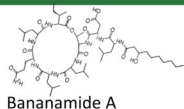




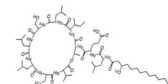
High-Throughput Screening of Promoters for Enhanced Cyclic Lipopeptide heterologous Production in *Pseudomonas*

1 Challenge of Cyclic Lipopeptides (CLiPs) Production



Bananamide A

An underexploited biotechnological potential → Why ??



Orfamide A



Low Yields

Low production by wild-type strains



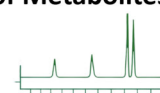
Cryptic Biosynthetic Gene Clusters

Native regulation limiting gene expression



Co-production of Metabolites

Complex mixtures of compounds

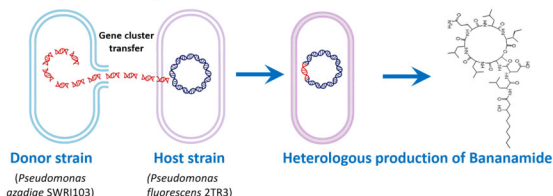


These limitations create major challenges for the purification, characterization, and individual valorization of CLiPs

2 Heteroclips Strategy

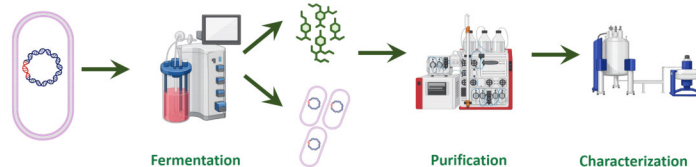
Heterologous expression

Introduce CLiPs biosynthetic gene clusters into an optimized host for controlled production



Process Engineering

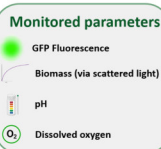
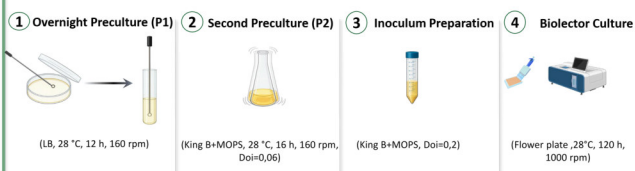
Scalable Production, Purification and Characterization of CLiPs



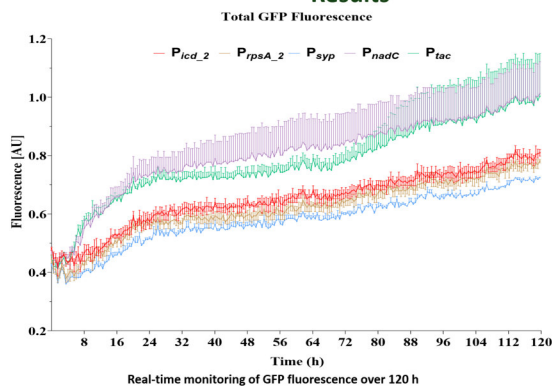
Combining heterologous expression and process engineering to unlock the production potential of CLiPs

3 Identification of Strong Promoters Using GFP Transcriptional Reporters

Workflow



Results

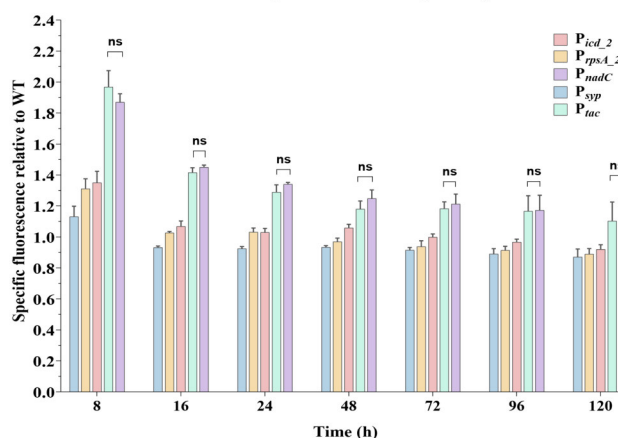


Key findings

- P_{nadC}** displayed the highest GFP fluorescence
- P_{tac}** exhibited strong GFP fluorescence
- P_{ica_2}, P_{rpsA_2}, P_{syp}** showed basal activity comparable to WT

Results

Relative specific fluorescence (WT = 1)



Comparison of promoter activities based on relative specific GFP fluorescence. Data represent the mean $\pm$ SD of three replicates. For clarity, only non-significant differences (ns) are indicated. All other comparisons were statistically significant ($p < 0.05$), as determined by two-way ANOVA followed by Tukey's multiple comparisons test

P_{nadC} and P_{tac} exhibited the highest promoter activities throughout cultivation, particularly during the early stages of growth

4 Take-Home Message



P_{nadC} and P_{tac} were identified as the strongest promoters for heterologous CLiPs production in *Pseudomonas*

5 Next steps



Integration of **P_{nadC}** and **P_{tac}** upstream of CLiPs biosynthetic clusters



Culture optimization



Scale-up in bioreactors



Purification and characterization



Biocontrol applications