Bacterial Strain Engineering To Enhance The Heterologous Production Of The Cyclic Lipopeptide Orfamide

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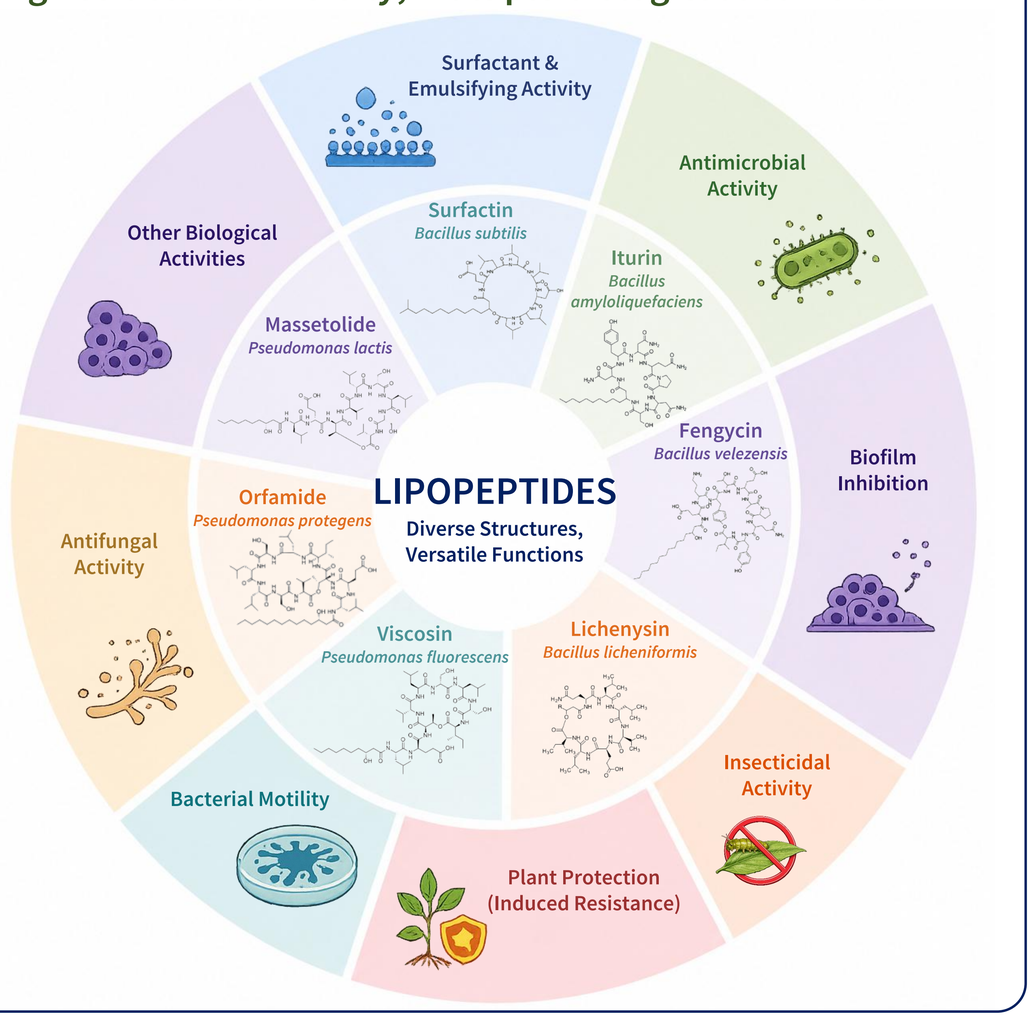
CLiPs: PROMISING BIOCONTROL MOLECULES

High structural diversity, multiple biological activities



A MAJOR CHALLENGE

- Large biosynthetic gene cluster
- Complex biosynthetic pathways requiring specialized host machinery
- Limited understanding of regulatory mechanisms
- Risk of instability and low expression

KEY ADVANTAGES

- Broad-spectrum antimicrobial activities
- Biosurfactant properties
- Induction of plant defense responses
- Eco-friendly alternatives to chemical pesticides

CURRENT LIMITATIONS

- Low natural production yields
- Cryptic or poorly expressed biosynthetic gene clusters
- Production as complex mixtures of structural variants

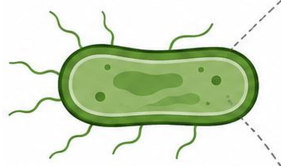
CONSEQUENCES

- Difficult characterization of bioactivities
- Challenging industrial-scale production
- Limited exploitation in biocontrol applications

