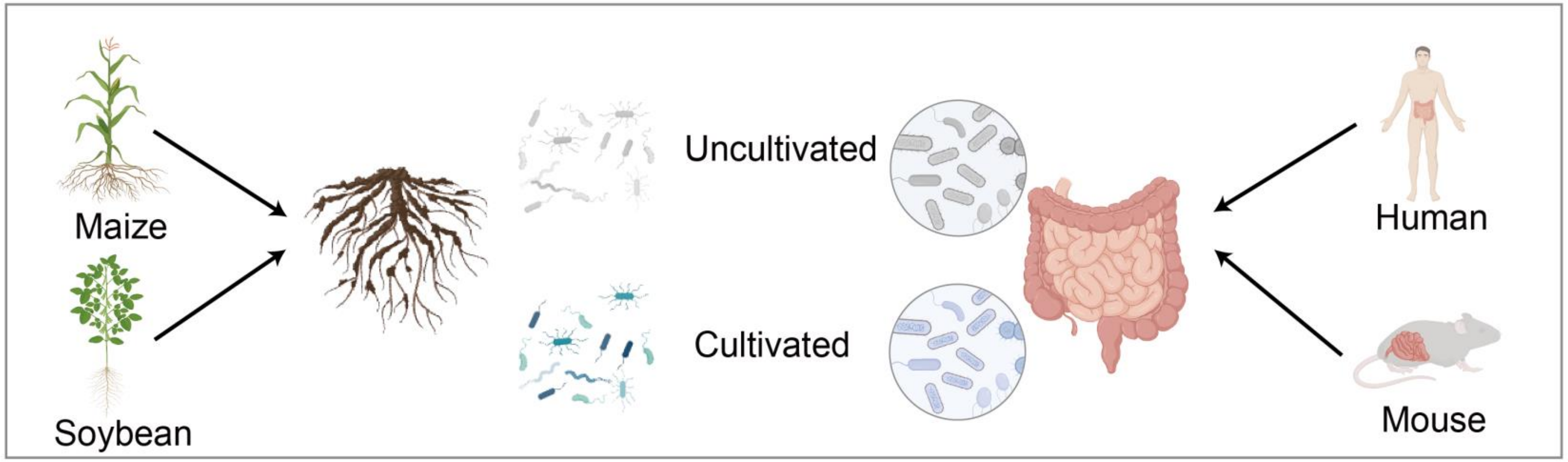
Ribosomal RNA operon copy number: a trait-informed framework to close the microbial cultivation gap

Yuxiao Huang[1#], Qingliu Wu[1#], Chang Liu[2] , Pugang Yu[1] ,
Hao Li[1] , Shengyue Tang[1] ,Fusuo Zhang[1] , Shuangjiang Liu[2,3,4] , Bin Ni[1*]

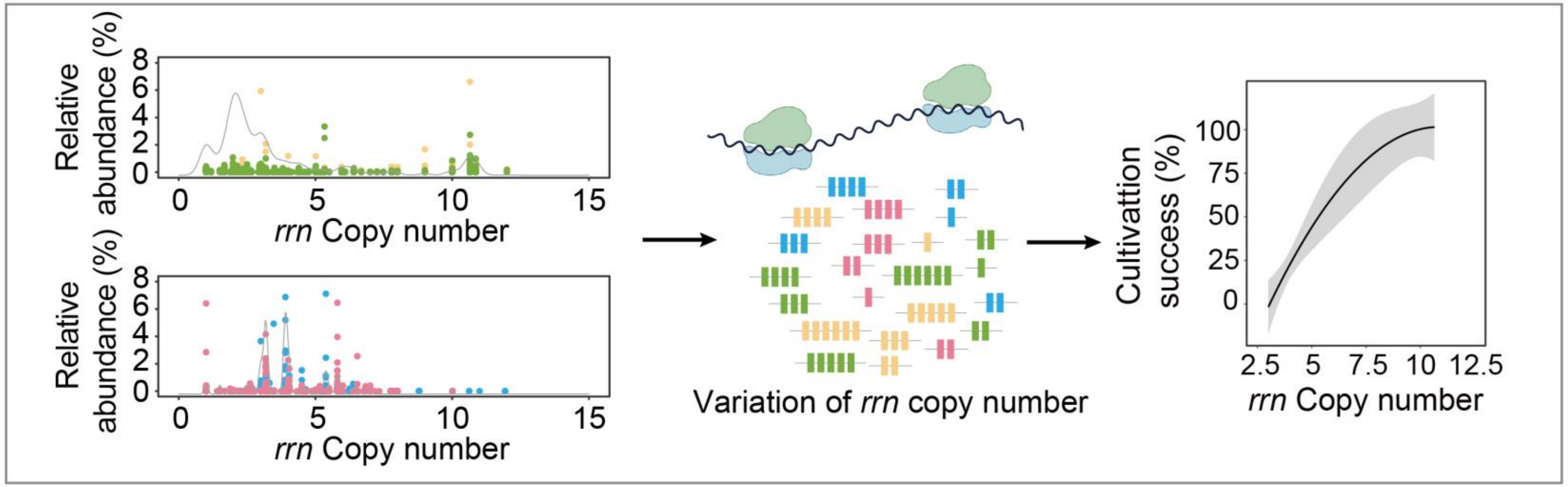
[1]Key Laboratory of Nutrient Use and Management, College of Resources and Environmental Sciences,
National Academy of Agriculture Green Development, China Agricultural University, Beijing, China
[2]State Key Laboratory of Microbial Technology,
Shandong University, Qingdao, Shandong, China
[3]State Key Laboratory of Microbial Diversity and Innovative Utilization,
Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[4]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China

