Antiviral defense systems drive persistence of antimicrobial-resistant bacteria but limit the transfer of antimicrobial resistance genes in anaerobic digestion

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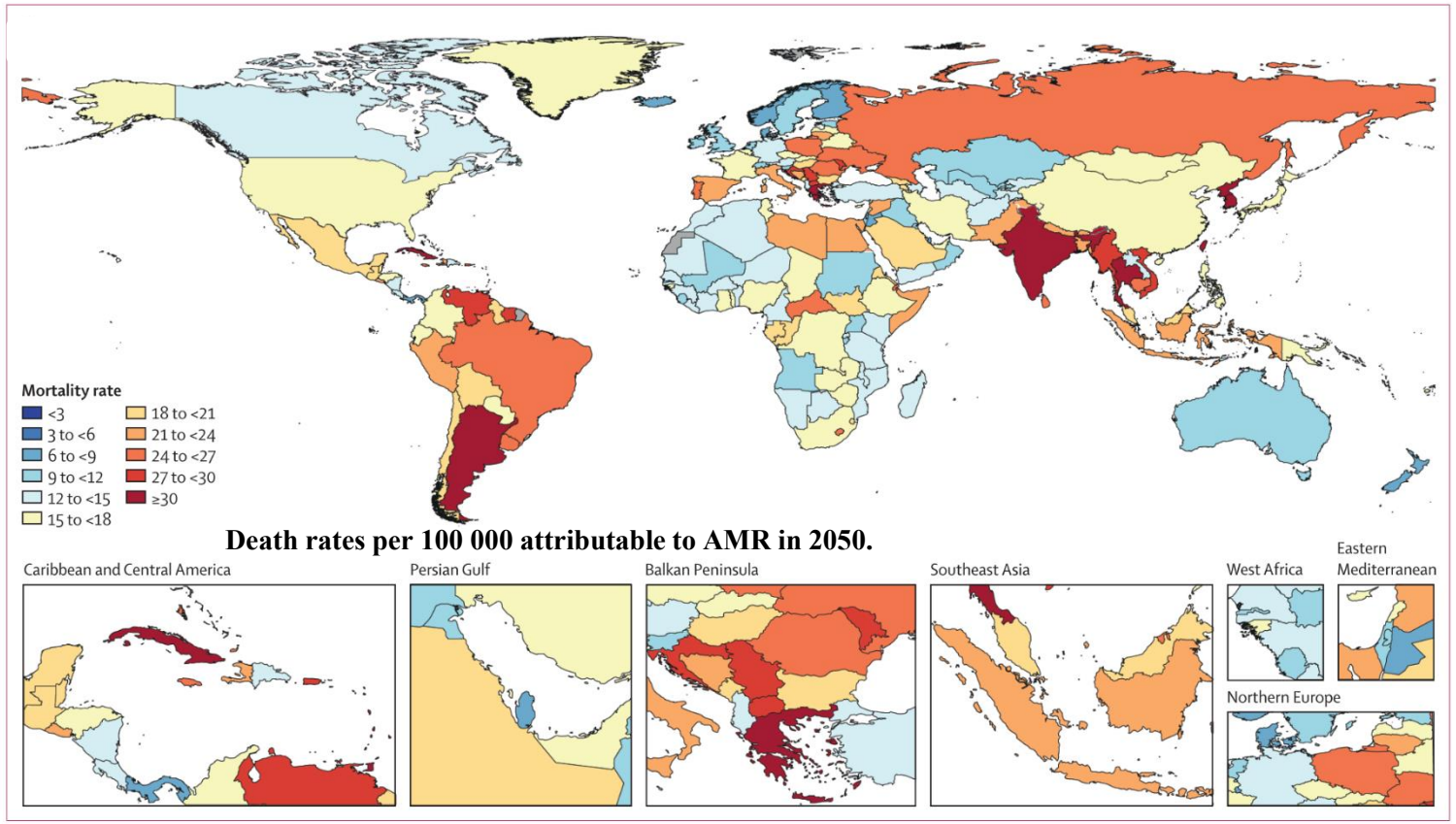
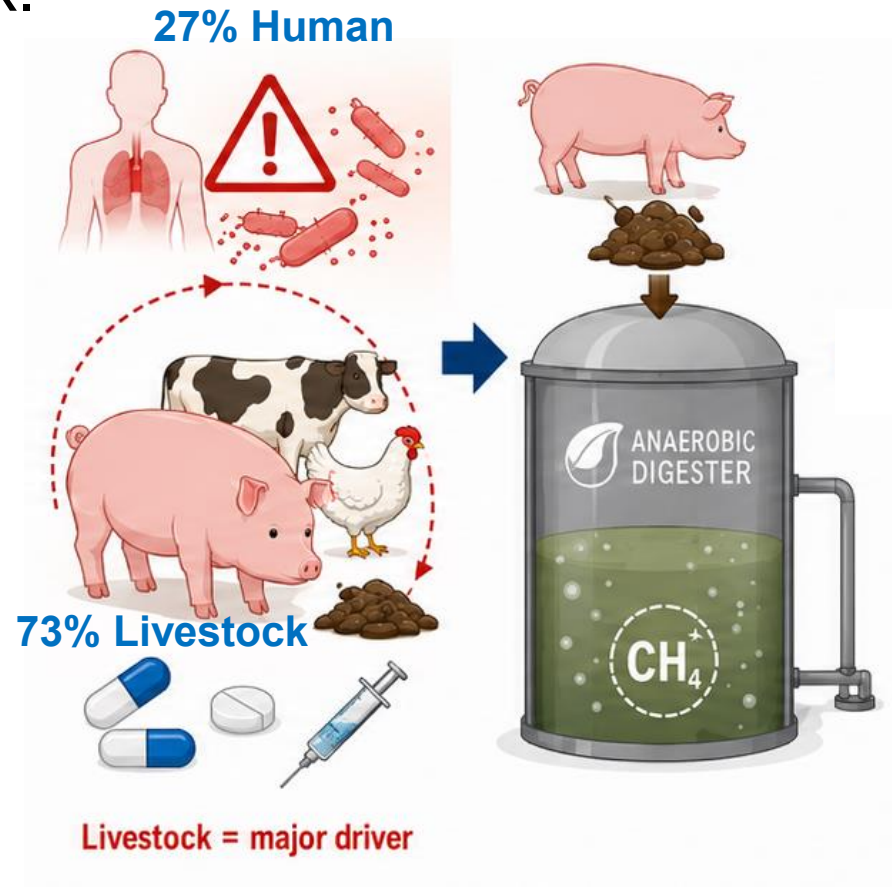
Background

