Dormancy in Spacecraft-Associated Actinobacteria: Implications for Mars Planetary Protection

June 1-2, 2026

Committee on Planetary Protection Virtual Meeting:
Limits of Terrestrial Life and the Probability of Growth on Mars,
Aeronautics, Space, and Astronomy
Center for Advancing Science and Technology (CAST)
National Academies of Sciences, Engineering, and Medicine

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College of Natural Sciences and Mathematics

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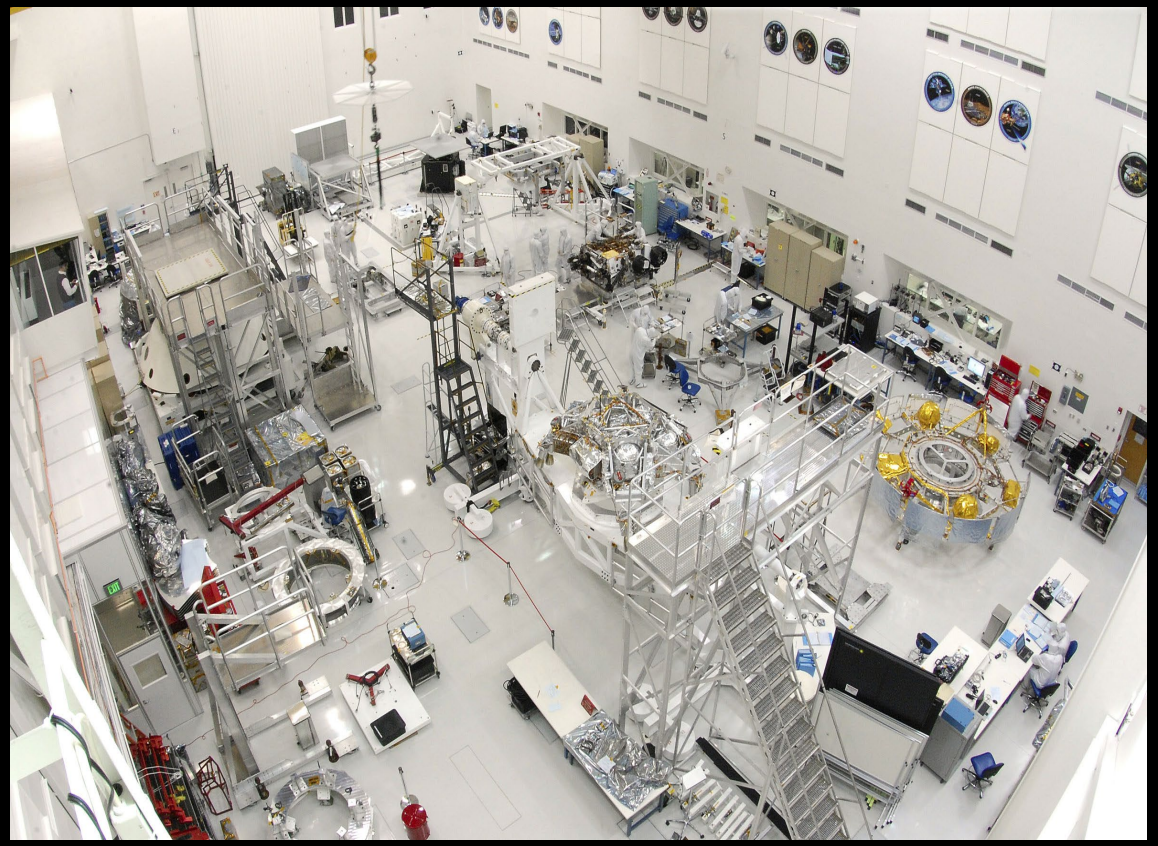
SPACECRAFT ASSEMBLY CLEAN ROOMS (SACs)/ FACILITY (SAF)

- low nutrients
- dry/controlled environments
- repeated cleaning/ decontamination



Selection pressure for microbial persistence

Bashir, M., et al (2016). *Frontiers in Microbiology,* PMID: 27667984
Ghosh, S., et al (2010). *Astrobiology*, PMID: 20446872
Moissl, C., et al (2007). *FEMS Microbiology Ecology*, PMID: 17655710

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Microbial contamination of spacecraft and associated environments

Predominant Spacecraft cleanroom contaminants are Gram-positive, spore-forming *Bacillus* species

Sporulation

Non-spore forming Gram-positive, Actinobacterial species

Non-cultivability/
Non-spore dormancy
less appreciated

Schultz J, *et al* (2025). Microbiome. PMID: 40350519

Moissl-Eichinger C, *et al* (2012). Astrobiology. PMID: 23121015

Muthamilselvi Sivabalan SK, et al (2026). Microbiology Spectrum. PMID: 42095681

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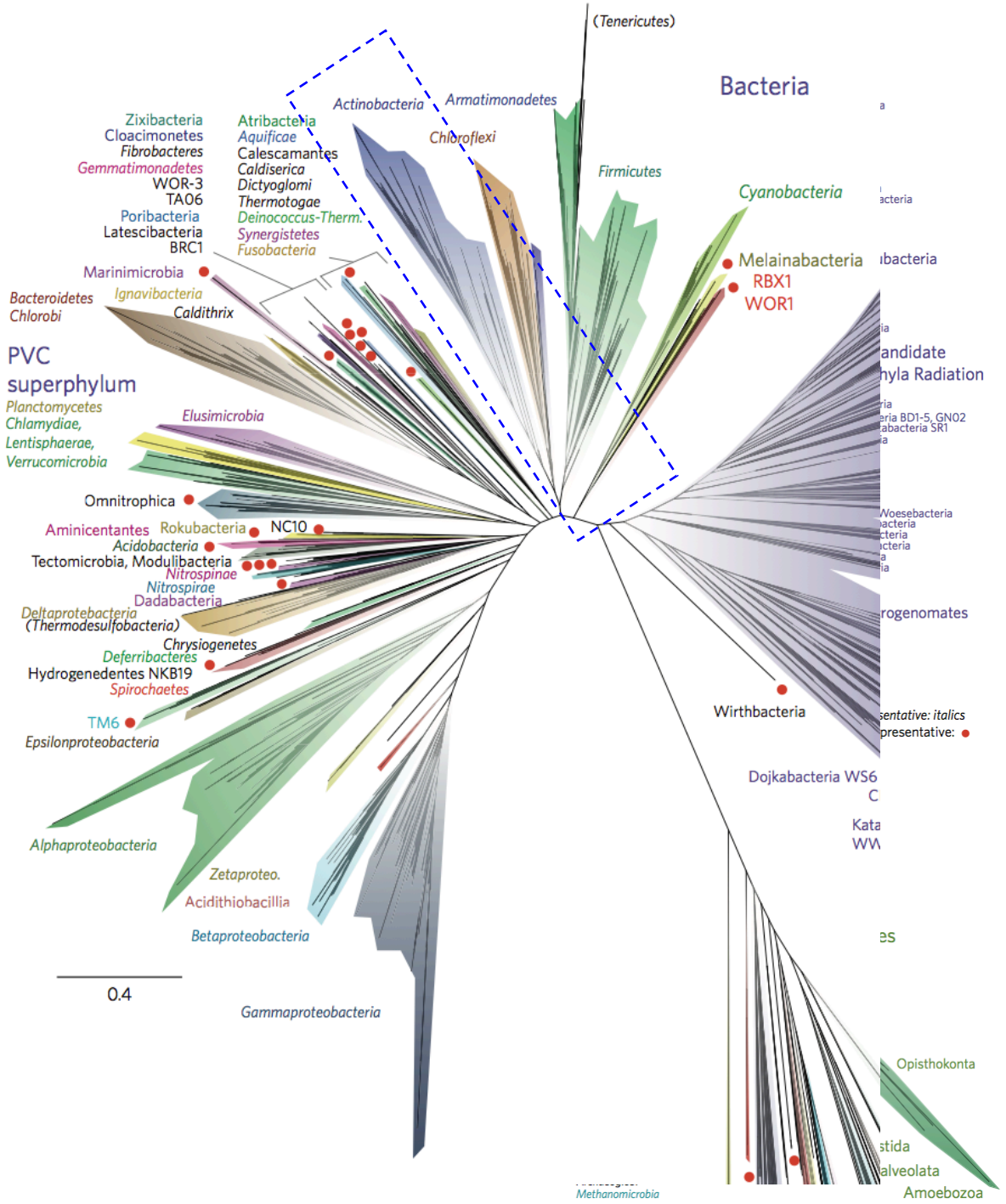

