A new transformation method of microorganisms with plasmid by alkali metal ions like K^+ and Na^+

MAKOTO SHODA AND TAKASHI ANO
Research Laboratory of Resources Utilization
Tokyo Institute of Technology

Summary

As transformation methods of microorganisms with plasmid DNA, two conventional methods have been popularly used, namely a competent cell method and a protoplast method. Each of these has its own advantages and disadvantages. We have isolated a bacterium, Bacillus subtilis which show a remarkable suppressive effect to phytopathogens. When we conducted the genetic analysis and genetic manipulation of the bacterium by applying the two methods, no transformation occurred. Then, we developed a new method which is applicable to the bacterium by using alkali metal ions like K^+ and Na^+.

The transformation treatment is briefly described as follows. The cells of B. subtilis were cultivated in L broth at 37 ° C overnight and the culture broth was centrifuged, followed by washing the precipitated cells with TE buffer. One ml of the suspension was mixed with various ion solutions and shaken at 30° C for 3 min. The solution of plasmids with different configurations was added and the mixture was stood still for 30 min. Then, 0.1 ml of polyethylene glycol(PEG)6000 solution was introduced into the mixture and gently mixed. After heating the solution at 42° C for 5 min, quick cooling at 30 ° C was carried out. Then, the solution was incubated at 37 ° C for 4 h for gene expression, and 0.1 ml of solution was spread onto L agar plates containing antibiotics as a genetic marker.

Among the ions tested, saturated solutions of alkali metal ions like K^+ and Na^+ showed a significantly high transformation frequency. The features of the new transformation are as follows. The optimal values of temperature; 30° C, cell concentration; 5×10^7 (cells/ml) and PEG;6000. The monomer and linear plasmids were efficiently transformed, which were impossible in the methods described above. This new method is easy to handle, safe and practical in the transformation of bacteria of which competency was hard to obtain.