0141	C
<i>barA</i>	HTZ92_RS04100	1.1965	V	<i>nuoH</i>	HTZ92_RS13855	-1.5542	C
<i>basI</i>	HTZ92_RS04115	1.3748	Q	<i>nuoG</i>	HTZ92_RS13860	-1.4471	C
<i>basJ</i>	HTZ92_RS04120	1.4499	HQ	<i>nuoF</i>	HTZ92_RS13865	-1.5915	C
<i>folE</i>	HTZ92_RS04155	-1.7268	H	<i>nuoE</i>	HTZ92_RS13870	-1.9065	C
<i>glnA</i>	HTZ92_RS04215	-2.2136	E	<i>nuoC</i>	HTZ92_RS13875	-1.2601	C
	HTZ92_RS04230	-1.6439	E		HTZ92_RS13880	-1.1400	C
	HTZ92_RS04265	-1.1006	-		HTZ92_RS13935	2.3496	K
	HTZ92_RS04275	1.5919	J		HTZ92_RS13940	1.9191	-
<i>ahcY</i>	HTZ92_RS04300	-1.2922	H		HTZ92_RS13965	2.2376	-
	HTZ92_RS04330	1.1878	P		HTZ92_RS13975	1.3719	K
	HTZ92_RS04335	-2.1133	P		HTZ92_RS13995	2.5298	K
<i>tsf</i>	HTZ92_RS04355	-1.8650	J	<i>tcuA</i>	HTZ92_RS14025	1.3513	C
	HTZ92_RS04385	-2.2435	M		HTZ92_RS14040	1.1362	K
	HTZ92_RS04400	-1.6588	-		HTZ92_RS14045	1.0834	C
<i>queF</i>	HTZ92_RS04405	-1.5678	-		HTZ92_RS14050	1.5487	G
	HTZ92_RS04430	1.8906	-		HTZ92_RS14055	2.2015	I
	HTZ92_RS04435	2.3905	-		HTZ92_RS14120	-3.0776	P
	HTZ92_RS04440	1.5140	-		HTZ92_RS14125	-2.8790	Q
	HTZ92_RS04445	2.7712	-		HTZ92_RS14130	-2.4300	-
	HTZ92_RS04450	2.4178	-		HTZ92_RS14175	1.1584	L
	HTZ92_RS04455	1.9580	-	<i>hisC</i>	HTZ92_RS14220	-1.0248	E
	HTZ92_RS04470	2.0521	-		HTZ92_RS14260	2.5526	-
	HTZ92_RS04475	2.3482	-		HTZ92_RS14265	1.5729	-
	HTZ92_RS04485	2.1788	T		HTZ92_RS14275	2.5998	-
	HTZ92_RS04495	1.2080	E	<i>gigA</i>	HTZ92_RS14285	-1.1922	KT
	HTZ92_RS04510	2.4366	-		HTZ92_RS14310	-1.3613	O
	HTZ92_RS04520	1.9577	-		HTZ92_RS14320	1.0558	-
	HTZ92_RS04525	1.8764	-	<i>trxA</i>	HTZ92_RS14365	1.5757	O
	HTZ92_RS04530	1.5534	L	<i>rho</i>	HTZ92_RS14370	-1.1538	K
	HTZ92_RS04535	2.2925	-		HTZ92_RS14375	-3.3728	-

	HTZ92_RS04540	1.8451	-		HTZ92_RS14380	-2.4978	-
	HTZ92_RS04550	1.9660	-		HTZ92_RS14385	1.2315	K
	HTZ92_RS04555	1.4559	-		HTZ92_RS14405	-1.1013	K
	HTZ92_RS04560	1.4406	-		HTZ92_RS14410	-2.3804	O
	HTZ92_RS04580	-2.1644	-		HTZ92_RS14435	-1.2030	O
	HTZ92_RS04585	-1.2136	-	<i>infC</i>	HTZ92_RS14440	2.1110	J
	HTZ92_RS04600	-1.5954	-		HTZ92_RS14450	-1.7110	IQ
	HTZ92_RS04605	1.0101	-	<i>pcnB</i>	HTZ92_RS14485	-1.2145	J
	HTZ92_RS04740	-1.9753	-		HTZ92_RS14530	-1.2618	I
	HTZ92_RS04755	1.4200	-		HTZ92_RS14555	-1.3072	C
	HTZ92_RS04805	-1.1792	E		HTZ92_RS14560	-1.2007	C
	HTZ92_RS04830	-5.4297	ET		HTZ92_RS14565	-1.3810	C
<i>hisQ</i>	HTZ92_RS04835	-2.9075	P		HTZ92_RS14610	-1.0726	J
	HTZ92_RS04840	-2.8700	P	<i>acnA</i>	HTZ92_RS14615	1.2785	C
<i>yegQ</i>	HTZ92_RS04860	1.1911	O		HTZ92_RS14645	-2.2658	K
	HTZ92_RS04875	-1.9534	C	<i>ilvC</i>	HTZ92_RS14660	-1.5954	H
	HTZ92_RS04885	2.1868	-	<i>ilvN</i>	HTZ92_RS14665	-1.6404	E
	HTZ92_RS04900	1.6477	K		HTZ92_RS14670	-1.3809	H
	HTZ92_RS04910	-2.6618	P		HTZ92_RS14675	-1.5106	-
	HTZ92_RS04945	-1.1672	U	<i>leuS</i>	HTZ92_RS14680	-1.5644	J
	HTZ92_RS05000	-1.1429	H		HTZ92_RS14710	-1.0212	I
	HTZ92_RS05010	-1.2827	-	<i>rsgA</i>	HTZ92_RS14725	-1.2163	-
	HTZ92_RS05045	-1.0843	I	<i>pilW</i>	HTZ92_RS14780	1.7456	NU
	HTZ92_RS05050	-1.1538	T	<i>rlmN</i>	HTZ92_RS14785	1.4317	J
	HTZ92_RS05085	1.1170	-	<i>ndk</i>	HTZ92_RS14790	-1.0028	F
	HTZ92_RS05160	-1.1177	E		HTZ92_RS14805	-1.1717	M
	HTZ92_RS05170	-1.2170	E		HTZ92_RS14820	-1.4845	Q
<i>csuB</i>	HTZ92_RS05270	-1.5882	-	<i>eda</i>	HTZ92_RS14850	-1.2428	G
<i>csuC</i>	HTZ92_RS05275	-3.1705	NU		HTZ92_RS14860	-1.2404	H
<i>csuD</i>	HTZ92_RS05280	-1.9772	NU	<i>pta</i>	HTZ92_RS14865	-1.6962	C
<i>uraH</i>	HTZ92_RS05345	-1.5499	-		HTZ92_RS14875	-1.0079	-
	HTZ92_RS05350	-2.5399	-		HTZ92_RS14880	1.0537	-
	HTZ92_RS05370	-2.2477	I	<i>leuA</i>	HTZ92_RS14910	1.0430	E
	HTZ92_RS05410	-2.3654	-		HTZ92_RS14935	-1.1390	-
	HTZ92_RS05415	-2.2489	-		HTZ92_RS14940	-4.7104	U
	HTZ92_RS05445	1.2007	EGP	<i>tatB</i>	HTZ92_RS14945	-5.8164	U
	HTZ92_RS05460	1.8579	-	<i>tatC</i>	HTZ92_RS14950	-2.9847	U
	HTZ92_RS05475	-1.1008	P		HTZ92_RS14955	-1.9424	-
	HTZ92_RS05520	-1.0591	K		HTZ92_RS14995	-1.6252	-
<i>alr</i>	HTZ92_RS05545	1.1017	E		HTZ92_RS15055	-1.0769	U
<i>rpsR</i>	HTZ92_RS05560	-1.7781	J		HTZ92_RS15065	-1.2863	M
<i>rpsF</i>	HTZ92_RS05565	-1.5097	J		HTZ92_RS15080	1.7844	C
<i>cyoB</i>	HTZ92_RS05585	-1.0256	C		HTZ92_RS15100	2.4736	-
<i>pgaB</i>	HTZ92_RS05615	-2.5038	G		HTZ92_RS15125	3.2176	-
<i>pgaD</i>	HTZ92_RS05625	-2.4687	-		HTZ92_RS15140	1.1478	-
	HTZ92_RS05630	-1.5969	P	<i>gltP</i>	HTZ92_RS15180	-1.1266	U
	HTZ92_RS05640	3.4436	-	<i>truA</i>	HTZ92_RS15210	1.3785	J
	HTZ92_RS05645	2.3104	-	<i>infA</i>	HTZ92_RS15220	-1.6579	J
	HTZ92_RS05670	-1.3869	K	<i>leuD</i>	HTZ92_RS15245	-2.6591	E
<i>kdpC</i>	HTZ92_RS05725	1.4674	P	<i>leuC</i>	HTZ92_RS15250	-2.3322	E

	HTZ92_RS05775	-1.4664	C		HTZ92_RS15265	1.5744	-
	HTZ92_RS05780	1.4542	-		HTZ92_RS15335	-1.1888	IQ
	HTZ92_RS05795	-2.2350	J		HTZ92_RS15340	-1.4594	-
	HTZ92_RS05825	-1.4262	K		HTZ92_RS15350	-1.0646	K
	HTZ92_RS05830	-1.9884	L		HTZ92_RS15375	-3.6050	I
	HTZ92_RS05860	1.0922	EG		HTZ92_RS15385	-1.2874	-
	HTZ92_RS05875	-1.9153	-		HTZ92_RS15395	-1.6785	-
	HTZ92_RS05890	-1.5790	F		HTZ92_RS15500	1.6594	O
	HTZ92_RS05910	-1.6855	C	<i>pnf</i>	HTZ92_RS15525	-1.6535	J
	HTZ92_RS05920	-1.4780	I	<i>rpsO</i>	HTZ92_RS15530	1.2309	J
	HTZ92_RS05935	1.7584	E		HTZ92_RS15565	-1.0697	-
	HTZ92_RS05975	-1.9978	-	<i>ubiE</i>	HTZ92_RS15570	-1.0921	H
	HTZ92_RS06020	1.1309	M		HTZ92_RS15585	-1.2768	CH
	HTZ92_RS06080	-6.3371	-	<i>rimP</i>	HTZ92_RS15650	-2.1563	-
	HTZ92_RS06185	-1.6676	-		HTZ92_RS15655	-1.3335	-
	HTZ92_RS06195	-1.4873	G		HTZ92_RS15705	-1.3677	-
	HTZ92_RS06200	-1.5337	T	<i>fadB</i>	HTZ92_RS15790	-1.3358	I
	HTZ92_RS06215	-1.1706	I		HTZ92_RS15800	1.6799	-
	HTZ92_RS06230	-2.3172	K		HTZ92_RS15815	1.2541	-
<i>accC</i>	HTZ92_RS06250	-1.6393	I		HTZ92_RS15845	1.8512	M
<i>accB</i>	HTZ92_RS06255	-2.2327	I		HTZ92_RS15865	3.6456	-
<i>aroQ</i>	HTZ92_RS06260	-1.2882	E		HTZ92_RS15870	1.7469	-
	HTZ92_RS06280	-2.4638	T	<i>rpoB</i>	HTZ92_RS15880	-1.3045	K
	HTZ92_RS06285	-6.3916	-	<i>rplJ</i>	HTZ92_RS15890	-1.3307	J
<i>nirB</i>	HTZ92_RS06290	-1.9005	C	<i>rplA</i>	HTZ92_RS15895	-1.8431	J
<i>nirD</i>	HTZ92_RS06295	-2.6080	CP	<i>rplK</i>	HTZ92_RS15900	-2.2470	J
	HTZ92_RS06300	-1.3816	C	<i>nusG</i>	HTZ92_RS15905	-1.0820	K
<i>moaA</i>	HTZ92_RS06315	-1.2371	H		HTZ92_RS15915	-1.3035	-
	HTZ92_RS06345	1.2863	K		HTZ92_RS15940	-1.0408	-
<i>tusD</i>	HTZ92_RS06370	1.4178	P		HTZ92_RS15975	-2.1591	-
	HTZ92_RS06415	-1.2001	T		HTZ92_RS16020	-1.9850	CO
<i>argF</i>	HTZ92_RS06420	-1.0516	E	<i>phoU</i>	HTZ92_RS16025	-3.5520	P
<i>glnG</i>	HTZ92_RS06430	-2.5257	T		HTZ92_RS16040	1.4809	K
	HTZ92_RS06435	-3.2378	T		HTZ92_RS16085	1.9860	-
<i>rimO</i>	HTZ92_RS06445	-1.1240	J		HTZ92_RS16090	1.4160	P
<i>pyrH</i>	HTZ92_RS06450	-1.4062	F		HTZ92_RS16105	-1.0481	O
<i>frf</i>	HTZ92_RS06455	-1.4541	J	<i>thrC</i>	HTZ92_RS16115	-1.0015	E
<i>bamA</i>	HTZ92_RS06480	-1.4451	M		HTZ92_RS16135	1.5728	T
	HTZ92_RS06485	-1.6437	M		HTZ92_RS16175	-2.3022	-
<i>recA</i>	HTZ92_RS06520	2.2780	L	<i>ribD</i>	HTZ92_RS16190	1.1324	H
	HTZ92_RS06540	-1.0270	-	<i>nrdR</i>	HTZ92_RS16195	1.2987	K
	HTZ92_RS06545	-2.9221	-		HTZ92_RS16200	-1.8413	P
	HTZ92_RS06600	1.0871	T		HTZ92_RS16235	-4.7092	-
	HTZ92_RS06605	-2.8660	-		HTZ92_RS16240	-1.4215	-
	HTZ92_RS06640	-2.2491	-		HTZ92_RS16250	-1.2021	-
	HTZ92_RS06645	-3.7389	-	<i>dinB</i>	HTZ92_RS16305	1.2526	L
	HTZ92_RS06685	-2.0417	-		HTZ92_RS16320	-1.4517	C
	HTZ92_RS06695	-3.0595	-		HTZ92_RS16330	1.0539	K
	HTZ92_RS06720	-1.1149	-		HTZ92_RS16335	1.1279	-
	HTZ92_RS06755	-1.0118	P		HTZ92_RS16355	-1.2918	-

<i>kdsA</i>	HTZ92_RS06885	-2.0835	M		HTZ92_RS16365	2.5431	-
<i>eno</i>	HTZ92_RS06890	-1.4356	G		HTZ92_RS16380	2.5921	-
	HTZ92_RS07005	1.5582	I		HTZ92_RS16390	2.0572	-
	HTZ92_RS07020	1.5028	-		HTZ92_RS16415	1.0466	-
<i>catA</i>	HTZ92_RS07130	-1.6000	Q		HTZ92_RS16430	-1.0288	EG
<i>catC</i>	HTZ92_RS07135	-2.7011	Q		HTZ92_RS16450	-1.8844	O
	HTZ92_RS07140	-1.2326	G	<i>atpD</i>	HTZ92_RS16465	-1.1291	C
	HTZ92_RS07160	1.9452	EG	<i>atpG</i>	HTZ92_RS16470	-2.1461	C
<i>abaQ</i>	HTZ92_RS07165	1.7970	G	<i>atpA</i>	HTZ92_RS16475	-1.7037	C
	HTZ92_RS07170	1.0572	-		HTZ92_RS16480	-3.1897	C
	HTZ92_RS07175	1.2527	C		HTZ92_RS16485	-2.8454	C
	HTZ92_RS07210	1.0133	-	<i>atpE</i>	HTZ92_RS16490	-2.6438	C
	HTZ92_RS07300	-1.2836	-		HTZ92_RS16550	-1.3683	O
	HTZ92_RS07305	-1.5274	-		HTZ92_RS16640	1.0695	T
	HTZ92_RS07340	1.5841	G		HTZ92_RS16650	1.4903	Q
	HTZ92_RS07350	2.0926	-		HTZ92_RS16660	1.6883	C
	HTZ92_RS07375	3.3275	C		HTZ92_RS16665	1.5997	IQ
	HTZ92_RS07380	3.4703	EG		HTZ92_RS16720	1.0044	K
	HTZ92_RS07385	3.6062	-		HTZ92_RS16735	1.4046	J
	HTZ92_RS07395	4.7376	M		HTZ92_RS16745	3.5058	C
	HTZ92_RS07400	4.7527	G		HTZ92_RS16750	1.7537	K
	HTZ92_RS07405	4.0865	I		HTZ92_RS16790	1.2006	-
	HTZ92_RS07430	1.2562	P		HTZ92_RS16805	1.7729	-
	HTZ92_RS07435	1.2691	P		HTZ92_RS16815	2.4260	I
	HTZ92_RS07460	3.6836	EF		HTZ92_RS16835	2.9395	-
	HTZ92_RS07465	2.6169	H		HTZ92_RS16855	-1.0414	-
	HTZ92_RS07470	1.0574	F		HTZ92_RS16940	-1.3847	M
<i>dcm</i>	HTZ92_RS07510	1.9438	L		HTZ92_RS16955	1.5933	M
	HTZ92_RS07560	1.0689	IQ	<i>tviB</i>	HTZ92_RS16985	-1.0943	M
	HTZ92_RS07625	1.3582	J		HTZ92_RS16990	-1.1272	M
	HTZ92_RS07645	3.4910	-		HTZ92_RS17000	-1.2448	D
<i>adeR</i>	HTZ92_RS07670	1.2125	KT	<i>nadC</i>	HTZ92_RS17025	1.5506	H
	HTZ92_RS07685	1.4118	L		HTZ92_RS17030	2.0198	-
	HTZ92_RS07700	-3.3929	M		HTZ92_RS17065	1.4622	-
	HTZ92_RS07740	-1.5515	-		HTZ92_RS17100	-1.5514	-
<i>pqqB</i>	HTZ92_RS07780	-1.4749	-		HTZ92_RS17110	2.6310	-
	HTZ92_RS07805	1.0563	Q		HTZ92_RS17115	2.2770	-
	HTZ92_RS07810	3.2738	IQ		HTZ92_RS17135	-1.1841	-
<i>modA</i>	HTZ92_RS07825	1.1260	P	<i>sul2</i>	HTZ92_RS17140	1.6061	H
	HTZ92_RS07830	2.1341	H		HTZ92_RS17145	1.3391	G
<i>antA</i>	HTZ92_RS07835	2.9856	P		HTZ92_RS17150	2.3310	M
<i>antB</i>	HTZ92_RS07840	2.1639	Q		HTZ92_RS17155	1.8506	-
	HTZ92_RS07850	1.3299	K		HTZ92_RS17160	2.6918	L
	HTZ92_RS07870	1.2640	IQ		HTZ92_RS17165	2.6128	K
	HTZ92_RS07875	1.4681	Q	<i>arsH</i>	HTZ92_RS17170	3.1314	-
	HTZ92_RS07960	-4.7052	-		HTZ92_RS17175	3.6140	T
	HTZ92_RS07965	-3.3403	-		HTZ92_RS17185	2.9036	-
	HTZ92_RS07985	-1.4048	-		HTZ92_RS17190	2.6073	-
	HTZ92_RS08020	-2.8331	K		HTZ92_RS17195	1.7779	-
	HTZ92_RS08095	-1.8033	C	<i>trbL</i>	HTZ92_RS17200	1.8402	U

	HTZ92_RS08100	-1.9602	C	<i>trbK</i>	HTZ92_RS17205	1.9835	-
	HTZ92_RS08105	-1.8974	C	<i>trbJ</i>	HTZ92_RS17210	1.6514	U
	HTZ92_RS08150	-1.4703	-		HTZ92_RS17220	2.5087	L
	HTZ92_RS08170	-1.2177	Q		HTZ92_RS17225	3.0889	L
	HTZ92_RS08205	-3.8919	M		HTZ92_RS17230	4.1444	K
	HTZ92_RS08245	1.2965	K		HTZ92_RS17235	1.9159	L
	HTZ92_RS08275	-1.5309	-		HTZ92_RS17270	-1.5004	-
	HTZ92_RS08290	-1.4561	-		HTZ92_RS17290	1.3093	C
	HTZ92_RS08340	-1.0904	P		HTZ92_RS17300	2.0132	K
	HTZ92_RS08345	3.0948	H		HTZ92_RS17325	3.1916	-
	HTZ92_RS08420	-1.5237	L		HTZ92_RS17340	-6.5372	P
	HTZ92_RS08425	-2.5237	-		HTZ92_RS17345	-4.8846	P
	HTZ92_RS08430	-1.2152	-	<i>ssuD</i>	HTZ92_RS17350	-5.6101	C
<i>iscU</i>	HTZ92_RS08445	1.7448	C	<i>ssuC</i>	HTZ92_RS17355	-5.3537	P
<i>iscA</i>	HTZ92_RS08450	1.1181	C		HTZ92_RS17360	-4.6618	P
	HTZ92_RS08505	-1.4128	GM		HTZ92_RS17405	-1.1105	-
<i>rpsT</i>	HTZ92_RS08510	-1.7745	J		HTZ92_RS17415	2.5312	-
	HTZ92_RS08520	1.1165	P		HTZ92_RS17420	1.3653	-
	HTZ92_RS08525	-2.0714	-		HTZ92_RS17430	2.6076	-
	HTZ92_RS08530	3.0772	-		HTZ92_RS17445	1.8901	-
	HTZ92_RS08545	-1.4644	P		HTZ92_RS17555	1.2357	-
	HTZ92_RS08555	-1.7874	E	<i>dmeF</i>	HTZ92_RS17565	3.2042	P
	HTZ92_RS08560	-1.0739	E	<i>yidC</i>	HTZ92_RS17580	-1.0134	U
	HTZ92_RS08570	-1.4454	P	<i>yidD</i>	HTZ92_RS17585	-1.0484	-
	HTZ92_RS08575	-1.2515	U	<i>rnpA</i>	HTZ92_RS17590	-1.6613	J
	HTZ92_RS08580	-1.5426	U	<i>rpmH</i>	HTZ92_RS17595	-2.8108	J
	HTZ92_RS08585	-2.9789	U	<i>cybC</i>	HTZ92_RS17620	1.0524	C
	HTZ92_RS08600	-1.4973	C		HTZ92_RS17625	-1.1937	-
<i>pyrF</i>	HTZ92_RS08625	-1.2202	F		HTZ92_RS17670	2.6140	-
	HTZ92_RS08650	-1.5915	I		HTZ92_RS17680	1.5325	-
	HTZ92_RS08690	-1.6810	U		HTZ92_RS17685	2.5457	-
	HTZ92_RS08770	-1.0498	M		HTZ92_RS17700	-1.4201	M
<i>fba</i>	HTZ92_RS08775	-1.0707	G		HTZ92_RS17770	-1.5957	-
	HTZ92_RS08780	-2.5072	-		HTZ92_RS17780	-2.2563	-
	HTZ92_RS08795	-1.0026	D	<i>dapB</i>	HTZ92_RS17785	1.0884	E
	HTZ92_RS08800	-1.4037	-		HTZ92_RS17795	1.8413	EGP
	HTZ92_RS08805	-1.3148	K		HTZ92_RS17805	1.0388	K
<i>putP</i>	HTZ92_RS08855	-2.0008	E		HTZ92_RS17810	1.5674	-
	HTZ92_RS08860	1.3198	K		HTZ92_RS17820	1.0827	K
<i>trmA</i>	HTZ92_RS08875	-1.8088	J		HTZ92_RS17830	-1.3094	L
	HTZ92_RS08915	1.9875	-		HTZ92_RS17865	1.5515	-
	HTZ92_RS08925	2.8549	-		HTZ92_RS17875	1.1522	L
	HTZ92_RS08930	-1.2324	M		HTZ92_RS17890	-1.0651	F
	HTZ92_RS08965	-2.1608	-		HTZ92_RS17955	1.7484	-
	HTZ92_RS08970	-3.8504	-	<i>zigA</i>	HTZ92_RS17960	1.6148	-
	HTZ92_RS09015	-1.4616	U	<i>hutH</i>	HTZ92_RS17985	-1.4972	E
	HTZ92_RS09100	1.6862	I		HTZ92_RS17990	-1.5636	E
	HTZ92_RS09125	-1.4550	P	<i>hutI</i>	HTZ92_RS17995	-1.1954	Q
	HTZ92_RS09130	-1.6934	P		HTZ92_RS18010	2.5483	-
	HTZ92_RS09145	-5.1148	I	<i>phoR</i>	HTZ92_RS18110	-2.0980	T

	HTZ92_RS09150	-4.5851	I	<i>phoB</i>	HTZ92_RS18115	-2.1149	K
	HTZ92_RS09155	-3.6845	C		HTZ92_RS18125	1.7381	-
	HTZ92_RS09160	-3.5192	P		HTZ92_RS18185	1.0550	-
	HTZ92_RS09170	-1.7197	P	<i>parE</i>	HTZ92_RS18190	1.1329	L
	HTZ92_RS09185	1.0178	-	<i>hpxO</i>	HTZ92_RS18210	-2.4850	CH
	HTZ92_RS09265	-1.7942	EJ		HTZ92_RS18215	-3.7444	M
<i>arsB</i>	HTZ92_RS09315	3.1054	P	<i>uraD</i>	HTZ92_RS18230	-3.0693	-
<i>arsC</i>	HTZ92_RS09325	-1.5008	P		HTZ92_RS18240	-2.0890	J
	HTZ92_RS09340	1.5207	Q		HTZ92_RS18275	-1.3292	P
	HTZ92_RS09350	1.2274	K	<i>ftsZ</i>	HTZ92_RS18330	1.1121	D
<i>tauD</i>	HTZ92_RS09365	-2.3636	Q		HTZ92_RS18355	1.5164	C
<i>tauC</i>	HTZ92_RS09370	-2.9962	P		HTZ92_RS18375	1.4493	C
	HTZ92_RS09375	-3.6460	P	<i>pdxH</i>	HTZ92_RS18395	1.4363	H
<i>tauA</i>	HTZ92_RS09380	-4.4793	P		HTZ92_RS18405	1.7276	-
	HTZ92_RS09385	1.0069	U	<i>sfnG</i>	HTZ92_RS18460	-5.6565	C
	HTZ92_RS09395	-1.8145	C	<i>msuE</i>	HTZ92_RS18465	-6.5929	-
	HTZ92_RS09400	-3.1108	I		HTZ92_RS18485	-2.6173	-
	HTZ92_RS09450	2.4063	H		HTZ92_RS18490	-2.3530	-
	HTZ92_RS09520	1.5106	K	<i>uvrA</i>	HTZ92_RS18515	2.5920	L
<i>yfcF</i>	HTZ92_RS09525	-1.5439	O		HTZ92_RS18520	1.7405	K
	HTZ92_RS09545	-3.4576	P		HTZ92_RS18525	1.4516	C
	HTZ92_RS09550	-4.5651	E	<i>ssb</i>	HTZ92_RS18555	1.8290	L
	HTZ92_RS09555	-4.5339	T		HTZ92_RS18580	-1.3635	L
	HTZ92_RS09560	-2.8410	E		HTZ92_RS18585	-1.7062	-
	HTZ92_RS09575	-2.1005	-		HTZ92_RS18610	1.0379	-
	HTZ92_RS09585	-3.6652	ET		HTZ92_RS18615	-2.9894	-
	HTZ92_RS09590	-2.7389	E		HTZ92_RS18620	2.6677	-
	HTZ92_RS09595	-3.6578	P		HTZ92_RS18630	1.6484	-
	HTZ92_RS09600	-3.1831	P		HTZ92_RS18635	1.8304	-
	HTZ92_RS09625	-2.0826	F		HTZ92_RS18645	1.1798	-
	HTZ92_RS09635	1.2295	E		HTZ92_RS18650	1.8558	-
<i>umuD</i>	HTZ92_RS09640	2.8031	KT		HTZ92_RS18675	1.3438	-
	HTZ92_RS09645	4.0251	-		HTZ92_RS18680	2.3438	-
	HTZ92_RS09660	1.8593	IQ		HTZ92_RS18690	2.2013	-
	HTZ92_RS09735	2.4305	K		HTZ92_RS18695	2.3428	-
	HTZ92_RS09825	1.1788	IQ		HTZ92_RS18700	1.5997	-
	HTZ92_RS09845	1.6539	EGP		HTZ92_RS18780	4.7498	-
	HTZ92_RS09865	1.9938	C	<i>rnpB</i>		2.5103	-
	HTZ92_RS09895	-1.4617	P	<i>ssrA</i>		2.4562	-
	HTZ92_RS09900	-2.7273	P	<i>ssrS</i>		2.0440	-
	HTZ92_RS09905	-3.0481	P				

Abbreviation: LFC, Log2FoldChange; COG, Cluster of Orthologous Group of proteins

Table S5. Distribution of DEGs across COG categories

COGs	Number of DEGs	
	Upregulated DEGs	Downregulated DEGs
-	165	185
C	47	24
CH	0	2
CI	1	0
CO	0	1
CP	0	2
D	6	5
E	33	14
EF	1	0
EG	4	1
EGP	7	2
EI	0	1
EJ	0	1
EM	1	0
ET	0	2
F	10	2
G	8	6
GM	0	1
H	17	10
HQ	1	0
I	19	11
IMU	0	1
IQ	8	4
J	53	9
K	25	45
KO	0	1
KT	3	2
L	9	26
M	26	7
MU	0	2
NU	4	5
O	15	7
OU	1	0
P	52	18
Q	8	11
T	10	5
U	18	4
UW	2	0
V	1	3

Abbreviation: DEGs, Differentially Expressed Genes; COG, Cluster of Orthologous Groups of proteins

Table S6. List of DEGs upregulated during resuscitation but downregulated after desiccation

Gene name	Locus Tag	LFC AD vs RES	LFC RES vs AD	COG category	Putative function
	HTZ92_RS15865	3.6456	-4.1466	-	YMGG-like glycine zipper-containing protein
	HTZ92_RS05640	3.4436	-4.3685	-	hypothetical protein
	HTZ92_RS15125	3.2176	-4.5045	-	HdeD family acid-resistance protein
	HTZ92_RS17325	3.1916	-4.5531	-	RcnB family protein
	HTZ92_RS16835	2.9395	-2.2159	-	DUF4126 domain-containing protein
	HTZ92_RS13795	2.9051	-2.5682	-	hypothetical protein
	HTZ92_RS08925	2.8549	-2.0111	-	FxsA family protein
	HTZ92_RS00930	2.8092	-4.0205	-	STAS domain-containing protein
	HTZ92_RS18620	2.6677	-1.1228	-	#N/D
uvrA	HTZ92_RS18515	2.5920	-2.0329	L	excinuclease ABC subunit UvrA
	HTZ92_RS00920	2.5894	-3.7460	Q	outer membrane lipid asymmetry maintenance protein MlaD
	HTZ92_RS00925	2.5669	-4.1493	Q	ABC transporter substrate-binding protein
	HTZ92_RS09735	2.4305	-1.4105	K	TetR/AcrR family transcriptional regulator
	HTZ92_RS09450	2.4063	-2.3020	H	malonate decarboxylase holo-ACP synthase
	HTZ92_RS05645	2.3104	-4.3217	-	hypothetical protein
	HTZ92_RS18690	2.2013	-1.6006	-	#N/D
	HTZ92_RS04885	2.1868	-1.2835	-	DUF4010 domain-containing protein
	HTZ92_RS00145	2.0714	-1.1060	-	hypothetical protein
	HTZ92_RS10105	2.0545	-1.8363	I	enoyl-CoA hydratase-related protein
	HTZ92_RS17030	2.0198	-3.3604	-	hypothetical protein
	HTZ92_RS08915	1.9875	-1.7094	-	hypothetical protein
paaG	HTZ92_RS10100	1.9715	-1.5229	I	2-(1,2-epoxy-1,2-dihydrophenylacetyl)-CoA isomerase PaaG
	HTZ92_RS17445	1.8901	-4.9156	-	hypothetical protein
	HTZ92_RS01770	1.8846	-4.1371	-	RcnB family protein
	HTZ92_RS10945	1.8756	-4.2419	-	hypothetical protein
	HTZ92_RS09660	1.8593	-1.9700	IQ	SDR family oxidoreductase
	HTZ92_RS05460	1.8579	-4.6514	-	hypothetical protein
	HTZ92_RS15845	1.8512	-3.6574	M	outer membrane beta-barrel protein
lolA	HTZ92_RS02590	1.8136	-4.4416	M	outer membrane lipoprotein chaperone LolA
	HTZ92_RS15080	1.7844	-2.5364	C	coniferyl aldehyde dehydrogenase
	HTZ92_RS15870	1.7469	-4.9085	-	hypothetical protein
pilW	HTZ92_RS14780	1.7456	-1.2560	NU	type IV pilus biogenesis/stability protein PilW
	HTZ92_RS18520	1.7405	-1.7156	K	TetR/AcrR family transcriptional regulator
	HTZ92_RS01335	1.7010	-1.7338	-	hypothetical protein
rplY	HTZ92_RS13500	1.6095	-1.5491	J	50S ribosomal protein L25
	HTZ92_RS11055	1.5887	-2.1357	-	hypothetical protein
	HTZ92_RS00730	1.5664	-3.9139	-	hypothetical protein
	HTZ92_RS13575	1.5536	-1.7484	J	L-threonylcarbamoyladenylate synthase
nadC	HTZ92_RS17025	1.5506	-1.6224	H	carboxylating nicotinate-nucleotide diphosphorylase
	HTZ92_RS09340	1.5207	-2.0102	Q	hydrolase
	HTZ92_RS18355	1.5164	-1.0727	C	2-oxo acid dehydrogenase subunit E2
clpB	HTZ92_RS11240	1.4950	-2.3887	O	ATP-dependent chaperone ClpB
	HTZ92_RS10095	1.4675	-1.8865	I	3-hydroxyacyl-CoA dehydrogenase
	HTZ92_RS03750	1.4521	-1.8116	-	CYTH and CHAD domain-containing protein
	HTZ92_RS18525	1.4516	-1.2367	C	zinc-binding dehydrogenase
tusD	HTZ92_RS06370	1.4178	-2.5405	P	sulfurtransferase complex subunit TusD
	HTZ92_RS07685	1.4118	-1.6269	L	excinuclease ABC subunit UvrA
	HTZ92_RS03365	1.4090	-2.8943	-	hypothetical protein
	HTZ92_RS16735	1.4046	-1.7655	J	RidA family protein

	HTZ92_RS03555	1.3929	-1.0410	K	hypothetical protein
	HTZ92_RS12610	1.3894	-2.9067	-	hypothetical protein
uvrB	HTZ92_RS03560	1.3786	-2.3902	L	excinuclease ABC subunit UvrB
	HTZ92_RS18675	1.3438	-2.9768	-	#N/D
	HTZ92_RS11060	1.3122	-3.1884	-	four-helix bundle copper-binding protein
	HTZ92_RS08245	1.2965	-1.1750	K	CerR family C-terminal domain-containing protein
acnA	HTZ92_RS14615	1.2785	-2.8199	C	aconitate hydratase AcnA
	HTZ92_RS15815	1.2541	-1.1054	-	hypothetical protein
lon	HTZ92_RS12330	1.2449	-1.1112	O	endopeptidase La
	HTZ92_RS17555	1.2357	-2.2250	-	GlsB/YeaQ/YmgE family stress response membrane protein
htpX	HTZ92_RS02950	1.2322	-1.0265	O	protease HtpX
	HTZ92_RS14385	1.2315	-1.1797	K	integration host factor subunit alpha
rpsO	HTZ92_RS15530	1.2309	-1.0197	J	30S ribosomal protein S15
	HTZ92_RS09350	1.2274	-1.1848	K	GntR family transcriptional regulator
	HTZ92_RS13440	1.2056	-1.0336	-	flavodoxin family protein
	HTZ92_RS16790	1.2006	-1.4099	-	hypothetical protein
	HTZ92_RS01200	1.1813	-1.8813	-	YheV family putative metal-binding protein
	HTZ92_RS03035	1.1597	-3.1457	C	FdhF/YdeP family oxidoreductase
	HTZ92_RS06020	1.1309	-1.2562	M	mechanosensitive ion channel
	HTZ92_RS00910	1.1054	-1.0106	Q	ABC transporter ATP-binding protein
dapB	HTZ92_RS17785	1.0884	-1.6808	E	4-hydroxy-tetrahydrodipicolinate reductase
	HTZ92_RS06600	1.0871	-1.0590	T	GGDEF domain-containing protein
	HTZ92_RS13280	1.0580	-1.1673	D	DNA translocase FtsK
	HTZ92_RS10005	1.0419	-3.8234	G	MFS transporter
	HTZ92_RS11275	1.0359	-1.5660	M	M48 family metalloproteinase
	HTZ92_RS07210	1.0133	-3.9716	-	type 1 glutamine amidotransferase domain-containing protein

Abbreviation: LFC, Log₂FoldChange; DEGs, Differentially Expressed Genes; AD, after 1-week desiccation; RES, Resuscitated; COG, Cluster of Orthologous Groups of proteins

Table S7. Distribution of the 75 DEGs across COG categories

COGs	Number of DEGs
-	34
C	5
D	1
E	1
G	1
H	2
I	3
J	4
K	6
L	3
M	4
O	3
P	1
Q	4
T	1
X	2

Abbreviation: COG, Cluster of Orthologous Groups of proteins; DEGs, Differentially Expressed Genes

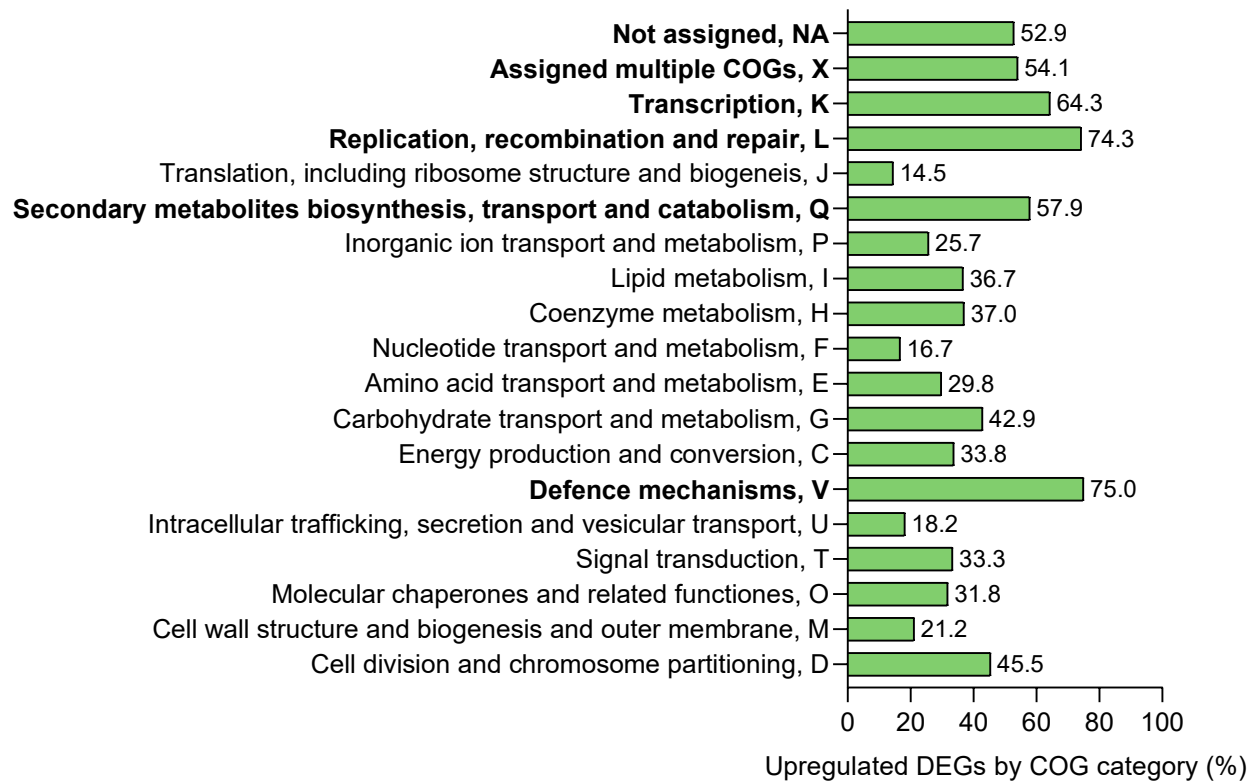


Figure S1 | Transcriptional analysis of *A. baumannii* ATCC 19606^T VBNC cells after resuscitation. Percentage of upregulated DEGs by COG category during resuscitation (AD vs RES). COGs categories with more than 50% of upregulated DEGs are highlighted in bold.