- **Antimicrobial resistance (AMR) is a severe "One Health" crisis**, associated with approximately 4.71 million deaths globally in 2021.
- Approximately **73% of global antibiotics are used in livestock farming**, playing a crucial role in the One Health framework.





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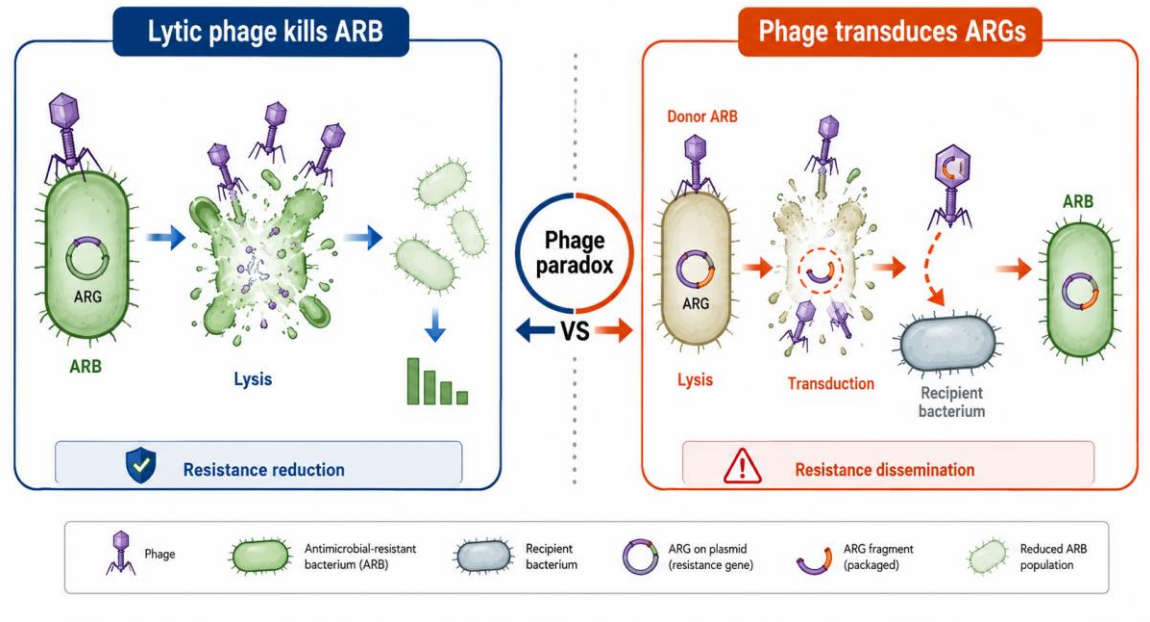
Zhang et al., Nature Sustainability 9, 65-76 (2026).