diverse phylum of Gram-positive bacteria

Hug, Laura A *et al* (2016), *Nature Microbiology* PMID 27572647

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Actinobacterial species

Non-spore forming Gram-positive, **Actinobacterial** species

Non-cultivability

Viable but nonculturable (VBNC) bacteria:

in a state of very low metabolic activity

do not divide,

but are alive and

can become culturable once resuscitated

Mali S, *et al* (2017) *J Bacteriol*. PMID: 28484042

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Why Dormancy Matters?

Dormancy = survival strategy under extreme stress

***Actinobacterial* strain *Micrococcus luteus* - revived from 125 million-year-old amber**

Dormancy of the tuberculosis causing *Mycobacterium tuberculosis*, which enters a state of clinical latency to evade the effects of antibiotics

Greenblatt C, *et al* (2004). Microb Ecol . PMID: 15164240

Gupta VK, *et al* (2018). Infect Dis (Lond). PMID: 28933243

Why Dormancy Matters for Mars Planetary Protection

VBNC = viable but non-culturable

Dormancy
Cells are alive but inactive

↓

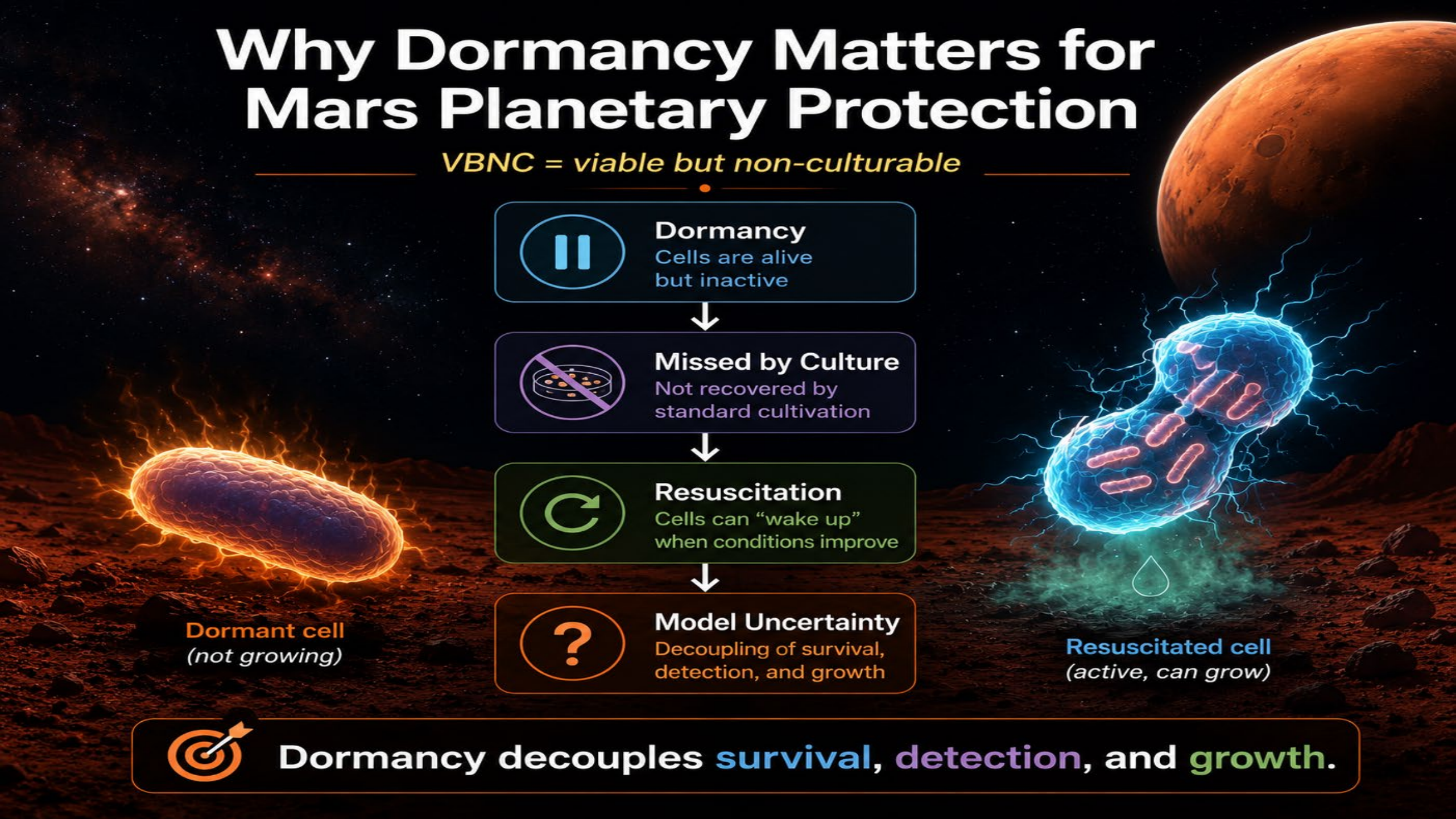
Missed by Culture
Not recovered by standard cultivation

↓

Resuscitation
Cells can "wake up" when conditions improve

↓

Model Uncertainty
Decoupling of survival, detection, and growth



Dormancy decouples survival, detection, and growth.

Actinobacteria Recur in Spacecraft-Associated Systems

› Astrobiology. 2010 Apr;10(3):325-35. doi: 10.1089/ast.2009.0396.

Recurrent isolation of extremotolerant bacteria from the clean room where Phoenix spacecraft components were assembled

Sudeshna Ghosh [1], Shariff Osman, Parag Vaishampayan, Kasthuri Venkateswaran

Affiliations + expand

PMID: 20446872 DOI: 10.1089/ast.2009.0396

Free article

Phylum	No of strains	%
Firmicutes	29	41
Actinobacteria	**25**	**36**
Proteobacteria	14	20
Others	2	3
Total	70	

Payload Hazardous Servicing Facility at NASA's Kennedy Space Center in Florida

https://www.nasa.gov/news-release/nasa-offers-media-access-to-cygnus-cargo-module-nov-13/

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

Microbiol Resour Announc. 2023 Feb 22;12(3):e01011-22. doi: 10.1128/mra.01011-22

Draft Genome Sequences of Spacecraft-Associated Microbes Isolated from Six NASA Missions

Michelle T Tran [a,*], Arman Seuylemezian [b], Alonna Wright [c], David Coil [c], Jonathan Eisen [c], Lisa Guan [b,✉]

Editor: Irene L G Newton[d]

Author information · Article notes · Copyright and License information

PMCID: PMC10019167 PMID: 36840549

Phylum	Count	%
Firmicutes	158	86%
Actinobacteria	**19**	**10%**
Proteobacteria	6	3%
Total	183	

Actinobacteria
Micrococcus luteus
Kocuria palustris
Rothia sp.
Microbacterium hydrocarbonoxydans
Microbacterium sp.
Curtobacterium sp.
Cellulosimicrobium funkei
Agromyces mediolanus
Janibacter melonis
Georgenia satyanarayanai

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Actinobacteria from spacecraft assembly clean rooms (SACs)