Yuxiao Huang, Qingliu Wu, Chang Liu, Pugang Yu, Hao Li, Shengyue Tang, Fusuo Zhang, et al. 2025.
Ribosomal RNA operon copy number: a trait-informed framework to close the microbial cultivation gap.
iMetaOmics 2:e70071. https://doi.org/10.1002/imo2.70071

Highlight

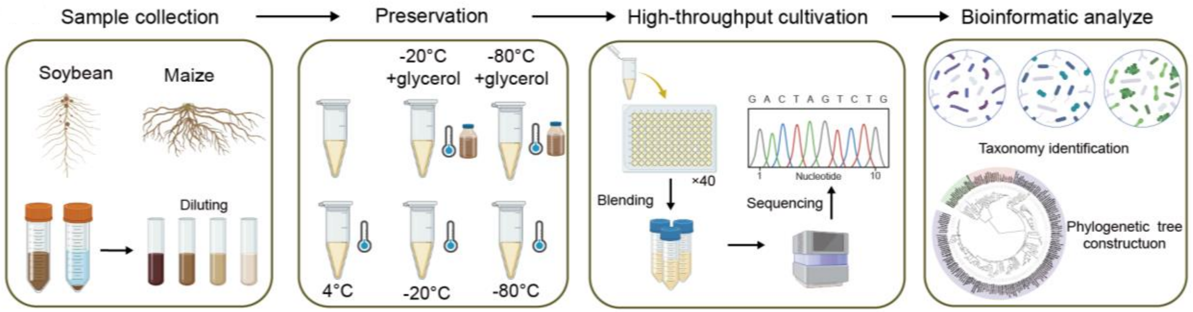


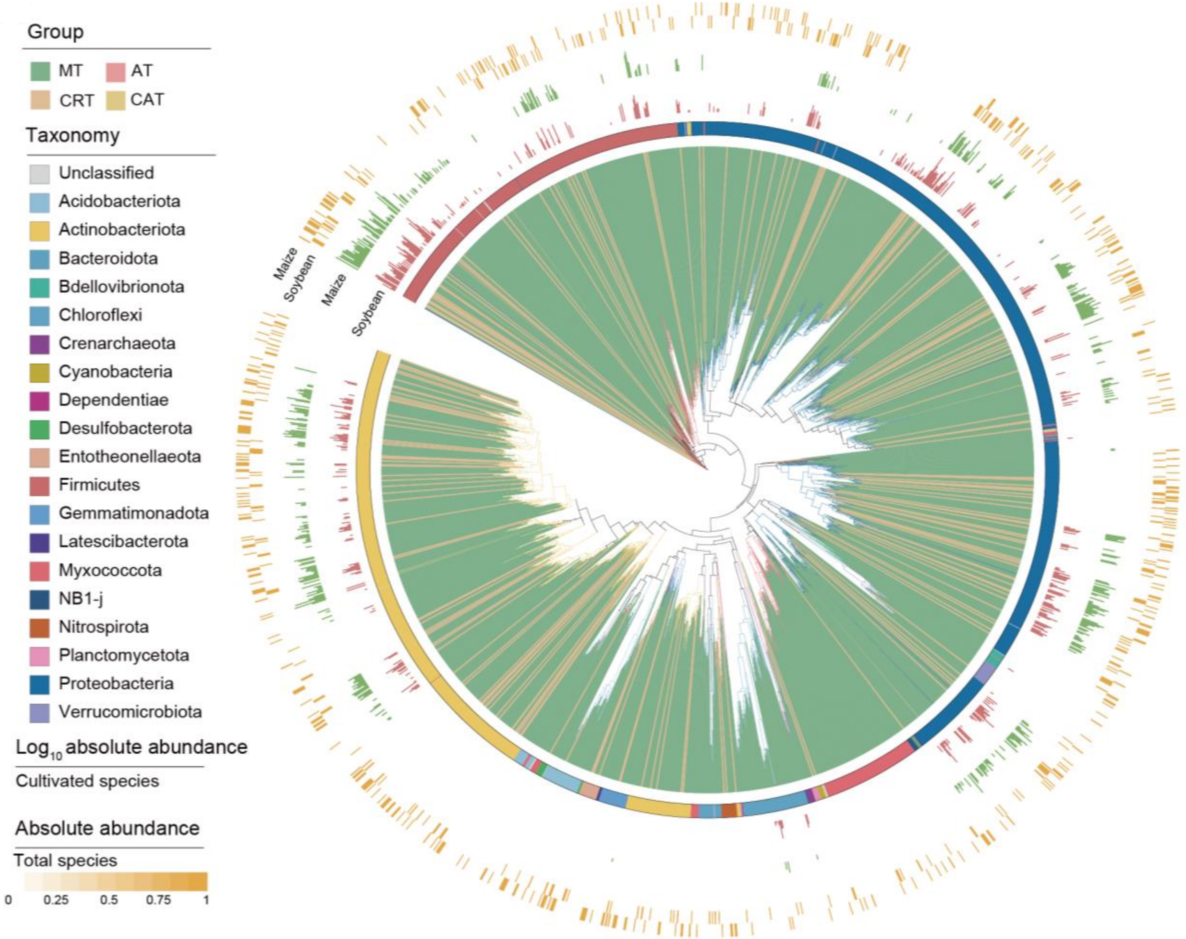
What factors govern the culturability of bacterial communities?



