BIOTECHNOLOGICAL APPLICATION OF *BACILLUS SUBTILIS* SPORE DISPLAY SYSTEM

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Key words: Bacillus subtilis, Spore, Surface display

Introduction. Bacterial surface display finds its important biotechnological application in the fields of screening tools of evolved enzyme, bioremediation, whole cell bioconversion and tool for live vaccine production. For the functional bacterial surface display of active enzyme of multimeric form, which is generally impossible due to molecular assembly of the monomer subunit subsequent to the secretion of displayed target protein outside the cell, a new surface display system based on *Bacillus subtilis* spore should be developed.

Here, we tried to develop a new bacterial surface display format for the efficient expression of multi-subunit enzyme.

Methods. Eleven *cot* genes were selected and tested for their anchoring efficiency for β-galactosidase expression.

Table 1. Anchoring motives used in this study

Gene	Size (kD)	Expression	Location	Comment
cotB	59	σ^K +GerE	OC	
cotC	12	σ^K +GerE	OC	
cotD	11	σ^K	OC	
cotE	24	σ^K,σ^K +spoIIID	OC	Outer coat assembly
cotG	24	σ^K +GerE	OC	CotB assembly
cotH	42.8	σ^K	IC	OC protein assembly
cotM	14	σ^K	OC	Outer coat assembly
cotV	14	σ^K, σ^K +GerE	-	Putative coat protein
cotX	18.6	σ^K, σ^K +GerE	OC	Outer coat assembly
cotY	17.9	σ^K, σ^K +GerE	OC	Outer coat assembly
spoIVA	55	σ^E	NCP	Attaches the precoat to the forespore

Single copy integration of Cot-LacZ expression vector onto the *amyE* site of *B. subtils* chromosome

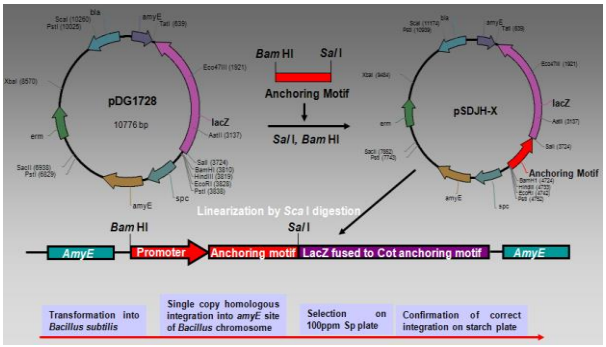

Fig.1 Construction of display vector for anchoring motif screening

Results. Among 11 spore coat proteins examined, *cotE* and *cotG* were selected. Using this motif, β-galactosidase, which is active only in tetrameric form, was functionally displayed on the surface of *Bacillus subtilis* spore.

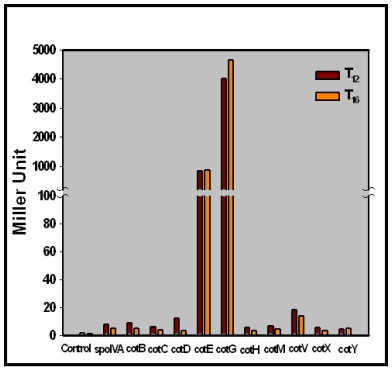

Fig.2 Screening of anchoring motifs by Miller assay

The surface localization of β-galactosidase was verified by enzymatic assay of purified spore, protease accessibility test and FACS analysis of spore expressing β-galactosidase. [1, 2]

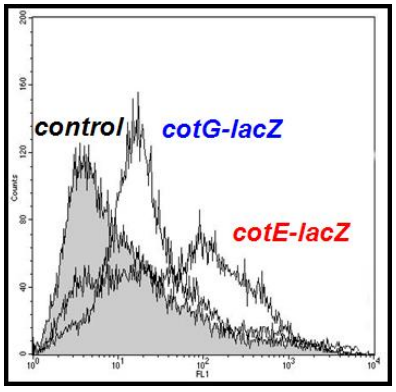

Fig.3 Verification of surface localization of fusion protein by FACS analysis of spore

Conclusions. Anchoring motifs were screened for the functional display of tetrameric β-galactosidase. FACS analysis, protease accessibility test verified correct, functional display of β-galactosidase on the surface of *B. subtilis* spore. Tetrameric Streptavidin and GFP_{UV}, were also successfully displayed.

Acknowledgements. This study was supported by research funds from Dong-A University and NRF (2012049613, 2012043375).

References.

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2. Hwang B, Pan J, Kim B, Kim J. (2013). *J. Nanosci. Nanotechnol. 13, 2313-2319*