

## Metagenomics and Digital Cell Modeling Facilitate Targeted High-Throughput Sorting of Anaerobic Hydrogen-Producing Microorganisms

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### Introduction



In traditional anaerobe sorting, a "cultivate first, followed by sort" strategy is often likened to "The Blind Men and the Elephant".

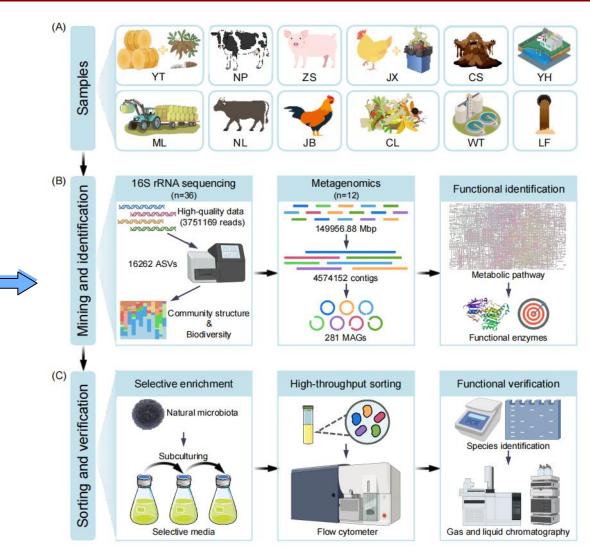
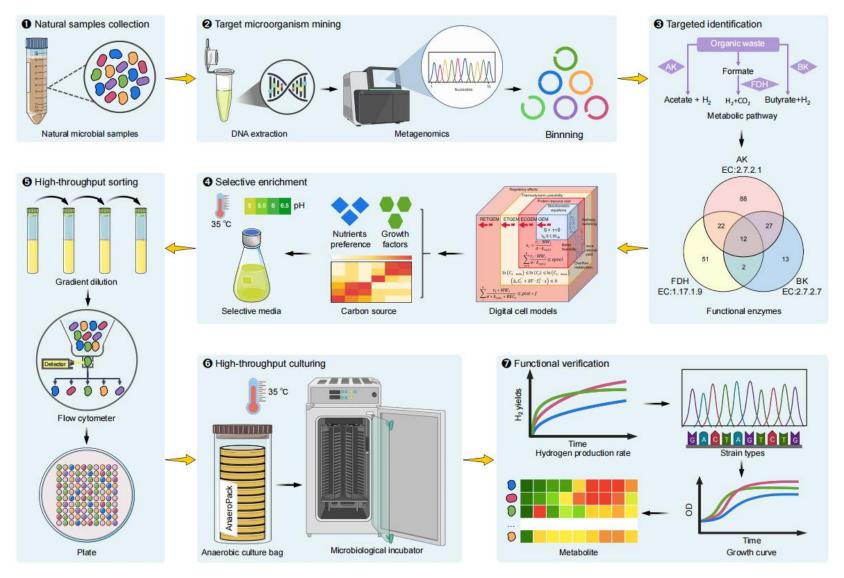


Figure 1A-1C. Experimental design and workflow



## **Highlights**



We propose a novel strategy, "prioritizing functional recognition, followed by targeted high-throughput sorting", for comprehensive, rapid, and efficient acquisition of target microorganisms.