Clean room at NASA's Kennedy Space Center

Case study

***Tersicoccus phoenicis* 1P05MA^T**
(= NRRL B-59547^T = DSM 30849^T)

Vaishampayan P, Moissl-Eichinger C, Pukall R, Schumann P, Spröer C, Augustus A, Roberts AH, Namba G, Cisneros J, Salmassi T, Venkateswaran K. Description of *Tersicoccus phoenicis* gen. nov., sp. nov. isolated from spacecraft assembly clean room environments. *Int J Syst Evol Microbiol*. 2013 Jul;63(Pt 7):2463-2471. doi: 10.1099/ijs.0.047134-0. Epub 2012 Dec 7. PMID: 23223813.

https://www.nasa.gov/image-article/new-horizons-spacecraft-clean-room-nasas-kennedy-space-center/

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Tersicoccus phoenicis DSM 30849 (1P05MA)

Non-spore-forming SAC isolate

Genome

- 3.21 Mb | 132 scaffolds | 3,185 genes
- 3,128 protein-coding | 46 tRNAs | 104 pseudogenes

Unique Genes (492)

- Toxin-antitoxin | Phage elements | Transporters
  DNA-binding | Hypothetical

- 2 full-length Rpf homologs → revive dormant cells

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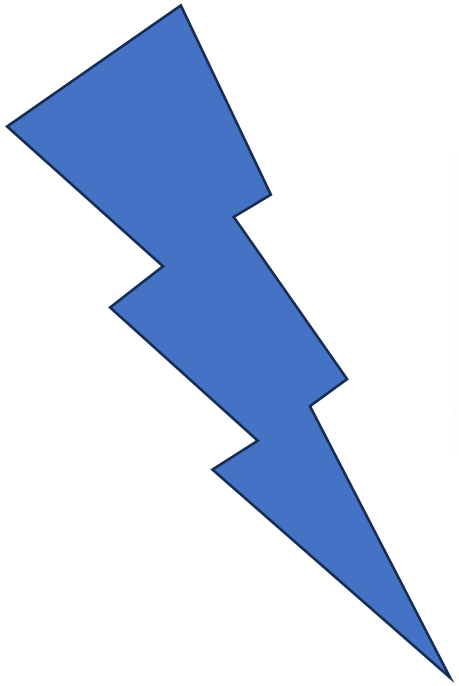
Tersicoccus phoenicis DSM 30849 (1P05MA)

Non-spore-forming SAC isolate

Our Research Question



- Hypothesis: enters & exits dormancy
- Experimentally testable?

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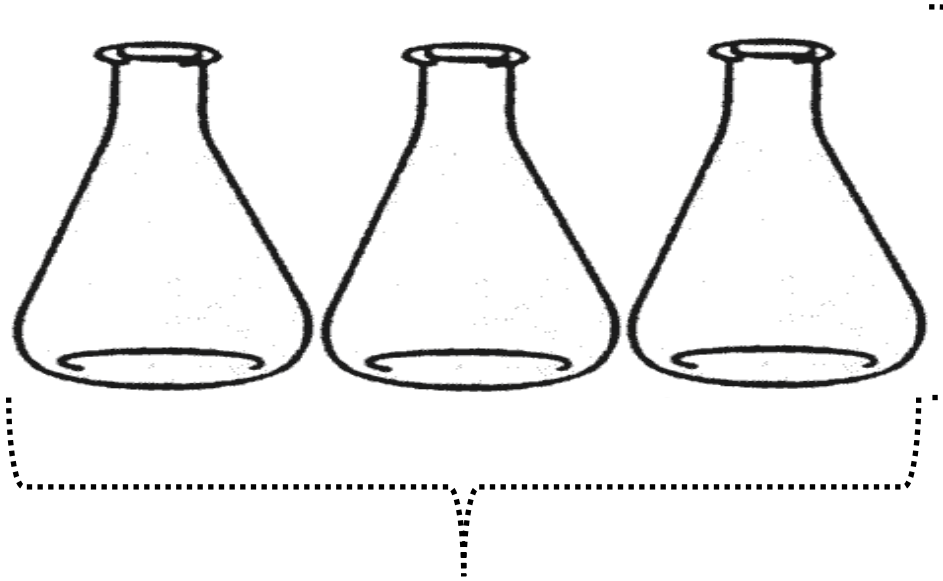
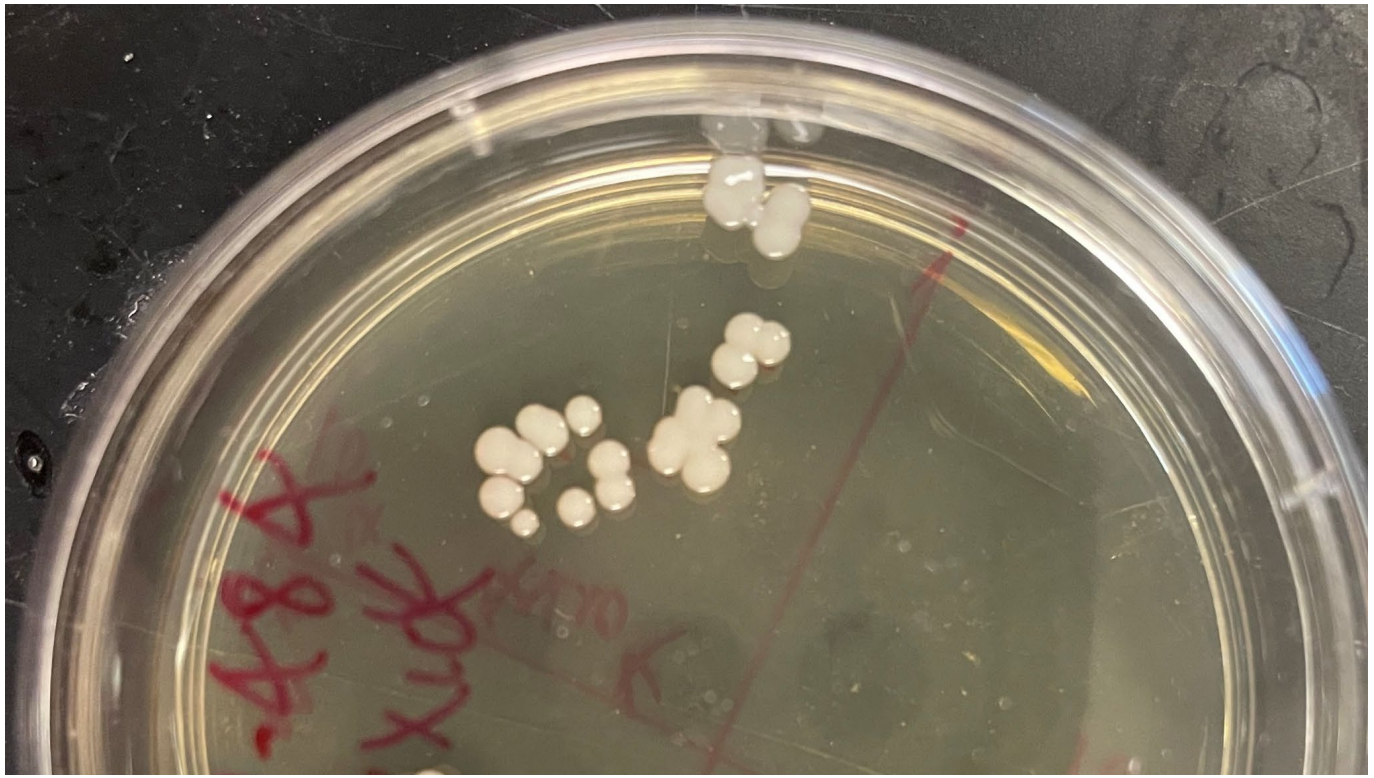
(= NRRL B-59547^T = DSM 30849^T)



Triplicates

Acetate minimal media (AMM) starvation model

- **Optical density (A600)**
- **Plated on LB agar plates - colony forming units (CFUs)**



Tirumalai M, Ali S, Fox GE, Widger W. *Tersicoccus phoenicis* (Actinobacteria), a spacecraft clean room isolate, exhibits dormancy. Microbiol Spectr. 2025 Sep 2;13(9):e0169225. doi: 10.1128/spectrum.01692-25. Epub 2025 Aug 11. PMID: 40788184; PMCID: PMC12403811.