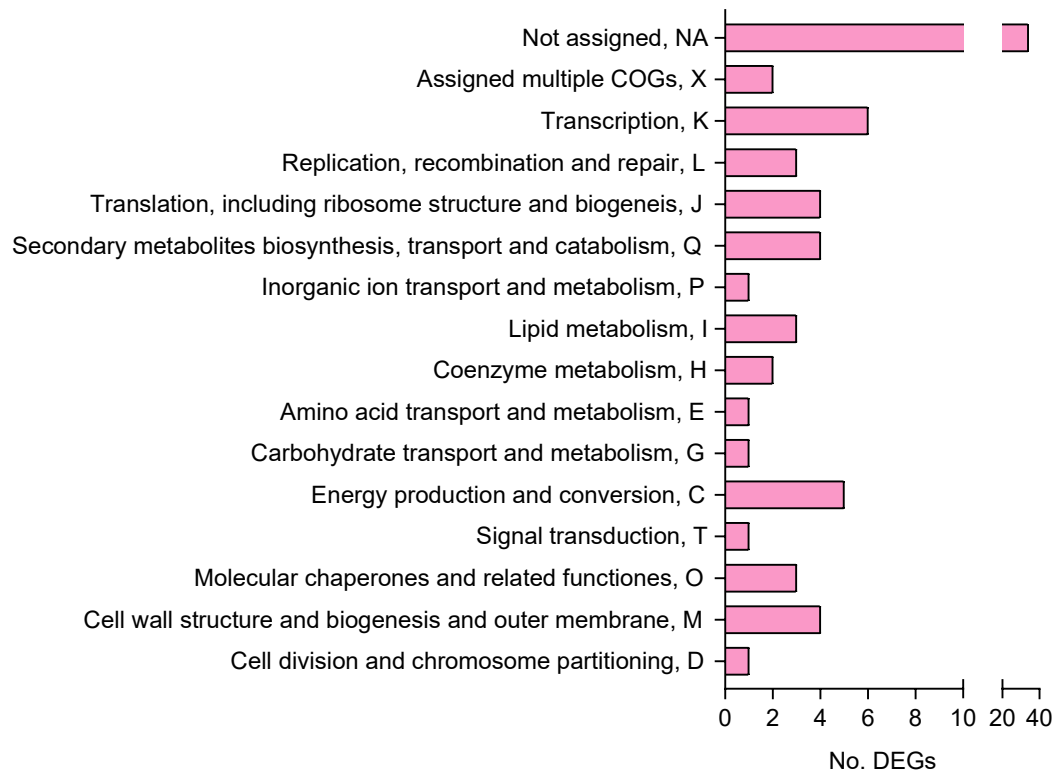


Figure S2 | COG functional analysis of DEGs specific to resuscitated *A. baumannii* ATCC 19606^T VBNC cells. Distribution of COG functional categories for 75 *A. baumannii* DEGs upregulated during resuscitation (AD vs RES) and downregulated after 1-week desiccation (BD vs AD).

TTT**CAT**GCATTCATCATAAACAACTATTAGACCAGTCGTCTAGTAAAATTA
TGTTGCTGGTTAAAATTTGCTTAGAGTAGAAATGTAGAATGAGGAATGTTA
AAAATACAACCTGCTTAATTAAACAAATTT**TTGTCG**TTTGTCTGTTTAAC**CGT**
CACAATTTGTCTGCTTTAAATCATTTTATACCACTTGAACAATTTTAAATCG
TTATCATATATAGGGTCTGTTGTTTATAACCAGTCACACCTCCCTGCGGCT
GATCACTCAACACCTAAATTTTCATCAATACACAATTGAGATGTTTT

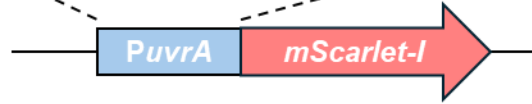


Figure S3 | Schematic illustration of the *PuvrA*::*mScarlet-I* transcriptional fusion. The sequence shown above corresponds to the cloned promoter fragment in the pWH1266-CG-VmS vector; the putative -10 and -35 RNA-polymerase binding sites are highlighted in bold.

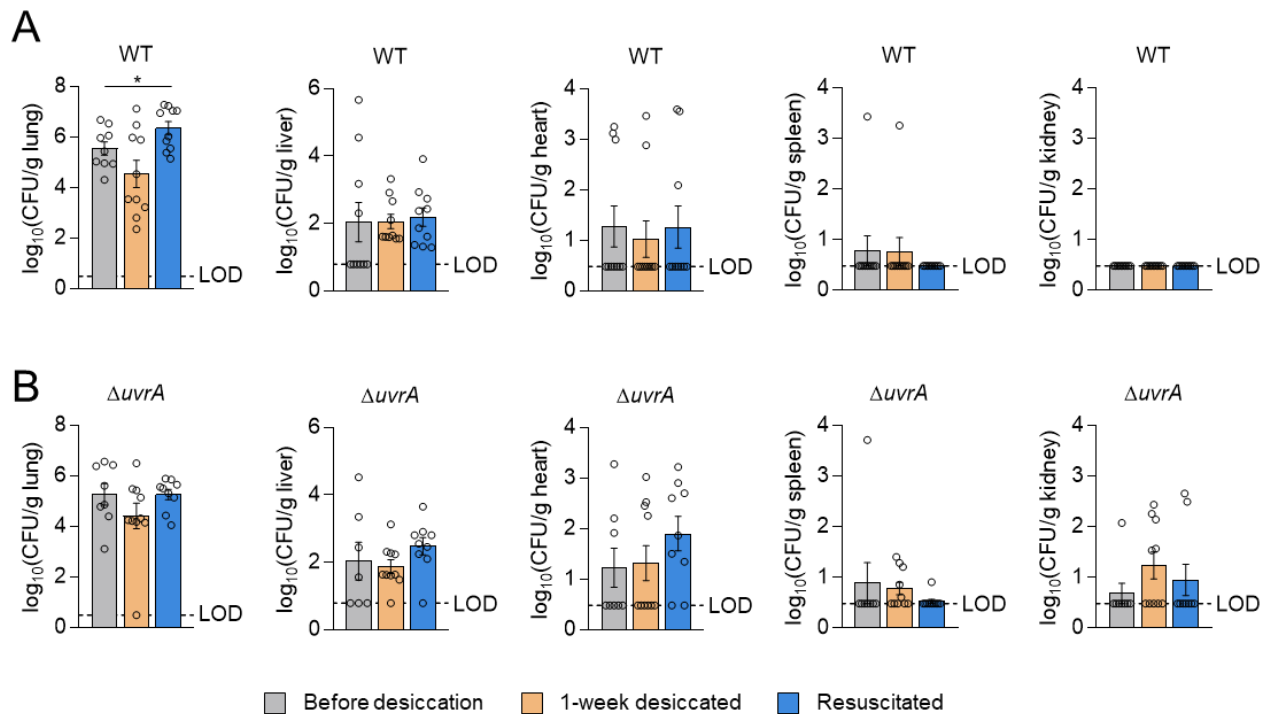


Figure S4 | Lung colonization and systemic dissemination of *A. baumannii* ATCC 19606^T in mice infected with bacteria in different physiological states. Female C57BL/6J mice (9–10 weeks old) were intranasally infected with *A. baumannii* WT or $\Delta uvrA$ strains, either before desiccation, after 1-week desiccation, or after resuscitation. At 36 hpi, mice were humanely euthanized, and WT (A) and $\Delta uvrA$ (B) bacterial burdens were quantified in multiple organs. Dashed lines represent the LOD of CFU counts (4 CFU/g for all organs except the liver, where it was 6 CFU/g). Each dot represents an individual mouse. Data are shown as mean $\pm$ SEM. Statistical significance was determined using unpaired t-tests * $P < 0.05$.

Chapter 5. ESKAPE Gram-negative bacteria escape culture-based detection upon desiccation on abiotic surfaces

Daniela Visaggio^{1,2,3*}, Massimiliano Lucidi^{1,2*}, Cinzia Spagnoli¹, Ilaria Ciccone¹, Francesco Imperi^{1,2,3}, Paolo Visca^{1,2,3}

¹Department of Science, Roma Tre University, Rome, Italy

²NBFC, National Biodiversity Future Center, Palermo, Italy

³Santa Lucia Foundation IRCCS, Rome, Italy

*Equally contributed

Microbiology Spectrum, 2026, e0335725 <https://doi.org/10.1128/spectrum.03357-25>

Publication synopsis

Hospital surfaces and medical devices are recognized as critical reservoirs for pathogen transmission, facilitating cross-contamination of patients through contact with healthcare personnel and equipment (Mitchell *et al.*, 2023). Desiccation represents a major challenge for bacterial survival on hospital surfaces, where prolonged dryness and low humidity prevail.

In previous chapters, it was demonstrated that *A. baumannii* can enter the VBNC state under desiccation and regain culturability when rehydrated in suitable buffers or biological fluids (Lucidi *et al.*, 2025). However, whether this strategy is widespread among the major nosocomial pathogens and how it impacts environmental monitoring protocols remains unclear.

ESKAPE bacteria, namely *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp, constitute a major threat in healthcare settings, owing to their role in healthcare-associated infections and their high levels of antimicrobial resistance (Woh & Zhang, 2025).

This chapter describes the ability of ESKAPE bacteria to survive desiccation on materials commonly found in hospital settings through transition to the VBNC state and subsequent resuscitation.

Following desiccation, all ESKAPE pathogens showed a marked reduction in cultivability, with variability depending on both species and surface type. However, Gram-positive ESKAPE species failed to recover cultivability upon resuscitation while Gram-negative species regained culturability, denoting a transition to the VBNC state in response to desiccation. It was also demonstrated that conventional culture-based methods failed to detect Gram-negative VBNC bacteria, which became detectable following a simple resuscitation step.

Overall, this work provides the first systematic assessment of VBNC induction and recovery among ESKAPE pathogens exposed to desiccation stress on various matrices found in hospitals. It highlights a critical limitation of current culture-based surveillance methods and proposes a simple modification to improve the accuracy of environmental monitoring in healthcare settings.



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ESKAPE Gram-negative bacteria escape culture-based detection upon desiccation on abiotic surfaces

Daniela Visaggio,^{1,2,3} Massimiliano Lucidi,^{1,2} Cinzia Spagnoli,¹ Ilaria Ciccone,¹ Francesco Imperi,^{1,2,3} Paolo Visca^{1,2,3}

AUTHOR AFFILIATIONS See affiliation list on p. 10.

ABSTRACT ESKAPE bacteria, namely *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., are leading causes of hospital-acquired infections and a major therapeutic challenge due to multidrug resistance. Hospital surfaces and medical devices are critical reservoirs for the transmission of these pathogens to patients. Standard methods for detecting microorganisms in the hospital environment are culture-based, so they cannot identify bacteria in the viable but non-culturable (VBNC) state. VBNC bacteria remain metabolically active and potentially infectious, but they fail to grow in conventional, nutrient-rich culture media. Reversion from the VBNC to the cultivable state is termed resuscitation. To assess whether ESKAPE species enter the VBNC state upon desiccation on abiotic materials commonly utilized in clinical facilities and can be resuscitated, bacterial cells were desiccated for 1 week on glass, different plastics, cotton, and titanium surfaces, then resuscitated in a carbon-free buffer. After desiccation, all ESKAPE pathogens exhibited reduced cultivability, with species- and surface-dependent variability. Gram-positive ESKAPE species did not regain cultivability after resuscitation. Conversely, Gram-negative species reverted to the cultivable state, indicating a transition to the VBNC state in response to desiccation. Compared to the standard methodology for biocontamination control (EN 17141:2020), the resuscitation step prior to culture yielded a significantly greater recovery of Gram-negative ESKAPE bacteria in the VBNC state from both experimentally contaminated samples and environmental surfaces. These findings pose the need for environmental monitoring approaches capable of detecting VBNC pathogens on abiotic hospital surfaces.

IMPORTANCE Accurate detection of microbial contamination in the hospital environment is fundamental for preventing nosocomial infections. Current protocols for environmental surveillance, however, rely almost exclusively on culture-based methods, which overlook bacteria in the viable but non-culturable (VBNC) state. This study demonstrates that clinically relevant Gram-negative ESKAPE pathogens can persist on hospital surfaces in the VBNC state, thereby evading conventional approaches for environmental control, resulting in substantial underestimation of the bacterial burden. We further show that a simple resuscitation step restores the cultivability of VBNC cells, improving their recovery rate, ultimately resulting in much greater sensitivity compared with conventional biocontamination control methods. These findings reveal a critical limitation of current environmental surveillance approaches and highlight the importance of integrating VBNC detection into monitoring protocols for achieving a more accurate assessment of surface contamination to strengthen infection prevention strategies.

KEYWORDS biocontamination, ESKAPE, hospital environment, resuscitation, sampling, VBNC

Editor Bobby G. Warren, Duke University, Durham, North Carolina, USA

Peer Reviewer Hajime Kanamori, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Address correspondence to Daniela Visaggio, daniela.visaggio@uniroma3.it, or Paolo Visca, paolo.visca@uniroma3.it.

Daniela Visaggio and Massimiliano Lucidi contributed equally to this article. The author order was determined by decreasing seniority and contribution to funding acquisition.

The authors declare no known conflict of interest or personal relationships that could have appeared to influence the work reported in this paper. A patent entitled "Metodo per la quantificazione di microrganismi patogeni su superfici abiotiche" was filed on 19 June 2025, application number: 10202500014596, Italian Patent Office (Ufficio Italiano Brevetti e Marchi).

See the funding table on p. 10.

Received 20 October 2025

Accepted 5 January 2026

Published 4 February 2026

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Hospital surfaces and medical devices are recognized as critical sources for pathogen transmission, facilitating cross-contamination of patients through various vectors, including healthcare personnel and medical equipment (1, 2). Although monitoring microbial contamination of hospital surfaces is crucial for containing an outbreak of infection when an environmental reservoir is suspected, contamination of the hospital environment is rarely investigated, and internationally agreed methodological guidelines are unavailable (3–5). Environmental sampling procedures in healthcare facilities may involve several methods and different devices (swabs, sponges, contact plates), but invariably rely on culture-based approaches, overlooking the ability of some bacterial species to enter a viable but non-culturable (VBNC) state (6–8). The VBNC state is a reversible condition in which viable bacterial cells lose cultivability upon exposure to an environmental stress. Reversion from VBNC to the culturable state is called resuscitation and occurs under conditions that vary between bacterial species and the type of stress (6, 9, 10).

In the hospital environment, bacterial contaminants of inanimate surfaces are exposed to multiple stresses, among which prolonged desiccation is particularly challenging. The progressive loss of water over extended periods of dryness threatens their survival, inducing biochemical, metabolic, and physiological changes (11).

Evidence has previously been provided that *Acinetobacter baumannii* strains lose cultivability after desiccation on abiotic surfaces by entering the VBNC state and can resuscitate when rehydrated in suitable buffers or biological fluids (12, 13). Therefore, transition to the VBNC state allows *A. baumannii* to persist on hospital surfaces as a concealed source of contamination, turning the inanimate environment into a hidden reservoir of infection. *A. baumannii* and other ESKAPE pathogens (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) are among the most dreaded bacteria responsible for healthcare-associated infections (HAI), being notorious for their high level of resistance to several antibiotics (14, 15). While ESKAPE pathogens have developed adaptations for survival in the modern health care setting (16) and are known to enter a VBNC state in response to several types of stress (Table S1), little is known about their behavior under desiccation stress. Here, we demonstrate that all Gram-negative ESKAPE bacteria can withstand desiccation by entering the VBNC state, and we propose a new environmental monitoring method that detects VBNC cells as well.

MATERIALS AND METHODS

Bacterial strains, sample preparation, and desiccation conditions

The bacterial strains used in this study are described in Table S2. Bacteria were pre-cultured in Luria Bertani broth (LB) at 37°C for 18 h, diluted 1:100 in fresh LB, and incubated at 37°C under shaking for 6 h. Bacterial cultures were harvested by centrifugation ($3,000 \times g$ for 5 min), washed twice with double-distilled water, and suspended in double-distilled water at an optical density at 600 nm (OD_{600}) of 1.0. Twenty-microliter aliquots of bacterial suspensions in double-distilled water were deposited onto 1.5 cm² surfaces of glass (GL), polyvinyl chloride (PVC), polypropylene (PP), polyester (PL), polystyrene (PS), polyethylene (PE), silicon (SL), cotton (CT), and titanium (Ti) and air-dried under the laminar flow hood at $25.2^\circ\text{C} \pm 1.5^\circ\text{C}$. These materials are broadly used in hospital settings as outlined in Table S3. After air-drying, bacterial cells were stored for 1 week in a 16 L-vacuum bell containing 50 g of silica gel at an average temperature and relative humidity of $20.9^\circ\text{C} \pm 0.6^\circ\text{C}$ and $13.0\% \pm 5.6\%$.

Bacterial resuscitation conditions

To assess bacterial cultivability after desiccation, air-dried samples were rehydrated in 2 mL of resuscitation buffer (RB, 6.8 g/L Na_2HPO_4 , 3 g/L KH_2PO_4 , 0.5 g/L NaCl, 1 g/L NH_4Cl , 0.02 g/L CaCl_2 , and 0.12 g/L MgSO_4 ; Table S4), incubated for 15 min at room

temperature, mixed by vortexing for 30 s, and 10-fold serially diluted in sterile saline for colony-forming unit (CFU) counts, as previously described (13). For resuscitation, the same tube from which the aliquot had been removed for the CFU counts was incubated at 37°C for 24 h under shaking, and CFU counts were performed on tryptic soy agar (TSA) plates.

Effect of different swabs and transport buffers on bacterial cultivability after desiccation

Fifty-microliter aliquots of bacterial suspensions prepared as described above were poured onto glass slides and desiccated for 1 week. Bacterial cells were harvested using different swabs (Table S4). The nylon swab (Copan) was placed in 5 mL of RB, whereas the commercial swabs (Table S4) were placed in 5 mL of their respective transport buffers (TB, Table S4), except for the LMS Amies swab, which contained 1 mL of Amies solution. All swabs were incubated for 15 min at room temperature, mixed by vortexing for 30 s, and the bacterial suspensions were 10-fold serially diluted in sterile saline for CFU counts, in order to determine the bacterial cultivability immediately after desiccation. Swabs placed in RB were incubated at 37°C for 24 h under shaking, whereas the commercial ones were maintained at 4°C for 24 h under static conditions. CFU counts were then performed on TSA plates to assess the bacterial cultivability. The resuscitation in Amies buffer was also evaluated by incubating the samples at 37°C under shaking for 24 h.

LIVE/DEAD staining for quantification of bacterial membrane integrity

Fluorescent labeling was performed using the LIVE/DEAD BacLight Bacterial Viability Kit (ThermoFisher Scientific), on bacterial suspensions before desiccation, after desiccation on glass Petri dishes, and after resuscitation, as previously described (13). The LIVE/DEAD BacLight Bacterial Viability Kit contains two nucleic acid dyes: SYTO 9, which penetrates all bacterial cells and fluoresces green upon DNA binding, and propidium iodide (PI), which enters only cells with damaged membranes, quenches the SYTO 9 emission, and emits red fluorescence. As PI quenches SYTO 9 fluorescence in non-viable cells, the green/red fluorescence ratio serves as a proxy for membrane integrity.

For microscopy, 20 μ L of each stained suspension was deposited onto a glass coverslip coated with 0.5% (wt/vol) agarose to immobilize bacterial cells. Samples were imaged using a Nikon A1R HD25 confocal laser scanning microscope equipped with an Apo TIRF 100 $\times$ oil immersion objective (numerical aperture = 1.49). For each condition, five images were acquired at a resolution of 512 $\times$ 512 pixels. Image analysis was conducted in ImageJ (Fiji) by manually selecting individual cells using the Multipoint Tool, adding the selected points to the ROI Manager, and exporting the pixel intensity values for red (R) and green (G) channels using a custom macro script (reported in Supplemental material).

Five randomly selected areas without cells were used to determine the background RG intensity ($RG_{\text{background}}$). For each cell, the green/red pixel intensity ratio was calculated and corrected by subtracting $RG_{\text{background}}$. Clusters in which single cells could not be clearly distinguished were excluded from the analysis. For each sample, >300 cells were analyzed.

Growth of ESKAPE bacteria in RB supplemented or not with a nutrient-rich medium

Bacterial strains were grown in LB at 37°C for 18 h. An aliquot of the bacterial cultures was washed and diluted to $OD_{600} = 0.01$ in a final volume of 200 μ L of RB. Alternatively, an aliquot was diluted to $OD_{600} = 0.02$ in 100 μ L of 2 $\times$ RB. Bacterial cultures were incubated at 37°C, and growth was monitored by recording the OD_{600} every 6 h for 24 h using a Spark 10 M microplate reader (Tecan). After 24 h, wells containing 100 μ L of bacterial cultures in 2 $\times$ RB were supplemented with 100 μ L of 2 $\times$ LB. Bacterial cultures were incubated at 37°C, and growth was monitored for additional 24 h.

Growth of ESKAPE bacteria in different transport buffers

Bacterial strains were grown in LB at 37°C for 6 h. An aliquot of the bacterial cultures was washed in double-distilled water and diluted to $OD_{600} = 0.001$ in a final volume of 100 μ L of D/E neutralizing broth (NB), maximum recovery diluent (MRD), neutralizing rinse solutions (NRS), and Amies solution. Bacterial cultures were incubated at 37°C, and growth was monitored by recording the OD_{600} every 4 h for 24 h using a Spark 10 M microplate reader (Tecan).

Environmental surface sampling

Laboratory benches, washbasins, mobile phones, and classroom desks were sampled using nylon swabs (Copan) pre-moistened in RB, LMS Swab Amies, or ESC Swab Maximum Recovery (Table S4). A 10 $\times$ 10 cm sampling mask (Copan) was used for all surfaces except for mobile phones, which were sampled using a 4 $\times$ 5 cm mask (Copan). The nylon swabs were placed in 2 mL of RB, whereas each commercial swab was placed in its respective buffer (Table S4). Samples were incubated at room temperature for 15 min, vortexed for 1 min, and 100- μ L aliquots were plated on TSA plates for CFU counts. Then, swabs immersed in RB were incubated at 37°C with shaking for 24 h, while those in Amies and MRD were stored at 4°C for 24 h. After incubation, serial dilutions in sterile saline solution were prepared, and 100 μ L aliquots were plated on TSA plates for CFU counts. The plates were incubated for 72 h at 37°C. For each surface type, six samples were collected and analyzed using nylon swab and RB, while three samples each were collected and analyzed using the LMS swab Amies and ESC Swab Maximum Recovery.

Statistical analysis

Statistical analysis was performed using the GraphPad InStat software v8.0. Data were analyzed by Student's *t*-test and one-way ANOVA, as indicated in the figure legends. Asterisks indicate the statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, ns = not significant.

RESULTS

Gram-negative ESKAPE bacteria survive desiccation by entering the VBNC state

Entry into a VBNC state in response to desiccation stress has only been reported for *A. baumannii* (13). This trait may enable bacteria to persist for extended periods and eventually spread in the hospital environment, thereby increasing the likelihood of patient exposure and the risk of healthcare-associated infections. Initially, we wondered whether the ability to withstand desiccation stress by entering the VBNC state is a common feature of ESKAPE pathogens. To this aim, one clinical isolate and one reference strain of ESKAPE species (Table S2) were desiccated for 1 week at room temperature on the surfaces of different materials frequently found in hospitals, namely glass, plastic polymers, cotton, and titanium (Table S3; Fig. 1A). The CFU counts revealed that cultivability after 1 week of desiccation varied among strains and was influenced by the type of material (Fig. 1B; Fig. S1). All ESKAPE bacteria, except *K. pneumoniae* and *A. baumannii*, exhibited a significant reduction in cultivability upon dehydration on almost all surfaces. This reduction was particularly pronounced for *P. aeruginosa* strains, as well as for clinical *Klebsiella aerogenes* (basonym *Enterobacter aerogenes*), and *S. aureus* isolates (Fig. 1B; Fig. S1 and S2). To differentiate cell death from entrance into the VBNC state, a resuscitation experiment was conducted. One-week desiccated bacterial cells were incubated for 24 h at 37°C in an isotonic buffer, named RB, which does not support bacterial growth due to the lack of any carbon sources (13) (Table S4). As expected, the absence of carbon sources impeded bacterial growth in RB (Fig. S3).

All Gram-negative bacteria fully or partly recovered cultivability after resuscitation, depending on the tested material (Fig. 1B; Fig. S1), denoting their transition to a desiccation-induced VBNC state, which was completely or largely reversed to a culturable

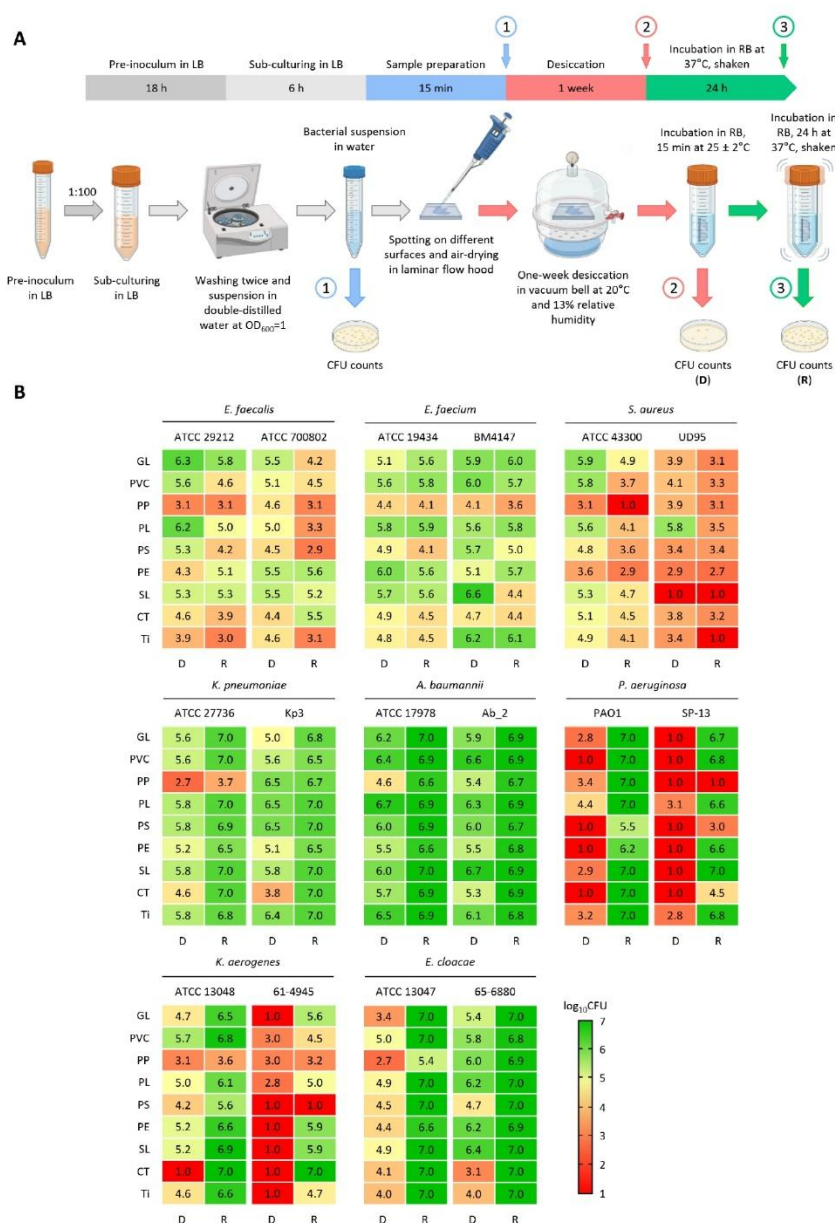


FIG 1 Cultivability of ESKAPE bacteria after desiccation on different surfaces and subsequent resuscitation. (A) Experimental timeline and schematization of desiccation resistance assay. The experiment comprises four sequential steps: (i) bacterial cultivation (gray), (ii) sample preparation (blue), (iii) desiccation (red), and (iv) resuscitation (green). The numbered arrows indicate the time points at which CFU counts were performed, i.e., before desiccation (1), after 1-week desiccation on different surfaces (2), and after resuscitation in RB at 37°C for 24 h under shaking (3). Panel A was created with Biorender. (B) Cultivability, expressed as log₁₀ CFU, after 1-week desiccation (D lane) and after resuscitation in RB (R lane). Data are the means of three independent experiments. Mean values and error bars are shown in Fig. S1. Abbreviations of surfaces: glass (GL), polyvinyl chloride (PVC), polypropylene (PP), polyester (PL), polystyrene (PS), polyethylene (PE), silicon (SL), cotton (CT), and titanium (TI).

state upon rehydration in RB. Conversely, the recovery of CFUs after incubation in RB was either equal to or lower than that observed after 1-week desiccation for all Gram-positive ESKAPE species (Fig. 1B; Fig. S1), suggesting that desiccation does not induce the

VBNC state in these pathogens, or the experimental conditions were unsuitable for their resuscitation.

To assess membrane damage following dehydration, cells were stained with the LIVE/DEAD dyes before desiccation, after 1-week desiccation, and after resuscitation. With the exception of *Enterococcus faecium* and *Enterococcus faecalis*, whose desiccated cells were not permeable to SYTO 9 (Fig. S4), all ESKAPE species showed significant loss of membrane integrity after desiccation (Fig. S4). After resuscitation, partial or no restoration of membrane integrity was observed in most species, with the only exception of *P. aeruginosa*, which displayed a nearly complete recovery of membrane integrity (Fig. S4). Therefore, no consistent correlation between the membrane integrity and cultivability was observed for almost all ESKAPE bacteria, indicating that Gram-negative ESKAPE bacteria can regain cultivability even without evident repair of the desiccation-induced membrane damage.

Bacteria in the VBNC state escape standard detection methods

According to the BS EN 17141:2020 standard procedure suited for “Cleanrooms and associated controlled environments—Biocontamination control”, after surface sampling with a swab moistened in a suitable TB, samples must be transported at a temperature between 1°C and 8°C. Then, an aliquot of the TB should be directly plated on TSA or stored at 3°C ± 2°C for no longer than 48 h before plating. Since Gram-negative ESKAPE bacteria enter the VBNC state upon desiccation, the BS EN 17141:2020 standard procedure was assessed in comparison with the resuscitation protocol for VBNC bacteria described in the previous section. Bacterial cells were desiccated on a glass slide for 1 week and harvested using a nylon swab soaked in RB or four types of commercial swabs soaked in the cognate TB (Table S4). The swab soaked in RB was incubated in 5 mL RB at 37°C for 24 h under shaking, whereas, according to the BS EN 17141:2020 recommendations, the commercial swabs were kept at 4°C under static conditions for 24 h in 5 mL of TB (Fig. 2A), except the LMS Amies swab, which contains 1 mL of Amies medium. Substantial differences were observed when CFUs were quantified after 24-h incubation in the different buffers (Fig. 2B; Fig. S5).

Regarding Gram-positive ESKAPE bacteria, incubation in RB resulted in a slight reduction of CFU counts compared to the commercial swabs, except for *E. faecium* ATCC 19436, which exhibited a moderate increase in CFU counts. Conversely, all Gram-negative ESKAPE bacteria were completely resuscitated in RB, but not in any of the TBs (Fig. S5). These results indicate that standard sampling methods fail to detect VBNC cells, leading to an underestimation of the number of viable bacteria on surfaces.

To verify whether bacterial resuscitation in a commercial buffer is influenced by incubation temperature, 1-week desiccated bacteria were harvested using a nylon swab soaked in Amies buffer and incubated at 37°C for 24 h under shaking. TBs other than Amies were not tested since they contain carbon sources (Table S4), which would sustain bacterial growth during incubation at 37°C (Fig. S6), therefore invalidating a direct comparison with the resuscitation procedure. Except for *P. aeruginosa*, which was partially resuscitated, none of the ESKAPE bacteria regained cultivability in Amies buffer. Conversely, all Gram-negative ESKAPE species were almost completely resuscitated in RB (Fig. 2C; Fig. S7).