## Mining of hydrogen-producing microorganisms

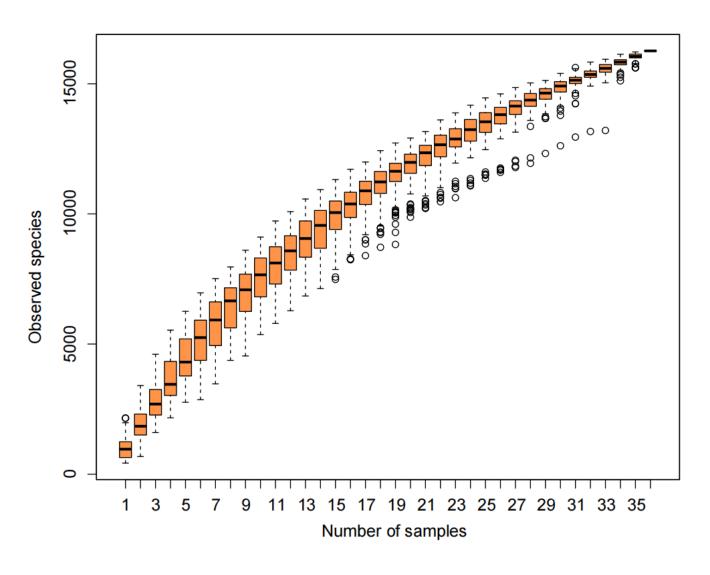


Figure S1. A total of 16,262 amplicon sequence variants (ASVs)

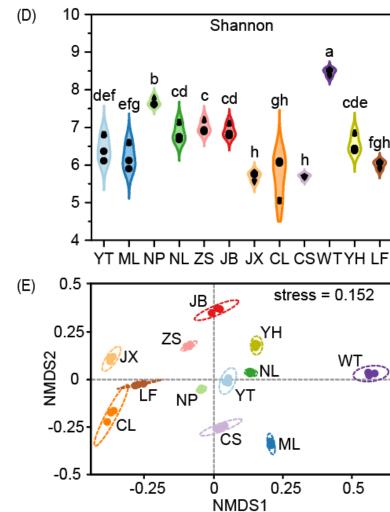


Figure 1D and 1E. Alpha diversity and Beta diversity of the microbial communities