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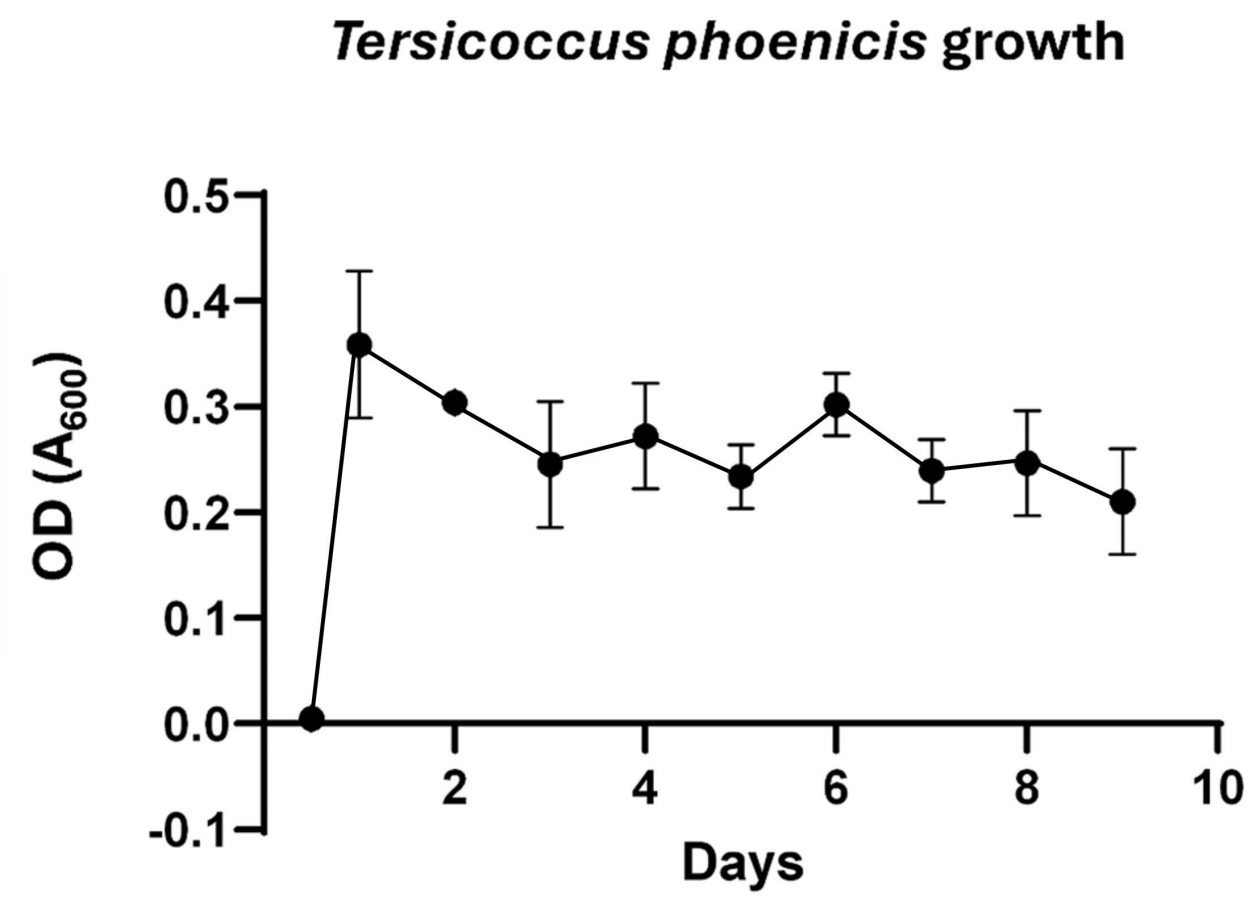

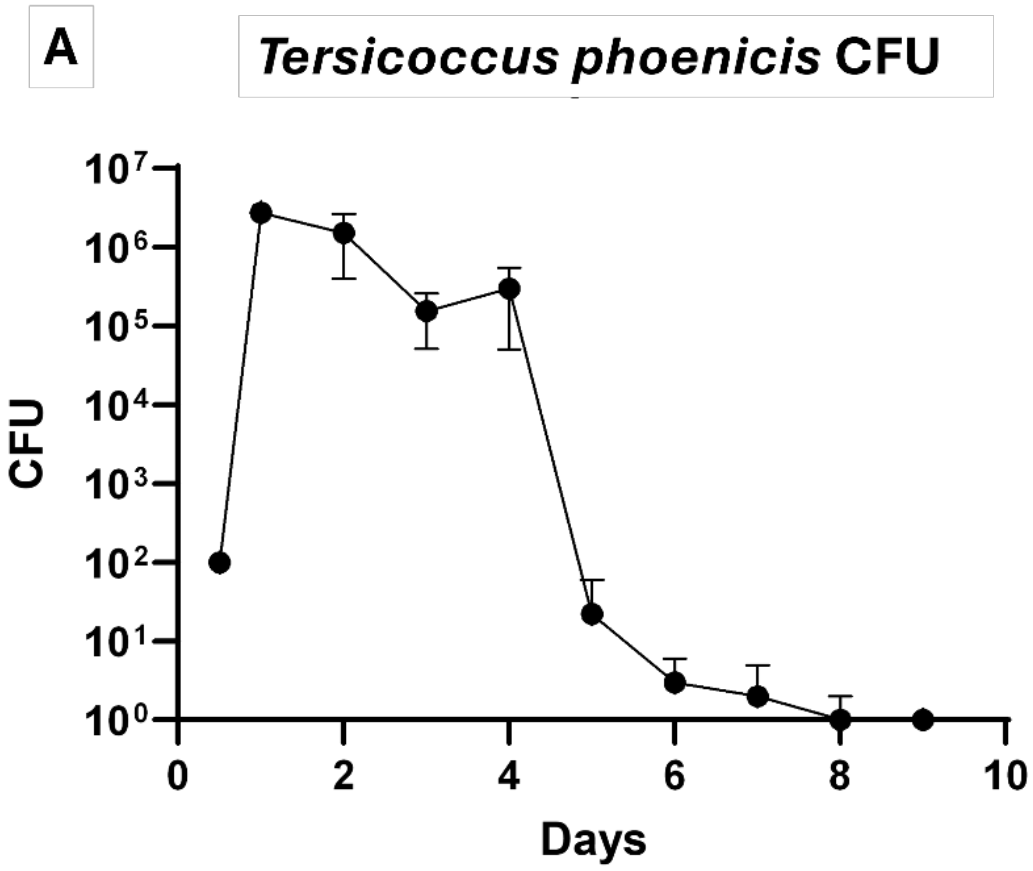

The OD reached a maximum of 0.25-0.3 by day 3 and was maintained at day 9

The viable counts reached a maximum between 1 and 2 days and rapidly decreased after day 4

SAC isolate *Tersicoccus phoenicis* 1P05MA^T enters dormancy after 4 days of growth