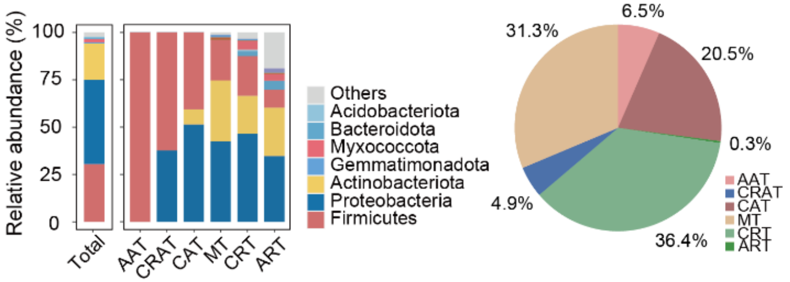
- Ribosomal RNA operon (*rrn*) copy number is closely correlated with cultivation biases across ecosystems.
- Conventional cultivation favors high-*rrn* taxa, while low-*rrn* oligotrophs are consistently underrepresented.
- Rhizosphere microbiomes harbor more high-*rrn* taxa, whereas gut microbiomes are enriched in low-*rrn* specialists.
- *rrn*-informed cultivation strategies improve recovery of elusive, functionally important microbes, bridging genomic traits with practical isolation methods.