HETEROLOGOUS PRODUCTION: A STRATEGY TO UNLOCK CLiPs BIOSYNTHESIS

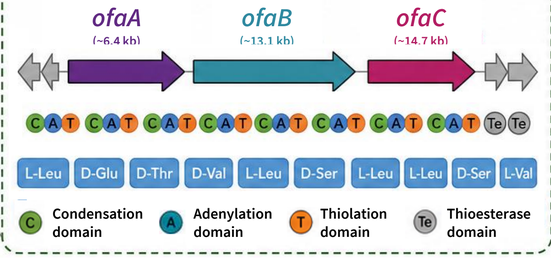
Example of orfamide

Native producer

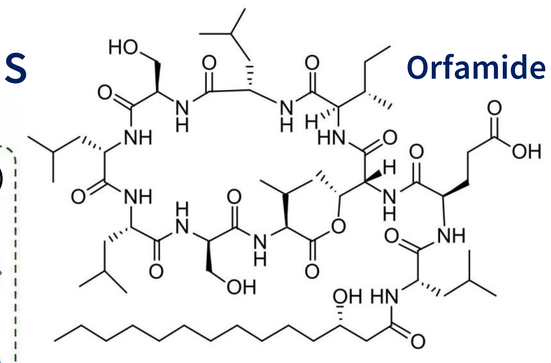


Pseudomonas protegens Fl5BN2

The *ofaABC* biosynthetic gene cluster (~34.2 kb)



Orfamide



Antifungal activity; Insecticidal activity; Biosurfactant properties; Induction of plant defense responses

MAIN STEPS OF HETEROLOGOUS PRODUCTION OF ORFAMIDE

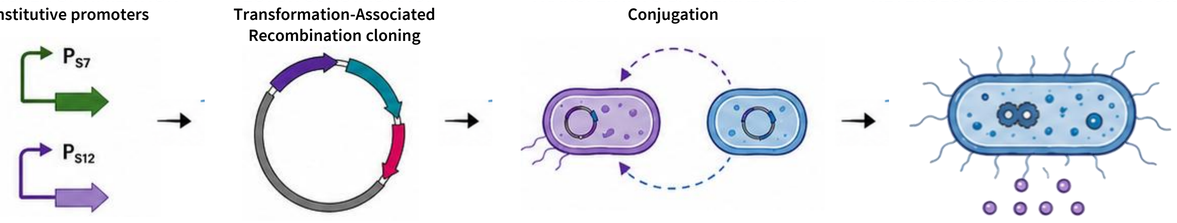
Goal : Develop an efficient heterologous system to produce and exploit orfamide for biocontrol applications

PROMOTER OPTIMIZATION
Constitutive promoters

VECTOR CONSTRUCTION
Transformation-Associated Recombination cloning

TRANSFER INTO BACTERIAL HOST
Conjugation

HETEROLOGOUS EXPRESSION OF ORFAMIDE



HOST SELECTION: *Paraburkholderia phytofirmans* PsJN

Selection criteria

Genetic tractability
Availability of genetic tools and efficient genome engineering capabilities

Robust growth and high biomass production
Rapid growth kinetics and high cell density under laboratory conditions

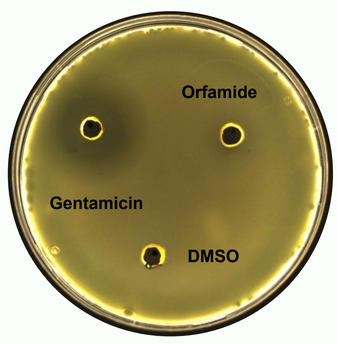
Intrinsic tolerance to the target lipopeptide
Resistance to the target lipopeptide to prevent self-toxicity during heterologous production

Low endogenous secondary metabolite production
Reduced production of native metabolites facilitating heterologous product detection and purification

Environmentally compatible and biosafe host
Suitable for sustainable production of biocontrol molecules

Tolerance to orfamide

Agar diffusion assay



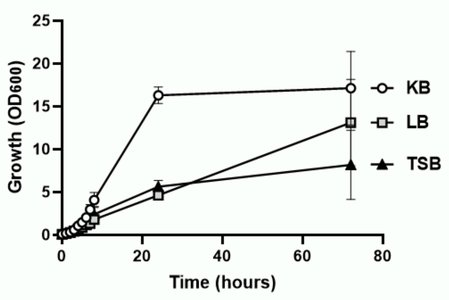
Orfamide : 1 g/L; Gentamicin : 50 µg/mL; DMSO: 10%