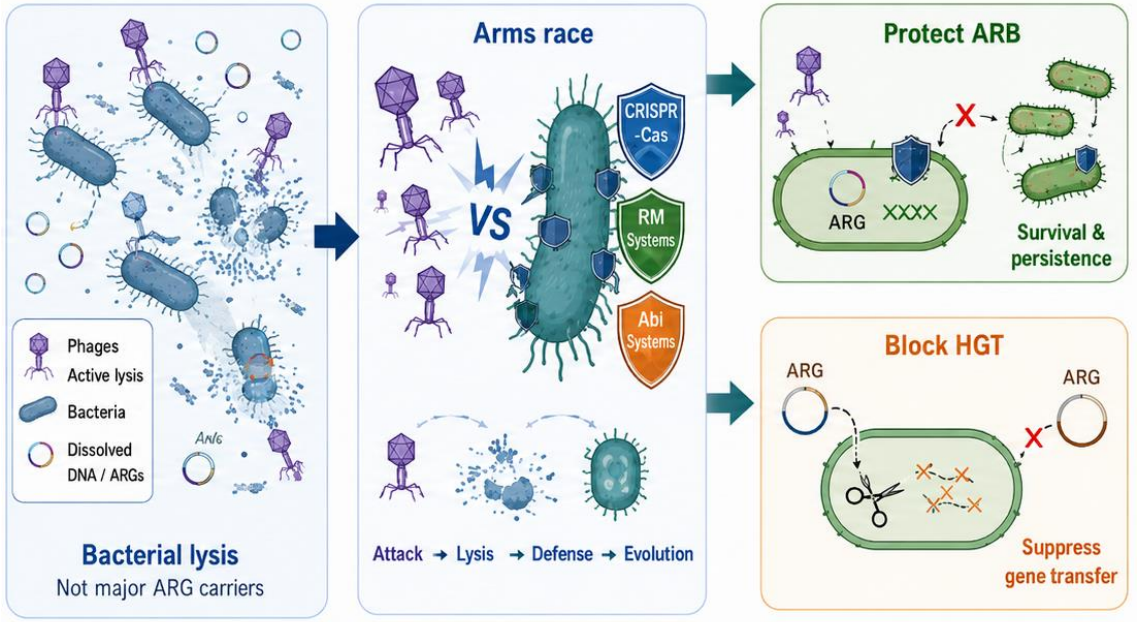
Background

The paradox of phages in AMR transmission: Lysis vs. Transduction



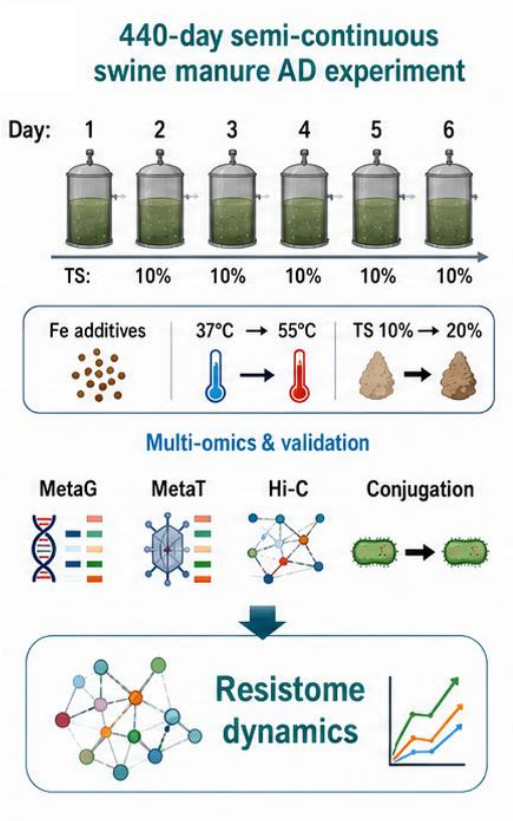
Which one dominates?

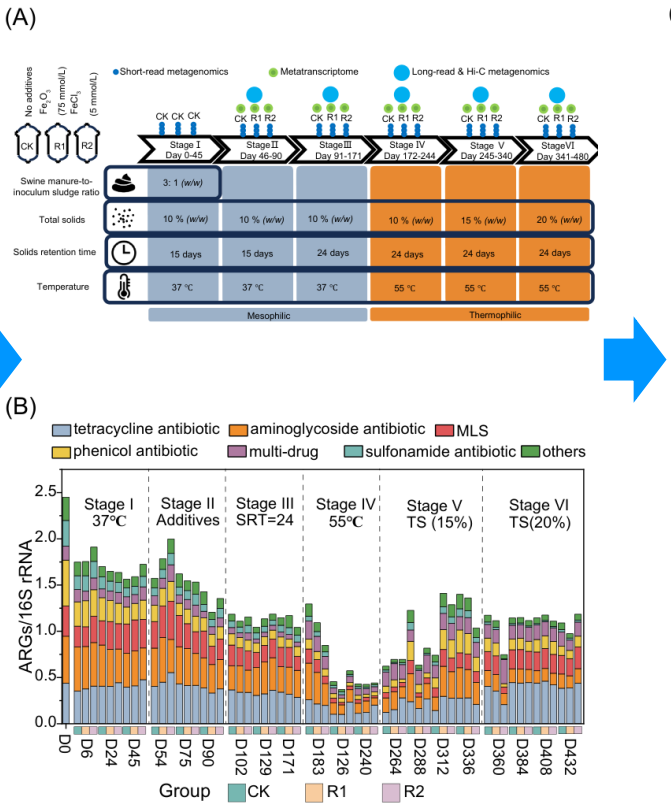
The duality of ADS in AMR transmission: Protecting ARB vs Suppressing HGT