Community composition and trait distributions in the rhizosphere

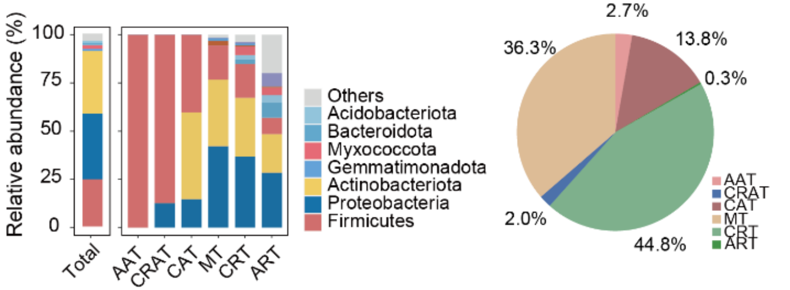




Soybean



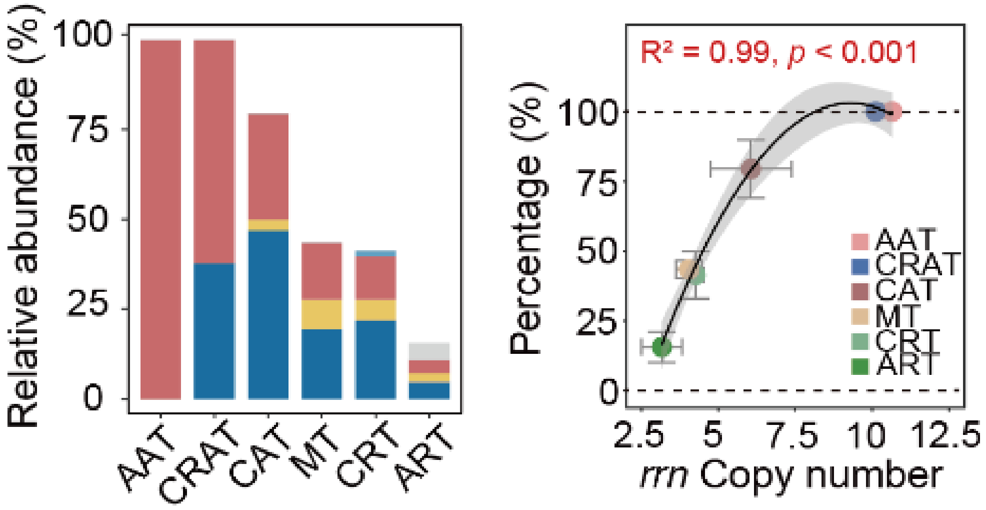
Maize



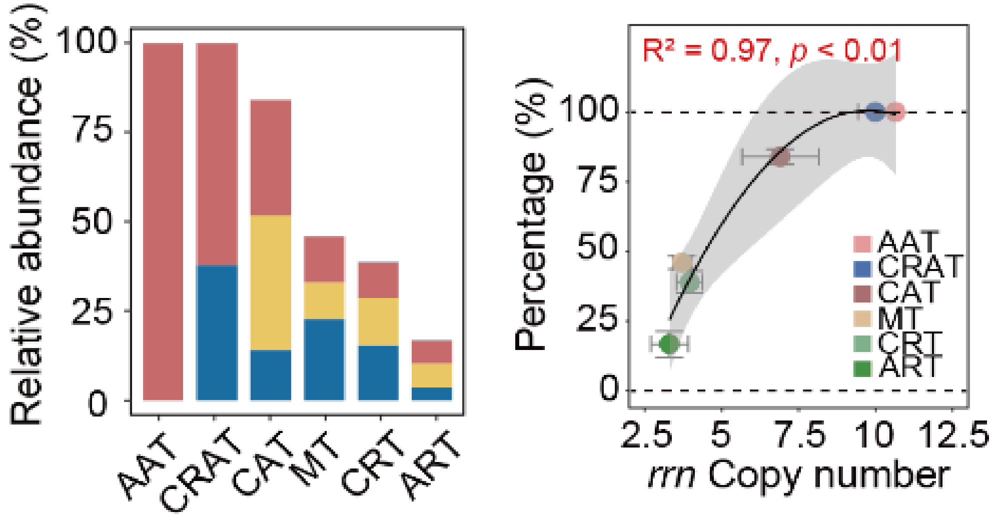
- ☐ The soybean rhizosphere prefers *Proteobacteria* and *Firmicutes*, while the maize rhizosphere has a higher proportion of *Actinobacteria*.
- ☐ The proportions of always rare taxa (ART) and moderate taxa (MT) in the maize rhizosphere are both higher than those in the soybean rhizosphere, whereas the soybean rhizosphere has a higher proportion of conditionally rare taxa (CRT).