Improved detection of the microbial load on dry surfaces through VBNC resuscitation

To assess the impact of the resuscitation step on bacterial quantification in a real-life environment, different surfaces within the Department of Science at Roma Tre University were sampled with three types of swab/buffer combinations, namely a nylon swab pre-moistened in RB, and the commercially available swabs LMS Swab Amies and ESC Swab MRD. Following surface sampling, an initial CFU count was performed (T_0). Subsequently, swabs were incubated for 24 h in RB at 37°C, or in Amies and MRD at 4°C (T_1) before CFU counting (Fig. 3A). CFU counts remained unchanged or even decreased

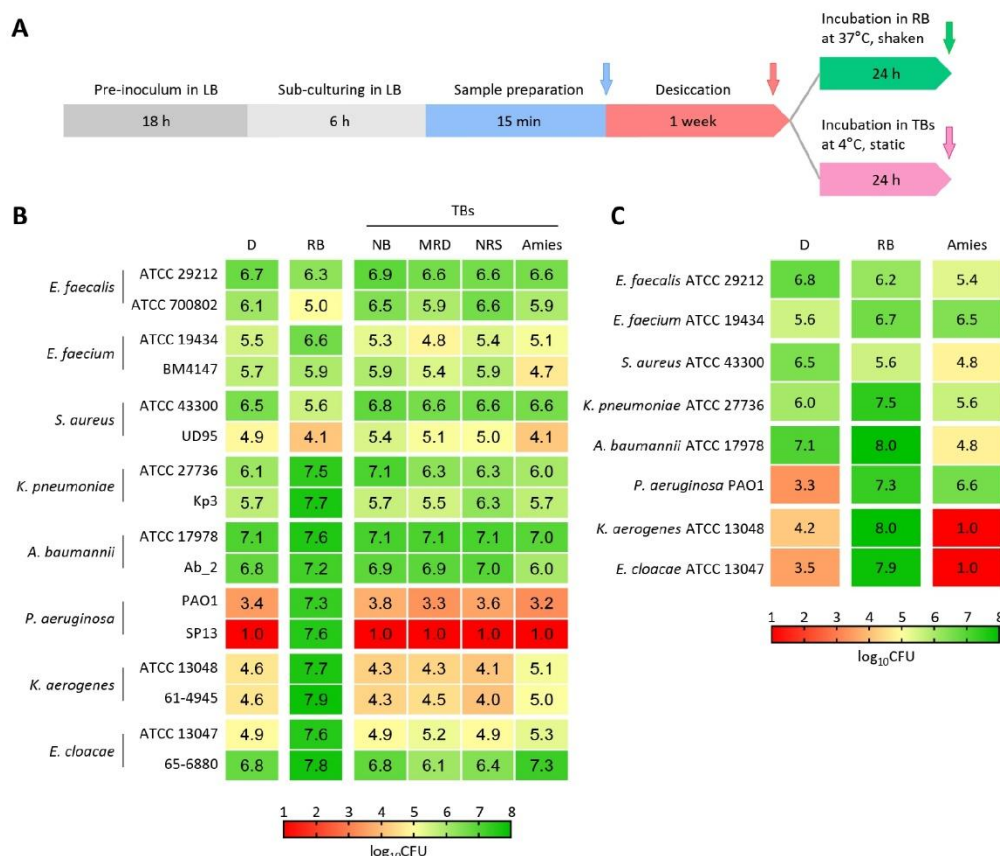


FIG 2 Comparison between the standard procedure for environmental contamination control and the procedure based on the resuscitation process. (A) Timeline of experimental procedure. The experiment comprises four sequential steps: (i) bacterial cultivation (gray), (ii) sample preparation (blue), (iii) desiccation on glass slides (red), and (iv) resuscitation in RB at 37°C for 24 h (green) or incubation in commercial TBs at 4°C for 24 h under static conditions (pink). The arrows indicate the time points at which CFU counts were performed. (B) Cultivability of bacteria determined after 1-week desiccation (D lane) and subsequent 24-h incubation at 4°C for all the TBs, or after 24-h incubation at 37°C for RB, and expressed as log₁₀ CFU. NB, neutralizing broth; MRD, maximum recovery diluent; NRS, neutralizing rinse solution. (C) Cultivability of ESKAPE bacteria after 1-week desiccation (D lanes) on glass slides and resuscitation in RB or Amies transport medium at 37°C for 24 h. Data in panels B and C are the means of three independent experiments. Mean values and error bars are shown in Fig. S4 and S6.

compared to the T₀ values for the commercial swabs, whereas incubation in RB markedly increased CFU counts, ranging from twofold up to 10⁶-fold relative to T₀ counts (Fig. 3B). These results suggest that conventional methods and commercially available swabs commonly used in surface sampling underestimate the actual bacterial load on abiotic surfaces.

DISCUSSION

Prevention and control of HAI remain significant medical challenges. Pathogens responsible for HAI, particularly ESKAPE bacteria, can spread indirectly through contact with fomites (17, 18). More than 40% of HAI are due to cross-contamination by health-care personnel, who can transfer pathogens via contaminated cloths, gloves, and frequently touched surfaces such as medical devices, tables, and beds (19, 20). The ability of pathogens to persist on surfaces depends not only on surface properties, such as porosity, hydrophobicity, roughness, and intrinsic antimicrobial activities, but also on specific characteristics of the pathogen itself (21). Environmental persistence has been extensively documented in healthcare settings for some ESKAPE species, such as *K.*

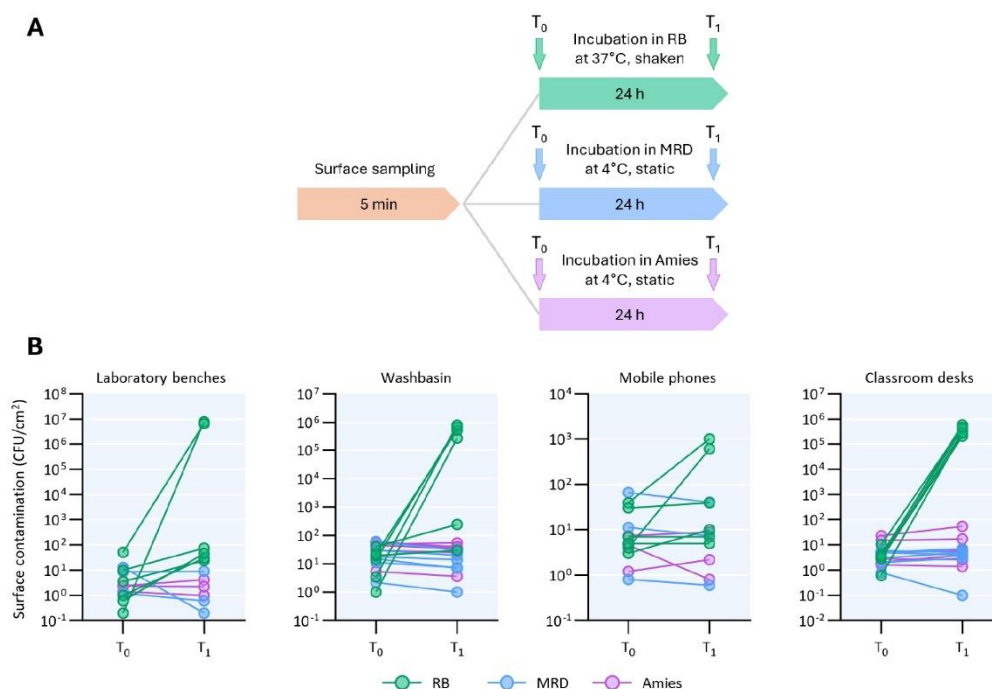


FIG 3 Sampling of different surfaces for bacterial detection using commercial swabs and swabs soaked in the RB. (A) Timelines of surface sampling with nylon swab soaked with RB (green), MRD (blue), and Amies (purple). Laboratory benches, washbasins, mobile phones, and classroom desks were sampled using appropriate sampling masks and pre-moistened swabs. Swabs were incubated at room temperature for 15 min, vortexed for 1 min, and 100 μ L were plated on TSA plates (T_0). Swabs immersed in RB were incubated at 37°C with shaking for 24 h, while those in Amies transport medium and MRD were stored at 4°C for 24 h before plating (T_1). (B) Surface contamination, expressed as the number of CFU per cm², determined at T_0 and T_1 . For each surface type, six samples were collected and analyzed using RB, while three samples each were collected and analyzed using either Amies or MRD.

pneumoniae, *A. baumannii*, and *S. aureus*, as opposed to *P. aeruginosa*, which is believed to survive for a very short time in the dry nosocomial environment (8, 22). However, standard culture-dependent methods for the assessment of surface contamination in hospitals do not detect bacteria in the VBNC state, likely underestimating the actual microbial burden. Moreover, despite the clinical relevance of *K. aerogenes* and *Enterobacter cloacae* (23, 24), no studies have so far investigated the ability of these species to enter the VBNC state in response to environmental stresses. Here, we demonstrate that desiccation on different substrates induces the transition of both *K. aerogenes* and *E. cloacae* to a VBNC state, resulting in a marked loss of cultivability that can be reversed by the resuscitation process.

Since desiccation is known to cause severe membrane damage in bacteria due to lipid oxidation, membrane protein misfolding, loss of fluidity, and, ultimately, disruption of the lipid bilayer (11, 25), the LIVE/DEAD stain has in the past been proposed as a tool to identify VBNC cells (6). Given that SYTO 9 permeates both viable and dead cells, while PI only stains cells with compromised membranes, the LIVE/DEAD stain is predicted to differentiate between actively growing bacteria (SYTO 9-positive), VBNC cells (both SYTO 9- and PI-positive), and dead cells (only PI-positive). However, in line with recent findings (12, 13), here we demonstrate that even apparently dead cells exhibiting strong PI uptake, indicative of severe membrane damage, can be resuscitated. This observation suggests that membrane integrity alone cannot serve as a critical marker for VBNC detection, and that complete regain of membrane integrity by Gram-negative ESKAPE species is not mandatory for recovery of cultivability after resuscitation.

The emerging notion that a portion of the bacterial population exposed to air desiccation can enter the VBNC state and elude detection calls into question the

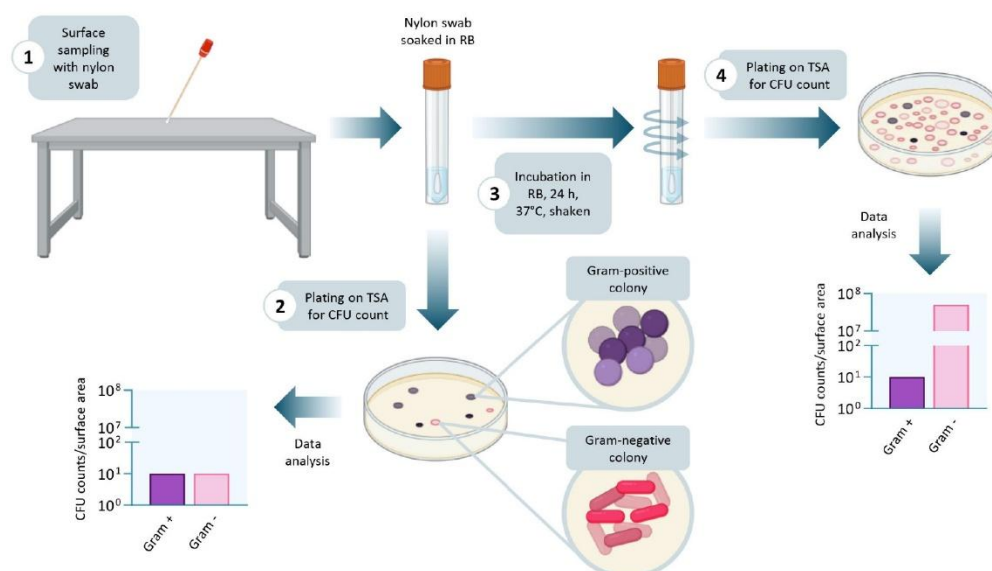


FIG 4 Graphical representation of the surface sampling procedure with or without the resuscitation step. The direct plating of the transport medium in which the swab is immersed after surface sampling allows the culture-based detection of a minority of ESKAPE Gram-negative bacteria, i.e., those that do not enter the VBNC state. Incubation of the swab in RB for 24 h at 37°C under shaking enables ESKAPE Gram-negative bacteria in the VBNC state to regain cultivability, for a more reliable assessment of bacterial contamination of the surface. The figure was created with Biorender.

reliability of current environmental monitoring methods. In Gram-negative ESKAPE pathogens, entry into the VBNC state appears to be a widespread strategy to withstand the desiccation-induced stress. Our study shows that the substrate material can influence bacterial survival and highlights the remarkable ability of certain pathogens, particularly *P. aeruginosa*, to elude direct culture-based detection once they enter the VBNC state.

Remarkably, we demonstrate that a simple resuscitation step involving 24-h incubation at 37°C in RB is enough to entirely recover the colony-forming ability of otherwise uncultivable Gram-negative ESKAPE bacteria (Fig. 4). The limits of traditional methods have been confirmed by comparative tests of individual ESKAPE species exposed to defined dehydration conditions. The higher sensitivity of the resuscitation protocol over traditional methods was also corroborated by a pilot environmental sampling experiment.

However, some limitations of this work should be considered when interpreting our findings. Our study has focused on a restricted set of pathogenic species, representative of the ESKAPE group; therefore, it cannot be assumed that the resuscitation protocol will work with other clinically relevant species. In addition, although reference and clinical isolates of each ESKAPE species displayed similar behavior, the limited number of strains examined does not allow us to rule out potential intraspecific variability. Another critical point is that the procedure proved unsuccessful in resuscitating Gram-positive ESKAPE species. Although these species can enter a VBNC state in response to different stressors (Table S1), the resuscitation protocol did not increase their cultivability, suggesting either an irreversible loss of cultivability after desiccation or an inadequacy of the RB for these species. Furthermore, although the method proved effective for Gram-negative bacteria that entered a VBNC state after desiccation, it remains unclear whether the same procedure can resuscitate VBNC cells arising within biofilms or induced by other stressors, including antimicrobial compounds or disinfectants. Finally, the main limitation of this work is that our proposed protocol for environmental sampling has not yet been validated in healthcare settings, where factors such as the use of disinfectants, the presence of organic material from patients' biological fluids, and different temperature and humidity levels may further modulate bacterial responses.

Despite these limitations, our results clearly call into question the reliability of conventional methodologies for environmental surveillance (e.g., BS EN 17141:2020) and raise important issues about bacteria previously thought incapable of surviving desiccation on hospital surfaces. Microbiological methods for environmental surveillance should therefore be implemented to unveil the elusive bacterial species capable of thriving in the VBNC state. According to the BS EN 17141:2020 standard procedure suited for “Cleanrooms and associated controlled environments—Biocontamination control”, environmental sampling can be performed using different devices, including contact plates, sponges, and swabs. Among these, the swab is the most frequently used device in healthcare settings (26, 27). Therefore, our protocol for surface sampling (Supplemental material and Fig. 4) can easily be implemented in existing environmental monitoring procedures, requiring only an additional step, consisting of 24-h incubation at 37°C in RB.

Conclusions

Based on our findings, we propose a simple method (protocol described on pages xviii–xix of the Supplemental material) for the recovery of bacteria on abiotic surfaces, which enables the detection of Gram-negative ESKAPE bacteria even in the VBNC state. The method is intended as an aid to infection control specialists to improve environmental surveillance standards.

ACKNOWLEDGMENTS

D.V., Writing—original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization | M.L., Writing—original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Data curation, Conceptualization | C.S., Methodology, Investigation, Data curation, Conceptualization | I.C., Methodology, Investigation, Data curation, Conceptualization | F.I., Writing—review and editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis | P.V., Writing—review and editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis.

AUTHOR AFFILIATIONS

¹Department of Science, Roma Tre University, Rome, Italy

²NBFC, National Biodiversity Future Center, Palermo, Italy

³Santa Lucia Foundation IRCCS, Rome, Italy

AUTHOR ORCIDs

Daniela Visaggio  <http://orcid.org/0000-0002-3192-8974>

Massimiliano Lucidi  <http://orcid.org/0000-0003-3238-9164>

Francesco Imperi  <http://orcid.org/0000-0001-5080-5665>

Paolo Visca  <http://orcid.org/0000-0002-6128-7039>

FUNDING

Funder	Grant(s)	Author(s)
Italian Ministry of Universities and Research (MUR)	Excellence Departments grant (art. 1, comma 314-337 Legge 232/2016)	Paolo Visca
Italian Ministry of Universities and Research (MUR)	PRIN 2022 grant (CUP F53D23000860001)	Daniela Visaggio
Italian Ministry of Universities and Research (MUR)	Rome Technopole, PNRR grant (M4-C2-Inv. 1.5 CUP F83B22000040006)	Paolo Visca

AUTHOR CONTRIBUTIONS

Daniela Visaggio, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft | Massimiliano Lucidi, Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft | Cinzia Spagnoli, Conceptualization, Data curation, Investigation, Methodology | Ilaria Ciccone, Conceptualization, Data curation, Investigation, Methodology | Francesco Imperi, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review and editing | Paolo Visca, Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review and editing

ETHICS APPROVAL

All experiments were conducted in accordance with institutional and national biosafety regulations. The clinical isolates were either previously published, as indicated in the references listed in Table S2, or obtained anonymously from the biobank of a regional hospital.

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Supplemental material (Spectrum03357-25-s0001.pdf). Tables S1 to S4, Fig. S1 to S7, and supplemental methods.

Open Peer Review

PEER REVIEW HISTORY (review-history.pdf). An accounting of the reviewer comments and feedback.

REFERENCES

- Loyola-Cruz MÁ, Durán-Manuel EM, Cruz-Cruz C, Márquez-Valdelamar LM, Bravata-Alcántara JC, Cortés-Ortiz IA, Cureño-Díaz MA, Ibáñez-Cervantes G, Fernández-Sánchez V, Castro-Escarpullí G, Bello-López JM. 2023. ESKAPE bacteria characterization reveals the presence of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* outbreaks in COVID-19/VAP patients. *Am J Infect Control* 51:729–737. <https://doi.org/10.1016/j.ajic.2022.08.012>
- Mitchell BG, McDonagh J, Dancer SJ, Ford S, Sim J, Thottiyil Sultanmhammed Abdul Khadar B, Russo PL, Maillard JY, Rawson H, Browne K, Kiernan M. 2023. Risk of organism acquisition from prior room occupants: an updated systematic review. *Infect Dis Health* 28:290–297. <https://doi.org/10.1016/j.idh.2023.06.001>
- Thomas RE, Thomas BC, Conly J, Lorenzetti D. 2022. Cleaning and disinfecting surfaces in hospitals and long-term care facilities for reducing hospital- and facility-acquired bacterial and viral infections: a systematic review. *J Hosp Infect* 122:9–26. <https://doi.org/10.1016/j.jhin.2021.12.017>
- van der Schoor AS, Boyle M, Voor in 't holt AF, Vos MC, Humphreys H. 2022. Environmental sampling of innate hospital surfaces: a survey of current practices and the need for guidelines. *J Hosp Infect* 128:92–95. <https://doi.org/10.1016/j.jhin.2022.07.024>
- Gastaldi S, Accorgi D, D'Ancona F. 2025. Tools and strategies for monitoring hospital environmental hygiene services. *J Hosp Infect* 159:52–61. <https://doi.org/10.1016/j.jhin.2025.01.011>
- Liu J, Yang L, Kjellerup BV, Xu Z. 2023. Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. *Trends Microbiol* 31:1013–1023. <https://doi.org/10.1016/j.tim.2023.04.009>
- Schapiro AJ, Dramé M, Olive C, Marion-Sanchez K. 2024. Bacterial viability in dry-surface biofilms in healthcare facilities: a systematic review. *J Hosp Infect* 144:94–110. <https://doi.org/10.1016/j.jhin.2023.11.004>
- Porter L, Sultan O, Mitchell BG, Jenney A, Kiernan M, Brewster DJ, Russo PL. 2024. How long do nosocomial pathogens persist on inanimate surfaces? A scoping review. *J Hosp Infect* 147:25–31. <https://doi.org/10.1016/j.jhin.2024.01.023>
- Li L, Mendis N, Trigui H, Oliver JD, Faucher SP. 2014. The importance of the viable but non-culturable state in human bacterial pathogens. *Front Microbiol* 5:258. <https://doi.org/10.3389/fmicb.2014.00258>
- Balogurusamy R, Gopi L, Kumar DSS, Viswanathan K, Meganathan V, Sathiyamurthy K, Athmanathan B. 2024. Significance of viable but non-culturable (VBNC) state in *Vibrios* and other pathogenic bacteria: induction, detection and the role of resuscitation promoting factors (Rpf). *Curr Microbiol* 81:417. <https://doi.org/10.1007/s00284-024-03947-8>
- Lebre PH, De Maayer P, Cowan DA. 2017. Xerotolerant bacteria: surviving through a dry spell. *Nat Rev Microbiol* 15:285–296. <https://doi.org/10.1038/nrmicro.2017.16>
- König P, Wilhelm A, Schaudinn C, Poehlein A, Daniel R, Wiedera M, Auerhoff B, Müller V. 2023. The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *mBio* 14:e0213923. <https://doi.org/10.1128/mbio.02139-23>
- Lucidi M, Capecchi G, Spagnoli C, Basile A, Artuso I, Persichetti L, Fardelli E, Capellini G, Visaggio D, Imperi F, Rampioni G, Leoni L, Visca P. 2025. The response to desiccation in *Acinetobacter baumannii*. *Virulence* 16:2490209. <https://doi.org/10.1080/21505594.2025.2490209>
- Venkateswaran P, Vasudevan S, David H, Shaktivel A, Shanmugam K, Neelakantan P, Solomon AP. 2023. Revisiting ESKAPE pathogens: virulence, resistance, and combating strategies focusing on quorum

- sensing. *Front Cell Infect Microbiol* 13:1159798. <https://doi.org/10.3389/fcimb.2023.1159798>
15. Woh PY, Zhang X. 2025. The burden of ESKAPE pathogen-related hospital-acquired infections: clinical and financial perspective from a systematic review. *J Hosp Infect* 162:377–385. <https://doi.org/10.1016/j.jhin.2025.06.006>
 16. Miller WR, Arias CA. 2024. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics. *Nat Rev Microbiol* 22:598–616. <https://doi.org/10.1038/s41579-024-01054-w>
 17. D'Souza AW, Potter RF, Wallace M, Shupe A, Patel S, Sun X, Gul D, Kwon JH, Andleeb S, Burnham C-AD, Dantas G. 2019. Spatiotemporal dynamics of multidrug resistant bacteria on intensive care unit surfaces. *Nat Commun* 10:4569. <https://doi.org/10.1038/s41467-019-12563-1>
 18. Kramer A, Lexow F, Bludau A, Köster AM, Misailovski M, Seifert U, Eggers M, Rutala W, Dancer SJ, Scheithauer S. 2024. How long do bacteria, fungi, protozoa, and viruses retain their replication capacity on inanimate surfaces? A systematic review examining environmental resilience versus healthcare-associated infection risk by "fomite-borne risk assessment". *Clin Microbiol Rev* 37:e0018623. <https://doi.org/10.1128/cmr.00186-23>
 19. Sukhum KV, Newcomer EP, Cass C, Wallace MA, Johnson C, Fine J, Sax S, Barlet MH, Burnham C-AD, Dantas G, Kwon JH. 2022. Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. *Commun Med (Lond)* 2:62. <https://doi.org/10.1038/s43856-022-00124-5>
 20. Sandu AM, Chifiriuc MC, Vrancianu CO, Cristian RE, Alistar CF, Constantin M, Paun M, Alistar A, Popa LG, Popa MI, Tantu AC, Sidoroff ME, Mihai MM, Marcu A, Popescu G, Tantu MM. 2025. Healthcare-associated infections: the role of microbial and environmental factors in infection control—a narrative review. *Infect Dis Ther* 14:933–971. <https://doi.org/10.1007/s40121-025-01143-0>
 21. Katzenberger RH, Rösel A, Vonberg RP. 2021. Bacterial survival on inanimate surfaces: a field study. *BMC Res Notes* 14:97. <https://doi.org/10.1186/s13104-021-05492-0>
 22. Akinbobola AB, Osunla AC, Bello OM, Ajayi OA. 2022. Study of the persistence of selected Gram-negative bacteria pathogens of health-care-associated infections on hospital fabrics. *Am J Infect Control* 50:755–757. <https://doi.org/10.1016/j.ajic.2021.11.023>
 23. Wesevich A, Sutton G, Ruffin F, Park LP, Fouts DE, Fowler VG Jr, Thaden JT. 2020. Newly named *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*) is associated with poor clinical outcomes relative to other *Enterobacter* species in patients with bloodstream infection. *J Clin Microbiol* 58:e00582-20. <https://doi.org/10.1128/JCM.00582-20>
 24. Girlich D, Ouzani S, Emeraud C, Gauthier L, Bonnin RA, Le Sache N, Mokhtari M, Langlois I, Begasse C, Arangia N, Fournier S, Fortineau N, Naas T, Dortet L. 2021. Uncovering the novel *Enterobacter cloacae* complex species responsible for septic shock deaths in newborns: a cohort study. *Lancet Microbe* 2:e536–e544. [https://doi.org/10.1016/S2666-5247\(21\)00098-7](https://doi.org/10.1016/S2666-5247(21)00098-7)
 25. Greffe VRG, Michiels J. 2020. Desiccation-induced cell damage in bacteria and the relevance for inoculant production. *Appl Microbiol Biotechnol* 104:3757–3770. <https://doi.org/10.1007/s00253-020-10501-6>
 26. Rawlinson S, Ciric L, Cloutman-Green E. 2019. How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. *J Hosp Infect* 103:363–374. <https://doi.org/10.1016/j.jhin.2019.07.015>
 27. Chen F, Li Y, Wang W, Li J, Wang D, Sun X, Peng Y, Deng J. 2024. Comparative performance of contact plate method and swab method for surface microbial contamination on medical fabrics. *BMC Infect Dis* 24:530. <https://doi.org/10.1186/s12879-024-09416-8>

Supplemental Material

ESKAPE Gram-negative bacteria escape culture-based detection upon desiccation on abiotic surfaces

Daniela Visaggio^{a,b,c*}, Massimiliano Lucidi^{a,b*}, Cinzia Spagnoli^a, Ilaria Ciccone^a, Francesco Imperi^{a,b,c}, Paolo Visca^{a,b,c}

^a Department of Science, Roma Tre University, Rome, Italy

^b NBFC, National Biodiversity Future Center, Palermo, Italy

^c Santa Lucia Foundation IRCCS, Rome, Italy

*Equally contributed

✉ Address for correspondence: D. Visaggio, Department of Science, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy (daniela.visaggio@uniroma3.it); P. Visca, Department of Science, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy (paolo.visca@uniroma3.it).

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Table S1. ESKAPE species capable of entering the VBNC state upon exposure to different types of stress

Bacterial species	Stress	Reference
<i>Enterococcus</i> spp.	Chlorination	Jiang et al., 2023
	Nutrient limitation	Zhang et al., 2024; Gin et al., 2013; Lleo et al., 2007; Lleo et al., 2005; Signoretto et al., 2005; Lleo et al., 2003; Heim et al., 2002; Lleo et al., 2001
<i>Staphylococcus aureus</i>	Chitooligosaccharides	Li et al., 2024
	High pressure	Yang et al., 2023
	Citral and cinnamaldehyde	Cheng et al., 2023
	Low temperature (Frozen)	Yan et al., 2021
	Nutrient limitation	Li et al., 2020
	Non-thermal plasma	Liao et al., 2020
	Citric acid	Bai et al., 2019
<i>Klebsiella pneumoniae</i>	Antibiotics	Pasquaroli et al., 2014; Pasquaroli et al., 2013
	High salinity and micro-aerophilic conditions	Zhao et al., 2024
	Formic acid and organic acids (food preservatives)	Yadav et al., 2022
	Oxidative stress (H ₂ O ₂)	Alveraz-Daza et al., 2021
<i>Acinetobacter baumannii</i>	Desiccation	Konig et al., 2023; Lucidi et al., 2025
	Formic acid and organic acids	Yadav et al., 2022
	Low oxygen	Kvich et al., 2019
<i>Pseudomonas aeruginosa</i>	Antibiotics	Mangiaterra et al., 2023; Mangiaterra et al., 2020
	Chlorine	Qi et al., 2023; Qi et al., 2022; Chiang et al., 2022; Wang et al., 2022
	UV	Wang et al., 2021; Zhang et al., 2015
	Copper toxicity	Dopp et al., 2017; Bedard et al., 2014

Table S2. Bacterial strains used in this study

Bacterial species	Strain	Country	Year	Source	Resistance	Reference
<i>Enterococcus faecalis</i>	ATCC 29212	ns	ns	urine	ns	American Type Culture Collection
<i>E. faecalis</i>	ATCC 700802	USA	1987	blood	VAN (<i>vanB</i>), GEN	Sahm et al., 1989
<i>Enterococcus faecium</i>	ATCC 19434 ^T	ns	ns	unknown	ns	Schleifer and Kilpper-Balz, 1984
<i>E. faecium</i>	BM4147	ns	ns	human clinical	VAN (<i>vanA</i>)	Bugg et al., 1991
<i>S. aureus</i>	ATCC 43300	USA	ns	human clinical	MRSA	American Type Culture Collection
<i>S. aureus</i>	UD95	Italy	2011	human clinical	MDR	Hijazi et al., 2018
<i>K. pneumoniae</i>	ATCC 27736	ns	ns	human clinical	ns	American Type Culture Collection
<i>K. pneumoniae</i>	Kp3	Italy	2011	blood	MDR	Villa et al., 2014
<i>A. baumannii</i>	ATCC 17978	France	1951	blood	SXT	Smith et al., 2007
<i>A. baumannii</i>	Ab_2	Italy	2020	blood	MDR	This work
<i>P. aeruginosa</i>	ATCC 15692 (PAO1)	ns	ns	infected wound	ns	American Type Culture Collection
<i>P. aeruginosa</i>	SP-13	Italy	ns	blood	MDR	Bonchi et al., 2015
<i>Enterobacter aerogenes</i>	ATCC 13048 ^T	USA	ns	sputum	ns	Bascomb et al., 1971
<i>E. aerogenes</i>	61-4945	Italy	2016	blood	-	This work
<i>Enterobacter cloacae</i>	ATCC 13047 ^T	USA	ns	spinal fluid	susceptible	Hormaeche and Edwards, 1960
<i>E. cloacae</i>	65-6880	Italy	2016	blood	-	This work

Abbreviations: GEN, gentamicin; MDR, multidrug-resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; ns, not specified. ^T type strain.

Table S3. Substrate materials used in desiccation assays

Materials	Abbreviation	Some examples of hospital devices
Glass	GL	IV bottles, ampoules, vials, blood collection tubes
Polyvinyl chloride	PVC	Primary IV tubes and bags, blood bags, oxygen masks, endotracheal tubes, drainage bags and tubes, respiratory tubing, infusion sets, dialysis bags and tubing
Polypropylene	PP	Disposable scrubs, suture materials, and medical packaging
Polyester	PL	Surgical masks, vascular grafts, surgical mesh, implantable textiles, medical tapes and bandages, pillow and mattress covers, surgical drapes, and sterilization wraps
Polystyrene	PS	Tubes, sample containers, trays and instrument holders, and disposable syringes
Polyethylene	PE	Urinal bottles, IV tubing, syringe components, drains, oxygen masks
Silicone	SL	Catheters, endotracheal tubes, ostomy bags, wound dressings, infusion devices
Cotton	CT	Lab coats, cotton balls, cotton swabs, cotton gauze pads, cotton bandages
Titanium	Ti	Prosthetics, orthopedic, dental, cardiovascular, craniofacial and maxillofacial, neurosurgical implants and surgical instruments

Abbreviations: IV, intravenous

Table S4. List of the swabs and chemical composition of the suspension media used in this study

Swab	Abbreviation	Material	Buffer composition (g/L)	Provider
FLOQSwabs	RB	Nylon	Na ₂ HPO ₄ (6.80) KH ₂ PO ₄ (3.00) NaCl (0.50) NH ₄ Cl (1.00) CaCl ₂ (0.02) MgSO ₄ (0.12)	Swab: Copan Salt powder: Merk
ESC Swab D/E Neutralizing Broth ^a	NB	Rayon	Enzymatic digest of casein (5.00) Yeast extract (2.50) Dextrose (10.00) Sodium thioglycolate (1.00) Na ₂ S ₂ O ₃ (6.00) NaHSO ₃ (2.50) Lecithin; (7.00) Polysorbate 80; (5.00) Bromocresol purple (0.02)	Liofilchem
ESC Swab Maximum Recovery Diluent ^b	MRD	Rayon	Enzymatic digest of casein (1.00) NaCl (8.50)	Liofilchem
ESC Swab Neutralizing Rinse Solutions ^c	NRS	Rayon	Casein peptone (1.00) Histidine (1.00) Lecithin (2.00) Tween 80 (30.00) Na ₂ S ₂ O ₃ (5.00) NaCl (8.50)	Liofilchem
LMS swab Amies ^d	Amies	Polyurethane foam	Na ₂ HPO ₄ (1.15) KH ₂ PO ₄ (0.20) NaCl (3.00) KCl (0.20) CaCl ₂ (0.10) MgCl ₂ (0.10) C ₇ H ₅ NaO ₂ S (1.00)	Heinz Herenz Medizinalbedarf GmbH

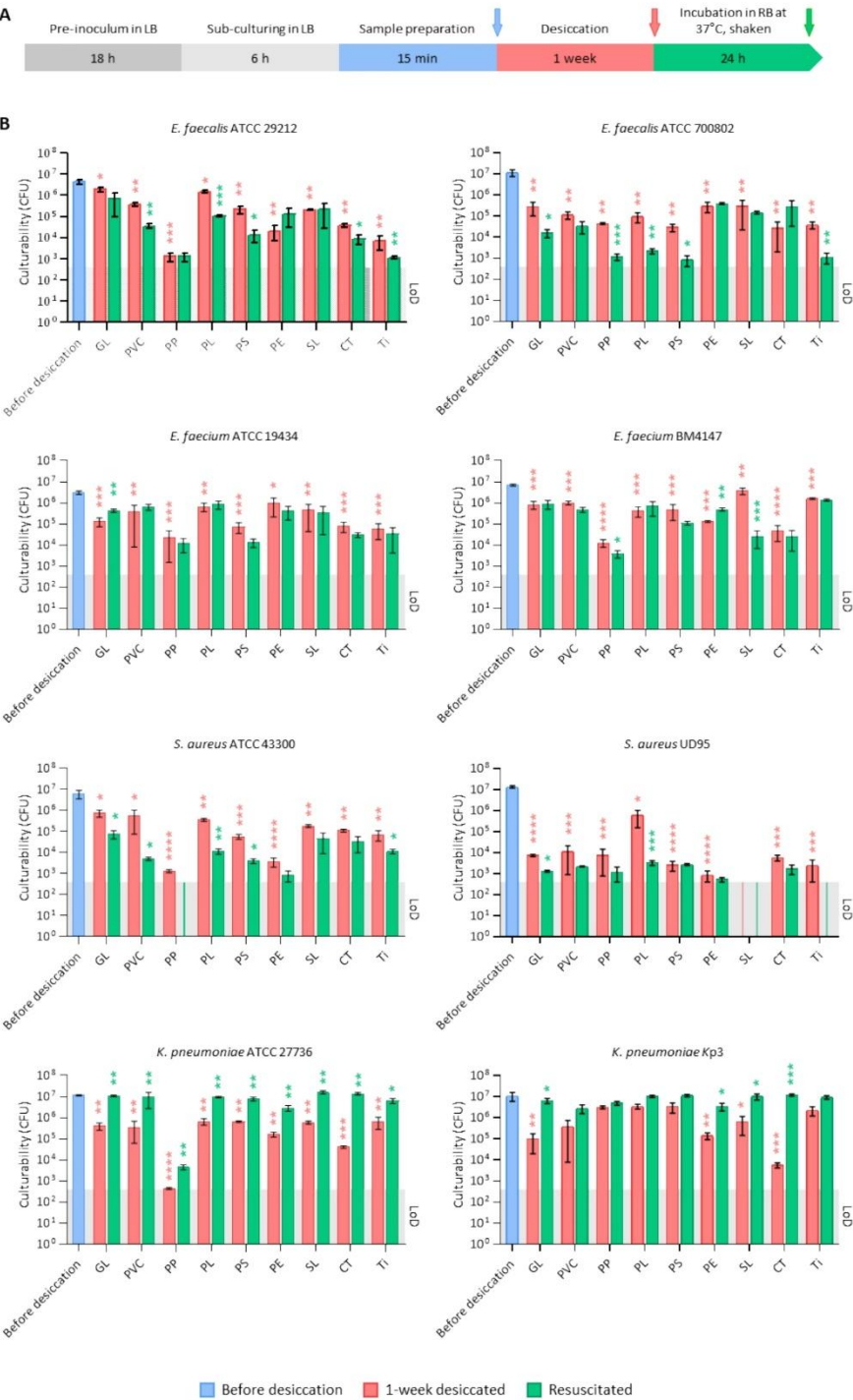
Abbreviations: ESC, easy surface checking; LMS, transport system with liquid medium.