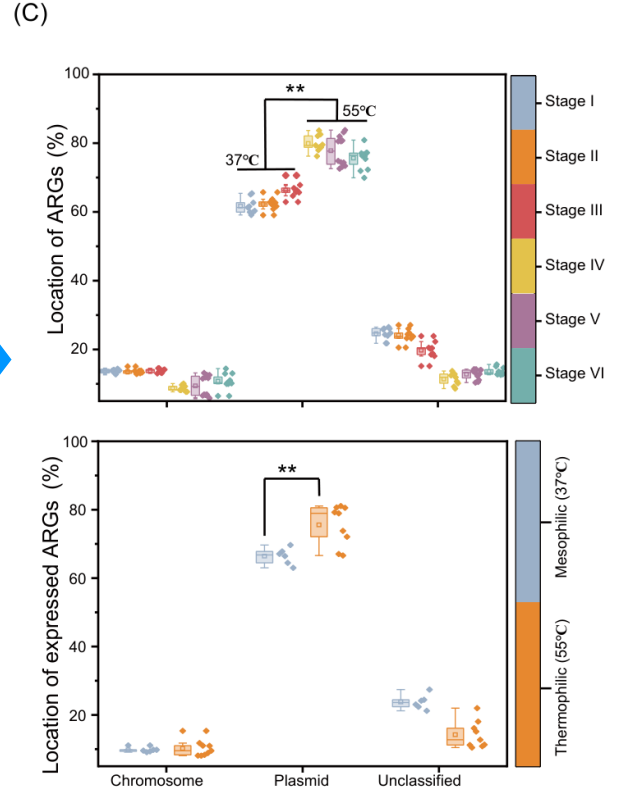


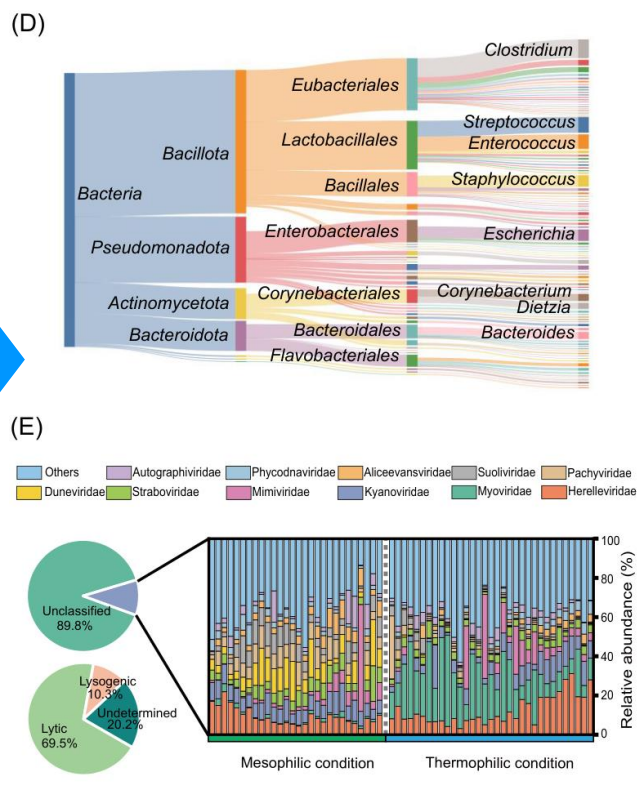
To be verified

Lysis overwhelmingly outweighs transduction









- **440-day** semi-continuous anaerobic digestion
- **Multi-omics and Hi-C**
- **Validation via qPCR and conjugation experiments**

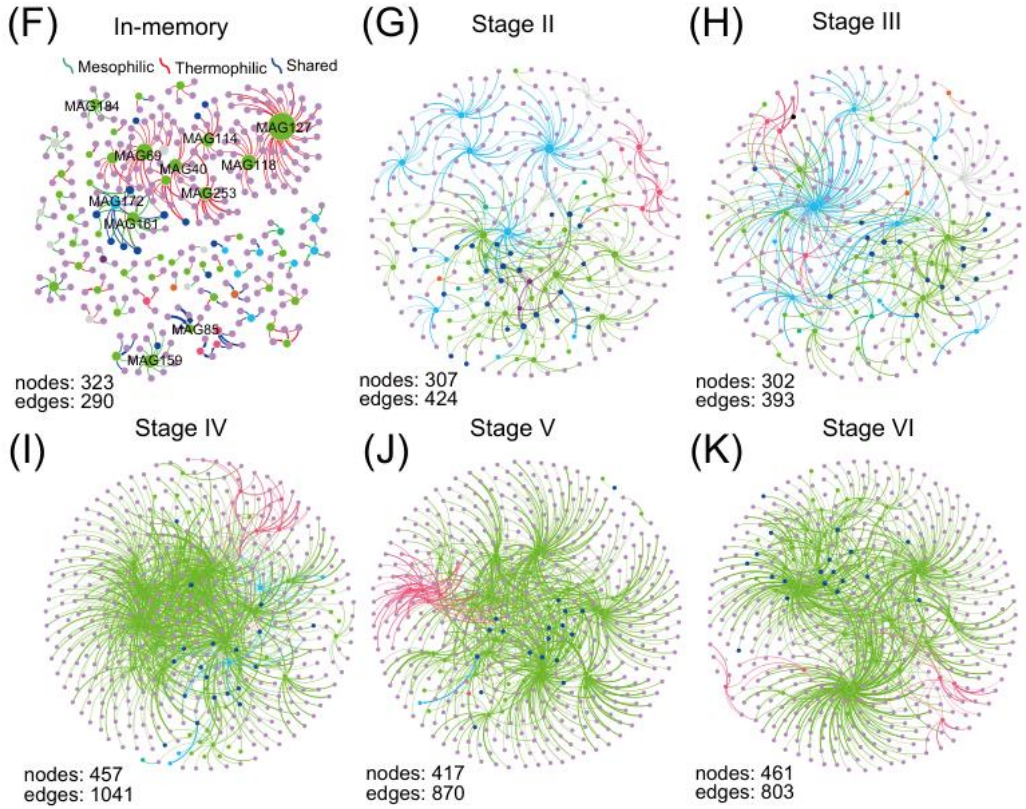
- **Significant changes in ARGs** with operational variations.
- **Thermophilic conditions** enhance ARG reduction, but **increased TS** leads to enrichment.