Quantitative relationship between *rrn* copy number and culturability

Soybean

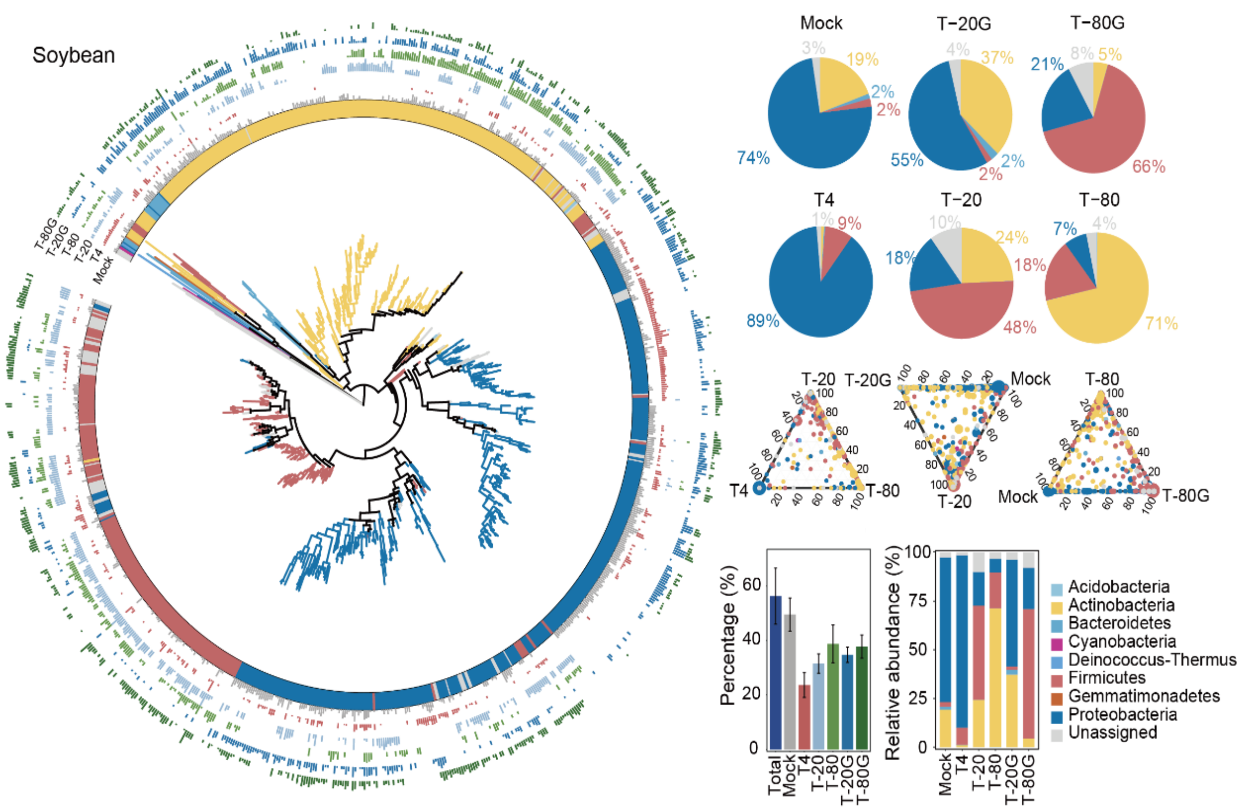


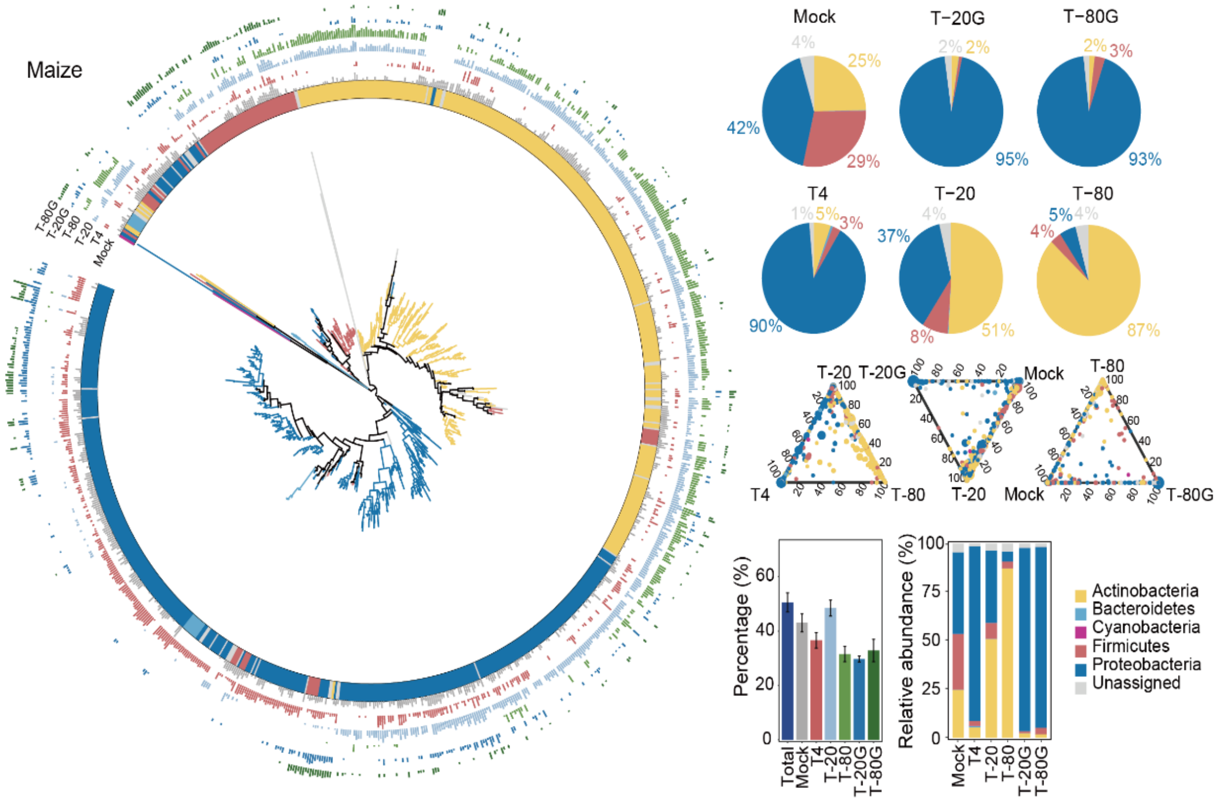
Maize



- A strong positive correlation was detected between *rrn* copy number and bacterial cultivability ($R^2 = 0.97$ in soybean and $R^2 = 0.99$ in maize), corresponding to the proportion of ASVs successfully isolated from the rhizosphere of both crops.
- At the phylum level, outcomes reflected this trend: *Proteobacteria* (generally high-*rrn*) exhibited high cultivability; *Firmicutes* (very high *rrn*) dominated AAT groups; *Actinobacteria* (intermediate *rrn*) showed genus-dependent variation.