^a the neutralizing agents in the medium inactivate residual antimicrobials. Sodium thiosulfate, sodium bisulfite, lecithin, and polysorbate 80 neutralize a broad spectrum of antiseptics and disinfectants, including quaternary ammonium compounds, phenolics, iodine, chlorine preparations, mercurials, formaldehyde, and glutaraldehyde.

^b the buffer contains an enzymatic digest of casein at low concentration and physiological saline, allowing the recovery of even stressed or injured cells.

^c the buffer contains the following neutralizers of disinfectants: L-histidine (inactivates aldehydes), lecithin (neutralizes the quaternary ammonium compounds), polysorbate 80 (inactivates phenolic compounds and mercurial derivatives), and sodium thiosulfate (neutralizes halogen compounds).

^d transport medium for the sampling and preservation of aerobic and anaerobic bacteria at room or refrigerated temperature.



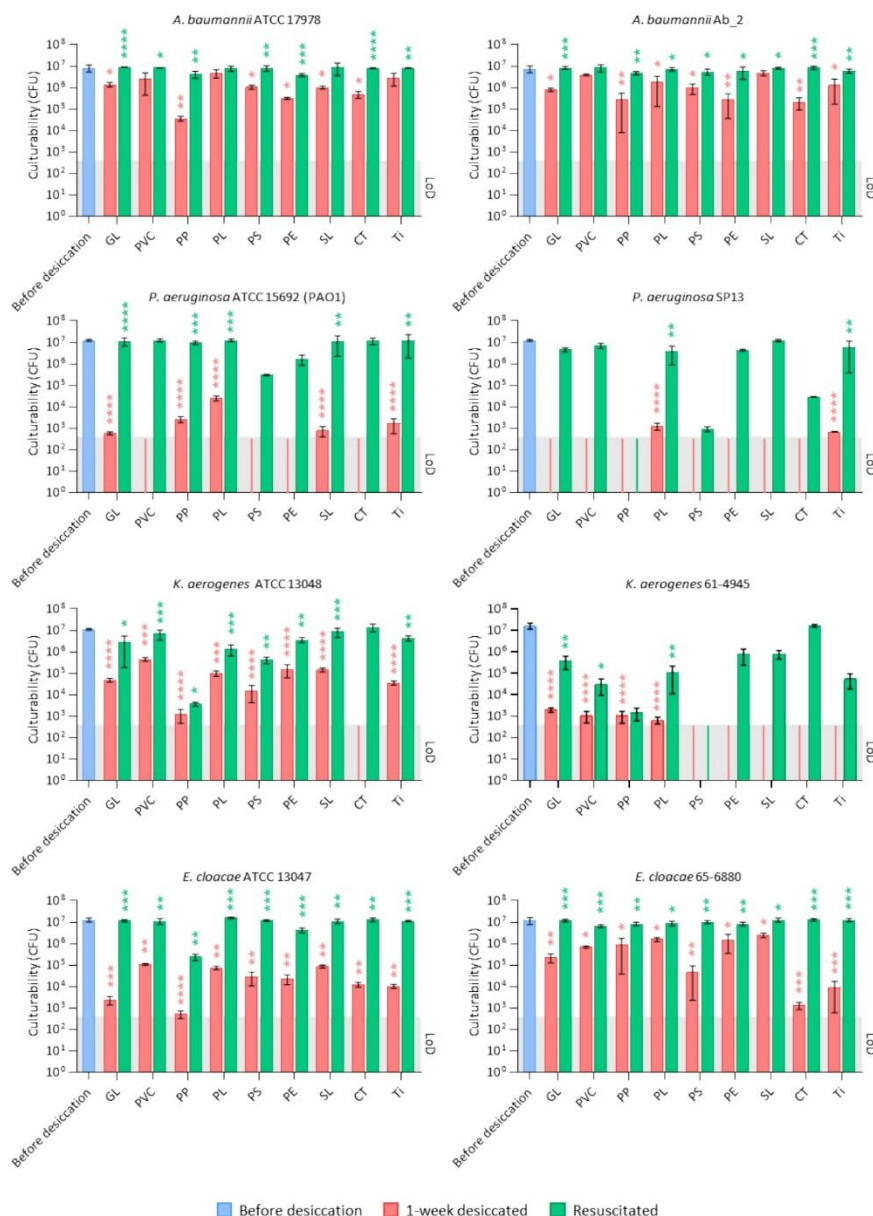
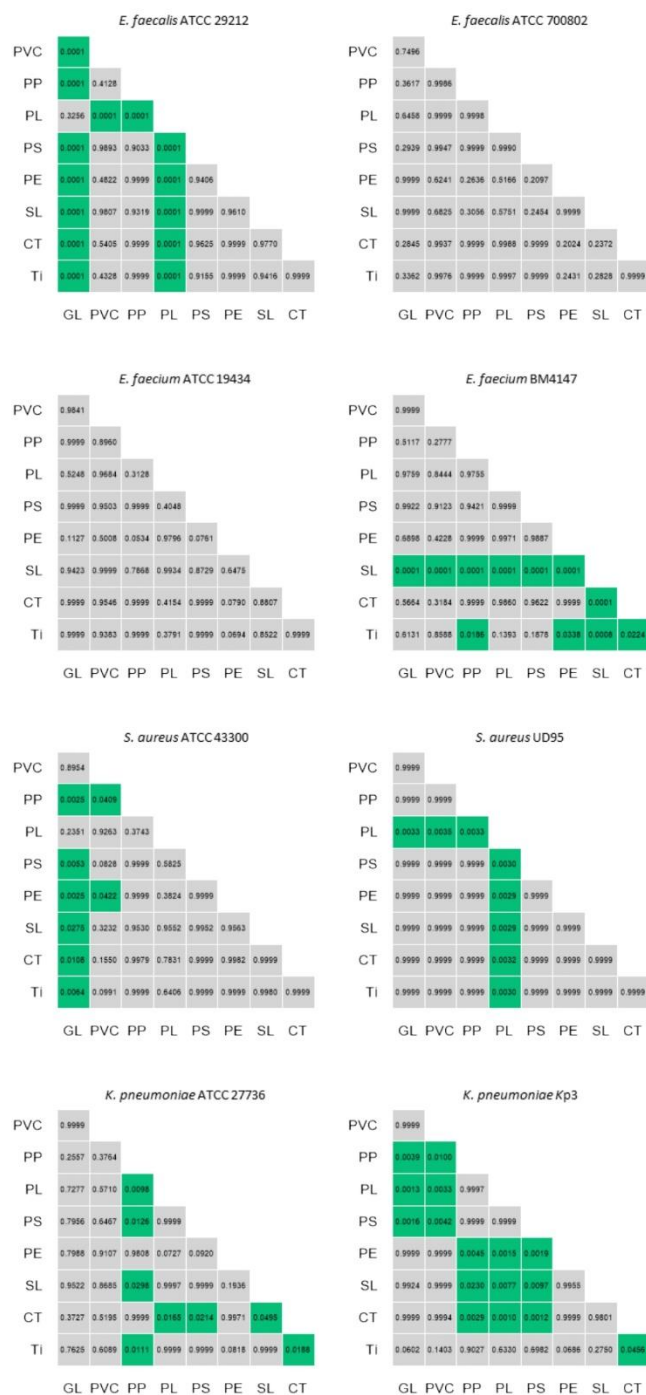


FIG S1 Resistance to desiccation and entrance into VBNC state after desiccation of ESKAPE bacteria. (A) Experimental timeline of the desiccation resistance assay. Colored arrows indicate the sampling times for CFU counts; cyan, after sample preparation in double-distilled water (before desiccation); red, after one-week desiccation; green, after resuscitation. **(B)** Bacterial strains were air-dried for one week on glass (GL), polyvinylchloride (PVC), polypropylene (PP), polyester (PL), polystyrene (PS), polyethylene (PE), silicon (SL), cotton (CT), and titanium (Ti). After desiccation, cells were suspended in RB and incubated at 37°C for 24 h with shaking. Cultivability was determined before desiccation, after one-week desiccation, and after resuscitation. The grey area indicates the limit of detection (LoD), corresponding to 4×10^2 CFU, with red and green segments representing CFU values below the LoD after desiccation and resuscitation, respectively. Data are the mean $\pm$ SD (error bars) of three independent experiments. Red asterisks indicate statistically significant differences between samples before and after desiccation for a given surface. Green asterisks indicate statistically significant differences between samples after desiccation on a given surface and after resuscitation. Statistically significant differences ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$) were determined using the unpaired *t*-test.



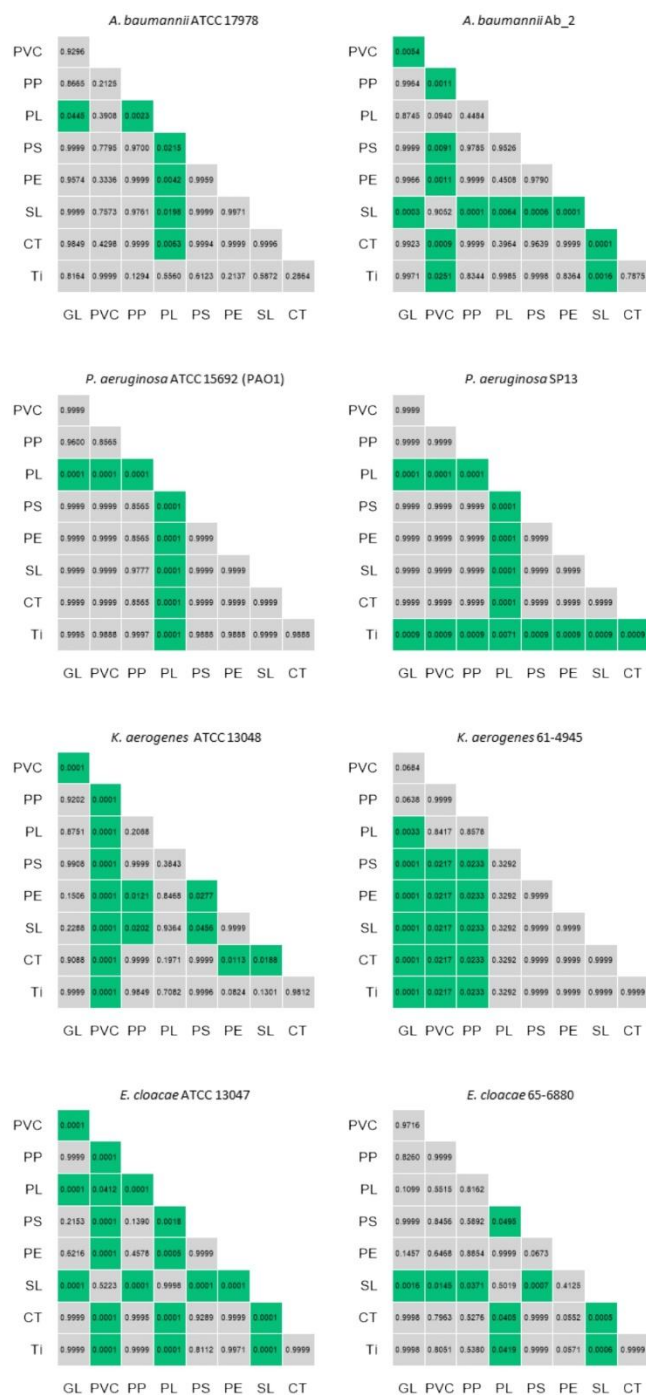


FIG S2. Statistical significance of the observed differences in bacterial cultivability after desiccation on different surfaces. One-way ANOVA followed by Tukey's HSD test was performed to compare the loss of cultivability after desiccation on the different surfaces, as illustrated by the red bars in Fig. S1. Numerical values of the post-hoc statistical comparisons are displayed; P values < 0.05 are highlighted in green, indicating statistically significant differences between surfaces in CFU counts of individual bacterial strains after desiccation.

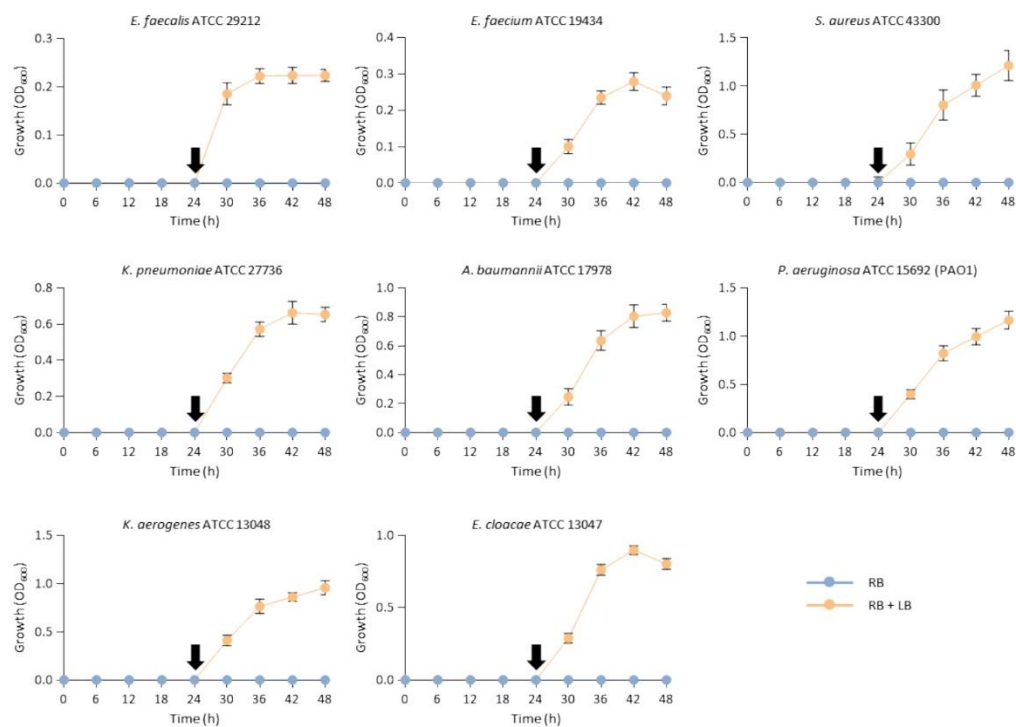
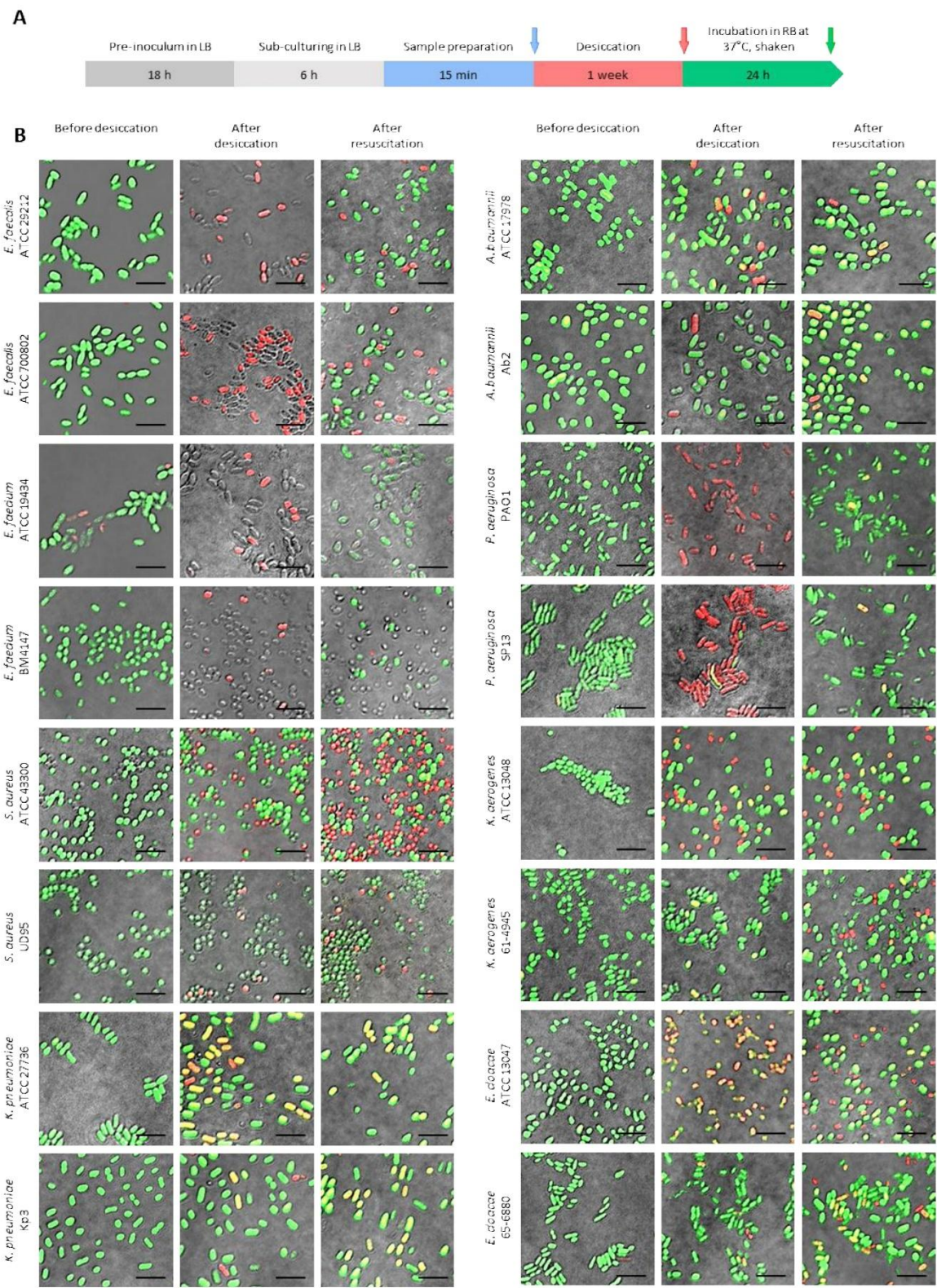


FIG S3 Growth of ESKAPE bacteria in RB supplemented or not with LB. The reference strains of ESKAPE bacteria were incubated at 37°C in RB (cyan) or in 2×RB supplemented, after 24 h, with an equal volume of 2×LB (orange). Black arrows indicate the time point at which 2×LB was added to 2×RB cultures. OD₆₀₀ was periodically measured for up to 48 h. Data are the mean ± SD (error bars) of three independent experiments.



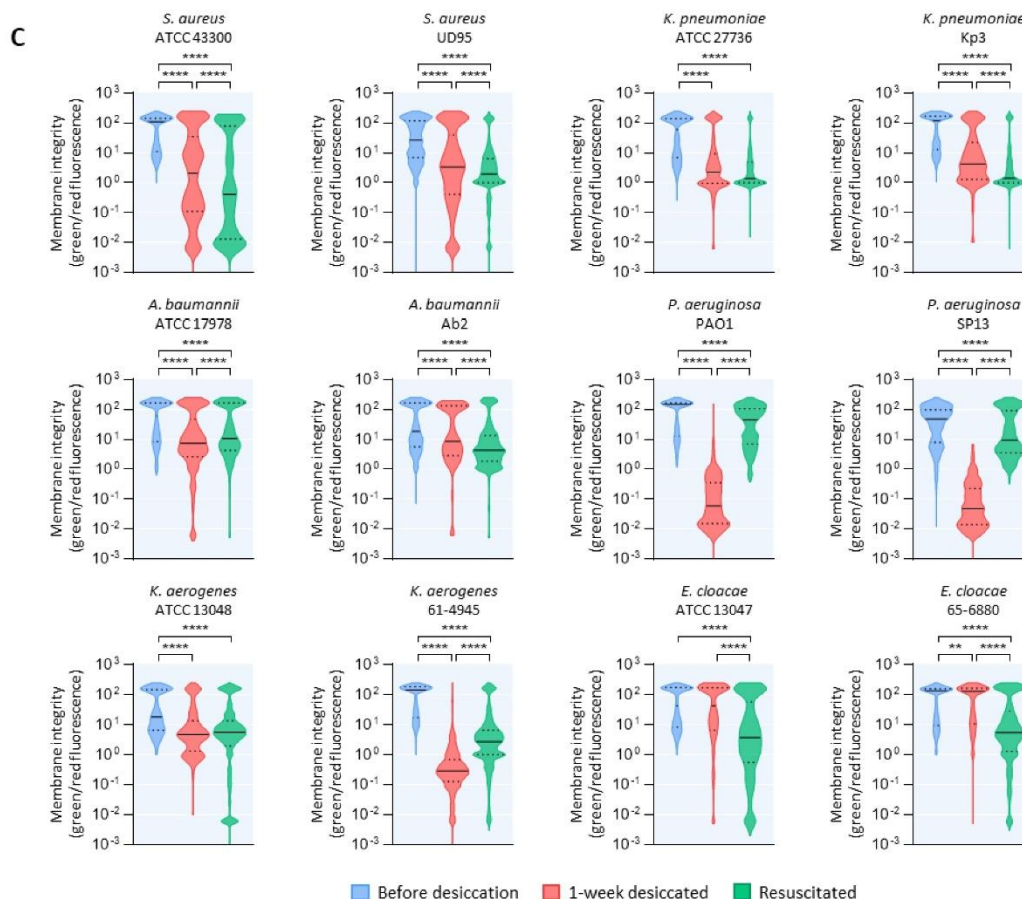
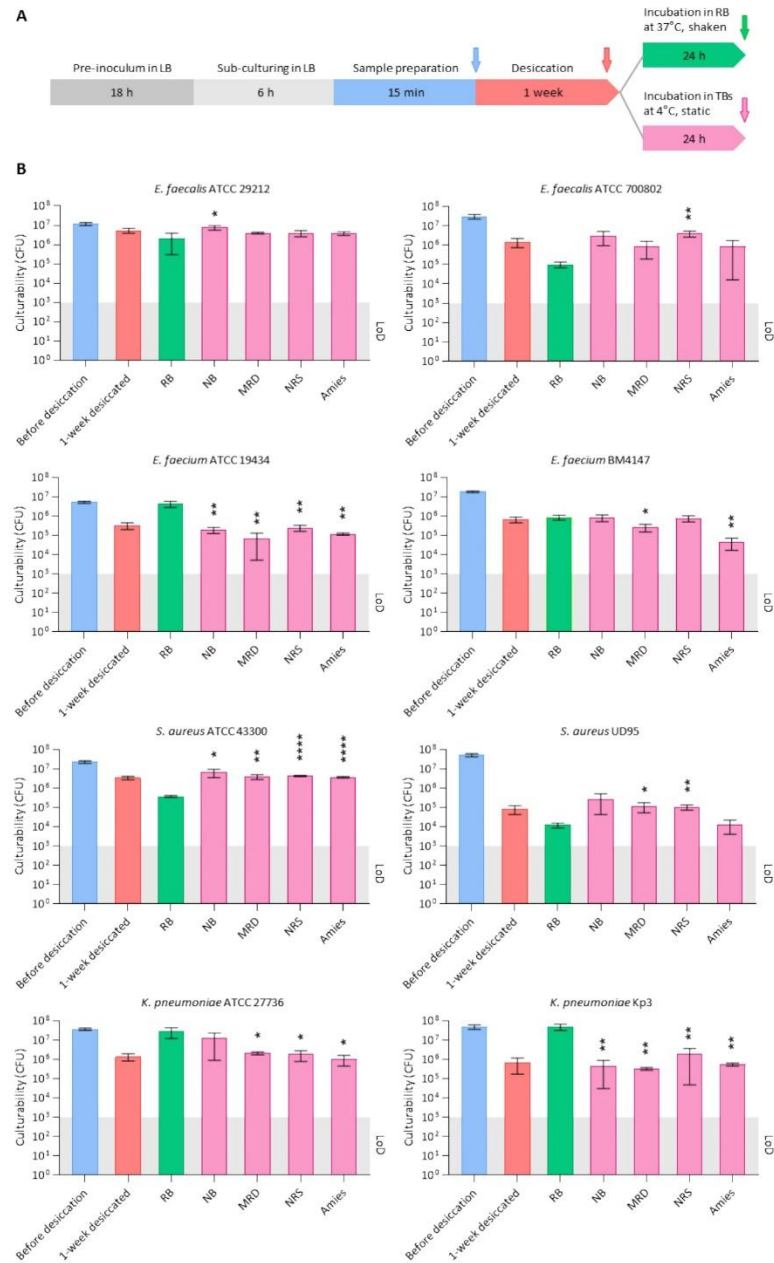


FIG S4 CLSM imaging and membrane integrity analysis of LIVE/DEAD-stained ESKAPE bacteria before and after desiccation, and after resuscitation. (A) Schematic of the experimental timeline. Colored arrows indicate the sampling times for LIVE/DEAD staining; cyan, after sample preparation in double-distilled water (before desiccation); red, after one week of desiccation on glass; green, after resuscitation. (B) Before desiccation, after desiccation, and after resuscitation, the bacterial suspensions were stained with 10 μ M SYTO 9 (green fluorescence) to label intact cells and 60 μ M PI (red fluorescence) to mark membrane-damaged cells. Stained bacterial cells were then examined using CLSM. Representative images display the merged channels of differential interference contrast (DIC), SYTO 9, and PI. Scale bar: 5 μ m. (C) From five CLSM images *per* sample, the green-to-red fluorescence ratio was calculated for each cell to quantitatively assess membrane integrity ($n > 350$ cells *per* sample). The resulting violin plots display the distribution of membrane integrity before desiccation (cyan), after desiccation (red), and following resuscitation (green). The median (filled lines) and interquartile ranges (dashed lines) are shown. Statistical significance was determined by the unpaired *t*-test (** $P < 0.01$; **** $P < 0.0001$).



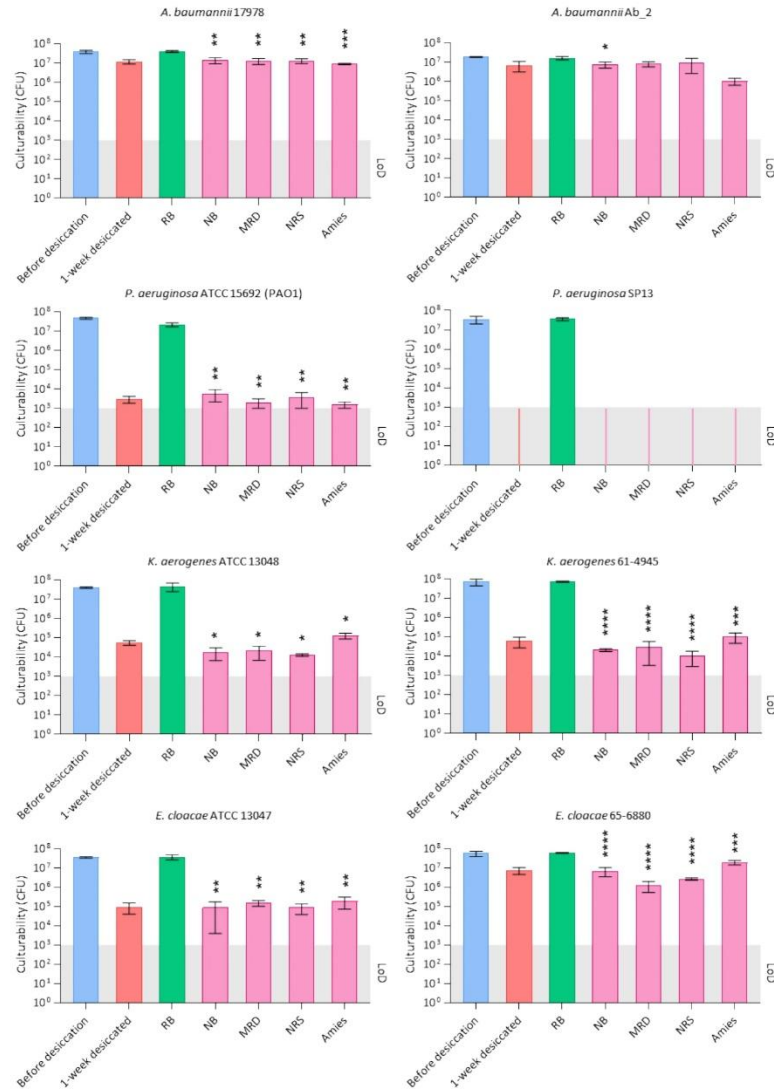


FIG S5. Comparison of the resuscitation procedure with the standard techniques used for environmental contamination control. (A) Experimental timeline. Bacterial strains were air-dried for one week on glass. After desiccation, cells were collected using a swab soaked in RB or different commercial swabs used for environmental contamination control. The swabs in RB were incubated at 37°C for 24 h with shaking (resuscitation) while commercial swabs were placed in their respective transport buffer and incubated at 4°C for 24 h in static conditions. Colored arrows indicate the sampling times for CFU counts: cyan, after sample preparation in double-distilled water (before desiccation); red, after one week of desiccation; green, after resuscitation in RB, and pink after incubation of commercial swabs at 4°C for 24 h in static. (B) Cultivability of cells after sample preparation in double-distilled water, after one-week desiccation, after resuscitation in RB, and after incubation at 4°C in transport buffer. Transport buffers: Neutralizing Broth (NB), Maximum Recovery Diluent (MRD), Neutralizing Rinse Solution (NRS), and Amies. The grey area indicates the limit of detection (LoD), corresponding to 1×10^3 CFU, with red and yellow segments representing CFU values below the LoD after desiccation and resuscitation, respectively. Data are the mean $\pm$ SD (error bars) of three independent experiments. Asterisks indicate statistically significant differences between CFU counts after resuscitation in RB and after the incubation at 4°C in the TBs. Statistically significant differences (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$) were determined using the unpaired *t*-test.

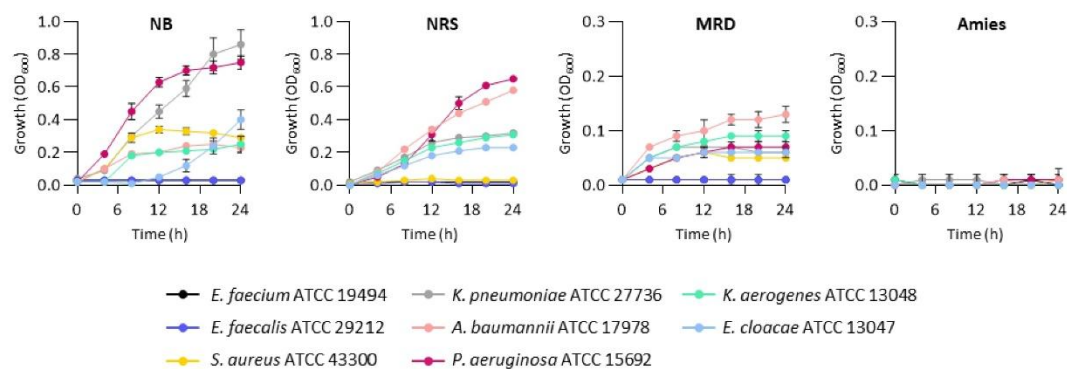


FIG S6. Growth profile of ESKAPE bacteria in different transport buffers. The reference strains of ESKAPE bacteria were inoculated at OD₆₀₀=0.001 in the indicated transport buffers and incubated at 37°C in static conditions. OD₆₀₀ was periodically measured for up to 24 h. Transport buffers: Neutralizing Broth (NB), Neutralizing Rinse Solution (NRS), Maximum Recovery Diluent (MRD), and Amies. Data are the mean $\pm$ SD (error bars) of three independent experiments.

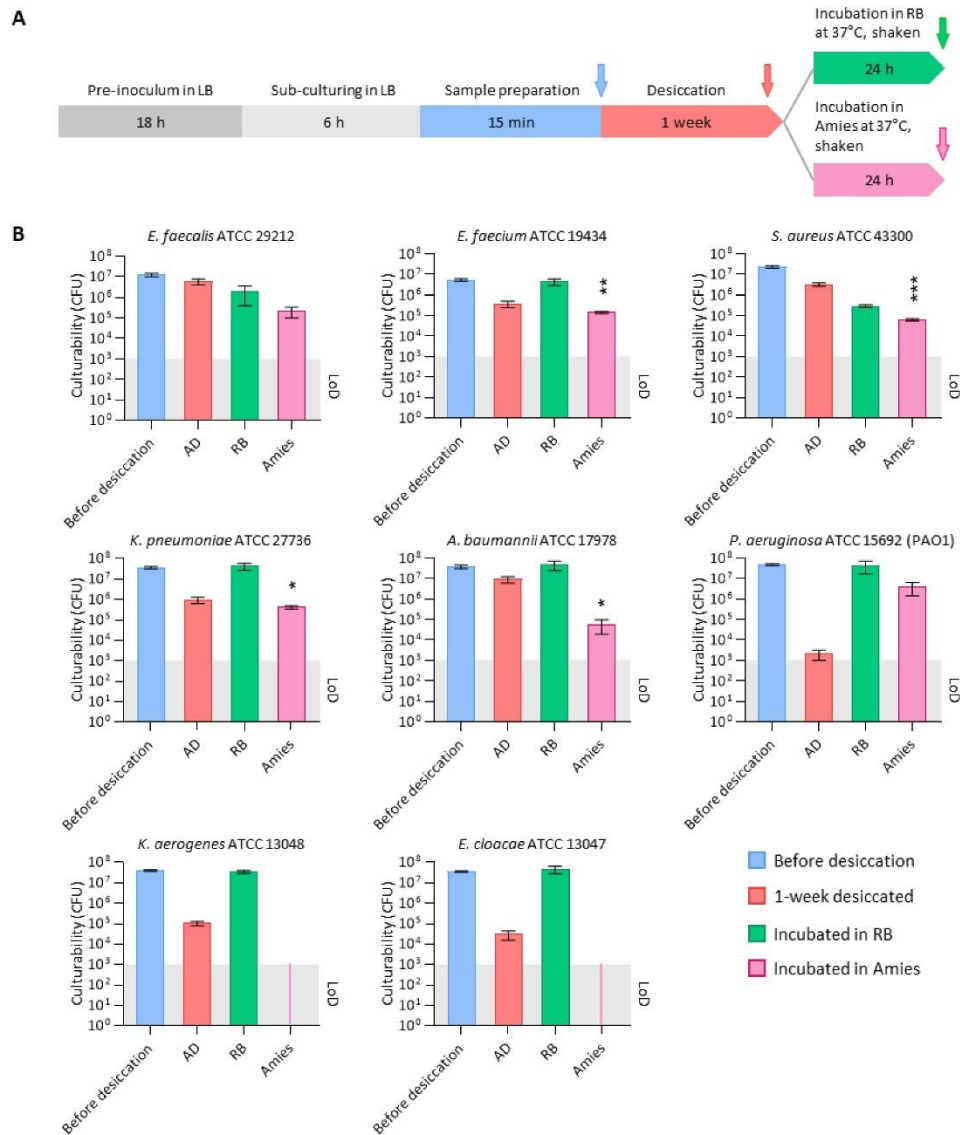


FIG S7. Resuscitation assay in RB and Amies solution. (A) Experimental timeline of the resuscitation assay in RB and in Amies. Bacterial strains were air-dried for one week on glass. After desiccation, cells were collected using a nylon swab soaked in RB or Amies and incubated at 37°C for 24 h with shaking (resuscitation). Colored arrows indicate the sampling times for CFU count: cyan, after sample preparation in double-distilled water; red, after one week of desiccation; green, after resuscitation in RB; pink, after resuscitation in Amies. (B) Cultivability of cells before desiccation (cyan), after one-week desiccation (AD, red), and after incubation at 37°C in the indicated buffers (RB, green; Amies, pink). The grey area indicates the limit of detection (LoD), corresponding to 1×10^3 CFU, with pink segments representing CFU values below the LoD after resuscitation. Data are the mean $\pm$ SD (error bars) of three independent experiments. Asterisks indicate statistically significant differences between CFU counts after resuscitation in RB and Amies. Statistically significant differences (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$) were determined using the unpaired t -test

Script for quantification of bacterial membrane integrity

```
// Check if a ROI exists
if (!isOpen("ROI Manager")) {
    exit("Open the ROI Manager and add a multipoint ROI.");
}

roiManager("select", 0); // Select the multipoint ROI
getSelectionCoordinates(x, y); // Get all the points from the ROI

n = x.length;

setResult("X", 0, "X");
setResult("Y", 0, "Y");
setResult("Red", 0, "Red");
setResult("Green", 0, "Green");

for (i = 0; i < n; i++) {
    px = getPixel(x[i], y[i]);
    red = (px & 0xff0000) >> 16;
    green = (px & 0x00ff00) >> 8;
    blue = (px & 0x0000ff);

    setResult("X", i+1, x[i]);
    setResult("Y", i+1, y[i]);
    setResult("Red", i+1, red);
    setResult("Green", i+1, green);
}

updateResults();
```


Protocol

Surface sampling for the detection of Gram-negative bacteria in VBNC state

Materials

Equipment and consumables

- Nylon swab (Copan) immersed in 2 mL RB
- Sampling templates (Copan), 10×10 cm (100 cm²) and/or 5×4 cm (20 cm²)
- Micropipette (20-200 µL) or plastic disposable graduated transfer Pasteur pipette (100 µL)
- Sterile disposable spatula
- TSA (Tryptic Soy Agar) plate
- Incubator set at 37°C with shaking
- Laminar flow hood or biosafety cabinet

Resuscitation Buffer (RB)

RB is the M9 basal salts solution (7 g/L Na₂HPO₄, 3 g/L KH₂PO₄, 0.5 g/L NaCl, 1 g/L NH₄Cl) supplemented with 0.02 g/L of CaCl₂ and 0.12 g/L MgSO₄ (Lucidi et al., 2025). Dispense 2 mL of RB to each swab-containing tube under sterile conditions (operate in a laminar flow hood or biosafety cabinet).