No inhibition zone observed with orfamide

PsJN is naturally resistant to orfamide

Robust growth

Growth curves in rich media (30°C)

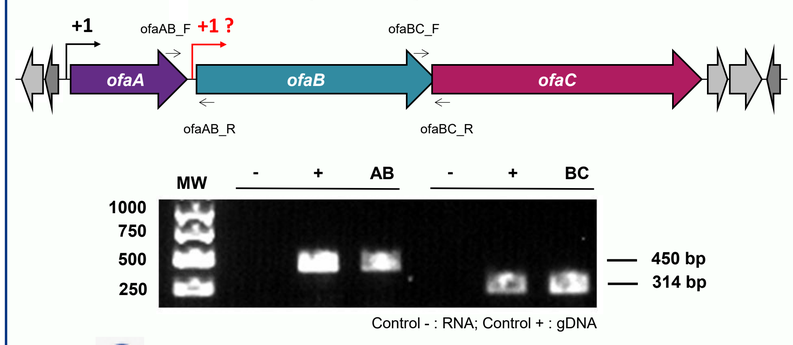


Rapid growth and high biomass accumulation

PROMOTER ENGINEERING

Orfamide synthetase genes organization

Reverse Transcription-Polymerase Chain Reaction

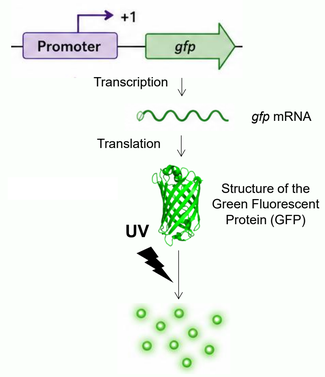


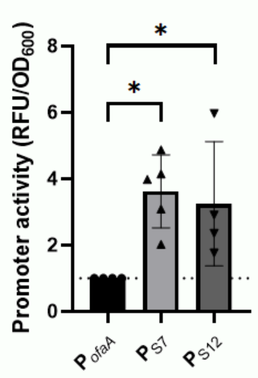
The *ofaA*, *ofaB*, and *ofaC* genes are co-transcribed

Promoter activity measurement

Transcriptional activity quantified using GFP reporter strains

- P_{S7} : constitutive promoter of the S7 ribosomal protein from the 30S ribosomal subunit of *P. phytofirmans* PsJN
- P_{S12} : constitutive promoter of the S12 ribosomal protein from the 30S ribosomal subunit of *P. phytofirmans* PsJN





Strong constitutive promoters enhance transcription of the *ofaABC* operon

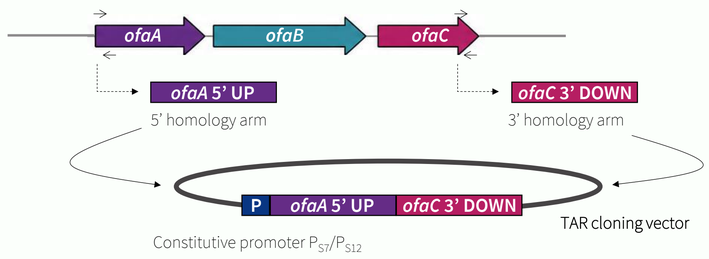
CLONING STRATEGY

Transformation-Associated Recombination cloning of the orfamide synthetase operon

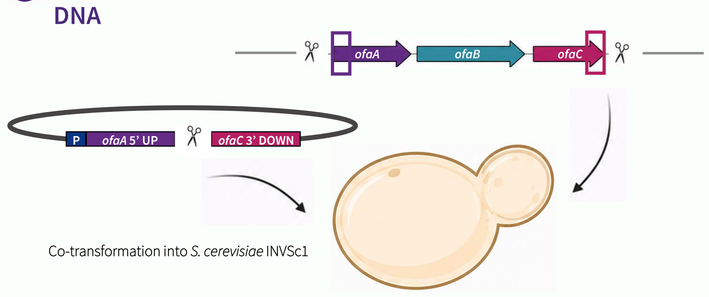
Assembly in the yeast *Saccharomyces cerevisiae* INVSc1

1 PCR construction of the TAR cloning vector

→ PCR amplification and insertion of the homology arms flanking the *ofaABC* operon, together with a strong constitutive promoter

