# Elucidating metabolic pathways in useful bacteria using CPP-PNA conjugates

Kakeru Mori*[1], Yugo Kawabuchi[1], Rei Suo[2], Shiro Itoi[2], Ryutaro Asano[1], and Tetsushi Mori†[1]

[1]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo – Japan

[2]College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa – Japan

**Abstract**

Bacterial mutants are crucial for the stable production of natural compounds, requiring the identification and inactivation of non-essential pathways or enzymes involved in target compound biosynthesis. However, conventional metagenesis approaches are labor-intensive and time-consuming. This study explores the use of cell-penetrating peptide-peptide nucleic acid (CPP-PNA) conjugates as a rapid in vivo tool for enzyme identification and functional evaluation in highly useful or hard-to-transform secondary metabolite producers. In this work, the *cyanobacteria*, *Synechocystis* sp. PCC6803 and a *Streptomyces* strain, *Streptomyces avermitilis*, were used as models. First, CPP-PNAs were synthesized to target the housekeeping genes of each strain and permeation efficiency was assessed by growth inhibition. Effective inhibition within 24-48 hours confirmed successful intracellular delivery. CPP-PNAs were designed to inhibit the translation of D-lactate dehydrogenase (Ddh) to assess its role in pyruvate accumulation for strain PCC6803, and biosynthetic enzymes in flaviolin production for *S. avermitilis*, respectively. Results showed that CPP-PNA effectively suppressed Ddh translation in strain PCC6803, leading to pyruvate accumulation within 24 hours. Interestingly, D-lactate also accumulated, indicating the presence of an alternative pathway for D-lactate biosynthesis. CPP-PNA regulation in *S. avermitilis* also showed promising results whereby reduction in the production of flaviolin was observed when the polyketide and cytochrome P450 was targeted. These findings position CPP-PNA as a rapid and efficient tool for enzyme function analysis and metabolic pathway elucidation, offering a novel strategy for bacterial strain engineering. Additionally, this method provides valuable insights into alternative biochemical routes, potentially advancing metabolic engineering in bacterial systems.

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*Speaker

†Corresponding author: moritets@go.tuat.ac.jp