Method

1. Selection of sampling area

Select the specified area of the surface to be examined and define the area using the sampling template (100 cm² or 20 cm², depending on the suspected level of contamination). The selected area should be representative of the sampled surface. For non-flat surfaces and surfaces not defined by a numerical size, the sampled area must be clearly described.

2. Sampling

2.1 Place the tube containing the swab in an upright position and ensure that the RB inside is at the bottom of the tube (if necessary, shake the tube downward to collect all the liquid at the bottom).

2.2 Press the tip of the swab against the inner wall of the tube to remove excess RB; the swab should be moist and evenly soaked with RB.

2.3 Place the sampling template (either 100 cm² or 20 cm²) on the surface to be sampled.

2.4 Place the swab tip on the surface and sample the defined area (e.g., ≤100 cm²) while rotating the swab between thumb and forefinger. For flat surfaces, the sampling should be performed

xviii

horizontally and vertically, *e.g.* 10 times in each direction. For small or hard-to-reach areas, ensure all parts, including crevices and gaps, are covered.

2.5 Return the swab to the sterile tube containing 2 mL of RB and close securely to keep it moist until analysis. Since RB contains no carbon source, samples do not require transport or storage at cold temperatures before analysis. Ensure that the transport or storage temperature is $\leq 37^{\circ}\text{C}$.

3. Resuscitation

Incubate the tube containing the swab immersed in RB at 37°C for 24 hours under shaking at 200 rpm.

4. Sample plating and incubation

4.1 Using a sterile pipette, transfer 100 μL of RB from point (3) onto a TSA plate and evenly spread the sample with a sterile spatula.

The volume of RB plated on the agar can be adjusted based on the operator's specific needs. For surfaces where high microbial contamination is expected, serial dilutions may be performed before plating. For surfaces where even the presence of a single bacterial cell may pose a risk, it is recommended to plate larger volumes.

Different culture media can also be used for plating according to the operator's needs. If the sampling is aimed at detecting a specific pathogen, selective media that allow isolation of the target bacterium can be used in addition to the TSA plate.

4.2 Incubate the plates at 37°C for up to 72 hours to allow colony formation.

The incubation temperature and time for the plates inoculated with RB can be adjusted depending on the bacterial species being selected.

5. Expression of results and calculation

5.1 Count the number of colonies (CFUs) on each plate.

5.2 Calculate the number of CFUs per sampled surface area (N_s) using the formula:

$$N_s = (N \times D) / A$$

Where:

- **N** is the number of CFUs counted on the Petri dish
- **A** is the sampled surface area in square centimeters (*e.g.* 100 cm^2 for the 10×10 cm template and 20 cm^2 for the 5×4 cm template)
- **D** is the inverse of the dilution plated (in case 100 μL of a total volume of 2 mL is plated, 1/20 of the sample is used, so $D = 20$).

References

- Alvear-Daza JJ, García-Barco A, Osorio-Vargas P, Gutiérrez-Zapata HM, Sanabria J, Rengifo-Herrera JA. Resistance and induction of viable but non culturable states (VBNC) during inactivation of *E. coli* and *Klebsiella pneumoniae* by addition of H₂O₂ to natural well water under simulated solar irradiation. *Water Res.* 2021 Jan 1;188:116499. doi: 10.1016/j.watres.2020.116499.
- Bai H, Zhao F, Li M, Qin L, Yu H, Lu L, Zhang T. Citric acid can force *Staphylococcus aureus* into viable but nonculturable state and its characteristics. *Int J Food Microbiol.* 2019 Sep 16;305:108254. doi: 10.1016/j.ijfoodmicro.2019.108254.
- Bascomb S, Lapage SP, Willcox WR, Curtis MA. Numerical classification of the tribe *Klebsiellae*. *J Gen Microbiol.* 1971 Jun;66(3):279-95. doi: 10.1099/00221287-66-3-279.
- Bédard E, Charron D, Lalancette C, Déziel E, Prévost M. Recovery of *Pseudomonas aeruginosa* cultivability following copper- and chlorine-induced stress. *FEMS Microbiol Lett.* 2014 Jul;356(2):226-34. doi: 10.1111/1574-6968.12494.
- Bonchi C, Frangipani E, Imperi F, Visca P. Pyoverdine and proteases affect the response of *Pseudomonas aeruginosa* to gallium in human serum. *Antimicrob Agents Chemother.* 2015 Sep;59(9):5641-6. doi: 10.1128/AAC.01097-15.
- Bugg TD, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry.* 1991 Oct 29;30(43):10408-15. doi: 10.1021/bi00107a007.
- Cheng S, Su R, Song L, Bai X, Yang H, Li Z, Li Z, Zhan X, Xia X, Lü X, Shi C. Citral and trans-cinnamaldehyde, two plant-derived antimicrobial agents can induce *Staphylococcus aureus* into VBNC state with different characteristics. *Food Microbiol.* 2023 Jun;112:104241. doi: 10.1016/j.fm.2023.104241.
- Dopp E, Richard J, Dwidjosiswojo Z, Simon A, Wingender J. Influence of the copper-induced viable but non-culturable state on the toxicity of *Pseudomonas aeruginosa* towards human bronchial epithelial cells in vitro. *Int J Hyg Environ Health.* 2017 Nov;220(8):1363-1369. doi: 10.1016/j.ijheh.2017.09.007.
- Gin KY, Goh SG. Modeling the effect of light and salinity on viable but non-culturable (VBNC) *Enterococcus*. *Water Res.* 2013 Jun 15;47(10):3315-28. doi: 10.1016/j.watres.2013.03.021.
- Heim S, Lleo M, Bonato B, Guzman CA, Canepari P. The viable but nonculturable state and starvation are different stress responses of *Enterococcus faecalis*, as determined by proteome analysis. *J Bacteriol.* 2002 Dec;184(23):6739-45. doi: 10.1128/JB.184.23.6739-6745.2002.
- Hijazi S, Visaggio D, Pirolo M, Frangipani E, Bernstein L, Visca P. Antimicrobial Activity of Gallium Compounds on ESKAPE Pathogens. *Front Cell Infect Microbiol.* 2018 Sep 10;8:316. doi: 10.3389/fcimb.2018.00316.
- Hormaeche E and Edwards PR. Proposal for the rejection of the generic name *Cloaca Castellani* and Chalmers, and proposal of *Enterobacter* as a generic name with designation of the type species and its type culture. *Int J Syst Evol Microbiol.* 1960. 10, 75-76. doi: 10.1099/0096266X-10-2-75

- Jiang Q, Li H, Wan K, Ye C, Yu X. Quantification and antibiotic resistance risk assessment of chlorination-residual viable/VBNC *Escherichia coli* and *Enterococcus* in on-site hospital wastewater treatment system. *Sci Total Environ*. 2023 May 10;872:162139. doi: 10.1016/j.scitotenv.2023.
- König P, Wilhelm A, Schaudinn C, Poehlein A, Daniel R, Widera M, Averhoff B, Müller V. The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *mBio*. 2023 Oct 31;14(5):e0213923. doi: 10.1128/mbio.02139-23.
- Kvich L, Fritz B, Crone S, Kragh KN, Kolpen M, Sønderholm M, Andersson M, Koch A, Jensen PØ, Bjarnsholt T. Oxygen Restriction Generates Difficult-to-Culture *P. aeruginosa*. *Front Microbiol*. 2019 Aug 29;10:1992. doi: 10.3389/fmicb.2019.01992.
- Li J, Liu C, Wang S, Mao X. *Staphylococcus aureus* enters viable-but-nonculturable state in response to chitooligosaccharide stress by altering metabolic pattern and transmembrane transport function. *Carbohydr Polym*. 2024 Apr 15;330:121772. doi: 10.1016/j.carbpol.2023.121772.
- Li Y, Huang TY, Mao Y, Chen Y, Shi F, Peng R, Chen J, Yuan L, Bai C, Chen L, Wang K, Liu J. Study on the Viable but Non-culturable (VBNC) State Formation of *Staphylococcus aureus* and Its Control in Food System. *Front Microbiol*. 2020 Nov 26;11:599739. doi: 10.3389/fmicb.2020.599739.
- Liao X, Liu D, Ding T. Nonthermal Plasma Induces the Viable-but-Nonculturable State in *Staphylococcus aureus* via Metabolic Suppression and the Oxidative Stress Response. *Appl Environ Microbiol*. 2020 Feb 18;86(5):e02216-19. doi: 10.1128/AEM.02216-19.
- Lleo M, Bonato B, Tafi MC, Caburlotto G, Benedetti D, Canepari P. Adhesion to medical device materials and biofilm formation capability of some species of enterococci in different physiological states. *FEMS Microbiol Lett*. 2007 Sep;274(2):232-7. doi: 10.1111/j.1574-6968.2007.00836.x.
- Lleò MM, Benedetti D, Tafi MC, Signoretto C, Canepari P. Inhibition of the resuscitation from the viable but non-culturable state in *Enterococcus faecalis*. *Environ Microbiol*. 2007 Sep;9(9):2313-20. doi: 10.1111/j.1462-2920.2007.01345.x.
- Lleò MM, Bonato B, Benedetti D, Canepari P. Survival of enterococcal species in aquatic environments. *FEMS Microbiol Ecol*. 2005 Oct 1;54(2):189-96. doi: 10.1016/j.femsec.2005.03.016. PMID: 16332318.
- Lleò MM, Bonato B, Signoretto C, Canepari P. Vancomycin resistance is maintained in enterococci in the viable but nonculturable state and after division is resumed. *Antimicrob Agents Chemother*. 2003 Mar;47(3):1154-6. doi: 10.1128/AAC.47.3.1154-1156.2003.
- Lleò MM, Bonato B, Tafi MC, Signoretto C, Boaretti M, Canepari P. Resuscitation rate in different enterococcal species in the viable but non-culturable state. *J Appl Microbiol*. 2001 Dec;91(6):1095-102. doi: 10.1046/j.1365-2672.2001.01476.x.
- Lucidi M, Capecchi G, Spagnoli C, Basile A, Artuso I, Persichetti L, Fardelli E, Capellini G, Visaggio D, Imperi F, Rampioni G, Leoni L, Visca P. The response to desiccation in *Acinetobacter baumannii*. *Virulence*. 2025 Dec;16(1):2490209. doi: 10.1080/21505594.2025.2490209.
- Mangiaterra G, Cedrarò N, Vaia sicca S, Citterio B, Frangipani E, Biavasco F, Vignaroli C. Involvement of Acquired Tobramycin Resistance in the Shift to the Viable but Non-Culturable State in *Pseudomonas aeruginosa*. *Int J Mol Sci*. 2023 Jul 18;24(14):11618. doi: 10.3390/ijms241411618.
- Mangiaterra G, Cedrarò N, Vaia sicca S, Citterio B, Galeazzi R, Laudadio E, Mobbili G, Minnelli C, Bizzaro D, Biavasco F. Role of Tobramycin in the Induction and Maintenance of Viable but Non-Culturable *Pseudomonas aeruginosa* in an In Vitro Biofilm Model. *Antibiotics (Basel)*. 2020 Jul 10;9(7):399. doi: 10.3390/antibiotics9070399.

- Pasquaroli S, Citterio B, Cesare AD, Amiri M, Manti A, Vuotto C, Biavasco F. Role of daptomycin in the induction and persistence of the viable but non-culturable state of *Staphylococcus aureus* biofilms. *Pathogens*. 2014 Sep 18;3(3):759-68. doi: 10.3390/pathogens3030759.
- Pasquaroli S, Zandri G, Vignaroli C, Vuotto C, Donelli G, Biavasco F. Antibiotic pressure can induce the viable but non-culturable state in *Staphylococcus aureus* growing in biofilms. *J Antimicrob Chemother*. 2013 Aug;68(8):1812-7. doi: 10.1093/jac/dkt086.
- Qi Z, Huang Z, Liu C. Metabolism differences of biofilm and planktonic *Pseudomonas aeruginosa* in viable but nonculturable state induced by chlorine stress. *Sci Total Environ*. 2022 May 15;821:153374. doi: 10.1016/j.scitotenv.2022.153374.
- Qi Z, Liu C. Metabolic characteristics and markers in viable but nonculturable state of *Pseudomonas aeruginosa* induced by chlorine stress. *Environ Res*. 2022 Nov;214(Pt 3):114111. doi: 10.1016/j.envres.2022.114111.
- Qi Z, Sun N, Liu C. Glyoxylate cycle maintains the metabolic homeostasis of *Pseudomonas aeruginosa* in viable but nonculturable state induced by chlorine stress. *Microbiol Res*. 2023 May;270:127341. doi: 10.1016/j.micres.2023.127341.
- Sahm DF, Kissinger J, Gilmore MS, Murray PR, Mulder R, Solliday J, Clarke B. In vitro susceptibility studies of vancomycin-resistant *Enterococcus faecalis*. *Antimicrob Agents Chemother*. 1989 Sep;33(9):1588-91. doi: 10.1128/AAC.33.9.1588.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual* (2nd ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y (1989)
- Schleifer, K. H. and Kilpper-Balz, R. Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the genus *Enterococcus* nom. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. Nov. *Int. J Syst Evol Microbiol*. 1984. 34, 31-34. <https://doi.org/10.1099/00207713-34-1-31>.
- Signoretto C, Burlacchini G, Pruzzo C, Canepari P. Persistence of *Enterococcus faecalis* in aquatic environments via surface interactions with copepods. *Appl Environ Microbiol*. 2005 May;71(5):2756-61. doi: 10.1128/AEM.71.5.2756-2761.2005.
- Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M, Snyder M. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev*. 2007 Mar 1;21(5):601-14. doi: 10.1101/gad.1510307.
- Villa L, Feudi C, Fortini D, García-Fernández A, Carattoli A. Genomics of KPC-producing *Klebsiella pneumoniae* sequence type 512 clone highlights the role of RamR and ribosomal S10 protein mutations in conferring tigecycline resistance. *Antimicrob Agents Chemother*. 2014;58(3):1707-12. doi: 10.1128/AAC.01803-13.
- Wang L, Ye C, Guo L, Chen C, Kong X, Chen Y, Shu L, Wang P, Yu X, Fang J. Assessment of the UV/Chlorine Process in the Disinfection of *Pseudomonas aeruginosa*: Efficiency and Mechanism. *Environ Sci Technol*. 2021 Jul 6;55(13):9221-9230. doi: 10.1021/acs.est.1c00645.
- Wang M, Ateia M, Hatano Y, Yoshimura C. Regrowth of *Escherichia coli* in environmental waters after chlorine disinfection: shifts in viability and cultivability. *Environ Sci (Camb)*. 2022 May 19;8(7):1521-1534. doi: 10.1039/d1ew00945a.
- Yadav M, Dhyani S, Joshi P, Awasthi S, Tanwar S, Gupta V, Rathore DK, Chaudhuri S. Formic acid, an organic acid food preservative, induces viable-but-non-culturable state, and triggers new

- Antimicrobial Resistance traits in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. *Front Microbiol.* 2022 Nov 24;13:966207. doi: 10.3389/fmicb.2022.966207.
- Yan H, Li M, Meng L, Zhao F. Formation of viable but nonculturable state of *Staphylococcus aureus* under frozen condition and its characteristics. *Int J Food Microbiol.* 2021 Nov 2;357:109381. doi: 10.1016/j.ijfoodmicro.2021.109381.
- Yang D, Jiang Z, Meng Q, Wang S, Pan H, Rao L, Liao X. Analyzing the pressure resistant, sublethal injury and resuscitable viable but non-culturable state population of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus amyloliquefaciens* and *Lactiplantibacillus plantarum* under high pressure processing. *Food Res Int.* 2023 Nov;173(Pt 1):113336. doi: 10.1016/j.foodres.2023.113336.
- Zhang B, Fu Y, Wang F, Jin P, Xu P, Li H, Xu X, Shen C. The risk of viable but non-culturable (VBNC) enterococci and antibiotic resistance transmission during simulated municipal sludge composting. *Waste Manag.* 2024 Jun 30;183:1-9. doi: 10.1016/j.wasman.2024.04.048.
- Zhang M, Wang X, Deng X, Zheng S, Zhang W, He JZ, Yu X, Feng M, Ye C. Viable but non-culturable state formation and resuscitation of different antibiotic-resistant *Escherichia coli* induced by UV/chlorine. *Water Res.* 2024 Sep 1;261:122011. doi: 10.1016/j.watres.2024.122011.7
- Zhang S, Ye C, Lin H, Lv L, Yu X. UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa*. *Environ Sci Technol.* 2015 Feb 3;49(3):1721-8. doi: 10.1021/es505211e.
- Zhao S, Dou C, Zhang J, Huang L, Gao Y, Du B, Cui X, Zhao H, Xue G, Ke Y, Gan L, Feng J, Feng Y, Cui J, Yan C, Xu Z, Fu T, Yu Z, Yang Y, Yuan J, You F. Multiple factors trigger the formation and resuscitation of the VBNC state in alcohol-producing *Klebsiella pneumoniae*. *Appl Environ Microbiol.* 2024 Jul 24;90(7):e0055724. doi: 10.1128/aem.00557-24.

Chapter 6. Improved method for quantitative detection of pathogenic microorganisms on abiotic surfaces

Daniela Visaggio^{1,2,3}, Massimiliano Lucidi^{1,2}, Cinzia Spagnoli¹, Francesco Imperi^{1,2,3}, Paolo Visca^{1,2,3}

¹Department of Science, Roma Tre University, Rome, Italy

²NBFC, National Biodiversity Future Center, Palermo, Italy

³Santa Lucia Foundation IRCCS, Rome, Italy

The patent was filed on June 19th, 2025, with the application number 102025000014596 at the Italian Patent Office (Ufficio Italiano Brevetti e Marchi).

Publication synopsis

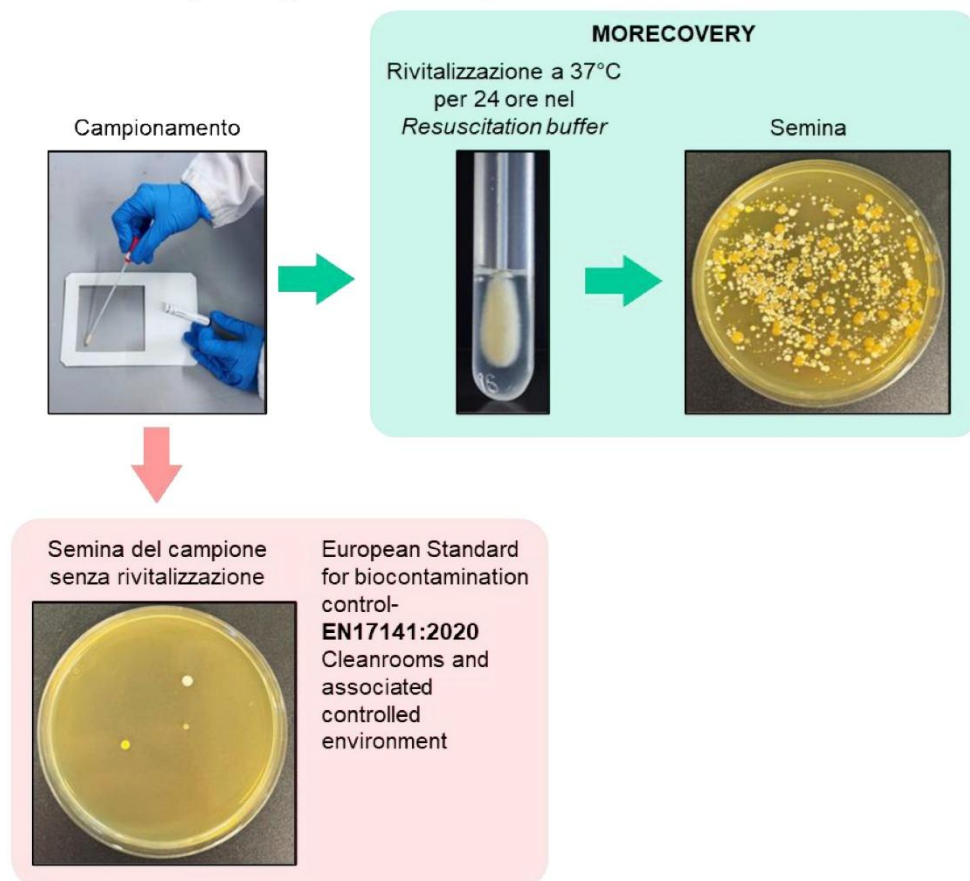
HAIs remain a major global health challenge, largely driven by multidrug-resistant pathogens that persist in hospital environments (Mitchell *et al.*, 2023; Macesic *et al.*, 2025). Hospital surfaces and medical devices act as critical reservoirs for microbial contamination, facilitating cross-transmission to patients through contact with healthcare personnel and equipment (Mitchell *et al.*, 2023). Effective environmental monitoring is therefore crucial for preventing infections. However, current protocols rely almost exclusively on culture-based methods, which fail to detect bacteria in the VBNC state (König *et al.*, 2023; Liu *et al.*, 2023). VBNC cells remain metabolically active yet unable to grow on conventional media, escaping routine surveillance and creating hidden reservoirs of infection risk. Current protocols, such as those described in EN 17141:2020 (Cleanrooms and associated controlled environments - Biocontamination control), rely almost exclusively on culture-based methods, which fail to detect bacteria in the VBNC state (van der Schoor *et al.*, 2022; Liu *et al.*, 2023). VBNC cells remain metabolically active but fail to grow on conventional culture media, thereby representing a hidden reservoir of infection that eludes routine surveillance. This limitation severely compromises the accuracy of standard environmental monitoring procedures, which only detect culturable cells. Consequently, the microbial burden on hospital surfaces is likely to be underestimated, hindering effective infection control strategies. Furthermore, previous studies have demonstrated that clinically relevant ESKAPE pathogens, except *Enterobacter* spp., can enter a VBNC state when exposed to environmental stresses, such as desiccation on abiotic surfaces (König *et al.*, 2023; Qi *et al.*, 2023; Zhao *et al.*, 2024; this thesis).

Evidence presented in earlier chapters confirmed this phenomenon in Gram-negative ESKAPE bacteria, highlighting the need for improved detection strategies. To overcome these limitations, a novel procedure called MORECOVERY was developed and patented.

This chapter outlines the rationale, development, and validation of the MORECOVERY method, incorporating a resuscitation step to restore culturability prior to plating. Comparative analyses with conventional protocols demonstrated that the inclusion of a resuscitation step significantly enhances the detection of Gram-negative ESKAPE pathogens after desiccation. By addressing a critical gap in current monitoring practices, MORECOVERY represents an innovative tool for infection prevention, with potential applications extending beyond healthcare, including food safety and sterility monitoring in pharmaceutical environments.

Invenzione **MORECOVERY**

**Nuovo metodo per la quantificazione di microrganismi
patogeni su superfici abiotiche**



INVENTORI:

Francesco Imperi
Massimiliano Lucidi
Cinzia Spagnoli
Daniela Visaggio
Paolo Visca



Al Magnifico Rettore
dell'Università degli Studi Roma Tre

RICHIESTA DI BREVETTAZIONE

A. TITOLO DELL'INVENZIONE

Ideare un breve titolo che descriva la natura dell'invenzione senza rivelare dettagli specifici che potrebbero consentire di riprodurre l'invenzione (ad esempio: nuovo composto anticancro, metodo per la fabbricazione di chip etc.) Limite di 60 caratteri

MORECOVERY

Nuovo metodo per la quantificazione di microrganismi patogeni su superfici abiotiche
Improved method for quantitative detection of pathogenic microorganisms on abiotic surfaces

B. INVENTORI APPARTENENTI AL PERSONALE DELL'ATENEO

Lista di tutto il personale dell'Ateneo che ha intellettualmente contribuito all'invenzione. Indicare altre collaborazioni o partecipazioni al progetto di ricerca di altre Istituzioni esterne. Nella parte "incarico nel progetto" indicare il ruolo che hanno ricoperto i partecipanti al progetto (ad esempio: inventore, co-inventore etc.)

Nominativo: Francesco Imperi	Qualifica: Professore Associato	Incarico nel progetto: co-inventore
Dipartimento: di Scienze	Indirizzo di lavoro: Viale G. Marconi 446	
Tel: 3409384385	Fax:	E-mail: francesco.imperi@uniroma3.it

Nominative: Massimiliano Lucidi	Qualifica: Ricercatore (RTDA)	Incarico nel progetto: co-inventore
Dipartimento: di Scienze	Indirizzo di lavoro: Viale G. Marconi 446	
Tel: 3450116470	Fax:	E-mail: massimiliano.lucidi@uniroma3.it

Nominative: Cinzia Spagnoli	Qualifica: Dottoranda	Incarico nel progetto: co-inventore
Dipartimento: di Scienze	Indirizzo di lavoro: Viale G. Marconi 446	
Tel: 3312785970	Fax:	E-mail: cinzia.spagnoli@uniroma3.it

Nominativo: Daniela Visaggio	Qualifica: Ricercatore (RTDA)	Incarico nel progetto: inventore
Dipartimento: Di Scienze	Indirizzo di lavoro: Viale G. Marconi 446	
Tel: 34028760806	Fax:	E-mail: daniela.visaggio@uniroma3.it

Nominativo: Paolo Visca	Qualifica: Professore Ordinario	Incarico nel progetto: co-inventore
Dipartimento: Di Scienze	Indirizzo di lavoro: Viale G. Marconi 446	
Tel: 3290570955	Fax:	E-mail: paolo.visca@uniroma3.it

Grado di Proprietà Intellettuale

Inventore	Proprietà intellettuale (in percentuale)
Francesco Imperi	10%
Massimiliano Lucidi	15%
Cinzia Spagnoli	10%
Daniela Visaggio	35%
Paolo Visca	30%

C. INVENTORI NON APPARTENENTI AL PERSONALE DELL'ATENEO*(in caso di co-inventore non appartenente al personale dell'Ateneo)*

Nominativo:	Qualifica:	Incarico nel progetto:
Tel:	Fax:	E-mail:
Descrivere brevemente il contributo del co-inventore		

Nominativo:	Qualifica:	Incarico nel progetto:
Tel:	Fax:	E-mail:

Descrivere brevemente il contributo del co-inventore:

D. TIPOLOGIA DI PROGETTO DAL QUALE È NATA L'INVENZIONE

Indicare la tipologia di progetto da cui è nata l'invenzione (ad esempio: CNR, EU, Ricerca Autonoma, Contratti di ricerca con partners privati, etc.)

TIPOLOGIA DI PROGETTO	PARTNERS DEL PROGETTO	CONTRIBUTO
Ricerca autonoma		

E. FONDI UTILIZZATI PER IL PROGETTO DAL QUALE È NATA L'INVENZIONE

Segnalare qualsiasi soggetto terzo che abbia contribuito con finanziamenti (senza la sottoscrizione di contratti di ricerca) o scambiando materiale soggetto a protezione della proprietà intellettuale

RICERCATORE	SPONSOR	CONTRIBUTO

F. ACCORDI, TRASFERIMENTI DI DOCUMENTAZIONE ED EVENTUALE CORRISPONDENZA

Segnalare ed allegare, se disponibili, copie della documentazione riguardante il progetto, eventualmente scambiata con enti e/o soggetti terzi.

/

G. DESCRIZIONE DEGLI EVENTI:

Queste informazioni sono importanti per capire la priorità dell'invenzione; spesso la **divulgazione di un'invenzione, resa accessibile ad un numero indeterminato di persone, è distruttiva del requisito della novità. Si ha divulgazione, ad esempio, quando l'invenzione è descritta nelle pubblicazioni scientifiche, nelle conferenze pubbliche, nelle esposizioni ufficiali, dissertazioni di tesi di laurea e/o di dottorato. Quindi condizione necessaria affinché un'invenzione sia brevettabile è che non sia stata divulgata in data anteriore al deposito della domanda di brevetto. Risulta importante evidenziare nell'area "riferimenti a documenti scritti/orali", soprattutto nei punti 4 e 5, quali informazioni siano state rese note, per valutare, in modo obiettivo, se il materiale diffuso sia sufficiente affinché un esperto del settore sia in grado di attuare l'invenzione in maniera completa.**

EVENTI	DATA	RIFERIMENTI A DOCUMENTI SCRITTI E/ORALI
1. Iniziale ideazione dell'invenzione	03/02/2025	Relazione del II anno di Dottorato della dottoranda Cinzia Spagnoli XVIII ciclo Le informazioni salienti relative all'invenzione sono state criptate.
2. Prima descrizione completa dell'invenzione		

3. Prima dimostrazione pratica di successo			
4. Il lavoro sarà:			
sottomesso alla pubblicazione? (S/N)	N		
accettato per la pubblicazione? (S/N)	N		
pubblicato?	N		
5. Se il lavoro è stato presentato ad una conferenza:			
È stato sottomesso un abstract? (S/N)	S	27/06/2024	Presentazione al convegno Cortona Procaroti 2024 Le informazioni salienti relative all'invenzione sono state criptate.
È stato pubblicato l'abstract? (S/N)	N		
La pubblicazione prevedeva una brochure? (S/N)	N		

H. DESCRIZIONE DELL'INVENZIONE

Descrivere sommariamente il funzionamento e il dispositivo dell'invenzione. Considerando che queste informazioni saranno usate per determinare la brevettabilità dell'invenzione, valutarne il potenziale e contattare potenziali clienti, è opportuno che tali informazioni siano il più possibile dettagliate. Se si è in possesso di un documento esemplificativo dell'invenzione, si chiede di compilare in modo sintetico gli spazi seguenti e di allegare una copia della relazione in proprio possesso al presente form.

1. In che cosa consiste esattamente l'invenzione?

Stato dell'arte

Le infezioni causate da batteri multiresistenti agli antibiotici sono una grave minaccia per la salute pubblica a livello globale. Negli USA, i batteri resistenti agli antimicrobici causano oltre 2,8 milioni di infezioni ogni anno, con un bilancio di oltre 35.000 decessi. Nell'Unione Europea, i batteri resistenti agli antimicrobici causano più di 670.000 infezioni e circa 33.000 decessi all'anno (Center for Disease Control, 2019; European Centre for Disease Prevention and Control, 2020).

Il fenomeno dell'antimicrobico-resistenza è particolarmente rilevante in ambito ospedaliero, dove il 71% delle infezioni correlate all'assistenza sanitaria (hospital-associated infections, HAI) è attribuibile a batteri resistenti agli antibiotici (<https://www.ecdc.europa.eu/en/healthcare-associated-infections>). Le infezioni nosocomiali da batteri multiresistenti sono una delle principali cause di morbidità e mortalità tra i pazienti ospedalizzati, oltre a rappresentare un ingente onere economico e gestionale per i sistemi sanitari. Negli Stati Uniti, il costo annuo per il trattamento di queste infezioni supera i 4,6 miliardi di dollari (Nelson et al., 2021). Tra le infezioni a maggiore impatto economico negli USA vi sono quelle causate da *Enterobacter spp.*, *Pseudomonas aeruginosa* e *Acinetobacter baumannii* resistenti agli antibiotici amikacina o imipenem, con un costo medio annuo stimato per paziente di 56.500 dollari (Nelson et al., 2021).

In Italia, il trattamento delle HAI dovute a batteri resistenti comporta un aumento significativo dei costi sanitari, con un incremento medio del 53,4%, variabile tra 3.744 € e 5.744 € per paziente (Orlando et al., 2024).

La pan-resistenza agli antibiotici è in costante crescita tra i batteri a circolazione ospedaliera (Karakonstantis et al., 2020) e le infezioni sostenute da questi batteri sono incurabili con le terapie antibiotiche convenzionali, ciò comportando gravissimi costi in termini di spesa sanitaria e vite umane

(Macesic et al., 2025).

Il contrasto alla diffusione dei microrganismi multiresistenti necessita l'adozione di strategie integrate che, oltre a ridurre l'uso inappropriato degli antibiotici, migliorino la sanificazione negli ambienti sanitari, minimizzando così la presenza di patogeni ospedalieri trasmissibili ai pazienti suscettibili (Mitchell et al., 2019; Peters et al., 2022; Leistner et al., 2023). Le superfici ospedaliere e i dispositivi medici sono ampiamente riconosciuti come serbatoi critici per la trasmissione di agenti patogeni e giocano un ruolo chiave nella contaminazione crociata tra pazienti (Mitchell et al., 2023). Numerosi studi hanno dimostrato che una sanificazione efficace degli ambienti ospedalieri è cruciale per la riduzione delle HAI (Otter, 2019; Mitchell et al., 2019; Peters et al., 2022; Leistner et al., 2023).

Tra gli elementi essenziali a garantire l'efficacia delle pratiche di sanificazione è il monitoraggio ambientale sistematico, finalizzato a valutare la presenza di microrganismi, in particolare di patogeni trasmissibili multiresistenti. Tuttavia, in Italia non esiste una normativa che imponga specifici obblighi per il campionamento ambientale routinario negli ospedali, che viene solitamente effettuato solo in risposta a focolai infettivi endemici o epidemici (van der Schoor et al., 2022).

Un'ulteriore criticità nel monitoraggio ambientale riguarda le metodologie impiegate per la rilevazione della contaminazione microbica. La European Committee for Standardization (CEN) ha recentemente introdotto il documento EN 17141:2020, che definisce le linee guida per il controllo della biocontaminazione in camere bianche e ambienti controllati associati (Cleanrooms and associated controlled environments – Biocontamination control).

Secondo quanto descritto nel documento EN 17141:2020 il campionamento ambientale nelle strutture sanitarie può essere effettuato mediante diversi dispositivi quali tamponi, spugne, piastre di contatto, e *strips* con terreno di coltura. Tuttavia, tali metodologie consentono di individuare esclusivamente i microrganismi in grado di replicarsi e crescere in terreni culturali subito dopo il campionamento. Tuttavia, alcune specie batteriche possono entrare in uno stato che, seppur vitale, non è coltivabile nei normali terreni culturali (stato denominato *viable but not culturable*, VBNC), risultando quindi non rilevabili con i tradizionali metodi di campionamento ambientale. Pertanto, le tradizionali metodiche di campionamento ambientale forniscono una sottostima della contaminazione microbica presente. È stato dimostrato che le specie batteriche comunemente associate alle infezioni ospedaliere come *A. baumannii*, *P. aeruginosa*, *Klebsiella pneumoniae* sono in grado di entrare nello stato di VBNC quando esposte a stress ambientali, quali ad esempio l'essiccamento su superfici abiotiche (Konig et al., 2023; Ni et al., 2025; Qi et al., 2023; Zhao et al., 2024). In particolare, studi condotti nel laboratorio di microbiologia dell'Università di Roma Tre hanno dimostrato che *A. baumannii* perde la coltivabilità in seguito all'essiccamento su superfici abiotiche entrando in uno stato VBNC e può tornare coltivabile se reidratato in una opportuna soluzione tampone o in fluidi biologici come saliva, urine e siero. Di conseguenza, quando *A. baumannii* si trova nello stato VBNC sulle superfici ospedaliere, non può essere rilevato dalle metodiche di monitoraggio ambientale tradizionali. Tuttavia, a seguito del contatto con l'ospite, può riacquistare la capacità di replicarsi e dare origine a un'infezione.