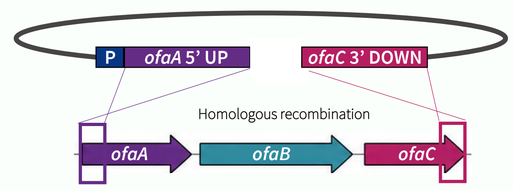


2 Linearization of the TAR cloning vector and donor genomic DNA

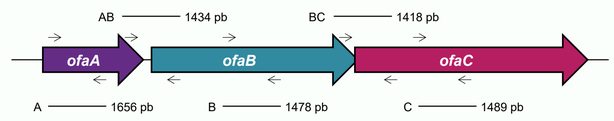


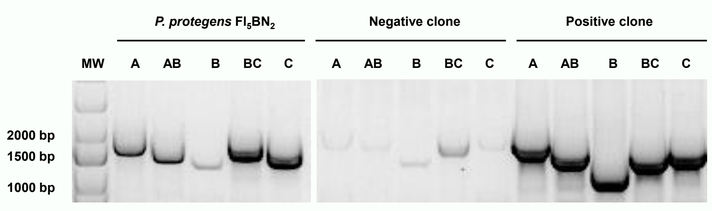
3 Homologous recombination in *S. cerevisiae* INVSc1

→ The *ofaABC* operon is captured through recombination at the *ofaA* 5′ and *ofaC* 3′ homology arms



4 PCR and sequence verification of the assembled TAR plasmid





Diagnostic PCR and sequencing confirm correct orfamide synthetase operon assembly

FROM GENOME MINING TO ALTERNATIVE HOSTS FOR HETEROLOGOUS PRODUCTION OF CLiPs

PSEUDOMONAS GENOME COLLECTION

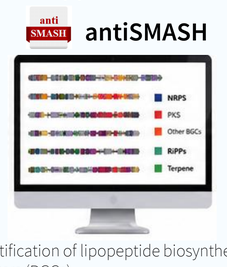
Thousands of publicly available *Pseudomonas* genomes

High taxonomic and ecological diversity

NCBI GenBank
EMBL European Molecular Biology Laboratory
DDBJ DNA Data Bank of Japan

LARGE-SCALE GENOME MINING

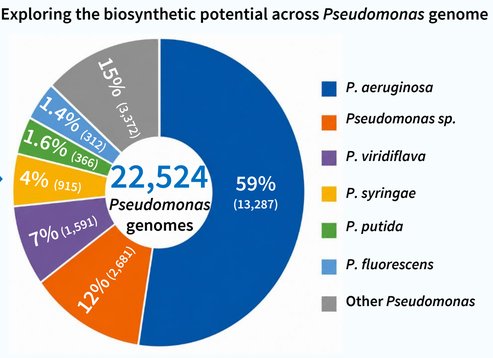
Automated pipeline with antiSMASH



- Identification of lipopeptide biosynthetic gene clusters (BGCs)
- Characterization of non-ribosomal peptide synthetase (NRPS) assembly lines
- Analysis of NRPS modules and domains
- Prediction of lipopeptide structures

PREDICTED CLiP BIOSYNTHETIC LANDSCAPE

Exploring the biosynthetic potential across *Pseudomonas* genome

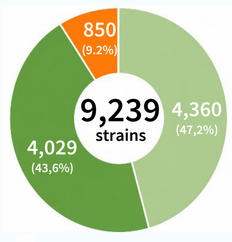


Distribution of genomes among the *Pseudomonas* genus

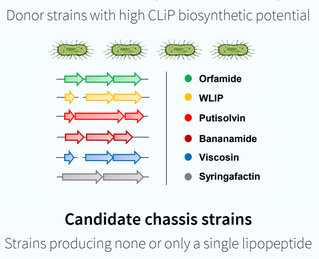
DONOR AND CHASSIS SELECTION

Reservoirs of biosynthetic pathways

Donor strains with high CLiP biosynthetic potential

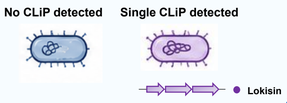


Predicted CLiP synthetases



Candidate chassis strains

Strains producing none or only a single lipopeptide



CONCLUSION & PERSPECTIVES

- ✓ *P. phytofirmans* PsJN is a promising heterologous host for orfamide production
- ✓ Promoter engineering and TAR cloning enable manipulation of large CLiP biosynthetic gene clusters
- ✓ Genome mining identified complementary donor and chassis strains for future CLiP production platforms
- ✓ Outlook: heterologous expression, production optimization, and biocontrol applications