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journal or publication title	Report of National Food Research Institute
volume	79
page range	80-80
year	2015-03-01
URL	http://doi.org/10.24514/00002952

doi: 10.24514/00002952

Genetic improvement of secondary metabolite production of an industrial bacterial strain

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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-gamma-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.