Phage-resistant E. coli strains developed to reduce fermentation failure

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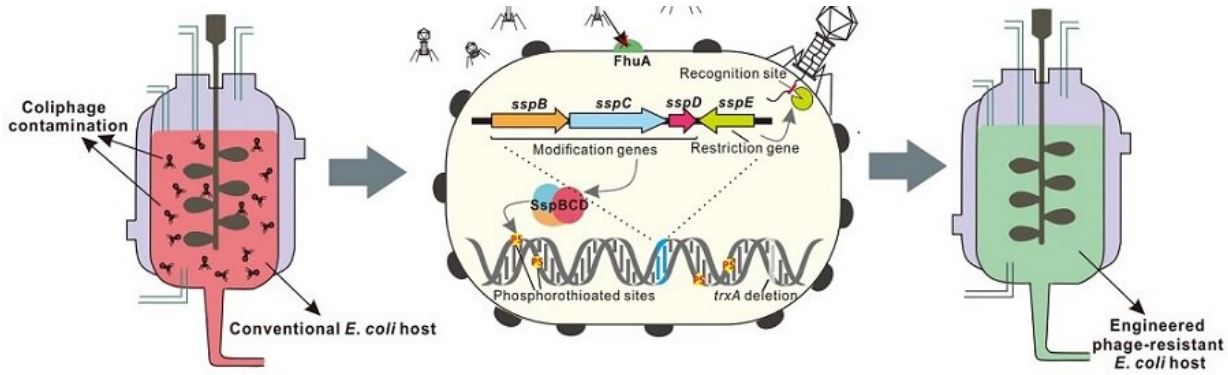

Fig. A schematic model of the systematic strategy for engineering phage-sensitive industrial E. coli strains into strains with broad antiphage activities. Through the simultaneous genomic integration of a DNA phosphorothioation-based Ssp defense module and mutations of components essential for the phage life cycle, the engineered E. coli strains show strong resistance against diverse phages tested and maintain the capabilities of producing example recombinant proteins, even under high levels of phage cocktail challenge. Credit: The Korea Advanced Institute of Science and Technology (KAIST)

A genome engineering-based systematic strategy for developing phage resistant Escherichia coli strains has been successfully developed through the collaborative efforts of a team led by Professor Sang Yup Lee, Professor Shi Chen, and Professor Lianrong Wang. This study by Xuan Zou et al was published in *Nature Communications* in August 2022 and

featured in the Editors' Highlights. The collaboration by the School of Pharmaceutical Sciences at Wuhan University, the First Affiliated Hospital of Shenzhen University, and the KAIST Department of Chemical and Biomolecular Engineering has made an important advance in the metabolic engineering and fermentation industry as it solves a big problem of phage infection causing fermentation failure.

Systems metabolic engineering is a highly interdisciplinary field that has made the development of microbial cell factories to produce various bioproducts including chemicals, fuels, and materials possible in a sustainable and environmentally friendly way, mitigating the impact of worldwide resource depletion and climate change. Escherichia coli is one of the most important chassis microbial strains, given its wide applications in the bio-based production of a diverse range of chemicals and materials. With the development of tools and strategies for systems metabolic engineering using E. coli, a highly optimized and well-characterized cell factory will play a crucial role in converting cheap and readily available raw materials into products of great economic and industrial value.

However, the consistent problem of phage contamination in fermentation imposes a devastating impact on host cells and threatens the productivity of bacterial bioprocesses in biotechnology facilities, which can lead to widespread fermentation failure and immeasurable economic loss. Host-controlled defense systems can be developed into effective genetic engineering solutions to address bacteriophage contamination in industrial-scale fermentation; however, most of the resistance mechanisms only narrowly restrict phages and their effect on phage contamination will be limited.

There have been attempts to develop diverse abilities/systems for environmental adaptation or antiviral defense. The team's collaborative efforts developed a new type II single-stranded DNA phosphorothioation

(Ssp) defense system derived from E. coli 3234/A, which can be used in multiple industrial E. coli strains (e.g., E. coli K-12, B and W) to provide broad protection against various types of dsDNA coliphages.

Furthermore, they developed a systematic genome engineering strategy involving the simultaneous genomic integration of the Ssp defense module and mutations in components that are essential to the phage life cycle. This strategy can be used to transform E. coli hosts that are highly susceptible to phage attack into strains with powerful restriction effects on the tested bacteriophages. This endows hosts with strong resistance against a wide spectrum of phage infections without affecting bacterial growth and normal physiological function. More importantly, the resulting engineered phage-resistant strains maintained the capabilities of producing the desired chemicals and recombinant proteins even under high levels of phage cocktail challenge, which provides crucial protection against phage attacks.

This is a major step forward, as it provides a systematic solution for engineering phage-resistant bacterial strains, especially industrial bioproduction strains, to protect cells from a wide range of bacteriophages. Considering the functionality of this engineering strategy with diverse E. coli strains, the strategy reported in this study can be widely extended to other bacterial species and industrial applications, which will be of great interest to researchers in academia and industry alike.

More information: Xuan Zou et al, Systematic strategies for developing phage resistant Escherichia coli strains, *Nature Communications* (2022). DOI: 10.1038/s41467-022-31934-9

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