# Optimization and scale-up of fengycins production by fermentation in chemically defined or co-products based media

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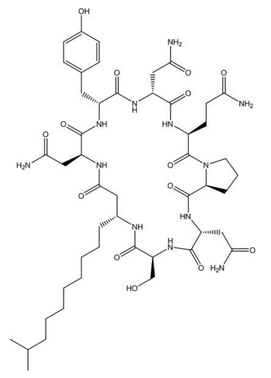
## 1 RESEARCH CONTEXT

Among all lipopeptides produced by *Bacillus* strains, we are interested in **fengycins**. These cyclic lipopeptides naturally produced by ***Bacillus subtilis*** are recognized for their strong antifungal, antibiofilm and surfactant properties. These properties lead to many interesting applications in the environmental, agricultural or health sectors.

However, due to their current high production costs, fengycins are in most applications not able to challenge the chemically synthesized counterparts economically. To improve their production yields and make fengycins cost-effective, we implemented an integrated strain engineering strategy. A first production strain (mono- and hyper-producer of fengycins) has been generated through UV-induced mutagenesis (Lipomme-bio project – See poster 1.12). Through CRISPR-Cas9 engineering (SURFs UP project), we then obtained a non-sporulating *B. subtilis* strain producing exclusively fengycins up to 1 g/L. This strain was subsequently used to **develop an efficient and scalable production process**.



Fengycin structure

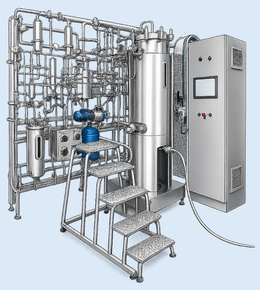
## 2 PRODUCTION PROCESS

### UPSTREAM PROCESS (USP)

1 Shaking flask preculture

2 Benchtop bioreactor preculture

3 Pilot-scale bioreactor culture



*Objectives : biomass growth and fengycins production*

### DOWNSTREAM PROCESS (DSP)

4 In-line centrifugation → 5 Ultrafiltration & diafiltration → 6 Falling-film evaporation

7 Purification

7.1 Acid & ethanol purification

7.1.1 Acid precipitation → 7.1.2 Centrifugation → 7.1.3 Ethanol resuspension


Fengycins and proteins precipitation → Fengycins enriched pellet recovery → Pellet resuspension and proteins precipitation

7.1.4 Centrifugation → 7.1.5 Rotary evaporation → 7.1.6 Freeze-drying → 7.1.7 Storage

Fengycins enriched supernatant recovery → Ethanol removal → Fengycins drying → Fengycins powder

OR

7.2 C18 matrix bulk purification

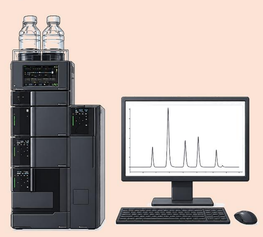
7.2.1 Binding → 7.2.2 Elution → 7.2.3 Freeze-drying → 7.2.4 Storage

Fengycins capture → Fengycins collect → Fengycins drying → Fengycins powder

*Objectives : fengycins production recovery, concentration, purification and freeze-drying*

### QUALITY CONTROL (QC)

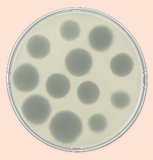
8 UPLC-DAD quantification



**Fengycins purity** levels reached :

- Acid & ethanol purification purity **≥ 90%**
- C18 bulk purification purity **≥ 99%**

9 Antifungal activity test



*Objectives : fengycins production quantification, purity evaluation and antimicrobial activity assessment*

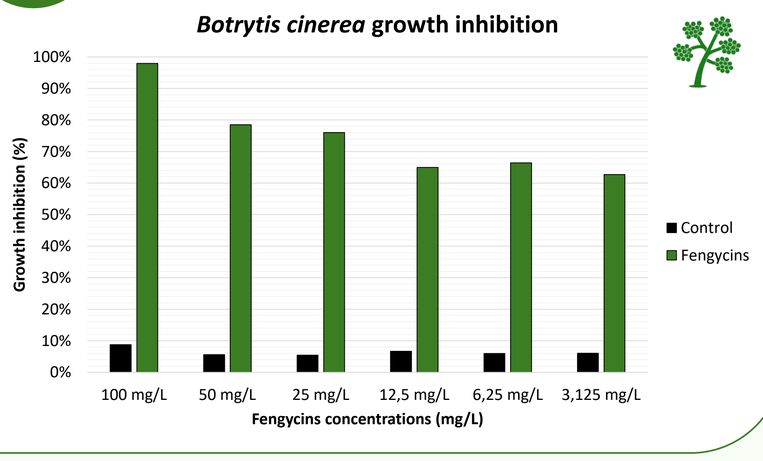
The **upstream process parameters** were first **optimized at bench scale** to identify the most efficient production conditions of our production strain in Landy2X medium.