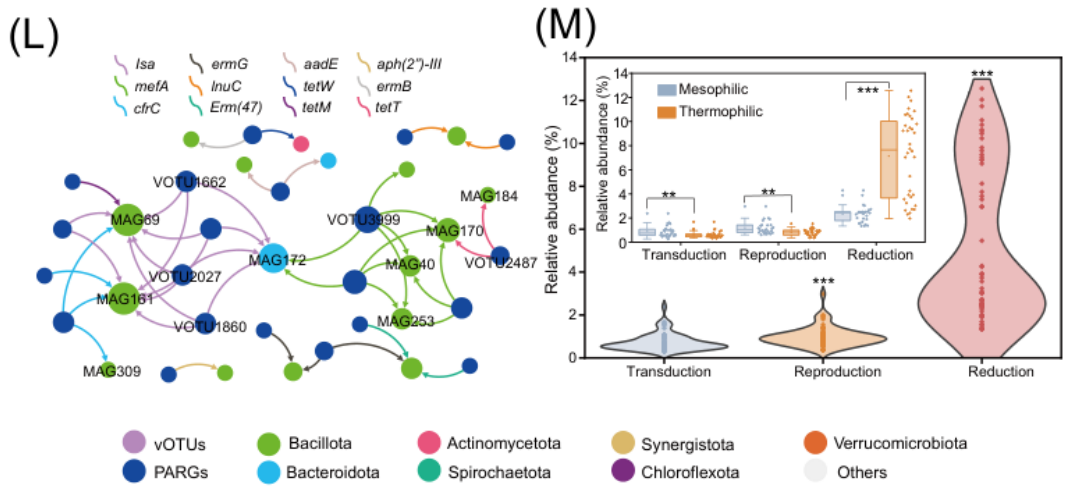
- 70.5%–77.6% of ARGs are located on **plasmids**.
- **Most ARGs are silent**; expressed ARGs are primarily plasmid-borne.

- ***Bacillota* are the main ARG hosts**, but actively expressed ARGs are concentrated in *Pseudomonadota*.
- Diverse unknown viral communities exist, with **69.5% being lytic phages**.

Lysis overwhelmingly outweighs transduction

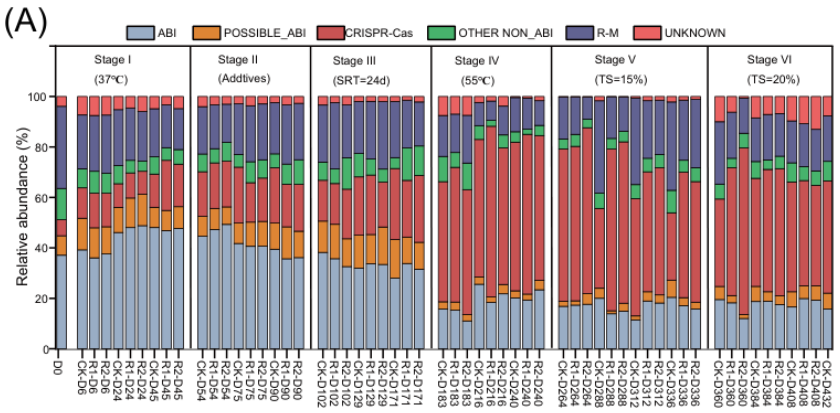
- **Reconstructed historical (In-memory) phage-host interactions** based on CRISPR-Cas, tRNA, and sequence homology.
- **Reconstructed on-going phage-host interactions** at different stages based on Hi-C.