Tirumalai M, Ali S, Fox GE, Widger W. 2025. *Microbiol Spectr* PMID: 40788184

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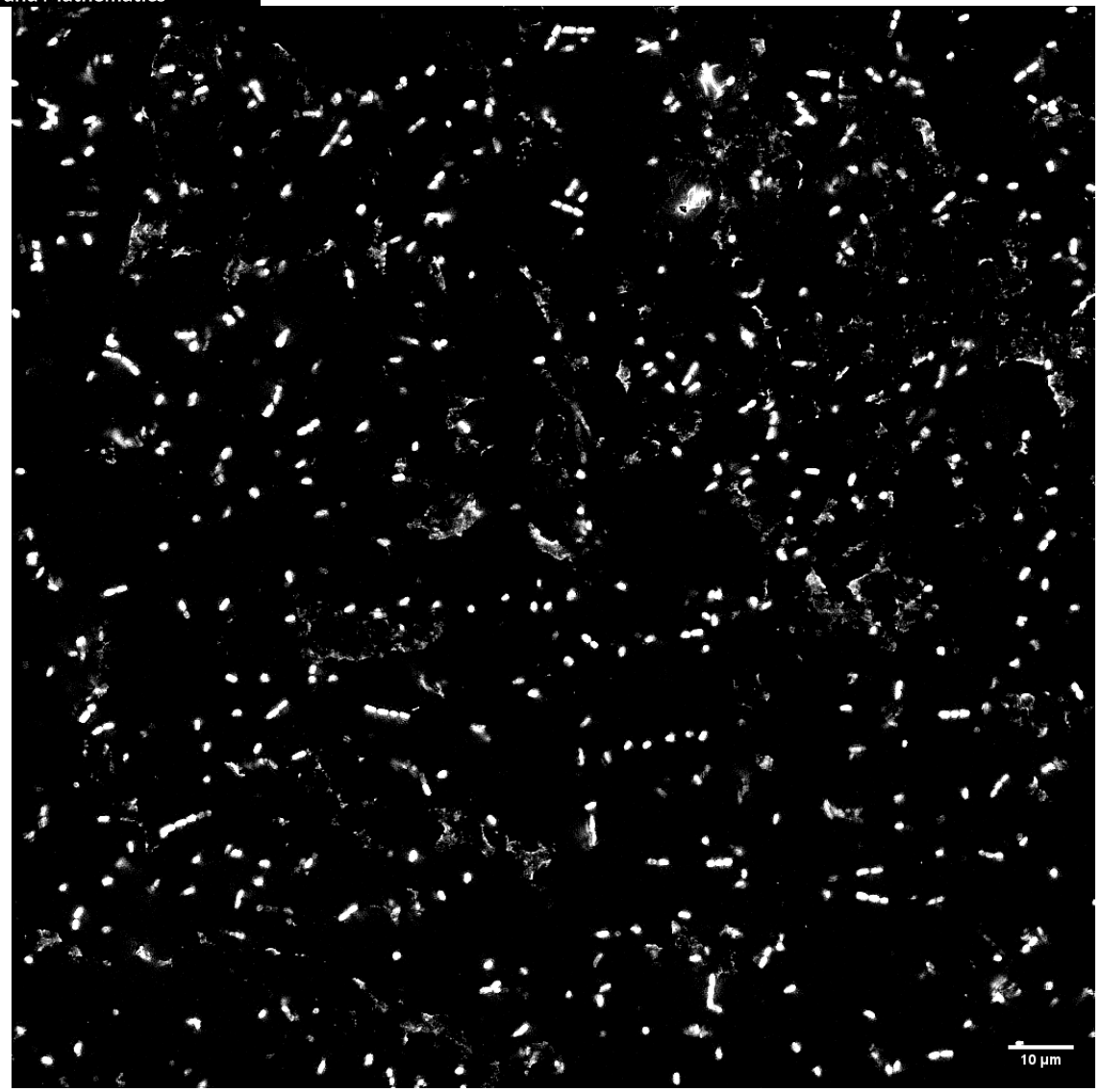
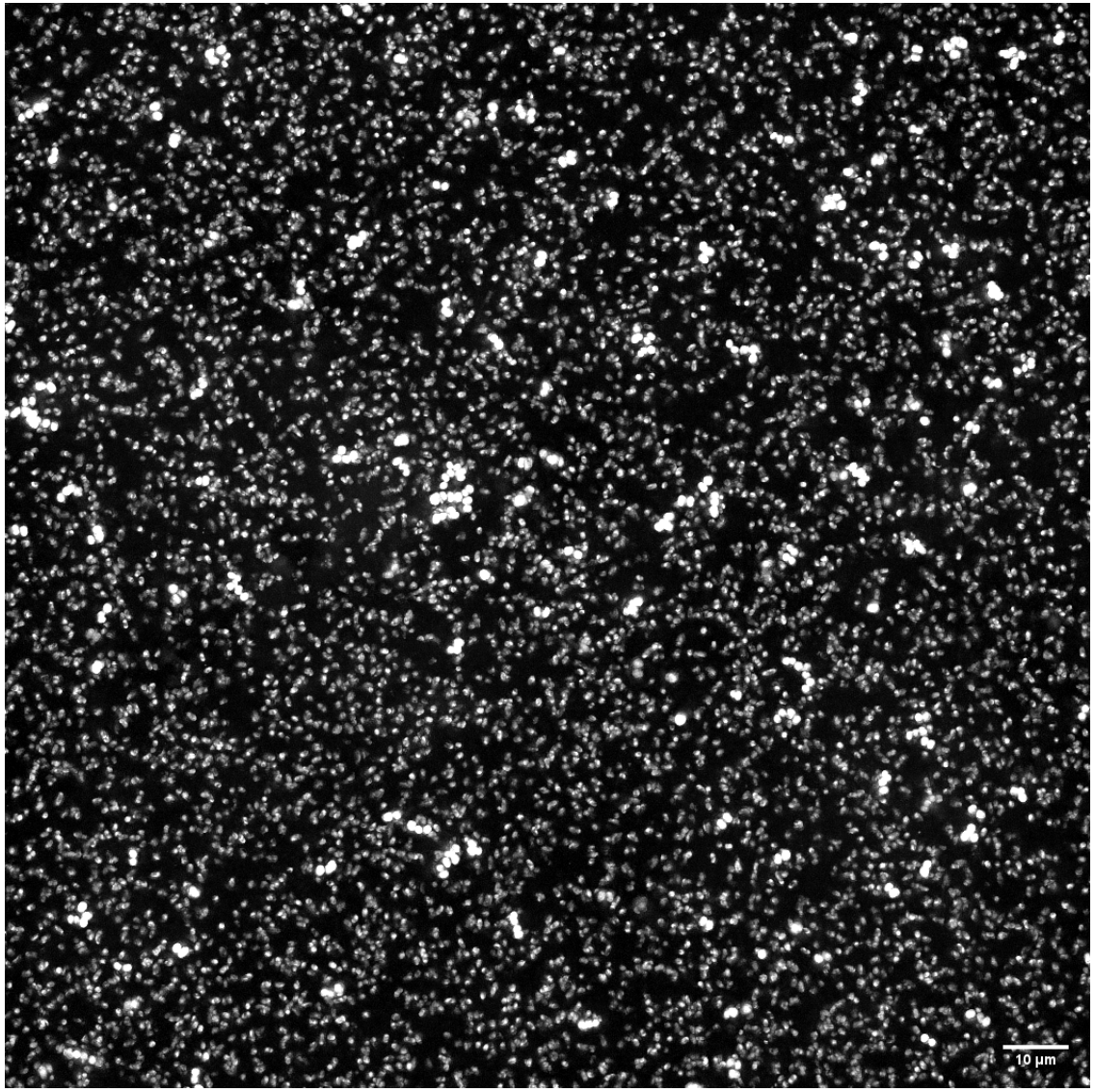
Micrographs of *Tersicoccus phoenicis* - images were captured on a Tomocube HT-X1 quantitative phase imaging microscope



