A new rapid and micro cell fusion method by alkali metal ions like K^+ and Na^+

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Summary

Since no transformation occurred by two conventional methods to a newly-isolated *Bacillus subtilis* which shows a broad suppressive spectrum to phytopathogens, we have developed a new transformation method by using alkali metal ions like K^+ and Na^+. We have simplified the procedure of this method and also analysed the mechanism of this new method.

The transformation treatment is briefly described as follows. 0.33 ml culture of *B. subtilis* grown in fresh L medium was transferred into polypropylene tube, pelleted by centrifugation and resuspended in 1ml of 410 mM KCl solution. An aliquot of this suspension (50 μl) was mixed with plasmid DNA (less than 5 μl), and then 50 μl of 70% PEG solution was added to this mixture. After PEG solution was diluted by the addition of 1 ml of LC medium, the cells were pelleted, resuspended in L medium and kept statically for 2h at 37°C for the expression, followed by being spread onto plates containing the antibiotic. Autolysis of whole cells in KCl solutions at 30°C was measured by monitouring the turbidity at 650 nm. Cell fusants were selected on the plates on which neither of the strains could grow because of their different genetic markers.

Simple and quick method for transformation of *B. subtilis* by plasmid DNA was developed. About 30 min was enough to complete the all steps before gene expression stage and several thousands of transformants were obtained from one μg of plamid DNA. The activation of autolysin(s) of the cells was strongly suggested to be involved in the mechanisms of this transformation. Colonies which expressed the completely different phenotype from that of parental strains were obtained at high frequency by this method, and cell fusions without protoplasting were considered to be the possible mechanism for this transformation. Applicatiuon of this new rapid and micro method to the industrially useful microorganisms will be promising.