【Grant-in-Aid for Transformative Research Areas（B）】

Bacteria UX: Universal transformation for genetic modification of any bacteria

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Project Information	Project Number : 25B207　Project Period (FY) : 2025-2027 Keywords : non-model bacteria, transformation, intracellular nucleases, cell membrane, artificial nucleic acids

Purpose and Background of the Research

●Outline of the Research

Life science research and biotechnology have long depended on the transformation of *Escherichia coli* (Fig. 1). However, some DNA sequences and proteins are difficult to handle in *E. coli*, limiting progress in the field. While *E. coli* is often considered easy to modify, this does not apply to most other bacteria, which remain challenging to transform. Fundamental, systematic research on transformation across diverse species is still lacking.

This project seeks to develop a "universal transformation" method that enables genetic modification of a wide range of bacteria, unlocking new microbial resources and advancing both basic and applied biosciences.

●Genetic Transformation is Similar to Viral Infection

From a bacterial perspective, introducing recombinant DNA is similar to a viral infection—foreign genetic material enters, replicates, and functions in the cell. In nature, bacteria are constantly exposed to bacteriophages (Fig. 2, left). To protect themselves, they have evolved defense systems that eliminate foreign DNA. Therefore, environmental bacteria are typically resistant to recombinant DNA, treating it like a phage and removing it (Fig. 2, right). So far, transforming such strains has relied on trial and error, without clear scientific guidance. Even in transformable bacteria, the reasons for success are often poorly understood. As the limitations of *E. coli* become more evident, basic research into bacterial transformation is increasingly vital for future progress in life science and biotechnology.

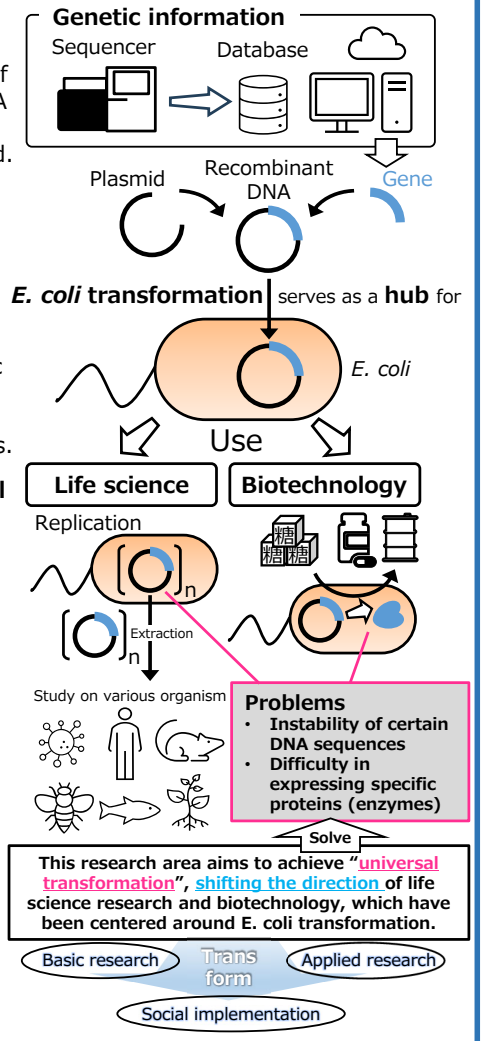

Figure 1. Outline of the research

Expected Research Achievements

●Overall Goal of this research area

This project focuses on fundamental research and technology development to overcome the "firewalls" that prevent the entry and replication of foreign DNA in bacteria. These barriers—① the cell membrane and ② intracellular nucleases (Fig. 2). By the end of this project, we aim to develop a scientifically grounded experimental framework and new techniques that will guide researchers in transforming bacteria other than *E. coli*.

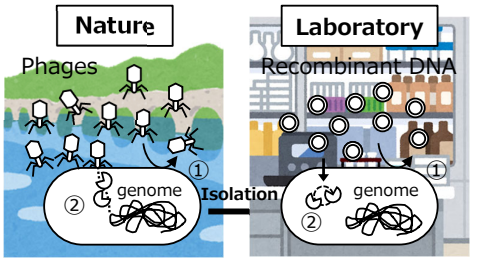

Figure 2 Bacterial firewalls

●Teams and Their Roles (Fig. 3)

Research Teams

Team A01: Molecular Bacteriology (Member: Ishikawa, Aoki, and Koiwai)
This team focuses on understanding and bypassing Firewall② intracellular nucleases.

Team A02: Nucleic Acid Chemistry (Member: Kamiya and Ariyoshi)
This team aims to inactivate Firewall② intracellular nucleases.

Team A03: Nano Bioengineering (Member: Tanaka and Mori)
This team efficiently circumvents Firewall① the bacterial cell membrane.

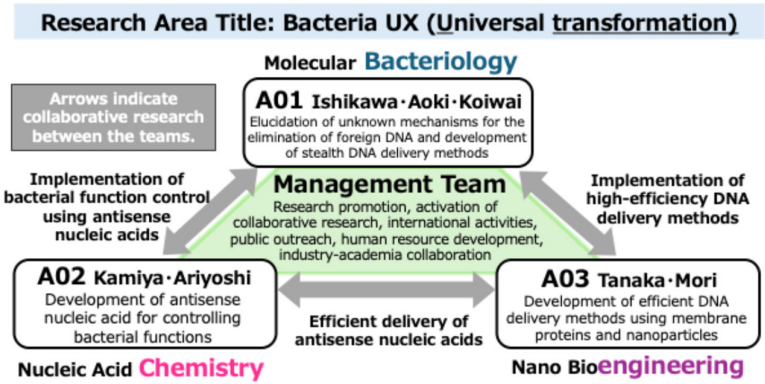

Figure 3　Research organization chart

●Future Outlook and Broader Impact

The continued dependence on *E. coli* as a transformation host limits the scope of recombinant DNA construction and biotechnological innovation.
The realization of universal transformation, enabling genetic modification across all bacterial species, will bring transformative change not only within microbiology but also across diverse scientific disciplines.
By creating new and interdisciplinary research areas, this initiative has the potential to:

- ☐ Uncover new biological phenomena and cellular functions (basic research)
- ☐ Foster innovative biotechnologies (applied research)
- ☐ Contribute to a more sustainable society (social implementation)

Homepage Address, etc.	http://bacteria-ux.jp/