***T. phoenicis* in exponential growth in acetate minimal media (AMM)**



***T. phoenicis* in the dormant state after 10 days of growth in acetate minimal media (AMM)**

Tirumalai M, Ali S, Fox GE, Widger W. 2025. *Microbiol Spectr* PMID: 40788184

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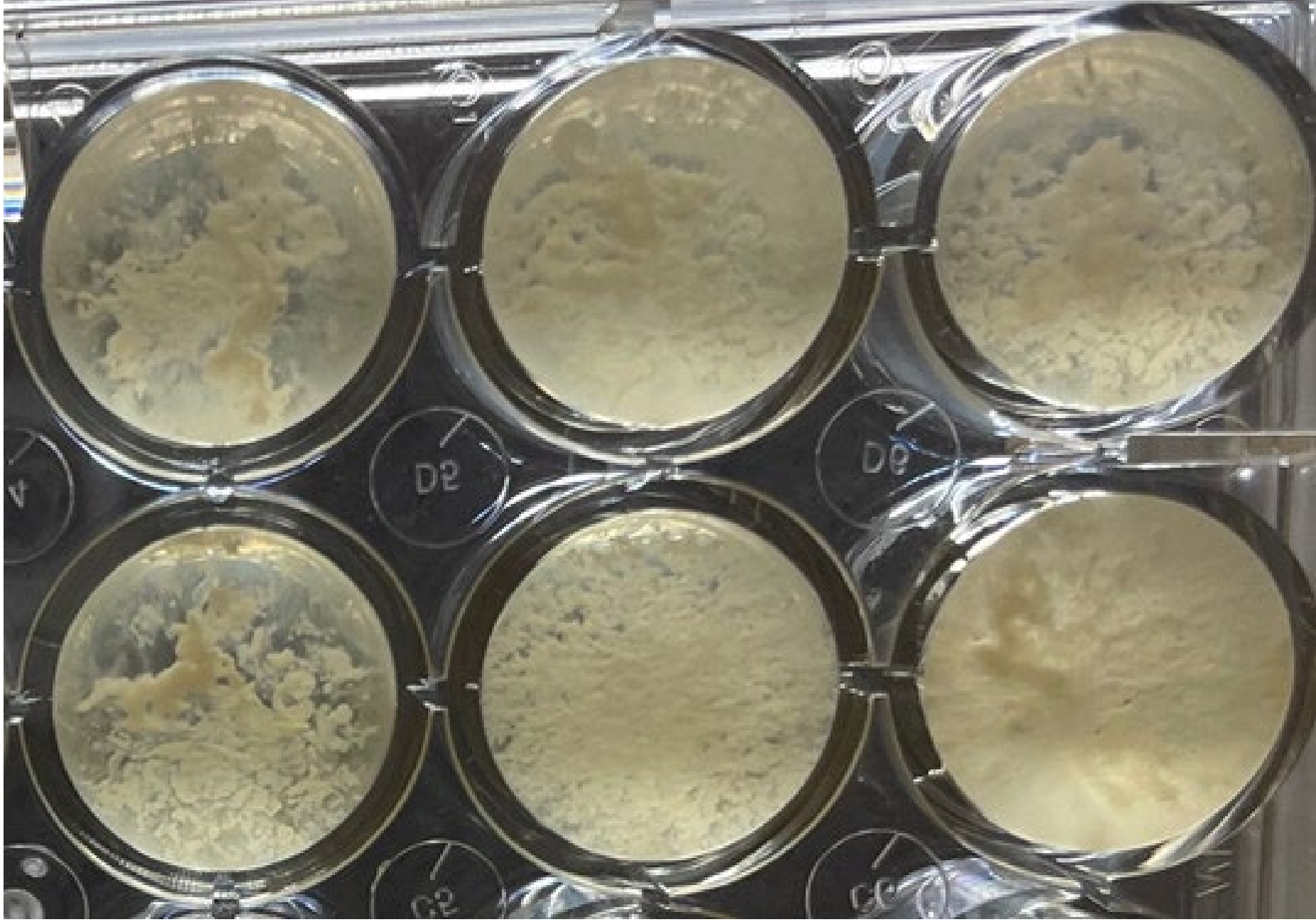
Tersicoccus phoenicis
- shows clustered aggregation when dormant - biofilm formation



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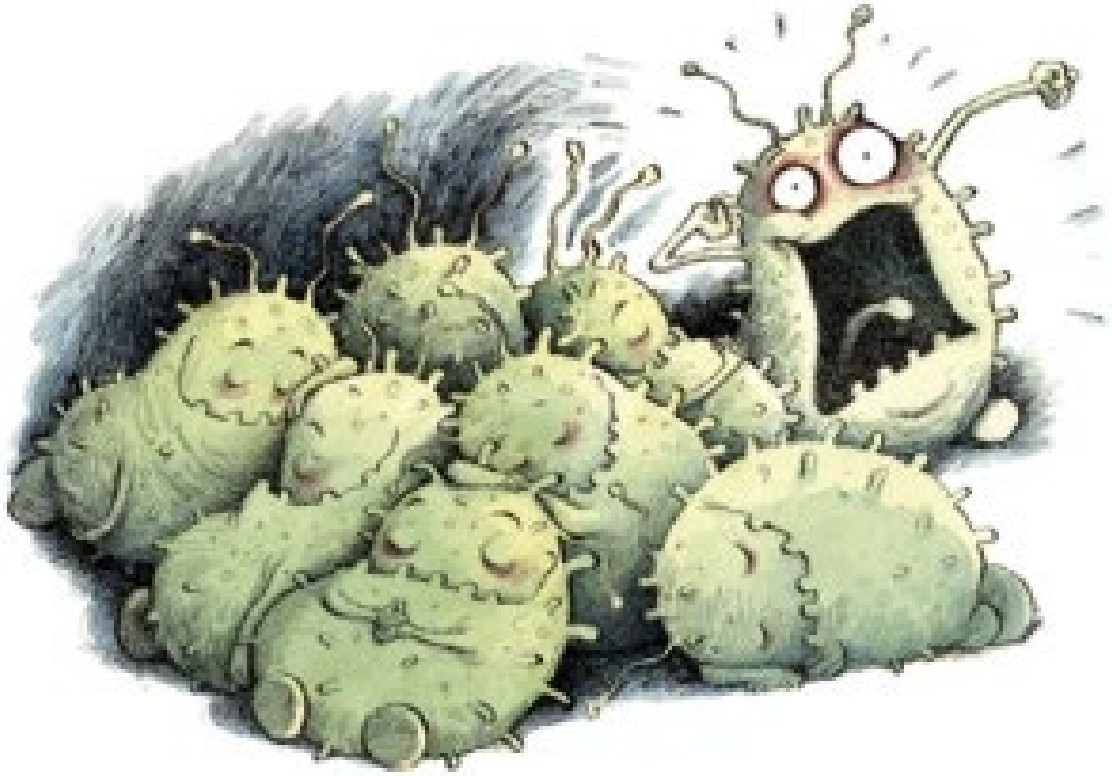
Can dormant *T. phoenicis* be revived using Resuscitation promoting factor?