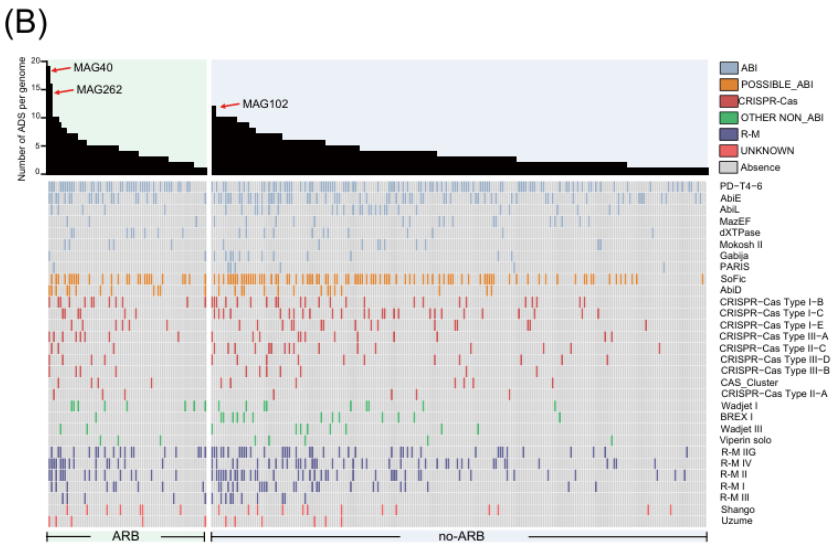




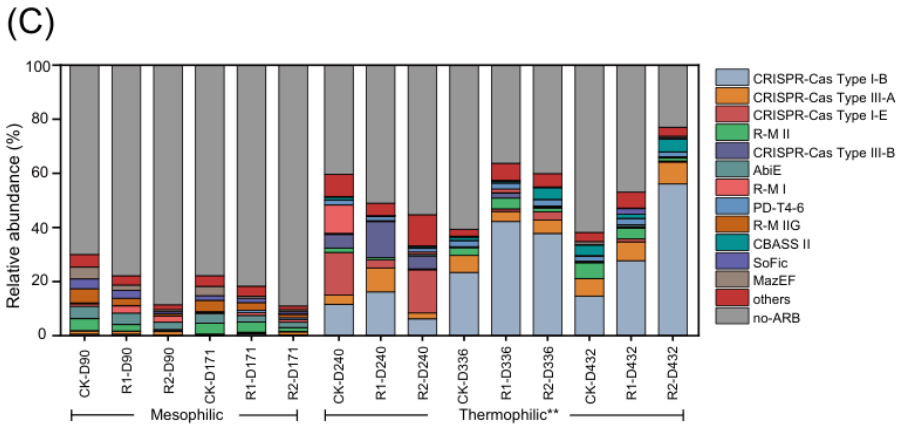
- **Identified 26 vOTUs carrying ARGs, accounting for 0.55 ± 0.32% of total ARGs** and 2.56 ± 1.04% of the viral community.
- Detected **45 transduction events**, including cross-phylum transfers.
- The abundance of **phages driving reduction** (5.16 ± 3.54%) was **significantly higher than** those involved in **transduction** (0.74 ± 0.39%) **and reproduction** (0.99 ± 0.45%) ($p < 0.001$).
- **Thermophilic anaerobic digestion significantly weakened the transduction and reproduction capabilities of phages for ARGs, but remarkably enhanced their reduction effect**.

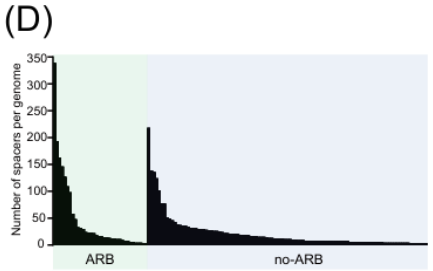
The dual role of antiviral defense systems

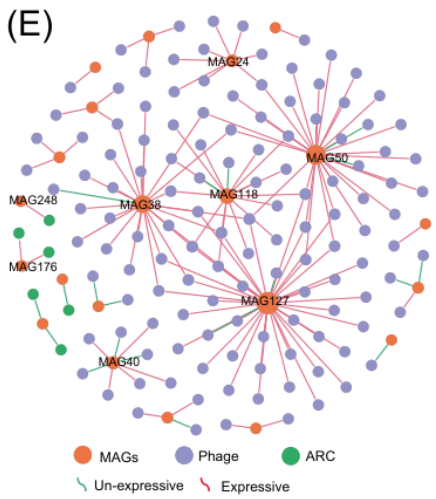


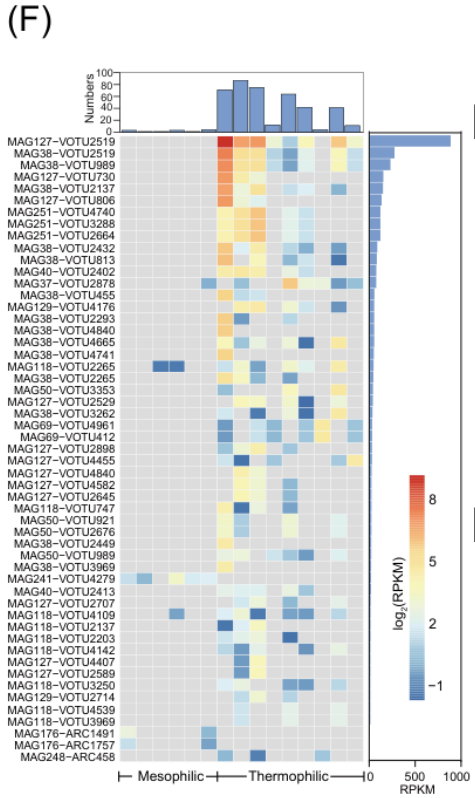


- **ADS are widely present**, dominated by Abi, CRISPR-Cas, and R-M systems, with **CRISPR-Cas being significantly enriched after entering the thermophilic stage**.
- On average, **each ARB carries 4.5 ADS, significantly higher than the 3.7 in non-ARB** ($p < 0.05$).







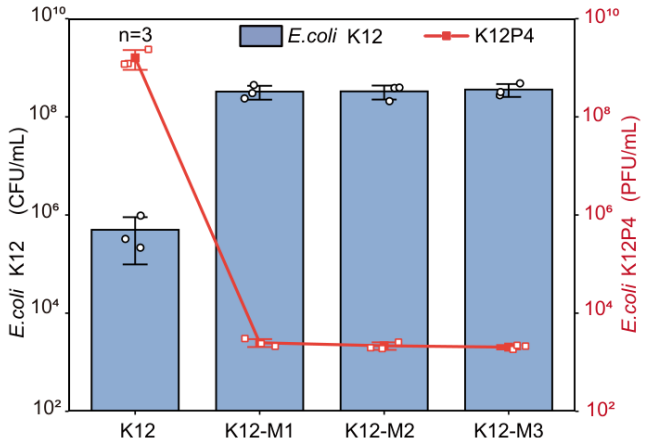


- The proportion of expressed ADS in ARB **increased from 27.5% to 46.4% in thermophilic condition**.
- **The number of CRISPR-Cas spacers in ARB is also significantly higher** than in non-ARB.
- The **CRISPR-Cas immune network demonstrates the immunity of ARB against phage lysis**; spacer expression confirms these defenses are actively occurring.
- Meanwhile, CRISPR-Cas also targets ARG-carrying contigs, **indicating its inhibitory role in the horizontal transfer of ARGs**.

