Fed-batch production of ice-binding protein of *Flavobacterium frigoris* PS1 by recombinant *Pichia pastoris*

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Abstract

Ice-binding proteins (IBPs) inhibit ice growth to permit the survival of polar organisms in the cold environments. The recombinant IBP from an Antarctic bacterium, *Flavobacterium frigoris* PS1, FfIBP (*Flavobacterium frigoris* ice-binding protein), was produced using *Pichia pastoris* expression system. The optimum fermentation temperature and pH for fed-batch production of FfIBP were 30℃ and 5, respectively. The maximal cell density and purified FfIBP were 112 g/L and 70 mg/L, respectively. The thermal hysteresis (TH) activity (0.85℃) of FfIBP obtained using a glycerol-methanol fed-batch culture system was 2-fold higher than that of the LeIBP (*Leucosporidium* ice-binding protein). This work allows for large-scale production of FfIBP, which could be extended to further application studies using recombinant IBPs.

Introduction

- ✓Ice-binding proteins (IBPs) can interact directly with ice.
- ✓Thermal hysteresis (TH): the difference between the melting and freezing points. TH occurs when IBPs bind to the ice crystal and inhibition of its growth.
- ✓We have isolated an ~25 kDa extracellular IBP (LeIBP) from the Arctic yeast *Leucosporidium* sp. AY30 (*Glaciozyma*).[1]
- ✓In the pilot-scale fermentation (700 L), the yield of rLeIBP was 300 mg/L.[2]
- ✓In the previous study, we provided a molecular basis for understanding the antifreeze mechanism of FfIBP and suggested new insights into the reasons for the higher TH activity of FfIBP (IBP from *Flavobacterium frigoris* PS1).[3]
- ✓This study aimed to find the optimal conditions for the production of recombinant FfIBP in Fed-batch culture of P. pastoris.

Materials & Methods

- ✓Bacterial strain

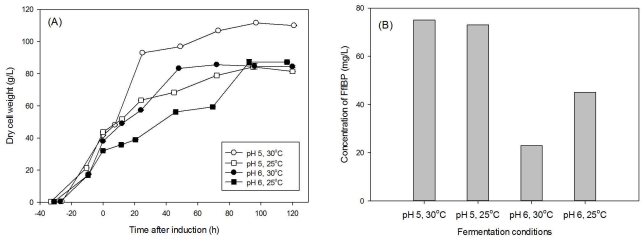
  FfIBP producing bacterium: *Pichia pastoris* X-33 (pPICZαA-FfIBP)
- ✓Composition of culture media (g/L) : FBS (Fermentation basal salts)

  glycerol, 40 g/L; $CaSO_4$, 0.93 g/L; K_2SO_4, 18.2 g/L; $MgSO_4 \cdot 7H_2O$, 14.9 g/L; KOH, 4.13 g/L; H_3PO_4 [85%], 26.7 ml/L; trace metal solution, 2.2 ml/L
- ✓ 7L jar fermenter (initial working volume = 3L)
- ✓DO-stat fed-batch fermentation
- ✓Induction: methanol feeding
- ✓Analysis

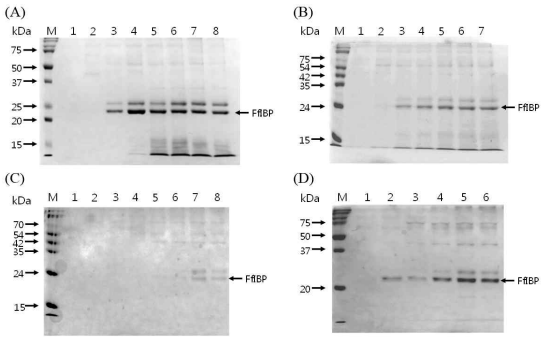
  Cell density: DCW and OD_{600}

  TH (Thermal hysteresis): measured using a nanoliter osmometer (Otago Osmometers, Dunedin, New Zealand)

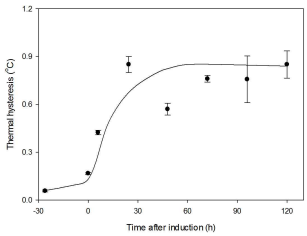
Results



Optimization of the FfIBP production condition in *P. pastoris* during fed-batch fermentation. (A) Time course of dry cell weight (B) Concentration of FfIBP produced from different culture conditions.



The amount of expressed FfIBP at different culture conditions. (A) pH 5, 30℃ (B) pH5, 25℃ (C) pH 6, 30℃ (D) pH 6, 25℃ .



Thermal hysteresis activity of FfIBP. Samples were taken from the culture broth of *P. pastoris* grown at 30℃, pH 5.

Summary

- ✓The optimal culture conditions for FfIBP production: 30℃, pH 5.
- ✓TH activity: 0.85 ℃

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References

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2. *Lee JH et al., Optimization of the pilot-scale production of an ice-binding protein by fed-batch culture of Pichia pastoris (2013) Appl Microbiol Biotechnol, 97, 3383-3393.*
3. *Do H et al., Structur-based characterization and antifreeze properties of a hyperactive ice-binding protein from the Antarctic bacterium Flavobacteium frigoris PS1 (2014) Acta Crystallogr Sec D, 70, 1061-1073.*