The **optimized upstream process** parameters were then successfully transferred to a 200L pilot-scale bioreactor, enabling the production of approximately **100 grams of fengycins** from 150L of Landy2X medium, demonstrating **strong robustness and scalability** under pre-industrial conditions.

The **developed downstream process** consists of a sequential workflow including clarification by centrifugation, concentration and purification by ultrafiltration and diafiltration (10kDa membrane), and concentration by evaporation steps. **Two different purification processes** yielding **high-purity fengycins** were implemented to further enrich the product, confirming the **efficiency and consistency** of the overall integrated process. Finally, the activity of fengycins is assessed through their antifungal activity.

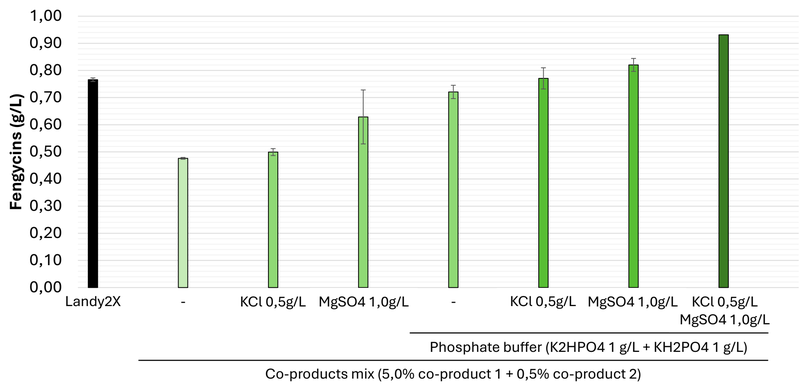
## 3 ANTIFUNGAL ACTIVITY

***Botrytis cinerea* growth inhibition**

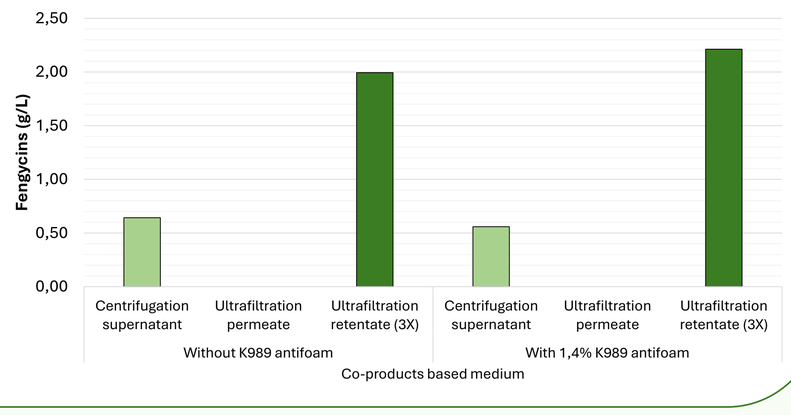



## 4 PRODUCTION MEDIA

**Production of fengycins in supplemented co-products mixes**




**Ultrafiltration of fengycins produced in co-products based media**




## 5 RESEARCH HIGHLIGHTS

- ✓ Generation of a non-sporulating *B. subtilis* strain, mono- and hyper-producer of fengycins.
- ✓ Development of an integrated USP–DSP process yielding ±100g of fengycins per batch with ≥90% purity.
- ✓ Confirmation of the antifungal activity of fengycins against *B. cinerea.*
- ✓ Development of a co-products based medium reducing medium cost by ±20 folds while maintaining high fengycins production levels.

## 6 PERSPECTIVES

- 🔍 Optimize fengycins production parameters in the developed co-products based medium.
- 🔍 Scale-up fengycins production process (USP & DSP) in this medium.

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