The dual role of antiviral defense systems

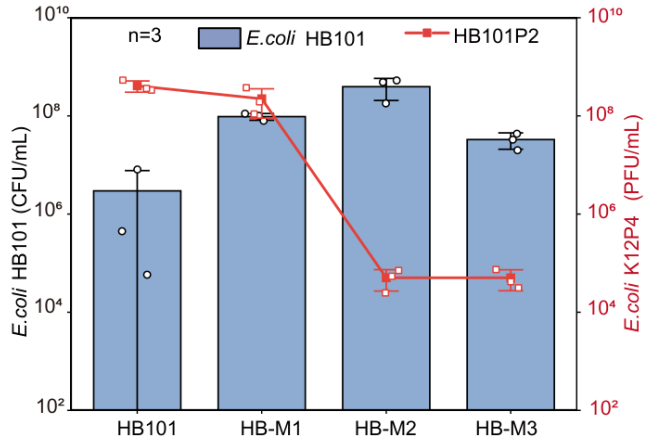
- **Donor:** Verification of antiviral defense *E. coli* K12 strains

(G)



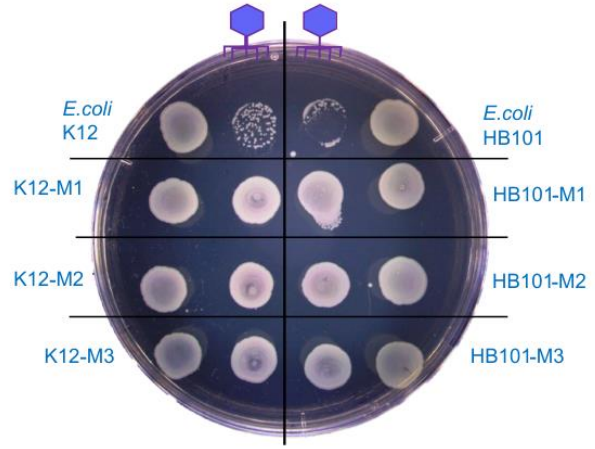
- **Recipient:** Verification of antiviral defense *E. coli* HB101 strains

(H)



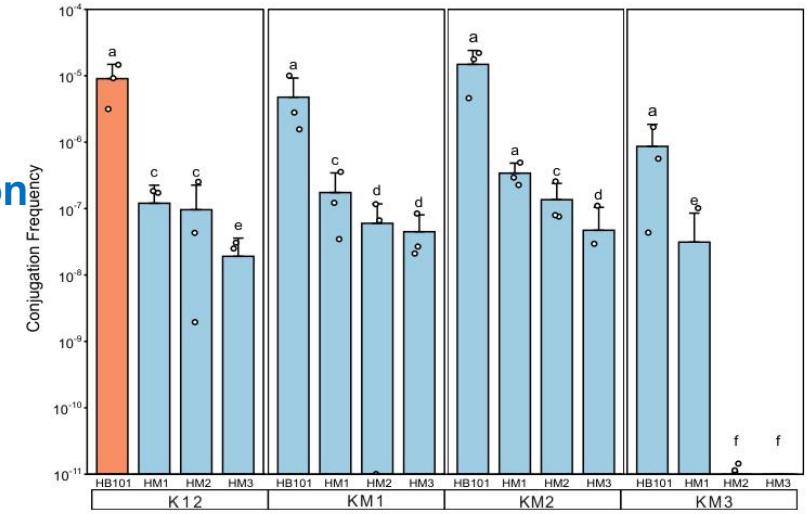
- **Phenotypic verification** of antiviral defense strains

(I)