## Mining of hydrogen-producing microorganisms

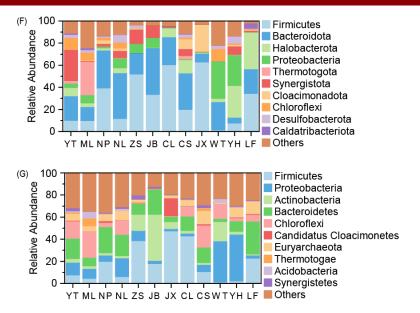


Figure 1F and 1G Community composition at the phylum level

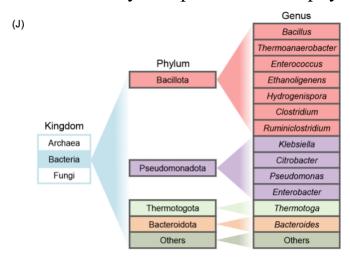


Figure 1J. Taxonomic classification of reported hydrogen-producing strains

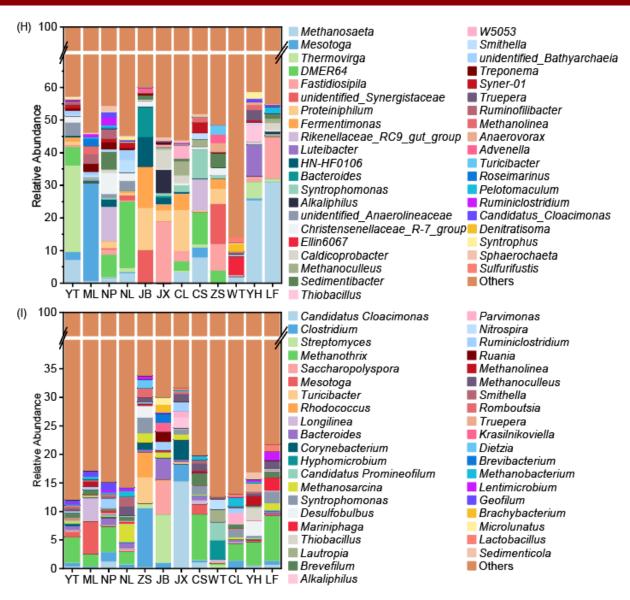


Figure 1H and 1I. Community composition at the genus level

# Identification of hydrogen-producing microorganisms

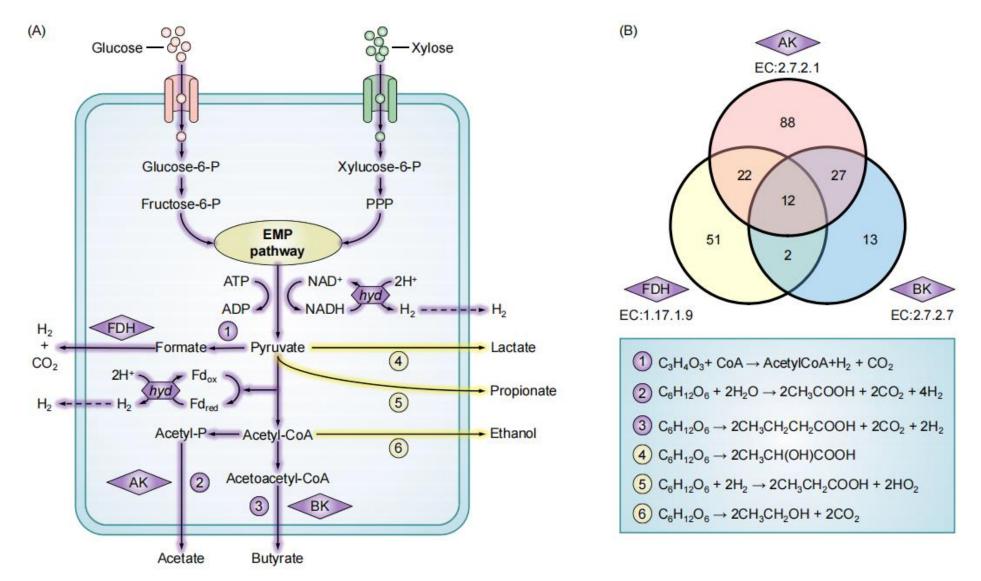


Figure 2A. The core metabolic pathways involved in anaerobic fermentation and hydrogen production

Figure 2B. Functional identification of potential anaerobic hydrogen-producing microorganisms

# Identification of hydrogen-producing microorganisms

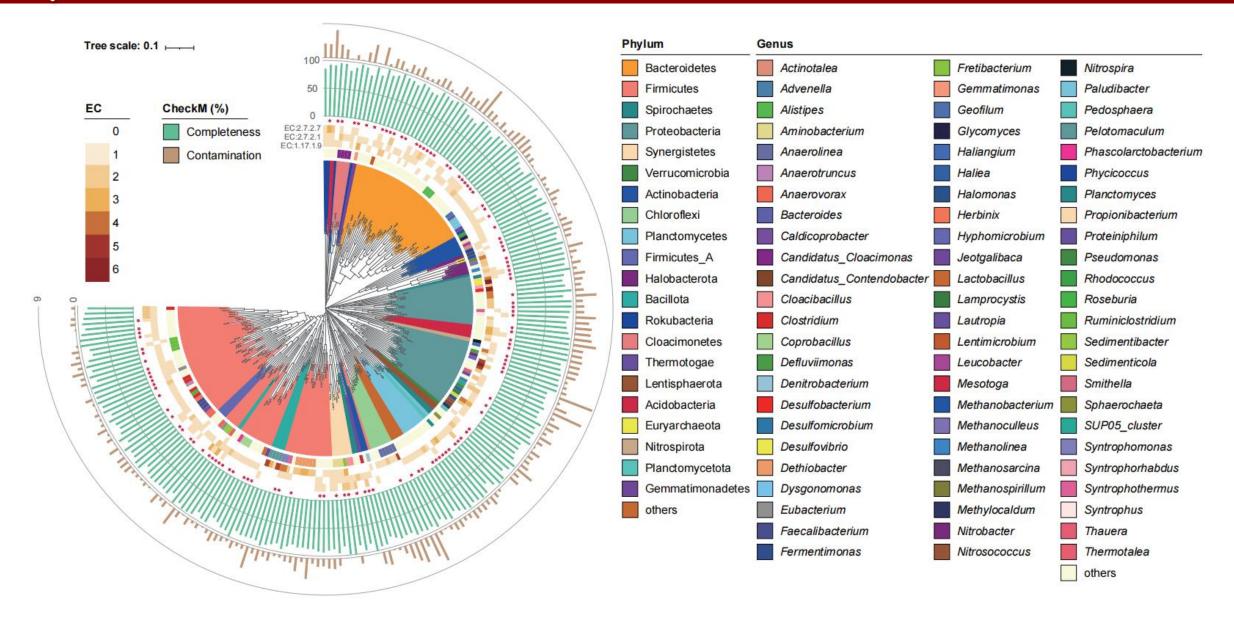


Figure 2C. Phylogenetic tree of 215 metagenome-assembled genomes (MAGs)



