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Genetic improvement of secondary metabolite production of an industrial bacterial strain

Dr. Le Thi Thu Hong
UNU-Kirin fellow from Vietnam

Applied Bacteriology Laboratory, Applied Microbiology Division
National Food Research Institute, NARO

Bacillus subtilis (natto) is an industrial fermentation strain that significantly increases the nutritional value of soybeans and develops a unique flavor and texture. *B. subtilis* (natto) produces extracellular poly- γ -glutamate (γ -PGA), a very viscous polymer of DL-glutamic acid linked by gamma peptide bonds. In *B. subtilis* (natto), γ -PGA is synthesized by *pgsBCA* operon. The expression of the *pgs* operon is regulated by quorum-sensing components, ComPA, DegQ, DegS, DegU and cell motility related SwrA. Disruption of *degQ* gene causes loss of ability of γ -PGA production, which is restored by mutations in *degS* as well as other unknown target genes. By whole genome sequencing analysis for the unknown targets revealed several candidate genes responsible to the mucoid colony phenotype, including a single point mutation occurred in *yxyZ* gene leading to alternation of an amino acid in the protein. We obtained evidence that single amino acid alteration of wild-type *yxyZ* plays an important role in restoring γ -PGA production that was abolished by disruption of *degQ*. In addition, it is noted that disruption of *yxyZ* gene not only effects on colony morphology relative to bacteria swarming mobility on solid surface but also reduces in *pgs* operon expression and exoprotease production. Furthermore, recombinant wild-type and the mutant YxyZ protein produced in *E. coli* cells behaved quite differently; wild-type YxyZ was stably expressed and effectively purified due to its good solubility whereas the mutant was very sensitive with changes of fermentation condition.