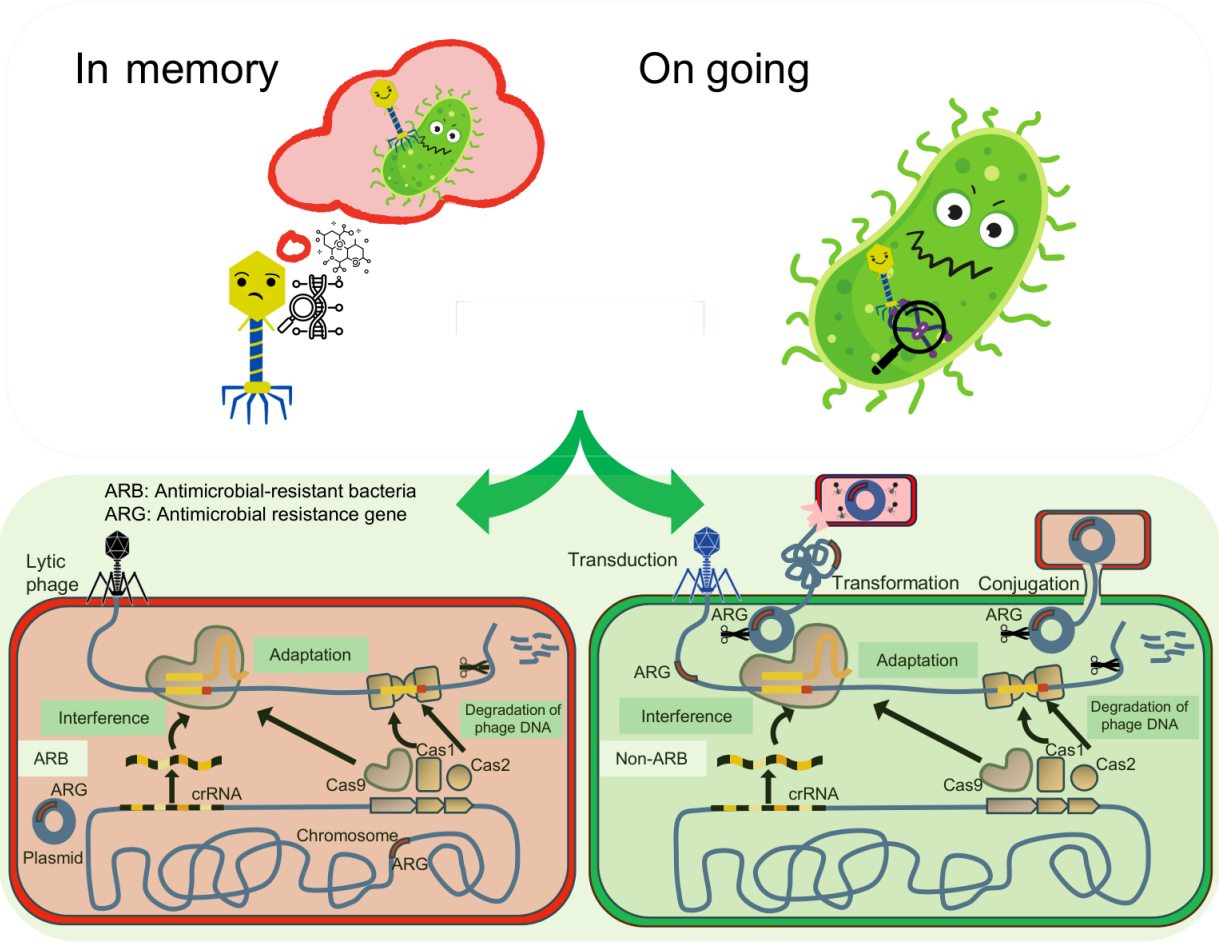
- Emergence of antiviral defense **significantly decreased conjugation frequency**

(J)



- After the emergence of phage immunity, compared to the wild-type pairs, **the frequency of acquiring the RP4 plasmid via conjugation significantly decreased by 98.7%–99.8%** (dropping from 9.05×10^{-6} to 1.91×10^{-8}, $p < 0.05$).

Take-home messages



- By integrating short/long-read metagenomics, metatranscriptomics, and *in situ* Hi-C interactions, combined with *in vitro* conjugation validation, **we systematically resolved the impact of the phage-host arms race on the fate of ARGs in anaerobic digestion**.

- We discovered that the proportion of phages carrying ARGs is extremely low, and transduction events are rare; **phages act more in reducing ARGs rather than as their transmission vectors.**

- **This study revealed the dual ecological role of ADS**: on one hand, protecting ARB from phage lysis and promoting their long-term persistence; on the other hand, inhibiting plasmid-mediated horizontal transfer of ARGs.

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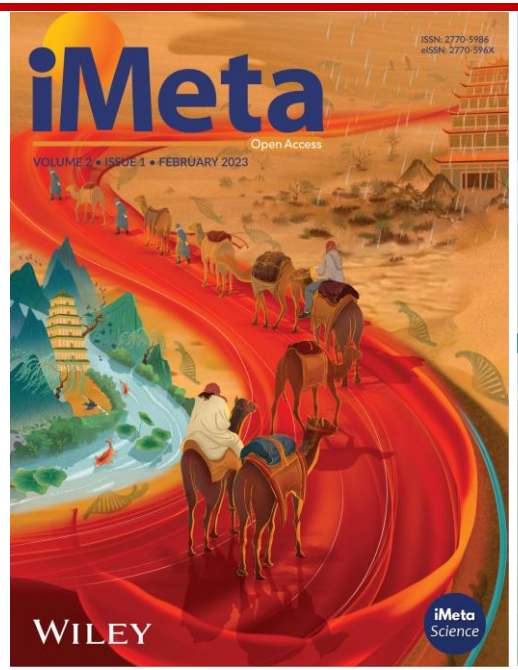

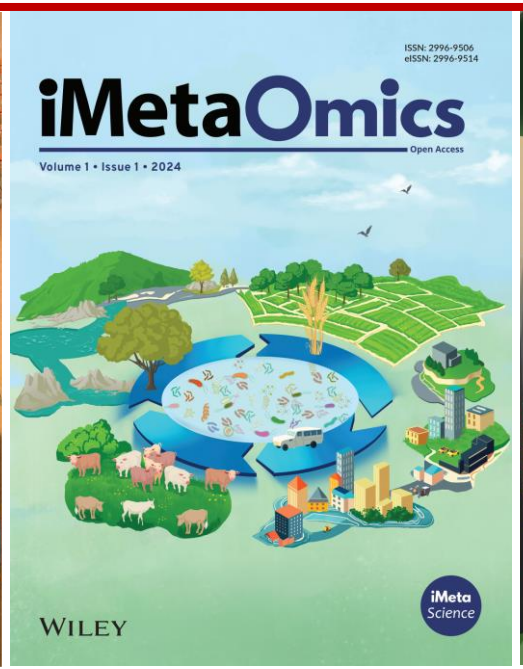

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