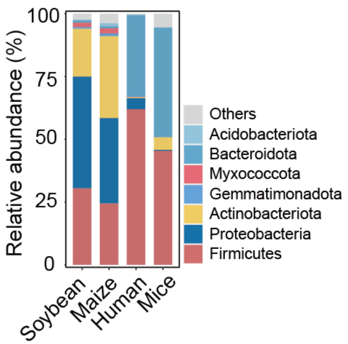
Preservation biases

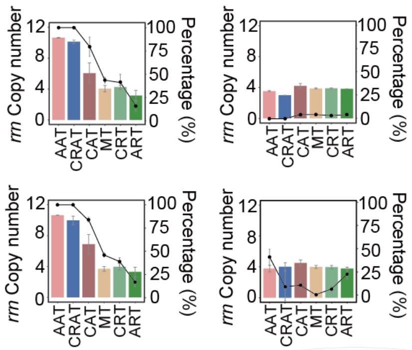


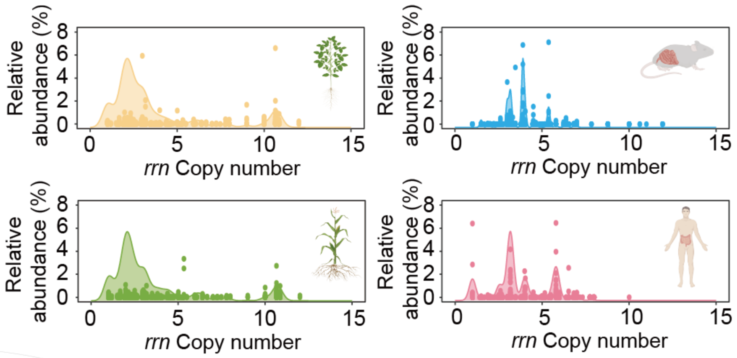


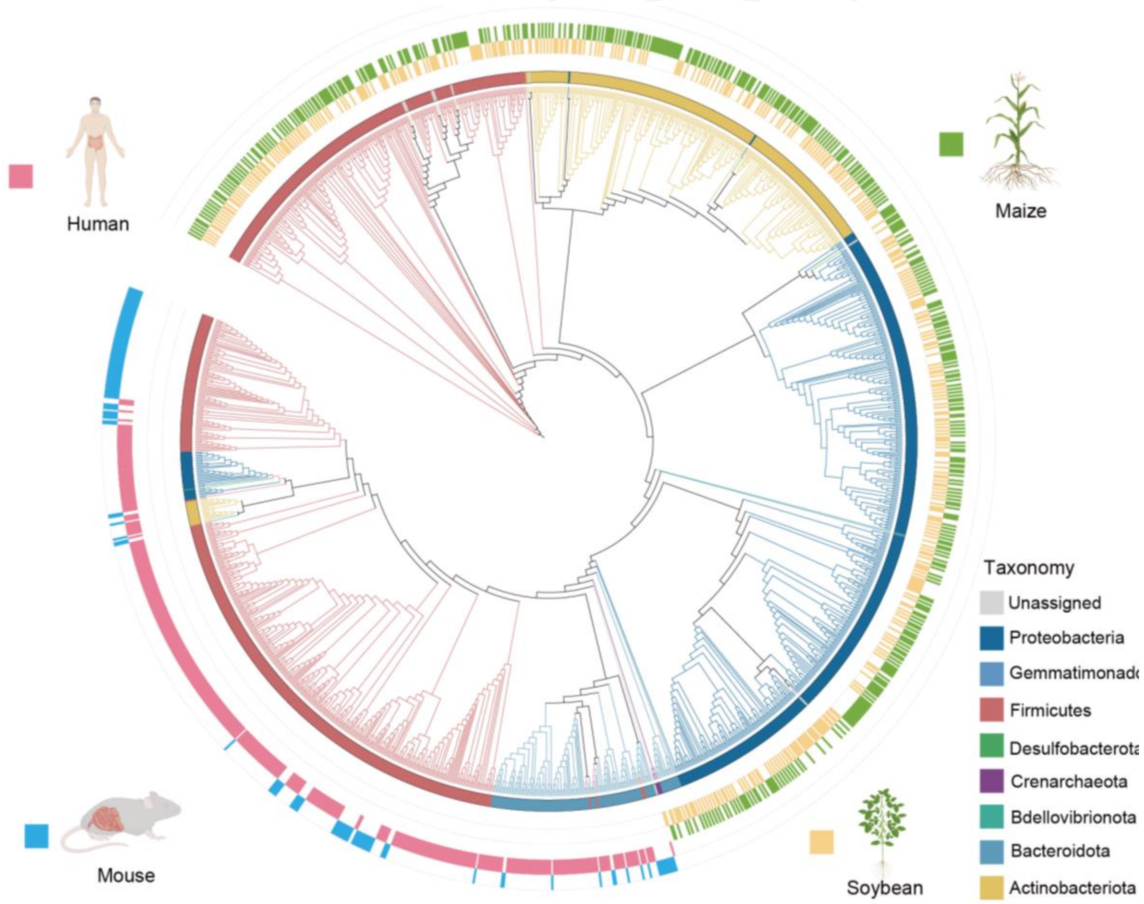
- Fresh samples captured the broadest diversity. Cold storage without cryoprotectant reduced Proteobacteria and enriched *Actinobacteria*, while glycerol partially restored *Proteobacteria* recovery. Low-*rrn* taxa declined under all storage regimes, highlighting compounding effects of cultivation and preservation biases.

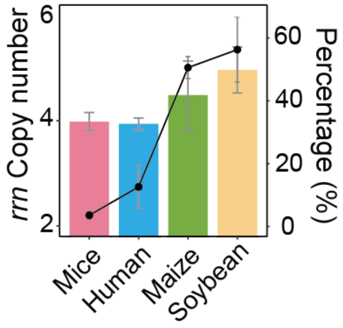
Cross-ecosystem comparison: rhizosphere vs. gut microbiomes

