Genetic approach for bacteriophage resistance of Bacillus subtilis (natto)

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Bacillus subtilis strains are the essential bacteria responsible for soybean fermentation. Bacteriophage contamination is a problem, which may occur during the fermentation and spoils the product. At present the *yueB* is the only gene reported as a bacteriophage receptor gene in *B. subtilis* and responsible for irreversible binding of bacteriophage SPP1 to cell surface. It is very interesting to study relationship between bacteriophage sensitivity and variation of *yueB* in *B. subtilis* species. With this preliminary study we obtained *yueB* gene from total 42 strains of *B. subtilis* including Japanese natto fermenting strain and strains isolated from Thua-nao of Thailand by PCR. The nucleotide and translated amino acid sequence of *yueB* gene were analyzed to examine phylogenetic relationship with each other. Deduced amino acid sequences of YueB revealed large diversity in the middle part of it which was supposed to be topologically exposed to cell surface. Moreover, we constructed the knock out mutant of *yueB* by insertion of erythromycin resistance cassettes and determine phage sensitivity in the absence of *yueB*. The phage sensitivity assay confirmed that the *yueB* gene knockout mutant of the laboratory strain *B. subtilis* 168 is resistant to SPP1 phage, but the mutant of natto strain *B. subtilis* NAFM5 was still sensitive to several phages including phages isolated from spoiled natto. These results suggest co-evolution of *yueB* and bacteriophages and *B. subtilis* might have bacteriophage receptor gene(s) other than *yueB*. The study on bacteriophage resistance in *B. subtilis* could be extremely useful and applicable in fermented food industry.

Keywords: *Bacillus subtilis*, *yueB* gene, natto, bacteriophage