Descrizione dell'invenzione

La presente invenzione consiste in un metodo innovativo che migliora la rilevazione e quantificazione di batteri patogeni contaminanti superfici abiotiche anche quando essi si trovano nello stato di VBNC. La presente proposta di brevetto si fonda su solide evidenze ottenute attraverso rigorosi esperimenti di laboratorio, che ne confermano l'efficacia e la validità.

In particolare, sono state selezionate 5 specie batteriche Gram-negative responsabili delle più frequenti infezioni correlate all'assistenza sanitaria, note per il loro elevato livello di resistenza (inclusa la pan-resistenza) agli antibiotici, segnatamente *A. baumannii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *K. pneumoniae* e *P. aeruginosa* (Macesic et al., 2025). Per ciascuna specie batterica è stato selezionato il ceppo di riferimento e un ceppo clinico. In primo luogo, è stato valutato se i ceppi appartenenti alle suddette specie fossero in grado di entrare in uno stato di VBNC qualora sottoposti a essiccamento su comuni superfici abiotiche ospedaliere (plastiche, vetro, cotone e titanio). I batteri sono stati precoltivati in Luria Bertani medium (LB) per 18 ore a 37°C, successivamente è stato fatto un rinfresco della coltura in LB (3 ml in provette da 15 ml), che è stata poi incubata per ulteriori 6 ore a 37°C in agitazione. La sospensione batterica è stata quindi lavata per due volte in acqua bidistillata sterile e portata ad una assorbanza a 600nm pari 1, corrispondente a circa 10⁸ Unità Formanti Colonia (UFC)/ml. Una aliquota di 20 µl contenente circa 2 × 10⁵ cellule batteriche è stata depositata su superfici sterili e piane di 1 cm x 1 cm dei seguenti materiali: vetro (GL), cloruro di polivinile (PVC), polipropilene (PP), poliestere (PL), polistirene (PS), polietilene (PE), silicone (SL), cotone (CT) e titanio (Ti). Le diverse superfici su cui sono stati depositi i batteri sono state poste sotto cappa a flusso laminare a 25,2±1,5 °C per consentire l'evaporazione dell'acqua e conservate per 1 settimana in una campana da vuoto da 16 L contenente 50

g di gel di silice, a una temperatura media di $20,9 \pm 0,6$ °C e un'umidità relativa del $13,00 \pm 5,6\%$. Dopo una settimana, ciascuna superficie è stata posta in provette da 50 ml contenete 2 ml di una soluzione tampone di rivitalizzazione, da noi sviluppato e denominato *resuscitation buffer* (RB).

La composizione dell'RB è 6.78 g/L Na_2HPO_4 , 3 g/L KH_2PO_4 , 0.5 g/L NaCl, 1 g/L NH_4Cl , CaCl_2 0.02 g/L, MgSO_4 0.12 g/L. L'assenza di una fonte di carbonio nell'RB non consente la crescita dei microrganismi. Il numero di unità formanti colonia (UFC) è stato definito sia dopo 15 minuti che dopo 24 ore a 37°C in agitazione a 180 rpm (Figura 1).

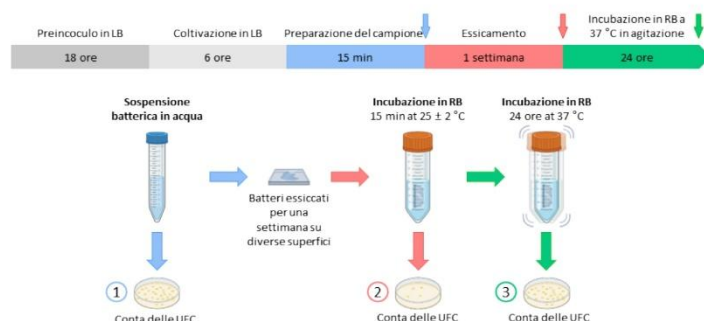
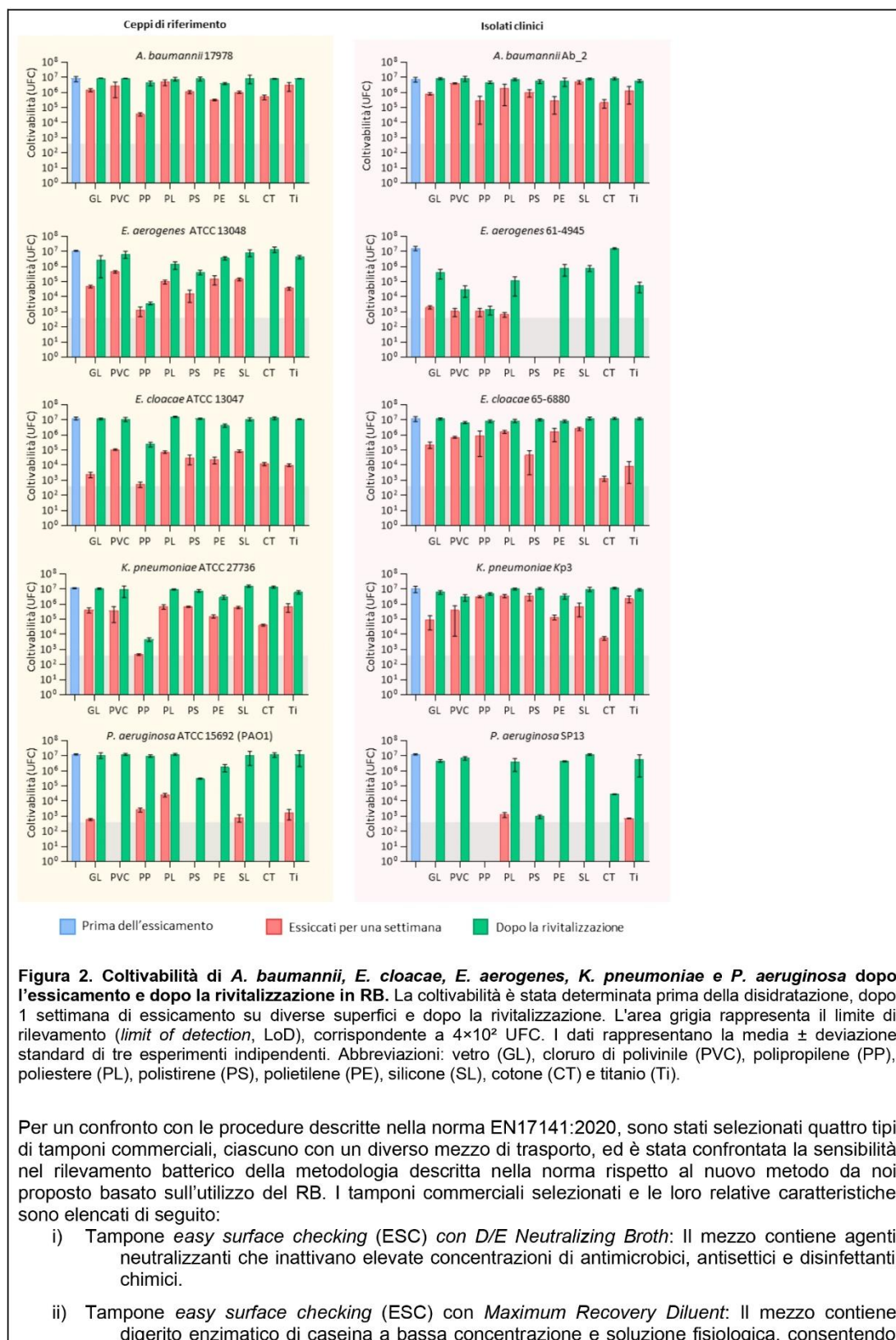


Figura 1. Procedura sperimentale per valutare la resistenza all'essiccamento dei batteri di interesse e di recupero della coltivabilità dopo l'incubazione in RB. Un'aliquota di 20 µl di sospensione batterica con densità ottica a 600 nm pari a 1, preparata in acqua bidistillata sterile, è stata serialmente diluita in base 10 in soluzione fisiologica (NaCl 9 g/L) sterile per definire il numero delle unità formanti colonia (1). Ulteriori aliquote da 20 µl in acqua sono state essiccate su diverse superfici. Dopo una settimana, le diverse superfici su cui sono state essiccate le cellule batteriche sono state incubate in RB, e la capacità dei batteri di replicarsi è stata valutata mediante conteggio delle UFC, sia dopo 15 minuti a 25 ± 2 °C (fase di essiccamento; 2) sia dopo 24 ore a 37°C in agitazione (fase di rivitalizzazione; 3).

L'essiccamento per una settimana sulle diverse superfici determina una drastica riduzione del numero di UFC rispetto al numero di cellule batteriche poste inizialmente sulle diverse superfici. Infatti, da circa il 60% al 100% delle cellule esposte a essiccamento diveniva non coltivabile su un terreno di coltura standard (LB agar o LA), come mostrato in Figura 2.

Tuttavia, una incubazione per 24 ore a 37°C in RB aumentava considerevolmente o ripristinava completamente la coltivabilità dei batteri di interesse (intesa come numero di UFC) sul terreno di coltura standard (LA). Tale risultato conferma che *A. baumannii*, *E. cloacae*, *E. aerogenes*, *K. pneumoniae* e *P. aeruginosa* possono entrare in uno stato di VBNC su diverse superfici abiotiche in seguito a disidratazione e che la loro successiva reidratazione nel RB li rende coltivabili e pertanto rilevabili con metodi colturali standard. Al fine di determinare se la metodica da noi proposta che si basa sull'utilizzo del RB sia più accurata nella quantificazione dei microrganismi su superfici abiotiche rispetto ai metodi tradizionali, le due procedure sono state messe sperimentalmente a confronto. In particolare, il nostro metodo di rivitalizzazione è stato confrontato con le indicazioni descritte nella norma EN17141:2020, relativa al controllo del biocontaminazione nelle camere bianche e ambienti controllati. Secondo la succitata norma, dopo il campionamento della superficie mediante tampone inumidito con *buffer* di trasporto, i campioni devono essere trasportati e tenuti a una temperatura compresa tra 1 e 8 °C e analizzati entro 24 ore. In caso contrario, i campioni devono essere conservati a 3 ± 2 °C per un massimo di 48 ore dopo il campionamento.



la rilevazione anche di cellule batteriche stressate o danneggiate.

- iii) Tampone *easy surface checking* (ESC) con *Neutralizing Rinse Solutions*: Il mezzo contiene neutralizzanti dei disinfettanti e fonti di carbonio a concentrazioni inferiori rispetto al tampone ESC con D/E Neutralizing broth.
- iv) Tampone *liquid medium swab* (LMS) con Amies: Il buffer è utilizzato per il campionamento e la conservazione di batteri aerobi e anaerobi a temperatura ambiente o refrigerata e non contiene alcuna fonte di carbonio.

La composizione dei diversi *buffer* dei tamponi commerciali selezionati è descritta in Tabella 1.

Tabella 1. Elenco dei tamponi e composizione dei mezzi di trasporto

Tampone	Abbreviazione	Materiale del tampone	Composizione del mezzo (g/L)	Distributore
ESC Swab D/E Neutralizing Broth ^a	NB	Rayon	Digerito enzimatico di caseina (5.00) Estratto di lievito (2.50) Destrosio (10.00) Tioglicolato di sodio (1.00) Na ₂ S ₂ O ₃ (6.00) NaHSO ₃ (2.50) Lecitina; (7.00) Polisorbato 80; (5.00) Porpora di bromocresolo (0.02)	Liofilchem
ESC Swab Maximum Recovery Diluent ^b	MRD	Rayon	Digerito enzimatico di caseina (1.00) NaCl (8.50)	Liofilchem
ESC Swab Neutralizing Rinse Solutions ^c	NRS	Rayon	Peptone di caseina (1.00) Istidina (1.00) Lecitina (2.00) Tween 80 (30.00) Na ₂ S ₂ O ₃ (5.00) NaCl (8.50)	Liofilchem
LMS Swab Amies ^d	Amies	Poliuretano	Na ₂ HPO ₄ (1.15) KH ₂ PO ₄ (0.20) NaCl (3.00) KCl (0.20) CaCl ₂ (0.10) MgCl ₂ (0.10) C ₂ H ₃ NaO ₂ S (1.00)	Heinz Herenz Medizinalbedarf GmbH

In particolare, le cellule batteriche preparate come precedentemente descritto sono state risospese in acqua bidistillata sterile a OD₆₀₀=1. Un'aliquota di 50 µL delle sospensioni batteriche è stata posta su una superficie di vetro di cm 1x1, lasciata essiccare sotto cappa a flusso laminare e incubata in una campana da vuoto da 16 L (20,9±0,6 °C e 13,00±5,6% di umidità) per una settimana. Le cellule batteriche sono state prelevate dal vetrino utilizzando diversi tamponi commerciali e un tampone in nylon (FloQ swab, Copan) pre-imbevuto di RB. Tutti i tamponi sono stati incubati nei rispettivi *buffer* per 15 minuti a temperatura ambiente, e le sospensioni batteriche sono state diluite serialmente (fattore 10) in soluzione fisiologica sterile per determinare le UFC, con lo scopo di definire la coltivabilità dopo una settimana di essiccamento.

Successivamente, i tamponi di nylon con l'RB sono stati incubati a 37°C in agitazione mentre i tamponi commerciali a 4°C in condizioni statiche per 24 ore. È stato quindi eseguito un ulteriore conteggio delle UFC per valutare la coltivabilità batterica.

Il numero delle UFC determinata dopo una settimana di essiccamento e semina dopo 15 minuti dalla raccolta del campione era comparabile, senza differenze significative. Differenze significative tra i diversi metodi di rilevamento si osservavano invece dopo 24 ore. In particolare, il numero di UFC rilevato con i tamponi commerciali dopo 24 ore di incubazione a 4°C era simile a quello rilevato dopo una settimana di essiccamento. Al contrario, il numero rilevato dopo l'incubazione in RB a 37°C in agitazione tornava ad essere simile al numero di cellule batteriche poste sul vetrino prima dell'essiccamento.

Da questo esperimento appare evidente come le procedure comunemente utilizzate per il monitoraggio dei batteri sulle superfici non siano in grado di quantificare con precisione il numero dei batteri presenti a causa della capacità di questi di entrare in uno stato di VBNC perdendo quindi la capacità di crescere su terreno solido (Figura 3).

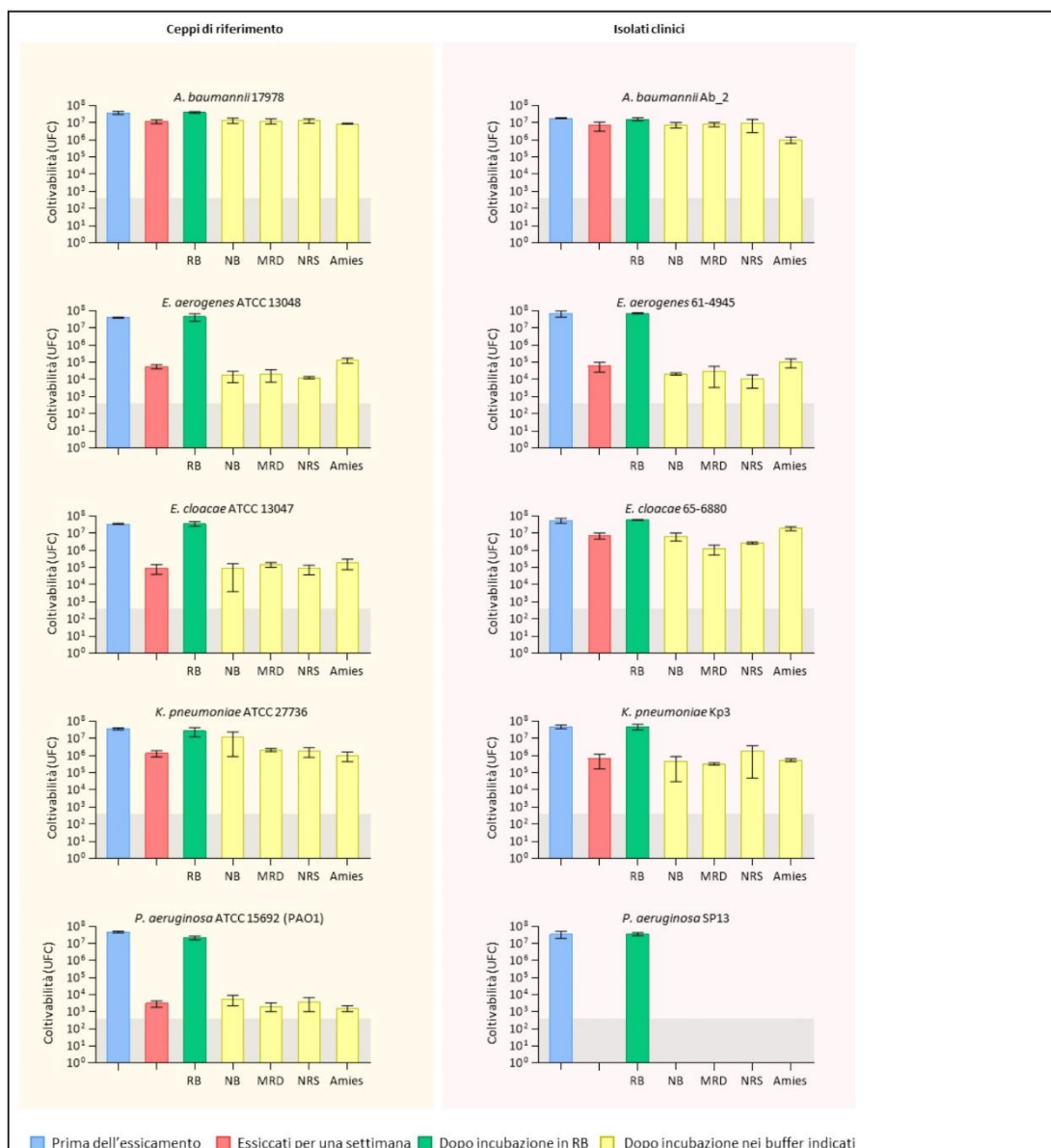


Figura 3. Confronto delle procedure standard e del metodo MORECOVERY per il controllo della contaminazione ambientale. Gli istogrammi mostrano la coltivabilità (UFC) delle cellule appartenenti a diverse specie e ceppi batterici dopo la preparazione del campione in acqua bidistillata sterile, dopo una settimana di essiccamento su vetro, dopo la rivitalizzazione in RB a 37°C per 24 ore oppure dopo l'incubazione a 4°C nei diversi buffer di trasporto (TB) per 24 ore. TB: Neutralizing Broth (NB), Maximum Recovery Diluent (MRD), Neutralizing Rinse Solution (NRS) e Amies. L'area grigia indica il limite di rilevamento (*limit of detection*, LoD), corrispondente a 4×10^2 UFC. I dati sono espressi come media $\pm$ deviazione standard di tre esperimenti indipendenti.

Al fine di comprendere se le differenze osservate fossero imputabili al materiale di cui è costituito il tampone, dalla composizione del mezzo di trasporto o dalla incubazione a 37°C è stato condotto un esperimento in cui i ceppi di riferimento per ciascuna specie batterica sono stati essiccati per una settimana su vetrino, come sopra descritto, e sono stati poi campionati con tamponi di nylon imbevuti in RB o Amies ed entrambi sono stati incubati nei suddetti mezzi a 37°C per 24 ore. Gli altri tamponi commerciali non sono stati utilizzati per questo esperimento dal momento che contengono una fonte di carbonio che determinerebbe crescita batterica a 37°C per 24 ore (Figura 4), inficiando la quantificazione del numero di cellule batteriche effettivamente presenti sulla superficie appena campionata.

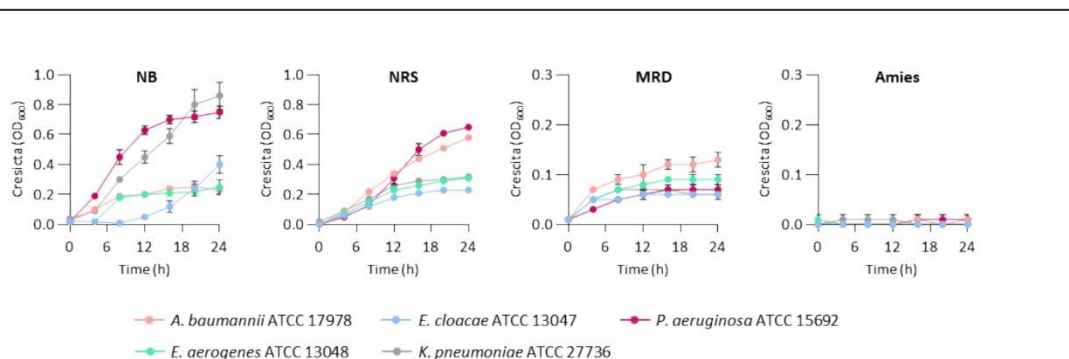


Figura 4. Profilo di crescita dei batteri appartenenti alle specie d'interesse in diversi tamponi di trasporto. I ceppi indicati sono stati inoculati a $OD_{600}=0,001$ nei diversi TB e incubati a 37°C . L' OD_{600} è stato misurato periodicamente fino a 24 ore. TB: Neutralizing Broth (NB), Maximum Recovery Diluent (MRD), Neutralizing Rinse Solution (NRS) e Amies. I dati sono espressi come media $\pm$ deviazione standard di tre esperimenti indipendenti.

Come si osserva in Figura 5, l'incubazione in Amies a 37°C per 24 ore determinava un aumento del numero di UFC rilevate, per *P. aeruginosa*, *A. baumannii* e *K. pneumoniae*, ma non il ripristino completo della coltivabilità, mentre nel caso di *Enterobacter spp.* l'incubazione in Amies a 37°C ne riduceva ulteriormente la coltivabilità. Come atteso, l'incubazione nel RB ripristinava completamente la coltivabilità di tutte le specie batteriche saggiate. I risultati ottenuti hanno dimostrato che la composizione dell'RB è cruciale per il processo di rivitalizzazione dei batteri.

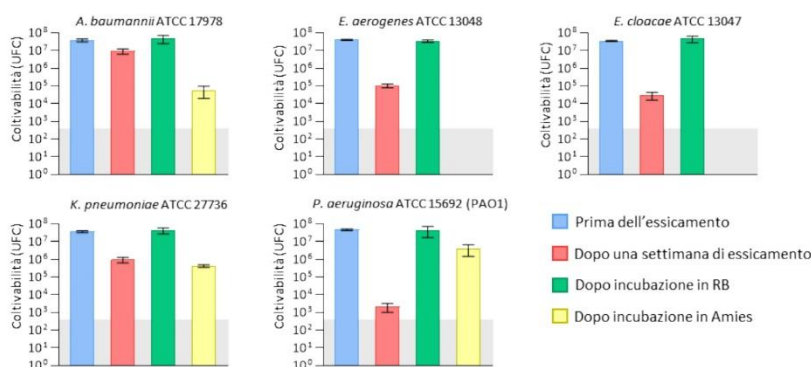


Figura 5. Rivitalizzazione delle cellule batteriche in RB e Amies. Coltivabilità delle cellule dopo la preparazione del campione in acqua bidistillata sterile, dopo una settimana di essiccamento su vetro e dopo la incubazione in RB o in Amies a 37°C per 24 ore. L'area grigia indica il limite di rilevamento (*limit of detection*, LoD), corrispondente a 4×10^2 UFC. I dati sono espressi come media $\pm$ deviazione standard di tre esperimenti indipendenti.

Alla luce dei risultati ottenuti, proponiamo un sistema di rilevamento basato sul processo di rivitalizzazione mediante l'utilizzo di RB, denominato MORECOVERY (da *more recovery*, a significare maggiore recupero).

In particolare, proponiamo un kit per il monitoraggio ambientale composto da:

1. Maschere di campionamento di due diverse dimensioni per il campionamento di superfici abiotiche;
2. Tampone in nylon con RB nel tubo di raccolta del tampone;
3. Piastre per la semina dei microrganismi (il kit di base contiene una piastra di Petri contenente il terreno ricco Tryptic Soy Agar [TSA], ma si potrebbero utilizzare anche piastre selettive per l'isolamento di patogeni specifici);
4. Pipetta Pasteur monouso sterile
5. Spatola sterile monouso
6. Protocollo con la procedura da eseguire per il monitoraggio ambientale.

L'allegato 1 contiene il protocollo dettagliato e le foto del kit.

Riferimenti bibliografici

Centeghe I, Norville P, Hughes L, Maillard JY. *Klebsiella pneumoniae* survives on surfaces as a dry biofilm. *Am J Infect Control*. 2023 Oct;51(10):1157-1162. doi: 10.1016/j.ajic.2023.02.009.

Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2019 [EB/OL]. Available at: <https://www.cdc.gov/drugresistance/biggest-threats.html> [access date June 30, 2023].

European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022—2020 data [EB/OL]. Available at: <https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data> [access date June 30, 2023].

Karakonstantis S, Kritsotakis EI, Gikas A. Pandrug-resistant Gram-negative bacteria: a systematic review of current epidemiology, prognosis and treatment options. *J Antimicrob Chemother*. 2020 Feb 1;75(2):271-282. doi: 10.1093/jac/dkz401. PMID: 31586417.

König P, Wilhelm A, Schaudinn C, Poehlein A, Daniel R, Widera M, Aeverhoff B, Müller V. The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *mBio*. 2023 Oct 31;14(5):e0213923. doi: 10.1128/mbio.02139-23.

Leistner R, Kohlmorgen B, Brodzinski A, Schwab F, Lemke E, Zakonsky G, Gastmeier P. Environmental cleaning to prevent hospital-acquired infections on non-intensive care units: a pragmatic, single-centre, cluster randomized controlled, crossover trial comparing soap-based, disinfection and probiotic cleaning. *EClinicalMedicine*. 2023 Apr 6;59:101958. doi: 10.1016/j.eclinm.2023.101958.

Macesic N, Uhlemann AC, Peleg AY. Multidrug-resistant Gram-negative bacterial infections. *Lancet*. 2025 Jan 18;405(10474):257-272. doi: 10.1016/S0140-6736(24)02081-6.

Mitchell BG, Hall L, White N, Barnett AG, Halton K, Paterson DL, Riley TV, Gardner A, Page K, Farrington A, Gericke CA, Graves N. An environmental cleaning bundle and health-care-associated infections in hospitals (REACH): a multicentre, randomised trial. *Lancet Infect Dis*. 2019 Apr;19(4):410-418. doi: 10.1016/S1473-3099(18)30714-X.

Mitchell BG, McDonagh J, Dancer SJ, Ford S, Sim J, Thottiyil Sultanmuhammed Abdul Khadar B, Russo PL, Maillard JY, Rawson H, Browne K, Kiernan M. Risk of organism acquisition from prior room occupants: An updated systematic review. *Infect Dis Health*. 2023 Nov;28(4):290-297. doi: 10.1016/j.idh.2023.06.001.

Nelson RE, Hatfield KM, Wolford H, Samore MH, Scott RD, Reddy SC, Olubajo B, Paul P, Jernigan JA, Baggs J. National Estimates of Healthcare Costs Associated With Multidrug-Resistant Bacterial Infections Among Hospitalized Patients in the United States. *Clin Infect Dis*. 2021 Jan 29;72(Suppl 1):S17-S26. doi: 10.1093/cid/ciaa1581.

Ni X, Yan C, Guo B, Han Z, Cui C. Occurrence, predictive models and potential health risk assessment of viable but non-culturable (VBNC) pathogens in drinking water. *Environ Pollut*. 2025 Mar 1;368:125794. doi: 10.1016/j.envpol.2025.125794.

Orlando S, Cicala M, De Santo C, Mosconi C, Ciccacci F, Guarente L, Carestia M, Liotta G, Di Giovanni D, Buonomo E, Riccardi F, Palombi L, Emberti Gialloreti L. The financial burden of healthcare-associated infections: a propensity score analysis in an Italian healthcare setting. *Infect Prev Pract*. 2024 Oct 10;7(1):100406. doi: 10.1016/j.infpip.2024.100406.

Otter JA. Can cleaning REACH further in reducing hospital infections? *Lancet Infect Dis*. 2019 Apr;19(4):345-347. doi: 10.1016/S1473-3099(18)30795-3.

Peters A, Schmid MN, Parneix P, Lebowitz D, de Kraker M, Sauser J, Zingg W, Pittet D. Impact of environmental hygiene interventions on healthcare-associated infections and patient colonization: a systematic review. *Antimicrob Resist Infect Control*. 2022 Feb 19;11(1):38. doi: 10.1186/s13756-022-01075-1.

Qi Z, Sun N, Liu C. Glyoxylate cycle maintains the metabolic homeostasis of *Pseudomonas aeruginosa* in viable but nonculturable state induced by chlorine stress. *Microbiol Res*. 2023 May;270:127341. doi: 10.1016/j.micres.2023.127341.

van der Schoor AS, Boyle M, Voor In 't Holt AF, Vos MC, Humphreys H; ESCMID Study Group for

Nosocomial Infections. Environmental sampling of innate hospital surfaces: a survey of current practices and the need for guidelines. J Hosp Infect. 2022 Oct;128:92-95. doi: 10.1016/j.jhin.2022.07.024.

Zhao S, Dou C, Zhang J, Huang L, Gao Y, Du B, Cui X, Zhao H, Xue G, Ke Y, Gan L, Feng J, Feng Y, Cui J, Yan C, Xu Z, Fu T, Yu Z, Yang Y, Yuan J, You F. Multiple factors trigger the formation and resuscitation of the VBNC state in alcohol-producing *Klebsiella pneumoniae*. Appl Environ Microbiol. 2024 Jul 24;90(7):e0055724. doi: 10.1128/aem.00557-24.

Possibili rivendicazioni del brevetto:

Con la presente richiesta di brevetto intendiamo proteggere:

- il metodo da noi sviluppato e la composizione del kit MORECOVERY per la rilevazione di microrganismi su ogni tipo di superficie;
- qualunque altro metodo di rilevamento in ambito sanitario, alimentare, farmaceutico, industriale e zootecnico che si basi sul protocollo di MORECOVERY compreso l'utilizzo dell'RB e incubazione del tampone a 37°C;
- l'utilizzo della procedura MORECOVERY per la rilevazione di microrganismi appartenenti a specie batteriche e fungine diverse da quelle indicate nella presente richiesta di brevetto.

Applicazione industriale:

Il potenziale mercato di MORECOVERY è da ricercare in ambito sanitario, anche se il suo uso è di sicuro interesse anche per i settori alimentare, farmaceutico, industriale e zootecnico.

Le HAI costituiscono il 71% dei casi di infezioni da batteri resistenti agli antibiotici, compresi i batteri resistenti agli antibiotici di ultima linea, come gli *Enterobacterales* resistenti ai carbapenemi. Tali patogeni, insieme a *A. baumannii* resistente ai carbapenemi e gli *Enterobacterales* resistenti alle cefalosporine di terza generazione sono stati posti nel 2024 dall'Organizzazione Mondiale della Sanità (OMS) in cima alla lista dei patogeni che rappresentano un problema sanitario a livello globale (<https://www.who.int/publications/i/item/9789240093461>). *P. aeruginosa* è un altro patogeno che l'OMS ha definito critico e che costituisce il principale responsabile delle infezioni polmonari in pazienti con fibrosi cistica. Per prevenire la diffusione di infezioni ospedaliere causate da patogeni multi-o pan-resistenti è essenziale che vengano messe in atto misure preventive e di controllo, incluse il monitoraggio della contaminazione batterica sulle superfici ospedaliere. Infatti, lo European Centre for Disease Prevention and Control (ECDC) ha stimato che fino al 50% delle infezioni correlate all'assistenza sanitaria possa essere prevenuto grazie all'adozione di adeguate misure di prevenzione e controllo delle infezioni negli ambienti sanitari (<https://www.ecdc.europa.eu/en/healthcare-associated-infections>). Tuttavia, le attuali misure di controllo della contaminazione batterica non tengono conto, della capacità di alcuni microrganismi di entrare in uno stato di VBNC e, pertanto, non consentono una accurata determinazione della presenza di microrganismi nell'ambiente campionato. Il kit MORECOVERY rappresenta una innovazione nell'ambito delle metodiche di campionamento e di controllo della diffusione dei principali patogeni in ambito clinico. Pertanto, tutte le aziende che forniscono dispositivi per il campionamento e il monitoraggio dei microrganismi nell'ambiente potrebbero essere potenzialmente interessate allo sviluppo e alla commercializzazione della presente invenzione. Inoltre, dal momento che MORECOVERY può essere applicato a qualsiasi ambito in cui sia necessaria una valutazione accurata della contaminazione microbica, la nostra invenzione potrebbe riscuotere interesse non solo in ambito sanitario ma anche in ambito agroalimentare, farmaceutico e industriale.

Limitazioni (tecniche, commerciali, legislative, ambientali):

Una possibile limitazione della nostra invenzione è che l'RB potrebbe non essere efficace nel rendere nuovamente coltivabili VBNC di microrganismi diversi da quelli analizzati.

2. Quali sono le componenti innovative ed uniche e in cosa si differenziano dall'attuale stato dell'arte?

Indicare le componenti frutto di una reale attività creativa e non di applicazioni di principi già noti. Questa parte permette di comprendere meglio gli aspetti innovativi dell'invenzione.

Miglioramenti e vantaggi rispetto alle tecnologie attuali o alternative:

Sebbene sul mercato siano presenti diversi kit e strumenti per il monitoraggio ambientale, nessuno di questi è in grado di rilevare i batteri quando questi si trovano in uno stato di VBNC. Molti microrganismi, inclusi i batteri Gram negativi di interesse clinico, come *A. baumannii*, *Enterobacter spp.*, *K. pneumoniae*, e *P. aeruginosa*, sono in grado di entrare in uno stato di VBNC in seguito alla esposizione a diversi tipi di

stress, incluso quello da essiccamento su superfici abiotiche. L'entrata nello stato di VBNC fa sì che questi batteri non possano essere rilevati con i metodi tradizionali che si basano tutti sulla coltivazione e che non prevedono la rivitalizzazione. Tuttavia, dal momento che lo stato di VBNC è uno stato transiente indotto da stress (ad esempio, l'essiccamento), quando i batteri quiescenti e non coltivabili si ritrovano in condizioni favorevoli possono tornare nuovamente a moltiplicarsi, come ad esempio quando entrano nell'organismo ospite. Pertanto, il vantaggio del metodo proposto consiste nella capacità di rilevare in maniera accurata i batteri Gram-negativi principalmente responsabili delle infezioni in ambito ospedaliero anche quando essi si trovano nello stato di VBNC. Al contrario i metodi tradizionali non sono in grado di rilevare i batteri nello stato di VBNC e pertanto determinano una sottostima o il mancato rilevamento del numero di batteri effettivamente presenti sulle superfici. Inoltre, le procedure attuali prevedono il trasporto dei campioni a una temperatura controllata compresa tra 1 e 8 °C mentre MORECOVERY non richiede il trasporto dei campioni a temperatura controllata ed è pertanto di più facile utilizzo per l'operatore che svolge il campionamento.

La nostra proposta rappresenta un'innovazione nel settore del monitoraggio ambientale, in quanto introduce un kit completo per il campionamento. Attualmente, sul mercato non sono disponibili soluzioni integrate. Le diverse componenti necessarie, come maschere di campionamento, piastre per la semina e tamponi, vengono vendute singolarmente e spesso da aziende diverse, rendendo l'acquisizione del materiale complessa e dispendiosa in termini di tempo. Il kit MORECOVERY, invece, fornisce in un'unica soluzione tutti gli strumenti necessari, semplificando il processo di campionamento e garantendo maggiore efficienza, riproducibilità e praticità.