## Selective medium and High-throughput sorting

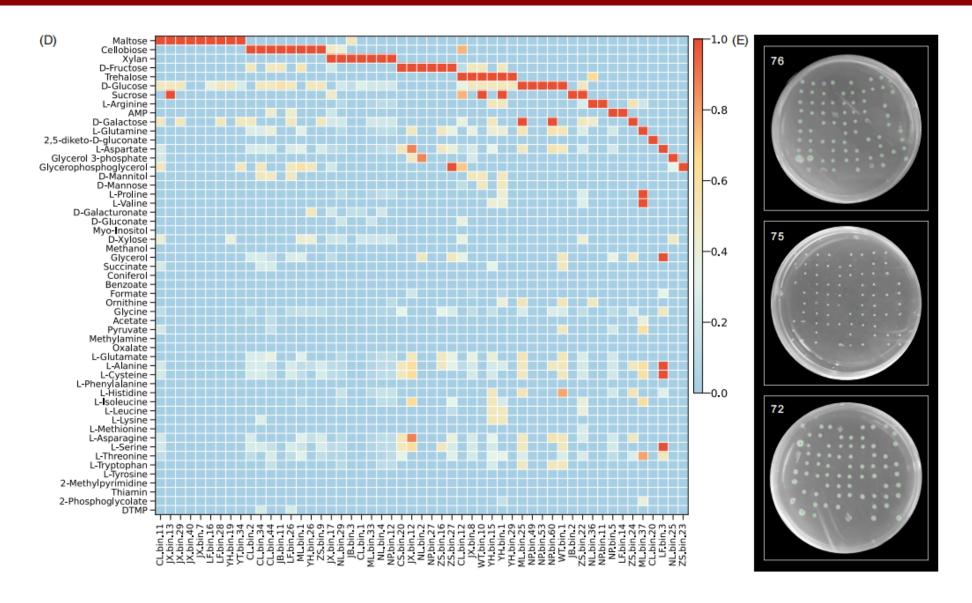


Figure 2D and 2E. Simulation prediction of digital cell model and high-throughput sorting of target strains



## **Summary**

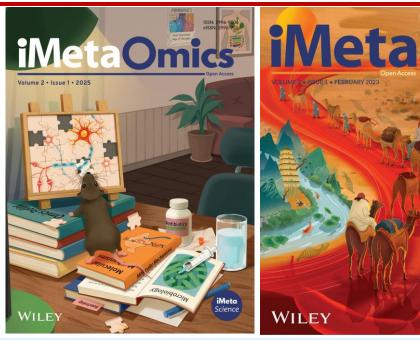
- □ We proposed a new strategy of "function recognition priority coupled with targeted high-throughput sorting", achieving a paradigm shift from experience-driven to data-driven approaches. This strategy demonstrates strong universality and broad application prospects.
- By constructing digital cell models based on metagenomic data, we accurately predicted the carbon source utilization profiles, nutritional requirements, and metabolic characteristics of target strains, providing a theoretical basis for the rational design of selective culture media.
- We established a comprehensive anaerobic microbial workflow encompassing high-throughput excavation, identification, cultivation, and sorting. The acquisition rate of target strains reached 37%, with a viability rate exceeding 70%. This achievement lays a critical foundation of key strains for the industrialization of synthetic microbiomes.

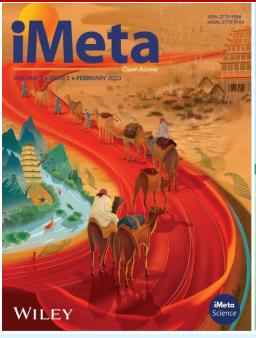
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