Dormant *Micrococcus luteus*
(well-known to be revived with Rpf)

SAC isolate
***T. phoenicis* 1P05MA^T**



Both have the gene for Rpf & hence should be capable of resuscitation

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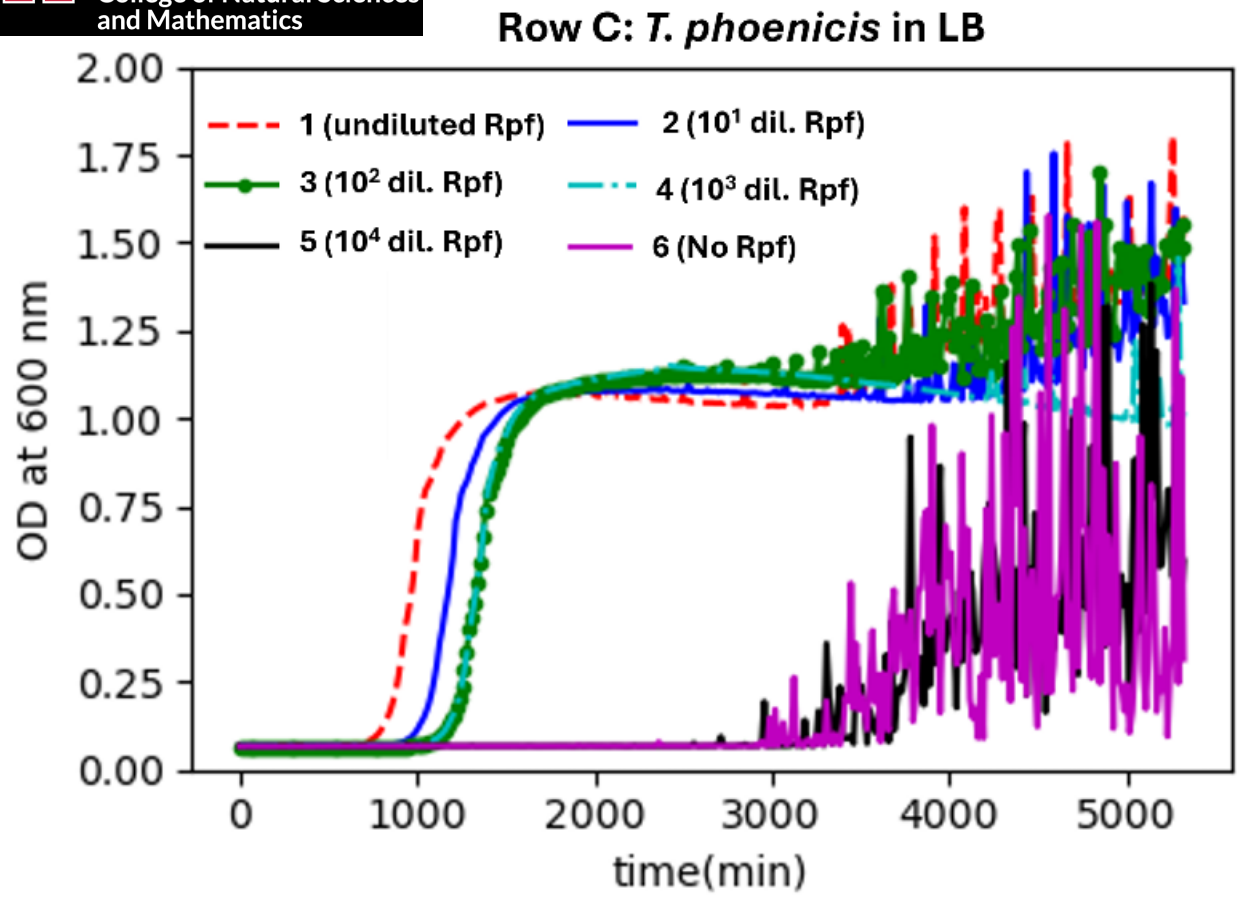
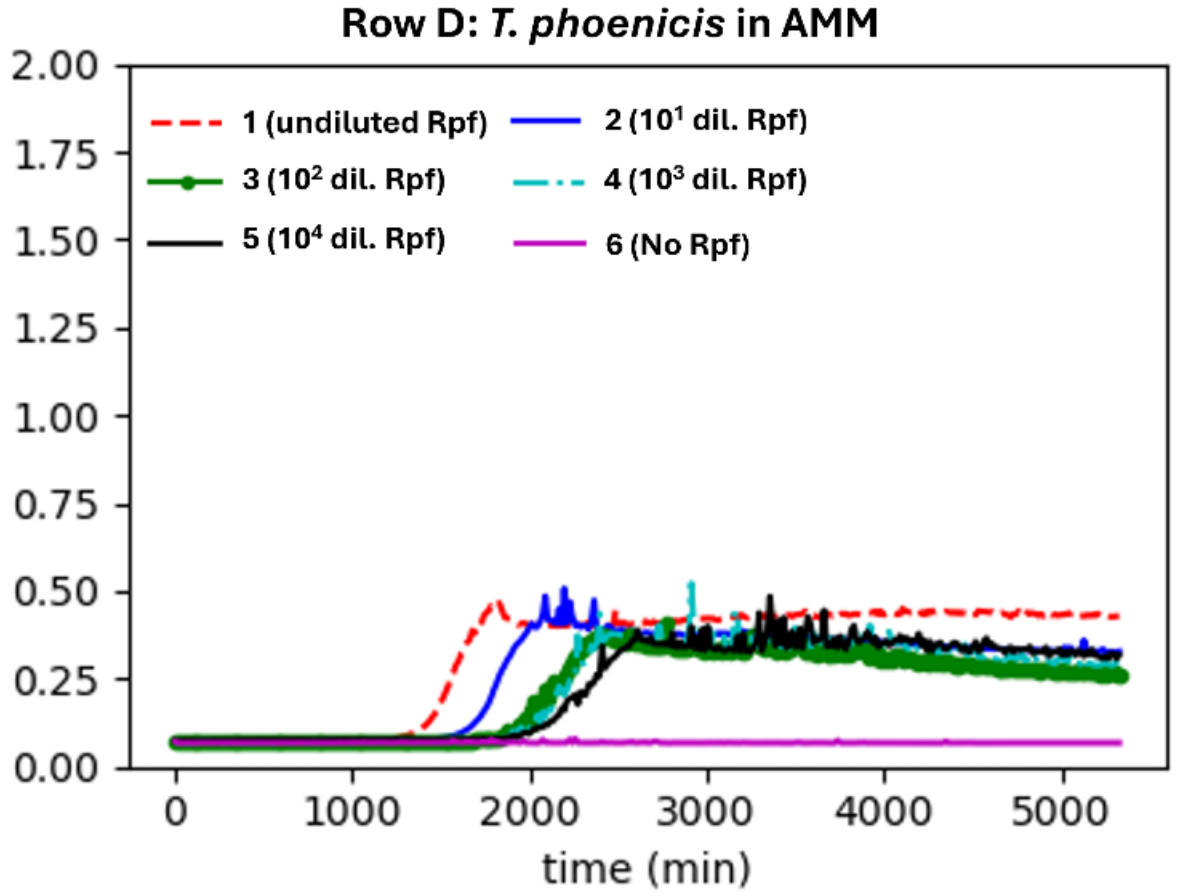




Dormant *T. phoenicis* showed negligible growth in the absence of Rpf in AMM.

Rpf revives dormant cells of the SAC isolate *Tersicoccus phoenicis*

Tirumalai M, Ali S, Fox GE, Widger W. 2025. *Microbiol Spectr* PMID: 40788184

Desiccation Drives Dormancy in a Spacecraft Clean-Room Isolate

Tersicoccus phoenicis (SAC isolate)

DAY 0
Before drying

3.4×10^6 CFU/ml
(Initial cell count)

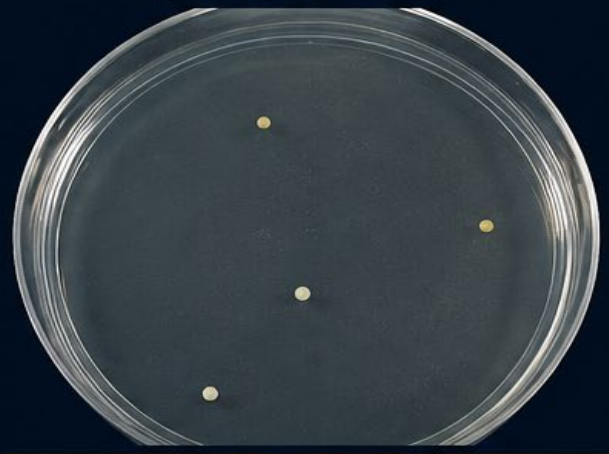
Active, culturable cells

DESICCATION
(7 days)