Pertanto, i vantaggi rispetto alle tecnologie attuali disponibili sono:

1. **Semplicità di esecuzione:** MORECOVERY viene fornito come kit utilizzabile anche da personale non specializzato.
2. **Maggiore accuratezza dei risultati:** MORECOVERY previene la sottostima della contaminazione batterica sulle superfici, migliorando l'affidabilità del monitoraggio ambientale.
3. **Kit completo e pronto all'uso:** MORECOVERY include tutte le componenti necessarie (maschere di campionamento, tamponi, il *Resuscitation Buffer* (RB) e piastre per la semina), eliminando la necessità di acquistare i materiali separatamente da fornitori diversi.
4. **Nessuna necessità di trasporto a temperatura controllata:** a differenza delle metodiche attuali, MORECOVERY non richiede il mantenimento dei campioni tra 1 e 8 °C, semplificando ulteriormente la gestione del campione.
5. **Costo estremamente contenuto del kit:** che lo rende accessibile a un'ampia gamma di utenti.

3. Sono state compiute indagini in merito all'attuale stato dell'arte che escludano tassativamente la presenza di dispositivi simili nel mercato di riferimento?

Indicare dettagliatamente quali ricerche sono state eseguite per confermare gli aspetti realmente innovativi ed originali dell'invenzione di cui si chiede la tutela brevettale e per avere la certezza che nessun dispositivo simile è stato oggetto di tutela.

Ricerche eseguite:

Da ricerche eseguite nella maggiore banca dati della letteratura scientifica biomedica (PubMed, <http://www.ncbi.nlm.nih.gov>) è emerso che vi sono diversi metodi per la rilevazione di cellule batteriche nello stato di VBNC. Tali metodiche consistono nell'utilizzo di due coloranti in combinazione (CTC e DAPI o SYTO9 e PI) che consentono di discriminare le cellule vive da quelle morte oppure su metodiche basate sulla reazione a catena della polimerasi (PCR) quantitativa. Entrambi le metodiche richiedono strumentazione adeguata, come ad esempio un microscopio a fluorescenza o un termociclatore per PCR quantitativa, e reagenti costosi (Liu J, Yang L, Kjellerup BV, Xu Z. Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. Trends Microbiol. 2023 Oct;31(10):1013-1023. doi: 10.1016/j.tim.2023.04.009.). Nessuno dei metodi descritti si basa sul processo di rivitalizzazione mediante incubazione in RB a 37°C. La metodica da noi proposta non solo è innovativa ma è anche di semplice esecuzione, in quanto non richiede l'utilizzo di strumentazione e reagenti specifici e costosi.

Inoltre, sono stati consultati anche i seguenti siti web:

1. *European Patent Office (EPO)* - <https://www.epo.org/searching-for-patents/technical/espacenet.html#tab-1>
2. *Ufficio Italiano Brevetti e Marchi* - <https://www UIBM.gov.it/biotech/dataset.html>

e non sono stati individuati brevetti che rivendicano la metodica o il kit da noi proposto per la determinazione quantitativa e accurata dei microrganismi su superfici.

Per una verifica di brevettabilità del kit MORECOVERY è raccomandata la consulenza dello Studio De Simone & Partners Via Vincenzo Bellini, 20 - 00198 Rome - Italy | info@desimonepartners.com | +39 06 853361 (Consulente dott.ssa Maria Paola Pace Napoleone)

4. **Descrivere il funzionamento dell'invenzione**, *inclusando schemi, disegni, calcoli etc. necessari per spiegarne il funzionamento. Se si è in possesso di un documento dettagliato, si chiede di descrivere sinteticamente nello spazio seguente il funzionamento dell'invenzione e di allegare il predetto documento alla presente richiesta.*

L'invenzione consiste in un kit per il monitoraggio della contaminazione microbica su superfici abiotiche ed è costituito da:

- Tampone in nylon immerso in 2 ml di *Resuscitation Buffer* (RB)
- Composizione del RB (per un litro di acqua bidistillata sterile):
- 6,78 g di Na_2HPO_4
 - 3,00 g di KH_2PO_4
 - 0,50 g di NaCl
 - 1,00 g di NH_4Cl
 - 0,02 g di CaCl_2
 - 0,12 g di MgSO_4
 - 2 Maschere di campionamento da 10 cm×10 cm e da 5×4 cm
 - Piastra Petri contenente il terreno nutritivo agarizzato TSA (Tryptic Soy Agar)
 - Pipetta Pasteur monouso sterile
 - Spatola monouso sterile

Le foto del kit da noi proposto e il protocollo relativo al metodo di rilevamento dei microrganismi sulle superfici è descritto dettagliatamente nell'Allegato 1.

5. **Descrivere lo stadio di sviluppo dell'invenzione** (*ad esempio: stadio concettuale, stadio sperimentale, simulazione del modello di funzionamento, prototipo funzionante etc.*) *allegando dati e fotografie indicanti lo stadio di sviluppo del prototipo.*

L'efficacia del metodo di rilevamento proposto è stata dimostrata attraverso la simulazione in laboratorio del suo funzionamento ed è stato generato un prototipo funzionante mediante l'utilizzo di singole componenti (tampone, *Resuscitation Buffer* RB, maschere per il campionamento e piastre di TSA) già presenti sul mercato. In particolare, è stato utilizzato un tampone di nylon (FloQ swab, Copan), i sali necessari per generare l'RB sono stati acquistati dalla ditta Merk, le piastre Petri contenenti TSA agar sono state preparate in laboratorio utilizzando piastre Sarstedt e terreno in polvere della ditta Neogen. L'invenzione si trova in Technology Readiness Levels 6 (TLR 6) e cioè la tecnologia è stata sviluppata oltre la fase sperimentale e saggiata in condizioni che simulano quelle reali, confermandone la fattibilità e l'efficacia, ma non è ancora completamente ottimizzata per la produzione su larga scala o l'implementazione definitiva.

Necessità di ulteriori sviluppi (*dove come e da chi dovrebbero essere fatti*)

Nel kit MORECOVERY è presente una piastra di TSA che è un terreno ricco e non selettivo per la crescita batterica. Il kit potrebbe essere implementato con l'aggiunta di piastre di terreni selettivi per l'isolamento di specifici batteri patogeni. A titolo esemplificativo ma non esaustivo, è possibile assemblare kit *ad hoc* per il rilevamento di specifici patogeni (*A. baumannii*, *P. aeruginosa*, *Enterobacterales*) o antibiotico resistenze (quelle mediate da Extended Spectrum Beta-lactamases):

MORECOVERY-ABAUM = Kit base + 1 piastra aggiuntiva di Leeds agar medium (Hardy diagnostics)

MORECOVERY-PSEUDO = Kit base + 1 piastra di Cetrimide agar medium (Thermofisher)

MORECOVERY-ENTERO = Kit base + 1 piastra di Hektoen enteric agar (Liofilchem)

MORECOVERY-ESBL = Kit base + 1 piastra di Brilliance (tm) ESBL agar medium (Thermofisher)

ETC.

Lo sviluppo di kit con piastre per la semina diverse in base alle esigenze dell'operatore e al sito da campionare potrebbe essere svolto in collaborazione con eventuali partner industriali.

MORECOVERY potrebbe inoltre rappresentare la base per lo sviluppo di mezzi per la rivitalizzazione di VBNC di patogeni diversi da quelli descritti nella presente proposta di brevettazione e che rappresentano

un problema rilevante in ambito sanitario, alimentare e farmaceutico.

Time to market previsto (*tempo che intercorre tra lo stadio attuale in cui si trova l'invenzione e l'entrata dell'invenzione nel mercato di riferimento*)

L'invenzione è a TRL 6 e il *time-to-market* previsto è 1-2 anni

6. Qual è il potenziale valore commerciale dell'applicazione brevettata?

Fornire indicazioni sui mercati potenzialmente interessati dall'invenzione; fornire indicazioni sul ciclo di vita del prodotto e sul rischio della tecnologia/implementazione al fine di valutare con maggiore obiettività il valore potenziale del brevetto; fornire indicazioni dettagliate, anche utilizzando apposite relazioni da allegare al presente form, del bilancio energetico collegato all'invenzione, redatte ponendo in particolare evidenza i costi delle materie prime e il normale grado di usura delle medesime, nonché le valutazioni di carattere economico scaturenti dalla immissione del dispositivo sul mercato di riferimento

Dimensione e crescita del mercato di riferimento:

La valutazione della carica microbica sulle superfici avviene attraverso metodiche che si basano sulla coltivabilità dei microrganismi. Tuttavia, molti microrganismi possono entrare in uno stato di VBNC perdendo la capacità di moltiplicarsi nei comuni terreni di laboratorio. In tale stato i batteri sono comunque vitali e pertanto sono potenzialmente in grado di causare infezioni e riprodursi all'interno dell'organismo ospite. In ambito ospedaliero, la capacità dei batteri di entrare in stato VBNC rappresenta una criticità rilevante, poiché li rende non rilevabili con i metodi di monitoraggio ambientale comunemente utilizzati. In particolare, nel laboratorio di Microbiologia dell'Università Roma Tre è stato dimostrato che i principali patogeni Gram-negativi responsabili di infezioni nosocomiali, tra cui *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* ed *Enterobacter spp.*, possono assumere lo stato di VBNC in seguito a disidratazione su superfici abiotiche, eludendo così i tradizionali sistemi di rilevamento.

Un monitoraggio efficace di questi microrganismi negli ambienti ospedalieri risulterebbe fondamentale per limitare la diffusione delle infezioni tra i pazienti, considerando che superfici e dispositivi medici sono noti serbatoi di patogeni e svolgono un ruolo chiave nella trasmissione di agenti infettivi.

La presente invenzione ha il potenziale di rivoluzionare il settore del monitoraggio ambientale, offrendo una soluzione innovativa, semplice ed efficace. Poiché il rilevamento dei microrganismi nell'ambiente è essenziale per prevenire la trasmissione di agenti infettivi non solo in ambito sanitario, ma anche nel settore alimentare e farmaceutico, questa tecnologia potrebbe trovare applicazione in un mercato estremamente vasto. Ne è ipotizzabile l'uso anche nell'implementazione dei processi di monitoring previsti dall'HACCP (Hazard Analysis and Critical Control Points). L'HACCP impone una serie di misure per prevenire, eliminare o ridurre a livelli accettabili i pericoli microbiologici in diversi ambiti da quello alimentare a quello farmaceutico. Uno dei principali obblighi previsti dal sistema HACCP è l'analisi dei pericoli microbiologici, ovvero l'identificazione dei microrganismi che potrebbero contaminare il prodotto nelle diverse fasi della sua produzione. Infatti, l'HACCP prevede l'individuazione dei punti critici di controllo (*Critical Control Point*, CCP), ossia le fasi del processo produttivo in cui il rischio microbiologico può essere monitorato ed eventualmente eliminato.

Il monitoraggio microbiologico prevede controlli su prodotti, superfici di lavoro e attrezzature mediante tamponi, piastre a contatto e test microbiologici specifici. In ambito alimentare ad esempio vengono adottati standard internazionali come la norma ISO 18593, che stabilisce le procedure per il campionamento delle superfici. Per garantire l'efficacia delle misure di controllo e sanificazione, vengono svolte verifiche periodiche, tra cui l'analisi microbiologica che è essenziale per mantenere elevati standard di sicurezza e qualità, prevenendo potenziali contaminazioni lungo tutta la filiera produttiva.

Un ulteriore aspetto distintivo della nostra invenzione è l'introduzione di un kit completo, che integra tutti gli strumenti necessari per il campionamento e la semina. Questa soluzione innovativa semplifica l'intero processo, migliorando l'efficienza operativa e la praticità per gli utenti.

Di conseguenza, le aziende specializzate nella produzione e commercializzazione di dispositivi medici, in particolare per il monitoraggio microbiologico nei settori sanitario, alimentare e farmaceutico, potrebbero essere fortemente interessate all'acquisizione del brevetto e allo sviluppo della tecnologia.

Distribuzione geografica del mercato di riferimento:

Il principale mercato di riferimento per la nostra invenzione potrebbe comprendere tutti i paesi con un elevato tasso di infezioni ospedaliere. Tuttavia, poiché il nostro kit è applicabile anche al monitoraggio dei microrganismi in altri settori, quali quello farmaceutico, alimentare e industriale, il potenziale di mercato si estende su scala globale.

Effetti economici, aumento del valore del prodotto dovuto a: (*diminuzione costo produzione unitario, aumento prestazioni, apertura nuove aree di mercato*)

Allo stato di prototipo di laboratorio, è stato stimato il costo del kit sulla base dei costi delle singole componenti disponibili on line:

1. **Tampone sterile in nylon Copan.** Una confezione da 100 pezzi ha un costo di 47,93 euro e pertanto il singolo tampone costa 0,48 euro. ([https://www.praxisdienst.it/it/Laboratorio/Supporti/Prelievo+di+campioni/eNAT+FLOQSwabs+regul ar+Tip+552C.html?speed=1&mtm_campaign=\(IT:ePMax\)%20low%20score&mtm_source=google&mtm_medium=cpc&mtm_content=PERFORMANCE_MAX&qad_source=1&gclid=Cj0KCQiAlbW-BhCMARIsADnwaspVJgB6jfYMOwiHR2ABm5wUPGceg0ATdbKlf0QWjp0Wk0Db_6_JYJkaAoxsEALw_wcB](https://www.praxisdienst.it/it/Laboratorio/Supporti/Prelievo+di+campioni/eNAT+FLOQSwabs+regul ar+Tip+552C.html?speed=1&mtm_campaign=(IT:ePMax)%20low%20score&mtm_source=google&mtm_medium=cpc&mtm_content=PERFORMANCE_MAX&qad_source=1&gclid=Cj0KCQiAlbW-BhCMARIsADnwaspVJgB6jfYMOwiHR2ABm5wUPGceg0ATdbKlf0QWjp0Wk0Db_6_JYJkaAoxsEALw_wcB))
2. **Resuscitation buffer (RB).** Il costo dell'RB è trascurabile considerando il costo dei salie la quantità necessaria per la produzione. Infatti, un kg di polvere di M9 salt ha un costo di 268,00 euro (<https://www.sigmaaldrich.com/IT/it/product/sigma/m6030?msocid=2bfecadea249632735b1dff3a3596220>). Con un kg di polvere si possono produrre un circa 90 L. Pertanto, considerando che per ogni kit sono necessari 2 ml di RB il costo è 0.0056 euro a tampone
3. **Maschere di campionamento.** Una confezione da 300 maschere ha un costo di 555,00 euro e pertanto 1,85 euro per singola maschera (<https://www.fishersci.pt/shop/products/x300-plastic-square-sampling-template-10cm-x-10cm/11775779>).
4. **Spatole monouso sterile per la semina.** Una confezione di 1000 spatole ha un costo di 222,00 euro e pertanto 0,22 euro per spatola. ([Copan Italia T-Shaped Plastic Spreaders Length: 171mm:Cell Culture Utensils, J Fisher Scientific](#))
5. **Pipetta Pasteur monouso sterile da 100 µl.** Una confezione da 1000 pipette ha un costo di 121,41 euro e pertanto 0,12 euro per pipetta (<https://www.cheimika.it/it-IT/articoli/Pipette-Pasteur--PE-HD--645.aspx>)
6. **Piastrine di TSA.** Una confezione di 20 piastrine costa 24.60 euro (<https://www.itwreagents.com/italy/it/product/tryptone-soy-agar-tsa-ph-eur-piastra-preparata-90-mm-per-microbiologia/453819>) e pertanto 1,24 euro a piastra.

Pertanto, il costo totale del kit è 5.76 euro (considerando due maschere di campionamento).

Il costo stimato del kit è stato calcolato sulla base dei prezzi di mercato reperibili presso fornitori indicati a titolo di esempio. Tuttavia, tali costi riflettono il prezzo finale al consumatore e non i costi di produzione effettivi sostenuti dalle aziende produttrici, che saranno sensibilmente inferiori. Di conseguenza, qualora il kit fosse prodotto su larga scala, il costo di produzione unitario potrebbe essere significativamente ridotto, consentendo margini di guadagno potenzialmente superiori, fino a 3-4 volte il costo di produzione.

Il kit MORECOVERY è pertanto estremamente economico in termini di materie prime utilizzate e pertanto potrebbe essere proposto a un prezzo competitivo, rendendolo estremamente richiesto nel mercato del campionamento ambientale.

Ciclo di vita del prodotto:

Per stimare il ciclo di vita della presente invenzione, è stato preso come riferimento un mezzo di trasporto ideato nel 1995 per il campionamento dei microrganismi in ambienti ospedalieri. Tale confronto permette di effettuare una proiezione realistica della durata e dell'evoluzione del prodotto nel tempo.

Nel 1995 è stato ideato un mezzo di trasporto per il rilevamento di *S. aureus* sulle superfici, che attualmente è ancora presente nel tampone commerciale ESC Swab Neutralizing broth (Dey BP and Engley FB Jr. Comparison of Dey and Engley (D/E) neutralizing medium to Lethen medium and standard methods medium for recovery of *Staphylococcus aureus* from sanitized surfaces. J Ind Microbiol. 1995 Jan;14(1):21-5. doi: 10.1007/BF01570061.). Tale mezzo di trasporto è ancora utilizzato per il rilevamento di microrganismi nonostante siano stati sviluppati negli anni molti altri *buffer* con la medesima finalità. Sulla base di questa premessa riteniamo che si possa prevedere che anche la nostra invenzione possa restare sul mercato per almeno 10 anni.

Valutazione sul rischio della tecnologia/implementazione:

La tecnologia da noi proposta non prevede alcun rischio dal momento che il mezzo di trasporto utilizzato non contiene nessuna sostanza tossica, infiammabile o inquinante.

I. AZIENDE

Basandosi sulla personale conoscenza, riportare i nominativi di aziende potenzialmente interessate all'utilizzo e/o sviluppo congiunto.

RAGIONE SOCIALE	Indirizzo
Liofilchem	Via Scozia, Zona Industriale; 64026, Roseto degli Abruzzi (TE)
Biosigma	Via Valletta, 6; 30010 Cona (VE)
Fisher Scientific Italia	Via San Bovio 3, 20054 Segrate (MI), Italia

Gli inventori sono alla ricerca di *venture capital* per la creazione di uno Spin-Off universitario per gli ulteriori passaggi di trasferimento tecnologico dell'invenzione.

J. Segnalare eventuali riferimenti presenti nella letteratura scientifica che descrivano lo stato dell'arte relativo all'invenzione.

Non sono presenti in letteratura articoli scientifici che descrivono lo stato dell'arte dell'invenzione

K. Ambito territoriale per il quale viene richiesta la tutela brevettuale

(barrare la casella interessata)

1. Nazionale ☐
2. Europeo ☐
3. Internazionale ☒

Modulo per la richiesta di brevettazione

Il sottoscritto Francesco IMPERI C.F. MPRFNC79R31H501V in servizio presso il Dipartimento di Scienze, Università degli Studi Roma Tre;

Il sottoscritto Massimiliano LUCIDI C.F. LCDMSM93M22H501O in servizio presso il Dipartimento di Scienze, Università degli Studi Roma Tre;

La sottoscritta Cinzia SPAGNOLI C.F. SPGCNZ97P56H501E in servizio presso il Dipartimento di Scienze dell'Università degli Studi Roma Tre;

La sottoscritta Daniela VISAGGIO C.F. VSGDNL84M44L109H in servizio presso il Dipartimento di Scienze dell'Università degli Studi Roma Tre;

Il sottoscritto Paolo VISCA C.F. VSCPLA56S01H501Z in servizio presso il Dipartimento di Scienze, Università degli Studi Roma Tre;

DICHIARO

- di accettare espressamente ed integralmente il Regolamento di Ateneo per la valorizzazione dell'attività scientifica, la tutela della proprietà industriale e intellettuale e il trasferimento dei risultati;
- che l'invenzione è stata conseguita nell'ambito di un progetto autonomamente elaborato dal sottoscritto e su di essa non grava alcun diritto di terzi committenti;
- di essere consapevole, nel rilasciare la presente dichiarazione ai sensi del D.P.R. 28 dicembre 2000, n. 445 e ss.mm.ii., delle sanzioni penali previste dall'articolo 76 della medesima norma per le ipotesi di falsità in atti e dichiarazioni mendaci ivi indicate;
- di essere informato, ai sensi e per gli effetti del D.Lgs. 30 giugno 2003, n. 196 e ss.mm.ii., che i dati personali raccolti saranno trattati, anche con l'ausilio di mezzi elettronici e strumenti informatici, esclusivamente nell'ambito del procedimento per il quale la presente dichiarazione viene resa

CHIEDO

- che l'invenzione sia brevettata a nome dell'Università degli Studi Roma Tre. Mi impegno a tal riguardo, a trasferire alla medesima Università i diritti che potranno derivare dalla brevettazione dell'invenzione e di non revocare tale impegno per un periodo di 90 giorni, a partire dalla data di sottoscrizione della presente istanza.

Roma li, 26/03/2025

Prof. Francesco Imperi



Dott. Massimiliano Lucidi



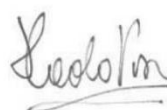
Dott.ssa Cinzia Spagnoli



Dott.ssa Daniela Visaggio



Prof. Paolo Visca



Il sottoscritto Prof. Giovanni Antonini Direttore del Dipartimento di Scienze certifica la disponibilità a contribuire alla copertura delle spese brevettuali sostenute dall'Ateneo con una quota di cofinanziamento pari a euro 800,00 (Ottocento) sul capitolo PRIN 2022 Visaggio "Development of novel antibacterial agents hijacking iron uptake systems: towards advanced "Trojan horse" strategies" (DAHIUS) CUP: F53D23000860001

Ai sensi dell'art. 10 del "Regolamento di Ateneo per la valorizzazione dell'attività scientifica, la tutela della proprietà industriale e intellettuale" tale quota non può essere inferiore al 10%.

Il Direttore del Dipartimento

Prof. Giovanni Antonini

Allegato 1

MORECOVERY

Nuovo metodo per la quantificazione di microrganismi patogeni su superfici abiotiche

Descrizione

MORECOVERY è un kit per il monitoraggio microbiologico delle superfici attraverso il metodo di campionamento con tampone. La composizione del mezzo di rivitalizzazione (*Resuscitation Buffer*, RB) e la procedura del saggio consentono di rendere coltivabili anche le cellule batteriche nello stato vitale ma non coltivabile (VBNC), e pertanto, di quantificare in maniera accurata la presenza di microrganismi su superfici abiotiche.

Contenuto del Kit

- Tampone in nylon immerso in 2 ml di *Resuscitation Buffer* (RB, Fig. 1A)
Composizione del RB (per un litro):
6,78 g di Na_2HPO_4
3,00 g di KH_2PO_4
0,50 g di NaCl
1,00 g di NH_4Cl
0,02 g di CaCl_2
0,12 g di MgSO_4
- 2 Maschere di campionamento da 10 cm×10 cm e da 5×4 cm (Fig. 1B e 1C)
- Pipetta Pasteur da 100 µl
- Spatola sterile monouso
- Piastra Petri contenente il terreno nutritivo agarizzato TSA (Tryptic Soy Agar, Fig. 1D)

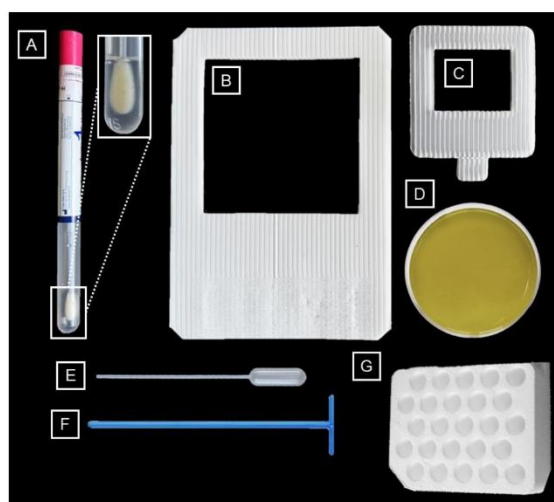


Figura A1. Componenti del kit MORECOVERY per la quantificazione di batteri patogeni contaminanti superfici abiotiche. A) Tampone in nylon immerso nel buffer RB. B) Maschera per il campionamento di una superficie pari a 10×10 cm. C) Maschera per il campionamento di una superficie pari a 5×4 cm. D) Piastra Petri contenente il terreno nutritivo agarizzato TSA (Tryptic Soy Agar) per la semina dei microorganismi. E) Pipetta Pasteur da 100 µL. F) spatola sterile monouso per la semina del campione. G) Portaprovette in polistirolo.

Procedura

1. Selezionare la superficie da campionare e delimitare un'area utilizzando una delle due maschere di campionamento in dotazione (100 cm² oppure 20 cm², a seconda del livello di contaminazione sospettata).
2. Disporre la provetta contenente il tampone in posizione verticale e verificare che il liquido (RB) al suo interno sia sul fondo della provetta (eventualmente scuotere la provetta verso il basso per raccogliere tutto il liquido sul fondo).
3. Premere la punta del tampone contro la parete della provetta per rimuovere l'eccesso di RB; il tampone deve essere umido e omogeneamente intriso di RB.
4. Disporre la maschera (a scelta 100 cm² o 20 cm²) sulla superficie da campionare.
5. Passare il tampone sulla superficie delimitata, assicurando un contatto uniforme e coprendo l'area con strisciate dapprima parallele e poi perpendicolari, ruotando il tampone durante la procedura. Per superfici piane, il campionamento deve essere eseguito orizzontalmente e verticalmente (10 volte in ciascuna direzione).

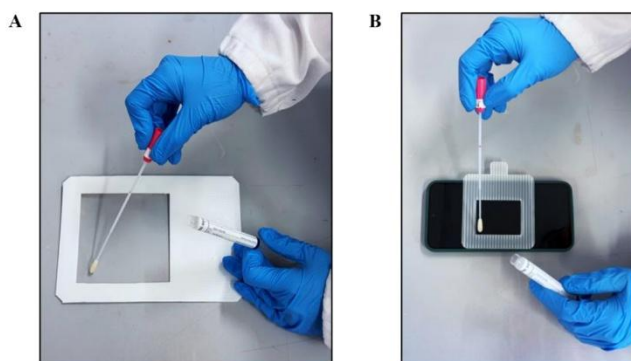


Figura A2. Campionamento di superfici abiotiche mediante l'utilizzo del tampone e delle maschere di campionamento. A) campionamento di una superficie di lavoro in melaminico utilizzando la maschera per il campionamento con superficie di 10×10 cm. B) campionamento di un telefono cellulare utilizzando la maschera per il campionamento con superficie di 5×4 cm.

6. Inserire il tampone nella provetta sterile contenente 2 mL di RB.
7. Incubare la provetta contenente il tampone a 37°C per 24 ore, agitandola manualmente di tanto in tanto.
8. Dopo l'incubazione, prelevare 100 µl del RB con una pipetta sterile e procedere con la semina su piastra Petri contenente il terreno TSA (Tryptic Soy Agar) utilizzando l'apposita spatola sterile monouso fornita col kit (Figura A3).

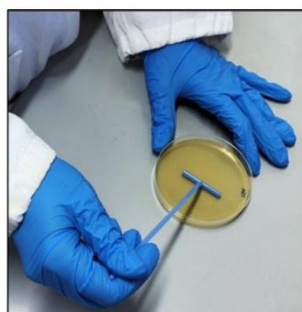


Figura A3. Semina del campione. Semina del campione su piastra Petri contenente il terreno TSA utilizzando l'apposita spatola sterile monouso.

9. Incubare a 37°C per 48 ore per consentire la formazione di colonie.
10. Dopo 48 ore di incubazione, contare il numero di unità formanti colonia (*colony forming unit*, CFU) su ogni piastra.
11. Calcolare il numero di CFU per superficie campionata (**Ns**) utilizzando la formula:

$$Ns = \frac{N \times D}{A}$$

Dove:

- **N** è il numero di CFU presenti sulla piastra Petri
- **A** è la superficie campionata, espressa in centimetri quadrati (100 cm² nel caso della maschera di campionamento 10 cm×10 cm e 20 cm² per la maschera di campionamento da 5×4 cm)
- **D** corrisponde all'inverso della diluizione che è stata seminata (dal momento che vengono seminati 100 µl del volume totale che è di 2 ml, è stato seminato 1/20 del campione e pertanto D è uguale a 20)

Note:

1. Il volume di RB che viene seminato sulla piastra può essere modificato in base alle esigenze specifiche dell'operatore. Per superfici in cui si prevede una elevata contaminazione microbica possono essere effettuate delle diluzioni seriali prima della semina. Per superfici in cui anche la presenza di una singola cellula batterica possa rappresentare un rischio, si consiglia la semina di volumi maggiori.
2. Il terreno su cui viene eseguita la semina può essere modificato in base alle esigenze dell'operatore. Se l'attività di campionamento è finalizzata alla ricerca di un patogeno specifico, oltre alla piastra di TSA possono essere utilizzate piastre selettive che consentono l'isolamento del microrganismo di interesse.
3. La temperatura e il tempo di incubazione delle piastre su cui è stato seminato l'RB possono essere modificate in base al microrganismo di interesse.

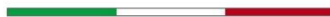


Ministero delle Imprese e del Made in Italy

Ricevuta di presentazione

per

Brevetto per invenzione industriale



Domanda numero: 102025000014596

Data di presentazione: 19/06/2025

Ricevuta di presentazione per Brevetto per invenzione industriale

DATI IDENTIFICATIVI DEL DEPOSITO

Ruolo	Mandatario
Depositante	Maria Pace Napoleone
Data di compilazione	19/06/2025
Riferimento depositante	ITPF9971/IT
Titolo	METODO PER LA QUANTIFICAZIONE DI MICRORGANISMI PATOGENI SU SUPERFICI ABIOTICHE
Carattere domanda	Ordinaria
Esenzione	NO
Accessibilità al pubblico	NO
Numero rivendicazioni	16
Autorità depositaria	

PRIVACY

Dichiaro di aver letto e compreso l'informativa sul trattamento dei dati personali trasmessi con il presente deposito, resa ai sensi dell'art. 13 del Regolamento (UE) 2016/679 e pubblicata all'interno del presente portale, oltre che sul sito istituzionale del Dipartimento Mercato e Tutela - Direzione Generale per la Proprietà Industriale Ufficio Italiano Brevetti e Marchi del Ministero delle Imprese e del Made in Italy

RICHIEDENTE/I

Natura giuridica	Persona giuridica
Denominazione	Università degli Studi Roma Tre
P.IVA/CF	04400441004
Tipo Società	le universita'
Nazione sede legale	Italia
Comune sede legale	Roma (RM)

Ricevuta di presentazione per Brevetto per invenzione industriale

Indirizzo	VIA OSTIENSE
Civico	133
CAP	00154
Telefono	
Fax	
Email	
Pec	
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Indirizzo	Via Giulio Caccini 1
Cap	00198
Nazione	Italia
Comune	Roma (RM)
PEC	patent@pec.desimonepartners.com

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Email	
Telefono	06 853361
Fax	

MANDATARI/RAPPRESENTANTI

Cognome	Nome
Pace Napoleone	Maria
Currado	Luisa

Ricevuta di presentazione per Brevetto per invenzione industriale

INVENTORI

Cognome	Nome	Nazione residenza
Visca	Paolo	Italia
Imperi	Francesco	Italia
Lucidi	Massimiliano	Italia
Spagnoli	Cinzia	Italia
Visaggio	Daniela	Italia

CLASSIFICAZIONI

Sezione	Classe	Sottoclasse	Gruppo	Sottogruppo
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DOCUMENTAZIONE ALLEGATA

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Rivendicazioni	NO	ITPF9971IT_Rivendicazioni.pdf.p7m hash: 123a529b23cca6e129680fb3f00660b4
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PAGAMENTI

Ricevuta di presentazione per Brevetto per invenzione industriale

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NOTE

Chapter 7. Concluding remarks

Bacteria exhibit remarkable resilience to environmental fluctuations, enabling them to persist in diverse and often hostile conditions. Among these stresses, desiccation represents one of the most relevant challenges in natural environments and hospital settings. The ability to withstand water loss is not merely an ecological adaptation, since it has profound implications for healthcare-associated infections (HAIs). HAIs are frequently driven by multidrug-resistant pathogens that persist on hospital surfaces and medical devices, acting as reservoirs for cross-transmission and facilitating outbreaks that elude infection control (Mitchell *et al.*, 2023; Macesic *et al.*, 2025). Among Gram-negative bacteria, *A. baumannii* has emerged as a paradigm of desiccation tolerance, maintaining culturability for extended periods on abiotic surfaces and thereby contributing to long-term contamination of the hospital environment (Giannouli *et al.*, 2013). Recent studies have revealed its ability to enter a reversible dormant state, known as Viable But Non-Culturable (VBNC), under extreme stresses such as osmotic imbalance and hypoxia (König *et al.*, 2023). In this state, cells remain metabolically active but lose culturability on standard media, regaining growth only when favourable conditions are restored. Currently, our understanding of the pathways and regulatory circuitries underlying *A. baumannii* desiccation resistance, VBNC cell formation, and resuscitation from the dormant state has only been partially uncovered.

To bridge this gap, the physiological and molecular basis of desiccation resistance in *A. baumannii* were initially investigated (Publication I, Chapter 3). Using ATCC 19606^T and ACICU strains, it was demonstrated that desiccation causes severe cellular damage, cell shrinkage, ATP depletion, and oxidative stress, resulting in over 90% loss of culturability within 24 hours. Remarkably, even cells with compromised membranes regained growth when resuspended in a carbon-free buffer under optimal conditions for membrane repair (37 °C, presence of divalent cations), revealing a reversible transition to the VBNC state. This phenomenon was confirmed across multiple clinical strains and other species within the genus, suggesting that induction of the VBNC state represents an effective survival strategy enabling *Acinetobacter* to persist under harsh conditions and thrive in diverse environments.

To elucidate the molecular mechanisms underlying the entry into the VBNC state, genome-wide transcriptomic profiling was performed on ATCC 19606^T and ACICU strains before and after 1-week desiccation. The analysis revealed extensive transcriptional remodelling, affecting approximately one-third of the genome, with notable strain-specific differences. The comparison of the transcriptomic profiles between the two *A. baumannii* strains allowed the prediction of a key molecular mechanism involved in the entry into the VBNC state. In particular, desiccated cells of ATCC 19606^T and ACICU, which are 99% in the VBNC state, modify their metabolism by

shortcutting the TCA cycle into the glyoxylate shunt (GS) pathway, via *aceA* upregulation, coding for the isocitrate lyase enzyme that produces glyoxylate and succinate from isocitrate. The crucial role of the GS pathway during desiccation has been confirmed by generating *aceA* knock-out mutants. The deletion of *aceA* reduced both desiccation resistance and resuscitation efficiency in ACICU, and completely abrogated resuscitation in ATCC 19606^T. These findings underscore the complexity of desiccation resistance in *A. baumannii* and highlight intra-species diversity. The activation of the GS pathway has been previously reported in other bacterial species during oxidative stress, antibiotic exposure, and host infection (Nandakumar *et al.*, 2014; Ahn *et al.*, 2016; Ha *et al.*, 2018; Park *et al.*, 2019). However, to our knowledge, this is the first evidence of GS pathway activation in *A. baumannii* during desiccation stress, highlighting its potential role as a versatile stress-response mechanism.

