

## **PRODUCTION OF ICE BINDING PROTEIN WITH INCREASED THERMAL HYSTERESIS ACTIVITY FROM RECOMBINANT *PICHIA PASTORIS***

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### **ABSTRACT**

Ice binding proteins (IBPs) are found in polar organisms such as fish, plants, and insects. IBPs assist organism to survive in cold environments. The functions of IBPs include adhesion to ice and inhibition of ice recrystallization. The FfIBP was isolated from Antarctic bacterium *Flavobacterium frigoris* PS1. A codon-optimized FfIBP was cloned and produced in *Pichia pastoris* using fed-batch fermentation with methanol feeding. The FfIBP secreted by *P. pastoris* has a glycosylation site, which reduces the thermal hysteresis activity of FfIBP. The FfIBP produced by *P. pastoris* showed a doublet on SDS-PAGE. The results of enzymatic digestion of glycosylated protein suggested that FfIBP has complex N-linked oligosaccharides. The non-glycosylated FfIBP expressed by site-direct mutagenesis exhibited a single band on SDS-PAGE and presented as high thermal hysteresis activity as expressed in *E. coli*. These results indicate that the glycan attached to asparagine at 203 position of FfIBP could disturb the binding of FfIBP to ice molecules. The findings of this study could be utilized to produce IBPs having hyperactivity on a large scale. [The research was supported by a grant from KOPRI (PE17100)]

### References

1. J. H. Lee et al., *Appl. Microbiol. Biotechnol.* 97, 3383-3393 (2013).