>99.999%
loss of culturability

DAY 7
After 7 days of drying

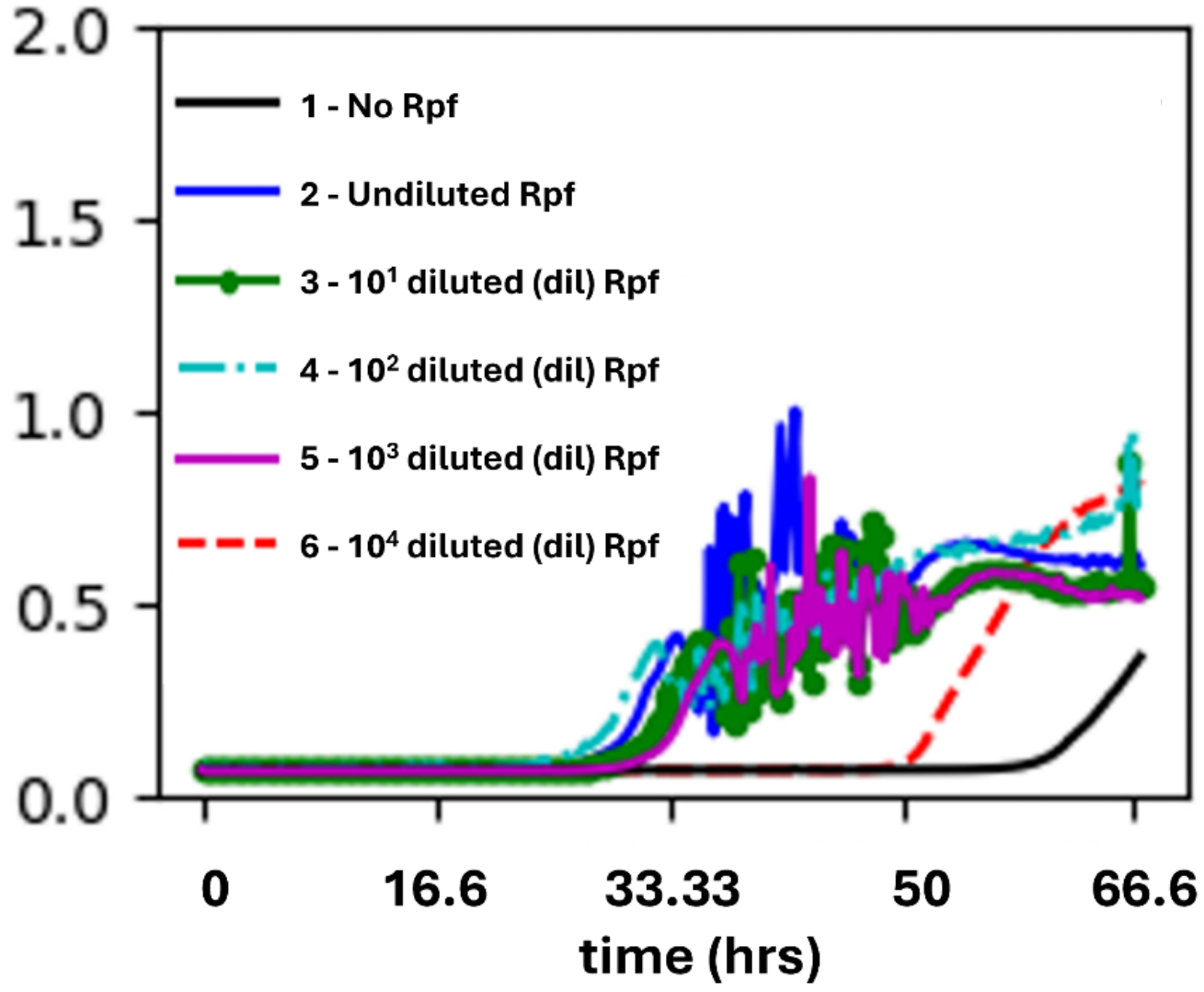
1–4 colonies detected
(in 5 µl plated)



Dormant / VBNC-like cells

Cells may persist despite becoming **largely undetectable** by standard cultivation.

CFU = colony forming units; VBNC = viable but non-culturable



Rpf revives *T. phoenicis* from dormancy after 168 hrs of air drying

Tirumalai M, Ali S, Fox GE, Widger W. 2025. *Microbiol Spectr* PMID: 40788184

Cassilly CD, Chander AM, Vaughn JA, Kunstman KJ, Green SJ, Venkateswaran K, Bertone PF, Bahr CW, Marcella SA, Morris HC (2025)

Effects of simulated space environmental conditions on cleanroom microbes.

***Front Microbiol*. 2025 Aug 19;16:1600106. doi: 10.3389/fmicb.2025.1600106. PMID: 40904680; PMCID: PMC12404038.**

Radiation-resistant strains reported include Actinobacteria

- *Micrococcus luteus* (Actinobacteria)
- *Kocuria rosea* (Actinobacteria)
- *Arthrobacter agilis* (Actinobacteria)

Three reported radiation-resistant isolates belong to the phylum **Actinobacteria**

Microbial archive at
the Jet Propulsion Laboratory (JPL) > 8,000 bacterial strains

Actinobacteria?

https://www.jpl.nasa.gov/news/nasas-jpl-seeking-applicants-for-first-space-accelerator/

https://planetaryprotection.jpl.nasa.gov/resources/pdf/Factsheet_web_1.pdf

Dormancy and Multi-Stress Survival

An Emerging Planetary Protection Question

WHAT WE KNOW

- Actinobacteria in spacecraft/space habitats
- Dormant/VBNC states occur
- Three reported radiation-tolerant strains are actinobacteria*

*Cassilly CD, Chander AM, Vaughn JA, Kunstman KJ, Green SJ, Venkateswaran K, Bertone PF, Bahr CW, Marcella SA, Morris HC. *Front Microbiol.* 2025; 16:1600106. doi: 10.3389/fmicb.2025.1600106.

Emerging directions for risk models

1. Treat dormancy as a **persistence state**, not growth.

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DDI
Drug Discovery Institute

ACKNOWLEDGMENTS

Supported in part by:

- **National Science Foundation Award NSF-MCB-EAGER 2227347**

  and

- **University of Houston's Drug Discovery Institute (DDI) Seed Grant.**

Dr. Parag Vaishampayan (NASA Ames Research Center),

and

Dr. Aaron Regberg (NASA Johnson Space Center).

For critical reviews-insightful comments on the published *T. phoenicis* dormancy manuscript

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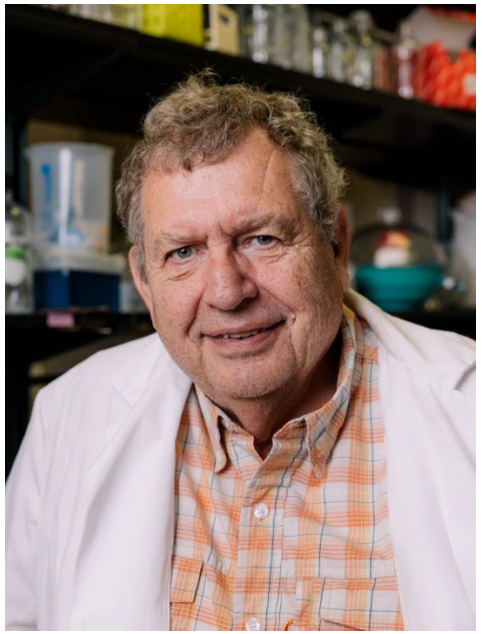
George E. Fox



William Widger

Sahar Ali

Ongoing collaboration:
Jason Hafner (Rice University)


Thank you!