While the previous section clarified the physiological and molecular changes underlying VBNC state entry, the process enabling cells to resuscitate was still a mystery. Therefore, the global transcriptional changes occurring during resuscitation of desiccation-induced VBNC cells of *A. baumannii* ATCC 19606^T were investigated (Manuscript II, Chapter 4). A genome-wide transcriptomic approach comparing VBNC cells with resuscitated ones, revealed a predominance of upregulated genes in COG category L, associated with replication, recombination, and repair. These findings strongly suggest that the repair of DNA, which is predictably damaged by the desiccation-induced oxidative stress (Grefte & Michiels, 2020), is a key process enabling the recovery from the VBNC state. Consistent with this hypothesis, we previously showed that desiccation promotes intracellular accumulation of reactive oxygen species (ROS), which damage macromolecules, including DNA, through oxidation and crosslinking (Publication I). To identify specific genes involved in the resuscitation process, differentially expressed genes (DEGs) from this study were compared with DEGs specific for the VBNC state entry identified in Chapter 3 (Publication I). During resuscitation, *uvrA* was identified as one of the most significantly induced genes. The gene *uvrA* encodes a component of the UvrABC nucleotide excision repair system, and it was downregulated during VBNC state entry. Previous studies have associated *uvrA* induction with DNA damage repair in *A. baumannii* under genotoxic stress, showing that its expression is regulated by RecA and triggered by DNA-damaging conditions such as UV irradiation and antibiotic exposure (Norton *et al.*, 2013). Consistent with these observations, Chapter 4 functionally validated the protective role of UvrA by assessing the phenotypic consequences of *uvrA* deletion. Exposure of the Δ *uvrA* strain under genotoxic stress, such as UV irradiation or oxidative stress induced by paraquat, markedly impaired its growth and survival. Furthermore, the contribution of *uvrA* to desiccation tolerance and recovery was evaluated, unveiling the inability of the Δ *uvrA* mutant strain to regain its culturability after resuscitation. Collectively,

these results underscore the essential role of DNA repair, and particularly of the UvrA protein, in resuscitation and highlight its pivotal contribution to desiccation stress resilience.

Finally, to determine how resuscitation and *uvrA* contribute to pathogenesis, a murine pneumonia model was employed to assess the pulmonary colonization capacity of *A. baumannii* wild-type and Δ *uvrA* strains before desiccation, after one week of desiccation, and following resuscitation. Both strains regained the ability to colonize the lungs after resuscitation; however, the wild-type strain achieved significantly higher pulmonary burdens than the Δ *uvrA* mutant, even exceeding pre-desiccation levels. These findings demonstrate that *uvrA* is essential not only for efficient recovery from VBNC state, but also for maintaining full virulence during subsequent host infection.

The two previous studies (Chapters 3 and 4) provide a comprehensive view of the mechanisms enabling *A. baumannii* to enter and recover from the VBNC state. It is worth noting that *A. baumannii* belongs to the ESKAPE group, composed of *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.. It is well established that all these species pose a critical threat to public health due to their high prevalence in HAIs and antimicrobial resistance (Woh & Zhang, 2025). Previous evidence indicated that ESKAPE pathogens, except *Enterobacter* spp., can enter the VBNC state under environmental stresses, but little was known about their behaviour under desiccation stress (König *et al.*, 2023; Qi *et al.*, 2023; Zhao *et al.*, 2024).

As anticipated, the VBNC state plays a concerning role in the persistence of pathogens within hospital environments, creating hidden reservoirs that contribute to the spread of HAIs. Hospital surfaces and medical devices act as key reservoirs for microbial contamination, enabling cross-transmission to patients through contact with healthcare personnel and equipment (Mitchell *et al.*, 2023).

Therefore, the ability of ESKAPE pathogens to adopt the VBNC strategy under desiccation stress was evaluated (Publication III, Chapter 5). Culturability after 1 week of desiccation on different abiotic surfaces commonly found in hospital environments was markedly reduced, varying among strains and depending on the type of surface material. Resuscitation in a carbon-free resuscitation buffer (RB) revealed that only Gram-negative strains regained culturability. In contrast, Gram-positive species did not resuscitate under the test conditions, highlighting fundamental differences in desiccation stress adaptation between these two groups.

Effective environmental monitoring is essential for infection prevention; however, current protocols, such as those described in BS EN 17141:2020 (Cleanrooms and associated controlled environments - Biocontamination control), rely almost exclusively on culture-based methods, which fail to detect VBNC cells (van der Schoor *et al.*, 2022; Liu *et al.*, 2023). To address this limitation, standard procedures were compared with the resuscitation protocol proposed in this thesis. After incubation in

RB, all Gram-negative ESKAPE bacteria were completely resuscitated, whereas Gram-positive species showed no recovery. Furthermore, none of the standard transport buffers enabled resuscitation, demonstrating that conventional sampling methods underestimate viable bacterial counts on surfaces. The superior sensitivity of the resuscitation protocol was confirmed through a pilot environmental sampling experiment on various surfaces within the Department of Science at Roma Tre University. Although this approach has not yet been validated in hospital settings, evidence presented in earlier chapters highlights the need for improved detection strategies. Based on these findings, a novel procedure named MORECOVERY was developed and patented, incorporating a resuscitation step to restore culturability before plating (Patent IV, Chapter 6).

In conclusion, this PhD thesis provides a comprehensive and integrated perspective on the adaptive strategies employed by *A. baumannii* to withstand desiccation, thereby shedding light on mechanisms that enable its persistence in healthcare environments. The findings presented here underscore the role of the VBNC state as a survival mechanism of *A. baumannii* and Gram-negative ESKAPE species, and its contribution to hidden reservoirs that escape conventional culture-based monitoring. These results are expected to impact infection control practices and lay the foundation for future studies aimed at unveiling additional aspects of bacterial adaptation to desiccation stress.

Further research should aim to identify common molecular pathways underlying VBNC transition across diverse bacterial species, as these mechanisms may share conserved features. Understanding these processes could enable the development of targeted strategies to prevent VBNC entry or resuscitation, thereby reducing the impact of the VBNC state on bacterial persistence in hospitals and infectious potential. Ultimately, integrating VBNC-specific protocols, such as the MORECOVERY, into routine monitoring could help mitigate the hidden threat posed by dormant pathogens in healthcare environments.

References

- Ahn, S., Jung, J., Jang, I.-A., Madsen, E. L., & Park, W. (2016). Role of glyoxylate shunt in oxidative stress response. *The Journal of Biological Chemistry*, 291(22), 11928–11938. <https://doi.org/10.1074/jbc.M115.708149>
- Almasaudi, S. B. (2018). *Acinetobacter* spp. as nosocomial pathogens: Epidemiology and resistance features. *Saudi Journal of Biological Sciences*, 25(3), 586–596. <https://doi.org/10.1016/j.sjbs.2016.02.009>
- Antunes, L. C. S., Visca, P., & Towner, K. J. (2014). *Acinetobacter baumannii*: evolution of a global pathogen. *Pathogens and Disease*, 71(3), 292–301. <https://doi.org/10.1111/2049-632X.12125>
- Asif, M., Alvi, I. A., & Rehman, S. U. (2018). Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infection and Drug Resistance*, 11, 1249–1260. <https://doi.org/10.2147/IDR.S166750>
- Ayrapetyan, M., Williams, T. C., Baxter, R., & Oliver, J. D. (2015). Viable but Nonculturable and persister cells coexist stochastically and are induced by human serum. *Infection and Immunity*, 83(11), 4194–4203. <https://doi.org/10.1128/IAI.00404-15>
- Ayrapetyan, M., Williams, T., & Oliver, J. D. (2018). Relationship between the Viable but Nonculturable state and antibiotic persister cells. *Journal of Bacteriology*, 200(20), e00249-18. <https://doi.org/10.1128/JB.00249-18>
- Balagurusamy, R., Gopi, L., Kumar, D. S. S., Viswanathan, K., Meganathan, V., Sathiyamurthy, K., & Athmanathan, B. (2024). Significance of Viable But Non-culturable (VBNC) state in vibrios and other pathogenic bacteria: induction, detection and the role of resuscitation promoting factors (Rpf). *Current Microbiology*, 81(12), 417. <https://doi.org/10.1007/s00284-024-03947-8>
- Ball, P. (2017). Water is an active matrix of life for cell and molecular biology. *Proceedings of the National Academy of Sciences of the United States of America*, 114(51), 13327–13335. <https://doi.org/10.1073/pnas.1703781114>
- Billi, D., & Potts, M. (2002). Life and death of dried prokaryotes. *Research in Microbiology*, 153(1), 7–12. [https://doi.org/10.1016/s0923-2508\(01\)01279-7](https://doi.org/10.1016/s0923-2508(01)01279-7)
- Boaretti, M., del Mar Lleo, M., Bonato, B., Signoretto, C., & Canepari, P. (2003). Involvement of *rpoS* in the survival of *Escherichia coli* in the viable but non-culturable state. *Environmental Microbiology*, 5(10), 986–996. <https://doi.org/10.1046/j.1462-2920.2003.00497.x>

-
- Boll, J. M., Tucker, A. T., Klein, D. R., Beltran, A. M., Brodbelt, J. S., Davies, B. W., & Trent, M. S. (2015). Reinforcing lipid A acylation on the cell surface of *Acinetobacter baumannii* promotes cationic antimicrobial peptide resistance and desiccation survival. *mBio*, 6(3), e00478-00415. <https://doi.org/10.1128/mBio.00478-15>
- Bollen, C., Louwagie, S., Deroover, F., Duverger, W., Khodaparast, L., Khodaparast, L., Hofkens, D., Schymkowitz, J., Rousseau, F., Dewachter, L., & Michiels, J. (2025). Composition and liquid-to-solid maturation of protein aggregates contribute to bacterial dormancy development and recovery. *Nature Communications*, 16, 1046. <https://doi.org/10.1038/s41467-025-56387-8>
- Bosch, J., Varliero, G., Hallsworth, J. E., Dallas, T. D., Hopkins, D., Frey, B., Kong, W., Lebre, P., Makhalanyane, T. P., & Cowan, D. A. (2021). Microbial anhydrobiosis. *Environmental Microbiology*, 23(11), 6377–6390. <https://doi.org/10.1111/1462-2920.15699>
- Brown, G. R., Sutcliffe, I. C., Bendell, D., & Cummings, S. P. (2000). The modification of the membrane of *Oceanomonas baumannii* when subjected to both osmotic and organic solvent stress. *FEMS Microbiology Letters*, 189(2), 149–154. <https://doi.org/10.1111/j.1574-6968.2000.tb09221.x>
- Cai, Y., Liu, J., Li, G., Wong, P. K., & An, T. (2021). Formation mechanisms of viable but nonculturable bacteria through induction by light-based disinfection and their antibiotic resistance gene transfer risk: a review. *Critical Reviews in Environmental Science and Technology*, 1–38. <https://doi.org/10.1080/10643389.2021.1932397>
- Campos, A., Lopes, M. S., Carnevalheira, A., Barbosa, J., & Teixeira, P. (2019). Survival of clinical and food *Acinetobacter* spp. Isolates exposed to different stress conditions. *Food Microbiology*, 77, 202–207. <https://doi.org/10.1016/j.fm.2018.09.009>
- Centers for Disease Control and Prevention (U.S.). (2019). Antibiotic resistance threats in the United States, 2019. *Centers for Disease Control and Prevention* (U.S.). <https://doi.org/10.15620/cdc:82532>
- Chapartegui-González, I., Lázaro-Díez, M., Bravo, Z., Navas, J., Icardo, J. M., & Ramos-Vivas, J. (2018). *Acinetobacter baumannii* maintains its virulence after long-time starvation. *PLoS ONE*, 13(8), e0201961. <https://doi.org/10.1371/journal.pone.0201961>
- Chin, C. Y., Tipton, K. A., Farokhyfar, M., Burd, E. M., Weiss, D. S., & Rather, P. N. (2018). A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. *Nature Microbiology*, 3(5), 563–569. <https://doi.org/10.1038/s41564-018-0151-5>
-

-
- Clancy, C. J., Schwartz, I. S., Kula, B., & Nguyen, M. H. (2021). Bacterial superinfections among persons with Coronavirus disease 2019: a comprehensive review of data from postmortem studies. *Open Forum Infectious Diseases*, 8(3), ofab065. <https://doi.org/10.1093/ofid/ofab065>
- Costa, O. Y. A., Raaijmakers, J. M., & Kuramae, E. E. (2018). Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. *Frontiers in Microbiology*, 9, 1636. <https://doi.org/10.3389/fmicb.2018.01636>
- Crowe, J. H., Carpenter, J. F., & Crowe, L. M. (1998). The role of vitrification in anhydrobiosis. *Annual Review of Physiology*, 60, 73–103. <https://doi.org/10.1146/annurev.physiol.60.1.73>
- Dewachter, L., Bollen, C., Wilmaerts, D., Louwagie, E., Herpels, P., Matthay, P., Khodaparast, L., Khodaparast, L., Rousseau, F., Schymkowitz, J., & Michiels, J. (2021). The dynamic transition of persistence toward the Viable but Nonculturable state during stationary phase is driven by protein aggregation. *mBio*, 12(4), e0070321. <https://doi.org/10.1128/mBio.00703-21>
- Di Martino, P. (2018). Extracellular polymeric substances, a key element in understanding biofilm phenotype. *AIMS Microbiology*, 4(2), 274–288. <https://doi.org/10.3934/microbiol.2018.2.274>
- Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nature Reviews Microbiology*, 5(12), 939–951. <https://doi.org/10.1038/nrmicro1789>
- Dong, K., Pan, H., Yang, D., Rao, L., Zhao, L., Wang, Y., & Liao, X. (2020). Induction, detection, formation, and resuscitation of viable but non-culturable state microorganisms. *Comprehensive Reviews in Food Science and Food Safety*, 19(1), 149–183. <https://doi.org/10.1111/1541-4337.12513>
- Dunn, M. F., Ramírez-Trujillo, J. A., & Hernández-Lucas, I. (2009). Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. *Microbiology (Reading, England)*, 155(Pt 10), 3166–3175. <https://doi.org/10.1099/mic.0.030858-0>
- Dworkin, J., & Shah, I. M. (2010). Exit from dormancy in microbial organisms. *Nature Reviews. Microbiology*, 8(12), 890–896. <https://doi.org/10.1038/nrmicro2453>
- Erkut, C., Gade, V. R., Laxman, S., & Kurzchalia, T. V. (2016). The glyoxylate shunt is essential for desiccation tolerance in *C. elegans* and budding yeast. *eLife*, 5, e13614. <https://doi.org/10.7554/eLife.13614>
- Esbelin, J., Santos, T., & Hébraud, M. (2018). Desiccation: an environmental and food industry stress that bacteria commonly face. *Food Microbiology*, 69, 82–88. <https://doi.org/10.1016/j.fm.2017.07.017>
-

-
- Ezraty, B., Gennaris, A., Barras, F., & Collet, J.-F. (2017). Oxidative stress, protein damage and repair in bacteria. *Nature Reviews Microbiology*, 15(7), 385–396. <https://doi.org/10.1038/nrmicro.2017.26>
- Fakruddin, Md., Mannan, K. S. B., & Andrews, S. (2013). Viable but Nonculturable bacteria: food safety and public health perspective. *International Scholarly Research Notices*, 2013(1), 703813. <https://doi.org/10.1155/2013/703813>
- Fardelli, E., Lucidi, M., Di Gioacchino, M., Bashiri, S., Persichetti, L., Capecchi, G., Gasperi, T., Sodo, A., Visca, P., & Capellini, G. (2022). Bio-physical mechanisms of dehydrating membranes of *Acinetobacter baumannii* linked to drought-resistance. *Biochimica Et Biophysica Acta. Biomembranes*, 1864(12), 184045. <https://doi.org/10.1016/j.bbamem.2022.184045>
- França, M. B., Panek, A. D., & Eleutherio, E. C. A. (2007). Oxidative stress and its effects during dehydration. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 146(4), 621–631. <https://doi.org/10.1016/j.cbpa.2006.02.030>
- Gayoso, C. M., Mateos, J., Méndez, J. A., Fernández-Puente, P., Rumbo, C., Tomás, M., Martínez de Ilarduya, Ó., & Bou, G. (2014). Molecular mechanisms involved in the response to desiccation stress and persistence in *Acinetobacter baumannii*. *Journal of Proteome Research*, 13(2), 460–476. <https://doi.org/10.1021/pr400603f>
- Geiser, F. (2013). Hibernation. *Current Biology: CB*, 23(5), R188-193. <https://doi.org/10.1016/j.cub.2013.01.062>
- Giannouli, M., Antunes, L. C. S., Marchetti, V., Triassi, M., Visca, P., & Zarrilli, R. (2013). Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78. *BMC Infectious Diseases*, 13, 282. <https://doi.org/10.1186/1471-2334-13-282>
- Giovannini, I., Boothby, T. C., Cesari, M., Goldstein, B., Guidetti, R., & Rebecchi, L. (2022). Production of reactive oxygen species and involvement of bioprotectants during anhydrobiosis in the tardigrade *Paramacrobiotus spatialis*. *Scientific Reports*, 12(1), 1938. <https://doi.org/10.1038/s41598-022-05734-6>
- Grant, W. D. (2004). Life at low water activity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359(1448), 1249–1267. <https://doi.org/10.1098/rstb.2004.1502>
- Green, E. R., Fakhoury, J. N., Monteith, A. J., Pi, H., Giedroc, D. P., & Skaar, E. P. (2022). Bacterial hydrophilins promote pathogen desiccation tolerance. *Cell Host & Microbe*, 30(7), 975-987.e7. <https://doi.org/10.1016/j.chom.2022.03.019>
-

-
- Grefte, V. R. G., & Michiels, J. (2020). Desiccation-induced cell damage in bacteria and the relevance for inoculant production. *Applied Microbiology and Biotechnology*, 104(9), 3757–3770. <https://doi.org/10.1007/s00253-020-10501-6>
- Grzyb, T., & Skłodowska, A. (2022). Introduction to bacterial anhydrobiosis: a general perspective and the mechanisms of desiccation-associated damage. *Microorganisms*, 10(2), 432. <https://doi.org/10.3390/microorganisms10020432>
- Ha, S., Shin, B., & Park, W. (2018). Lack of glyoxylate shunt dysregulates iron homeostasis in *Pseudomonas aeruginosa*. *Microbiology (Reading, England)*, 164(4), 587–599. <https://doi.org/10.1099/mic.0.000623>
- Harding, C. M., Hennon, S. W., & Feldman, M. F. (2018). Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nature Reviews. Microbiology*, 16(2), 91–102. <https://doi.org/10.1038/nrmicro.2017.148>
- Huang, M., & Hull, C. M. (2017). Sporulation: how to survive on planet Earth (and beyond). *Current Genetics*, 63(5), 831–838. <https://doi.org/10.1007/s00294-017-0694-7>
- Iii, J. M. F., Wells, G., & Pesci, E. C. (2018). Desiccation tolerance in *Acinetobacter baumannii* is mediated by the two-component response regulator BfmR. *PLOS ONE*, 13(10), e0205638. <https://doi.org/10.1371/journal.pone.0205638>
- Kelly, S., Schnugh, D., & Thomas, T. (2022). Effectiveness of ultraviolet-C vs aerosolized hydrogen peroxide in ICU terminal disinfection. *The Journal of Hospital Infection*, 121, 114–119. <https://doi.org/10.1016/j.jhin.2021.12.004>
- König, P., Wilhelm, A., Schaudinn, C., Poehlein, A., Daniel, R., Widera, M., Averhoff, B., & Müller, V. (2023). The VBNC state: a fundamental survival strategy of *Acinetobacter baumannii*. *mBio*, 14(5), e0213923. <https://doi.org/10.1128/mbio.02139-23>
- Lebre, P. H., De Maayer, P., & Cowan, D. A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, 15(5), 285–296. <https://doi.org/10.1038/nrmicro.2017.16>
- Lennon, J. T., den Hollander, F., Wilke-Berenguer, M., & Blath, J. (2021). Principles of seed banks and the emergence of complexity from dormancy. *Nature Communications*, 12(1), 4807. <https://doi.org/10.1038/s41467-021-24733-1>
- Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: The ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, 9(2), 119–130. <https://doi.org/10.1038/nrmicro2504>
-

-
- Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, 5, 258. <https://doi.org/10.3389/fmicb.2014.00258>
- Liu, J., Yang, L., Kjellerup, B. V., & Xu, Z. (2023). Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. *Trends in Microbiology*, 31(10), 1013–1023. <https://doi.org/10.1016/j.tim.2023.04.009>
- Lopalco, P., Stahl, J., Annese, C., Averhoff, B., & Corcelli, A. (2017). Identification of unique cardiolipin and monolysocardiolipin species in *Acinetobacter baumannii*. *Scientific Reports*, 7(1), 2972. <https://doi.org/10.1038/s41598-017-03214-w>
- Lucidi, M., Capecchi, G., Spagnoli, C., Basile, A., Artuso, I., Persichetti, L., Fardelli, E., Capellini, G., Visaggio, D., Imperi, F., Rampioni, G., Leoni, L., & Visca, P. (n.d.). The response to desiccation in *Acinetobacter baumannii*. *Virulence*, 16(1), 2490209. <https://doi.org/10.1080/21505594.2025.2490209>
- Lucidi, M., Visaggio, D., Migliaccio, A., Capecchi, G., Visca, P., Imperi, F., & Zarrilli, R. (2024). Pathogenicity and virulence of *Acinetobacter baumannii*: factors contributing to the fitness in healthcare settings and the infected host. *Virulence*, 15(1), 2289769. <https://doi.org/10.1080/21505594.2023.2289769>
- Macesic, N., Uhlemann, A.-C., & Peleg, A. Y. (2025). Multidrug-resistant Gram-negative bacterial infections. *Lancet (London, England)*, 405(10474), 257–272. [https://doi.org/10.1016/S0140-6736\(24\)02081-6](https://doi.org/10.1016/S0140-6736(24)02081-6)
- Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18(3), 268–281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>
- McDonald, M. D., Owusu-Ansah, C., Ellenbogen, J. B., Malone, Z. D., Ricketts, M. P., Frolking, S. E., Ernakovich, J. G., Ibba, M., Bagby, S. C., & Weissman, J. L. (2024). What is microbial dormancy? *Trends in Microbiology*, 32(2), 142–150. <https://doi.org/10.1016/j.tim.2023.08.006>
- Mitchell, B. G., McDonagh, J., Dancer, S. J., Ford, S., Sim, J., Khadar, B. T. S. A., Russo, P. L., Maillard, J.-Y., Rawson, H., Browne, K., & Kiernan, M. (2023). Risk of organism acquisition from
-

-
- prior room occupants: an updated systematic review. *Infection, Disease & Health*, 28(4), 290–297. <https://doi.org/10.1016/j.idh.2023.06.001>
- Nandakumar, M., Nathan, C., & Rhee, K. Y. (2014). Isocitrate lyase mediates broad antibiotic tolerance in *Mycobacterium tuberculosis*. *Nature Communications*, 5(1), 4306. <https://doi.org/10.1038/ncomms5306>
- Niu, H., Gu, J., & Zhang, Y. (2024). Bacterial persisters: Molecular mechanisms and therapeutic development. *Signal Transduction and Targeted Therapy*, 9(1), 174. <https://doi.org/10.1038/s41392-024-01866-5>
- Norton, M. D., Spilkia, A. J., & Godoy, V. G. (2013). Antibiotic resistance acquired through a dna damage-inducible response in *Acinetobacter baumannii*. *Journal of Bacteriology*, 195(6), 1335–1345. <https://doi.org/10.1128/JB.02176-12>
- Oliver, J. D. (2010). Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiology Reviews*, 34(4), 415–425. <https://doi.org/10.1111/j.1574-6976.2009.00200.x>
- Palethorpe, S., Farrow, J. M., Wells, G., Milton, M. E., Actis, L. A., Cavanagh, J., & Pesci, E. C. (2022). *Acinetobacter baumannii* regulates its stress responses via the BfmRS two-component regulatory system. *Journal of Bacteriology*, 204(2), e0049421. <https://doi.org/10.1128/JB.00494-21>
- Park, C., Shin, B., & Park, W. (2019). Alternative fate of glyoxylate during acetate and hexadecane metabolism in *Acinetobacter oleivorans* DR1. *Scientific Reports*, 9(1), 14402. <https://doi.org/10.1038/s41598-019-50852-3>
- Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews*, 21(3), 538–582. <https://doi.org/10.1128/CMR.00058-07>
- Potts, M. (1994). Desiccation tolerance of prokaryotes. *Microbiological Reviews*, 58(4), 755–805. <https://doi.org/10.1128/mr.58.4.755-805.1994>
- Potts, M., Slaughter, S. M., Hunneke, F.-U., Garst, J. F., & Helm, R. F. (2005). Desiccation tolerance of prokaryotes: application of principles to human cells. *Integrative and Comparative Biology*, 45(5), 800–809. <https://doi.org/10.1093/icb/45.5.800>
- Qi, Z., Sun, N., & Liu, C. (2023). Glyoxylate cycle maintains the metabolic homeostasis of *Pseudomonas aeruginosa* in viable but nonculturable state induced by chlorine stress. *Microbiological Research*, 270, 127341. <https://doi.org/10.1016/j.micres.2023.127341>
-

-
- Ramamurthy, T., Ghosh, A., Pazhani, G. P., & Shinoda, S. (2014). Current perspectives on Viable but Non-Culturable (VBNC) pathogenic bacteria. *Frontiers in Public Health*, 2, 103. <https://doi.org/10.3389/fpubh.2014.00103>
- Ramos, J. L., Gallegos, M. T., Marqués, S., Ramos-González, M. I., Espinosa-Urgel, M., & Segura, A. (2001). Responses of Gram-negative bacteria to certain environmental stressors. *Current Opinion in Microbiology*, 4(2), 166–171. [https://doi.org/10.1016/s1369-5274\(00\)00183-1](https://doi.org/10.1016/s1369-5274(00)00183-1)
- Rawlinson, S., Ciric, L., & Cloutman-Green, E. (2019). How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. *The Journal of Hospital Infection*, 103(4), 363–374. <https://doi.org/10.1016/j.jhin.2019.07.015>
- Rittershaus, E. S. C., Baek, S.-H., & Sassetti, C. M. (2013). The normalcy of dormancy: common themes in microbial quiescence. *Cell Host & Microbe*, 13(6), 643–651. <https://doi.org/10.1016/j.chom.2013.05.012>
- Rockinger, U., Funk, M., & Winter, G. (2021). Current approaches of preservation of cells during (freeze-) drying. *Journal of Pharmaceutical Sciences*, 110(8), 2873–2893. <https://doi.org/10.1016/j.xphs.2021.04.018>
- Russo, A., Bruni, A., Gulli, S., Borrazzo, C., Quirino, A., Lionello, R., Serapide, F., Garofalo, E., Serraino, R., Romeo, F., Marascio, N., Matera, G., Longhini, F., Trecarichi, E. M., & Torti, C. (2023). Efficacy of cefiderocol- vs colistin-containing regimen for treatment of bacteraemic ventilator-associated pneumonia caused by carbapenem-resistant *Acinetobacter baumannii* in patients with COVID-19. *International Journal of Antimicrobial Agents*, 62(1), 106825. <https://doi.org/10.1016/j.ijantimicag.2023.106825>
- Russotto, V., Cortegiani, A., Raineri, S. M., & Giarratano, A. (2015). Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *Journal of Intensive Care*, 3, 54. <https://doi.org/10.1186/s40560-015-0120-5>
- Sandu, A. M., Chifiriuc, M. C., Vrancianu, C. O., Cristian, R.-E., Alistar, C. F., Constantin, M., Paun, M., Alistar, A., Popa, L. G., Popa, M. I., Tantu, A. C., Sidoroff, M. E., Mihai, M. M., Marcu, A., Popescu, G., & Tantu, M. M. (2025). Healthcare-Associated Infections: the role of microbial and environmental factors in infection control—a narrative review. *Infectious Diseases and Therapy*, 14(5), 933–971. <https://doi.org/10.1007/s40121-025-01143-0>
- Setlow, P. (2014). Germination of spores of *Bacillus* species: what we know and do not know. *Journal of Bacteriology*, 196(7), 1297–1305. <https://doi.org/10.1128/JB.01455-13>
-

-
- Signoretto, C., del Mar Lleò, M., Tafi, M. C., & Canepari, P. (2000). Cell wall chemical composition of *Enterococcus faecalis* in the Viable but Nonculturable state. *Applied and Environmental Microbiology*, 66(5), 1953–1959.
- Singer, S. J., & Nicolson, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science (New York, N.Y.)*, 175(4023), 720–731. <https://doi.org/10.1126/science.175.4023.720>
- Strich, J. R., & Palmore, T. N. (2017). Preventing transmission of multidrug-resistant pathogens in the Intensive Care Unit. *Infectious Disease Clinics of North America*, 31(3), 535–550. <https://doi.org/10.1016/j.idc.2017.05.010>
- Sukhum, K. V., Newcomer, E. P., Cass, C., Wallace, M. A., Johnson, C., Fine, J., Sax, S., Barlet, M. H., Burnham, C.-A. D., Dantas, G., & Kwon, J. H. (2022). Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. *Communications Medicine*, 2, 62. <https://doi.org/10.1038/s43856-022-00124-5>
- Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., ... Zorzet, A. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, 18(3), 318–327. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3)
- Valcek, A., Kröger, C., de Dios, R., McCarthy, R. R., Coenye, T., Trent, M. S., Karah, N., Uhlin, B. E., Classen, A. Y., Higgins, P. G., Ebersberger, I., Laaberki, M.-H., Charpentier, X., Visca, P., Rather, P. N., & Van der Henst, C. (2025). Inter- and intra-bacterial strain diversity remains the “elephant in the (living) room”. *Npj Antimicrobials and Resistance*, 3, 67. <https://doi.org/10.1038/s44259-025-00138-8>
- van der Schoor, A. S., Boyle, M., Voor In 't Holt, A. F., Vos, M. C., Humphreys, H., & ESCMID Study Group for Nosocomial Infections. (2022). Environmental sampling of innate hospital surfaces: a survey of current practices and the need for guidelines. *The Journal of Hospital Infection*, 128, 92–95. <https://doi.org/10.1016/j.jhin.2022.07.024>
- Visca, P., Seifert, H., & Towner, K. J. (2011). Acinetobacter infection – an emerging threat to human health. *IUBMB Life*, 63(12), 1048–1054. <https://doi.org/10.1002/iub.534>
- Wang, T.-Y., Libardo, M. D. J., Angeles-Boza, A. M., & Pellois, J.-P. (2017). Membrane oxidation in cell delivery and cell killing applications. *ACS Chemical Biology*, 12(5), 1170–1182. <https://doi.org/10.1021/acscchembio.7b00237>
-

-
- Wang, X., Cole, C. G., DuPai, C. D., & Davies, B. W. (2020). Protein aggregation is associated with *Acinetobacter baumannii* desiccation tolerance. *Microorganisms*, 8(3), E343. <https://doi.org/10.3390/microorganisms8030343>
- Weber, D. J., Rutala, W. A., Anderson, D. J., Chen, L. F., Sickbert-Bennett, E. E., & Boyce, J. M. (2016). Effectiveness of ultraviolet devices and hydrogen peroxide systems for terminal room decontamination: Focus on clinical trials. *American Journal of Infection Control*, 44(5 Suppl), e77-84. <https://doi.org/10.1016/j.ajic.2015.11.015>
- Woh, P. Y., & Zhang, X. (2025). The burden of ESKAPE pathogen-related hospital-acquired infections: clinical and financial perspective from a systematic review. *Journal of Hospital Infection*, 162, 377–385. <https://doi.org/10.1016/j.jhin.2025.06.006>
- Xu, H.-S., Roberts, N., Singleton, F. L., Attwell, R. W., Grimes, D. J., & Colwell, R. R. (1982). Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. *Microbial Ecology*, 8(4), 313–323. <https://doi.org/10.1007/BF02010671>
- Zeidler, S., Hubloher, J., König, P., Ngu, N. D., Scholz, A., Averhoff, B., & Müller, V. (2018). Salt induction and activation of MtlD, the key enzyme in the synthesis of the compatible solute mannitol in *Acinetobacter baumannii*. *MicrobiologyOpen*, 7(6), e00614. <https://doi.org/10.1002/mbo3.614>
- Zeidler, S., & Müller, V. (2019a). Coping with low water activities and osmotic stress in *Acinetobacter baumannii*: significance, current status and perspectives. *Environmental Microbiology*, 21(7), 2212–2230. <https://doi.org/10.1111/1462-2920.14565>
- Zeidler, S., & Müller, V. (2019b). The role of compatible solutes in desiccation resistance of *Acinetobacter baumannii*. *MicrobiologyOpen*, 8(5), e00740. <https://doi.org/10.1002/mbo3.740>
- Zhang, J., Wang, L., Shi, L., Chen, X., Chen, C., Hong, Z., Cao, Y., & Zhao, L. (2020). Survival strategy of *Cronobacter sakazakii* against ampicillin pressure: induction of the viable but nonculturable state. *International Journal of Food Microbiology*, 334, 108819. <https://doi.org/10.1016/j.ijfoodmicro.2020.108819>
- Zhang, S., Ye, C., Lin, H., Lv, L., & Yu, X. (2015). UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa*. *Environmental Science & Technology*, 49(3), 1721–1728. <https://doi.org/10.1021/es505211e>
- Zhao, S., Dou, C., Zhang, J., Huang, L., Gao, Y., Du, B., Cui, X., Zhao, H., Xue, G., Ke, Y., Gan, L., Feng, J., Feng, Y., Cui, J., Yan, C., Xu, Z., Fu, T., Yu, Z., Yang, Y., ... You, F. (2024). Multiple factors trigger the formation and resuscitation of the VBNC state in alcohol-producing *Klebsiella*
-

pneumoniae. *Applied and Environmental Microbiology*, 90(7), e0055724.
<https://doi.org/10.1128/aem.00557-24>

Zhou, M., Huang, Y., Zhang, Y., Wang, Q., Ma, Y., & Shao, S. (2022). Roles of virulence regulator ToxR in viable but non-culturable formation by controlling reactive oxygen species resistance in pathogen *Vibrio alginolyticus*. *Microbiological Research*, 254, 126900.
<https://doi.org/10.1016/j.micres.2021.126900>

List of publications

Lucidi, M., Capecchi, G., **Spagnoli, C.**, Basile, A., Artuso, I., Persichetti, L., Fardelli, E., Capellini, G., Visaggio, D., Imperi, F., Rampioni, G., Leoni, L., & Visca, P. (2025). The response to desiccation in *Acinetobacter baumannii*. *Virulence*, 16(1), 2490209. <https://doi.org/10.1080/21505594.2025.2490209>

Visaggio, D., Lucidi, M., **Spagnoli, C.**, Ciccone, I., Imperi, F., & Visca, P. (2026). ESKAPE Gram-negative bacteria escape culture-based detection upon desiccation on abiotic surfaces. *Microbiology Spectrum*, e0335725. <https://doi.org/10.1128/spectrum.03357-25>

Spagnoli, C., Kunkle, D. E., Visaggio, D., Visca, P., Skaar, E. P. Transcriptomic landscape associated with the resuscitation of viable but not culturable *Acinetobacter baumannii* cells. Manuscript in preparation.

List of publications not included in this thesis

Lucidi, M., Imperi, F., Artuso, I., Capecchi, G., **Spagnoli, C.**, Visaggio, D., Rampioni, G., Leoni, L., & Visca, P. (2024). Phage-mediated colistin resistance in *Acinetobacter baumannii*. *Drug Resistance Updates*. 73, 101061. <https://doi.org/10.1016/j.drug.2024.101061>

Participation in national and international conferences

C. Spagnoli, D. Visaggio, P. Visca, E. P. Skaar. Molecular mechanisms implicated in the resuscitation of *Acinetobacter baumannii* VBNC cells induced by desiccation - *Acinetobacter* Online Conference. November 25th, 2025, online – **Flash presentation.**

C. Spagnoli, D. Visaggio, P. Visca, E. P. Skaar. Molecular mechanisms implicated in the resuscitation of *Acinetobacter baumannii* VBNC cells induced by desiccation - XXXV SIMGBM Congress. September 17-20, 2025, Rome, Italy – **Oral presentation.**

C. Spagnoli, D. Visaggio, P. Visca, E. P. Skaar. Molecular mechanisms implicated in the resuscitation of *Acinetobacter baumannii* VBNC cells induced by desiccation - *Acinetobacter* symposium “Biology of *Acinetobacter* XIV”. July 6 - 10, 2025, Niagara Falls, New York – **Poster.**

C. Spagnoli, G. Capecchi, M. Lucidi, D. Visaggio, P. Visca. Transition to the viable but not culturable state as an adaptive strategy of bacterial pathogens to survive desiccation in hospital settings - Cortona Prokaryotes 2024, Cortona (Italy), 2024 – **Oral presentation.**

G. Capecchi, M. Lucidi, D. Visaggio, I. Artuso, C. Spagnoli, L. Persichetti, E. Fardelli, G. Capellini, G. Rampioni, L. Leoni, F. Imperi, P. Visca. Desiccation induces apparent death in the pathogenic bacterium *Acinetobacter baumannii*. XXXIV SIMGBM CONGRESS - MICROBIOLOGY 2023, Cagliari (Italy), 2023 – **Attendee.**