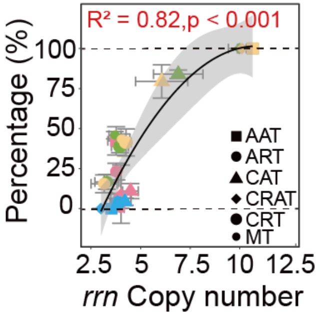






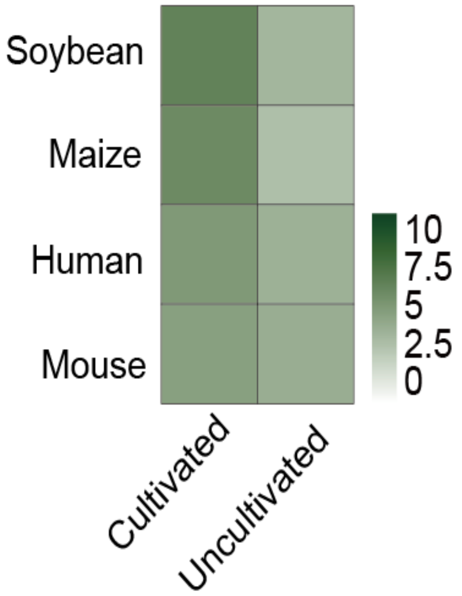


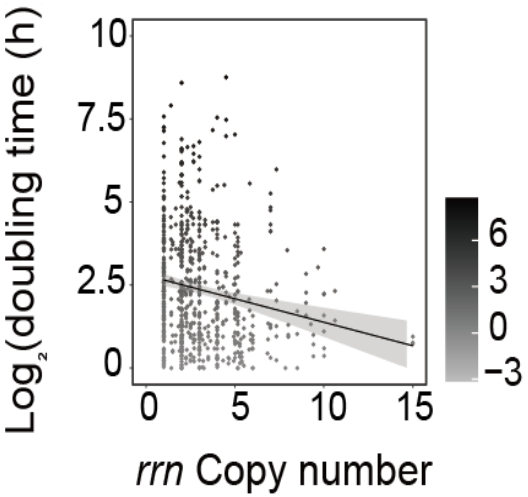


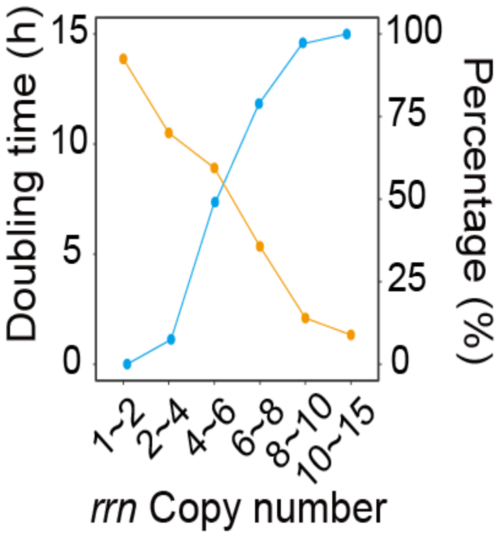


- ☐ The rhizosphere community has a higher average *rrn* copy number (approximately 6-8), while the gut community has a lower average *rrn* copy number (approximately 3-5).
- ☐ The proportion of high-rrn copy number taxa in the rhizosphere community is higher than that in the gut, whereas low- *rrn* copy number taxa are consistently underrepresented.
- ☐ The cultivable proportion of taxa with different rarity levels shows a significant positive correlation with the average *rrn* copy number.

Cultivation bias and trait-based corrections







- The average *rrn* copy number of cultivable taxa is consistently higher than that of uncultivated taxa.
- The growth rates of 700 bacterial species and 190 archaeal species are negatively correlated with *rrn* copy number.
- As the *rrn* copy number of microorganisms increases, the time required for growth decreases.

***rrn*-informed corrections to cultivation protocols:**

- Media design: Adopt oligotrophic/root exudate-based media for low-*rrn* taxa; use chemically defined media to reduce fast-grower dominance.
- Incubation regimes: Extend periods for slow growers; apply fluctuating nutrients to mimic natural environments; adjust temperature/oxygen to match ecological niches.
- Co-culture & in situ cultivation: Use diffusion chambers/iChips to preserve microbial interactions; pair target taxa with helper strains for cross-feeding or detoxification.
- Preservation methods: Apply cryoprotectants and slow cooling to minimize selective losses; reduce freeze–thaw cycles for sensitive low-*rrn* taxa.

Conclusion

- ☐ Microbes' "great plate count anomaly" and conventional cultivation biases hinder culturing most slow-growing specialists, limiting applications and biodiversity conservation.
- ☐ *rrn* copy number predicts cultivation biases; trait-based optimization boosts isolation of abundant and elusive microbes.
- ☐ As a strong predictor, *rrn* copy number should be combined with other genomic features for comprehensive cultivation guidance.
- ☐ This trait-informed framework bridges sequencing and cultivation to advance related fields; future studies need to clarify the *rrn* copy number–cultivability causality.

Yuxiao Huang, Qingliu Wu, Chang Liu, Pugang Yu, Hao Li, Shengyue Tang, Fusuo Zhang, et al. 2025.
Ribosomal RNA operon copy number: a trait-informed framework to close the microbial cultivation gap.
iMetaOmics 2:e70071. https://doi.org/10.1002/imo2.70071

iMeta: To be top journals in biology and medicine

WILEY

"***iMeta***" launched in 2022 by iMeta Science Society, impact factor (IF) **33.2**, ranking top 65/22249 in world and 2/161 in the microbiology. It aims to publish innovative and high-quality papers with broad and diverse audiences. **Its scope is similar to *Cell*,** *Nature Biotechnology/Methods/Microbiology/Medicine/Food*. Its unique features include video abstract, bilingual publication, and social media with 600,000 followers. Indexed by **SCIE/ESI**, **PubMed**, **Google Scholar** etc.

"***iMetaOmics***" launched in 2024, with a **target IF>10, and its scope is similar to *Nature Communications*, *Cell Reports***, *Microbiome, ISME J, Nucleic Acids Research, Briefings in Bioinformatics,* etc.

"***iMetaMed***" launched in 2025, with a **target IF>15, similar to *Med, Cell Reports Medicine***, *eBioMedicine, eClinicalMedicine* etc.

Society: http://www.imeta.science
Publisher: https://wileyonlinelibrary.com/journal/imeta
iMeta: https://wiley.atyponrex.com/journal/IMT2
Submission: iMetaOmics: https://wiley.atyponrex.com/journal/IMO2
iMetaMed: https://wiley.atyponrex.com/journal/IMM3

iMetaScience

iMetaScience

office@imeta.science
imetaomics@imeta.science

Promotion Video

Update
2025/7/6