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A novel gene delivery system for yeast, animals and plants using bio-active beads

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We have produced micrometer-sized calcium alginate beads referred to as "bio-active beads" that encapsulate DNA molecules from the sizes of plasmid (few kb) to those of yeast artificial chromosomes (YACs, ca. 500kb). In order to evaluate the efficiency of the bio-active beads in genetic transformation, yeast spheroplasts, human cells, and plant protoplasts isolated from different materials were transformed with bio-active beads containing genetic materials of plasmids or YACs. In the case of tobacco BY-2 cells, approximately ten-fold higher GFP expression was observed than the conventional method using a naked plasmid solution. YAC with ca. 500 kb in size was also transformed to yeast spheroplasts. HeLa cells were also successfully transfected by using the bio-active beads.

Introduction

A highly efficient and versatile genetic transfer system is an important key technology in biotechnology. Demands for simultaneous transformation of a large number of genes are increasing for functional analysis of genetic networks. The difficulty in introducing large DNA molecules and/or a large quantity of DNA molecules into cells, especially plant cells lies in the lack of appropriate and efficient methods for this purpose. Among yeast, animals and plants, transformation system for plants is much behind to former two organisms, although several methods have been developed for genetic transformation of plant cells.

We, therefore, examined the effects of the micrometer-sized alginate beads in genetic transformation. Alginate is a kind of hydrophilic polysaccharide that gelates in the presence of Ca^{2+} ions. Alginate is harmless to yeast, animal and plant cells and has been used as a material to immobilize bacteria in bioreactors and to encapsulate plant somatic embryos in artificial seeds (Kersulec et al. 1993). When a fine water/oil type emulsion of sodium alginate and some kind of organic solvent are mixed with a solution containing Ca^{2+} ions, the alginate solidifies as uniformly small and spherical particles. When hydrophilic molecules such as DNA, chromosomes or nuclei are added to the emulsion, they would be entrapped within the solidified calcium alginate beads (Sone et al. 2002).

Using these micrometer-sized alginate beads with genetic materials, we examined the

transformation efficiency in yeast, human cells, and several plant cells.

Methods for production of bio-active beads

We examined two different methods for bio-active beads production. The first one was the method using a tube mixer and the second one was the sonication method as shown in Figure 1. As a result, bio-active beads with 1 - 100 μm in diameter were produced by the

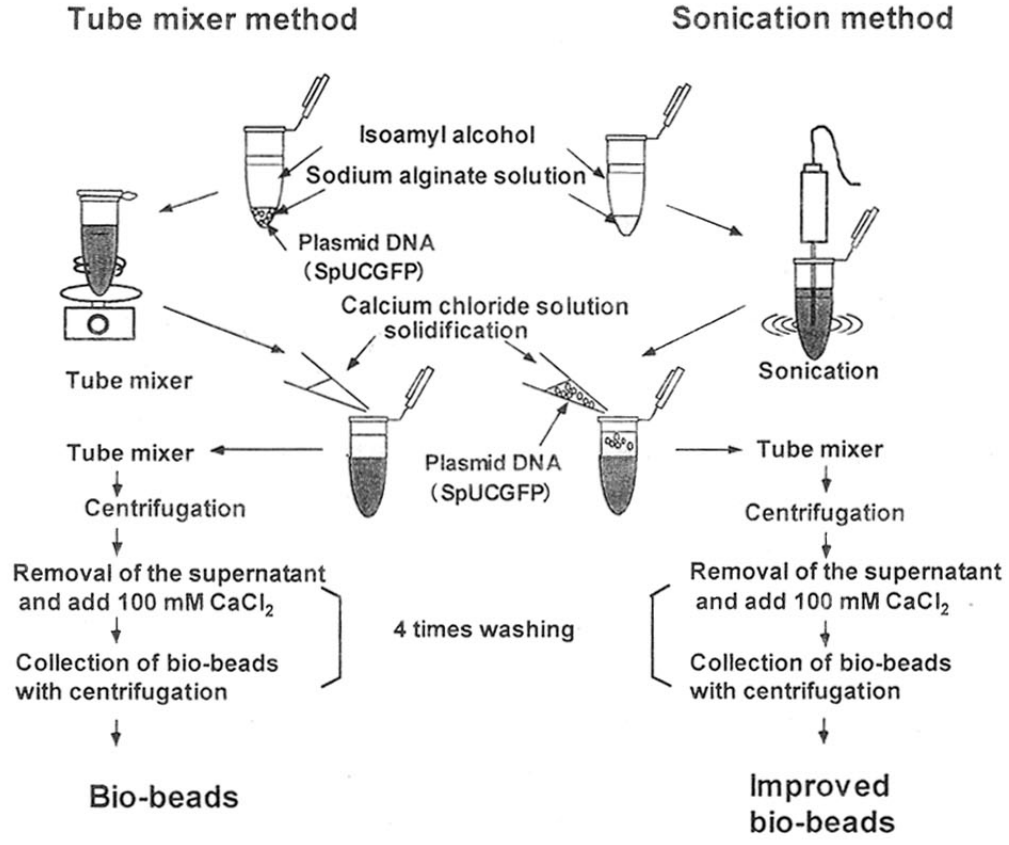

Fig. 1. Two methods for producing bio-active beads.

tube mixer method. Entrapment of plasmid DNAs in the bio-active beads was confirmed by staining DNA with YOYO-1. The sizes of the bio-active beads dispersed in a wide range and the beads had teardrop shapes. On the other hand, the sonication method produced finer and more uniform emulsion than the tube mixer method. With this method, the finer bio-beads (0.1-15 μm) with plasmid DNAs entrapped on the surface were obtained. By using the same methods, yeast chromosomes and YACs with the size up to ca. 500 kb were entrapped on the bio-active beads (Sone et al. 2002).

Transformation of yeast cells and transfection of HeLa cells with bio-active beads

Yeast cells were transformed by using yeast specific chromosomes with the sizes of 185 kb,

389 kb, and 468 kb. Each chromosome has its own biochemical marker for the selection of the transformants. Bio-active beads and spheroplasts of a yeast line without those chromosomes were mixed at the presence of polyethylene-glycol (PEG). Figure 2 shows the pals-field gel electrophoresis (PFG) patterns clearly indicating successful transformation of each yeast chromosome to the recipient yeast cells (Mizukami et al. 2003).

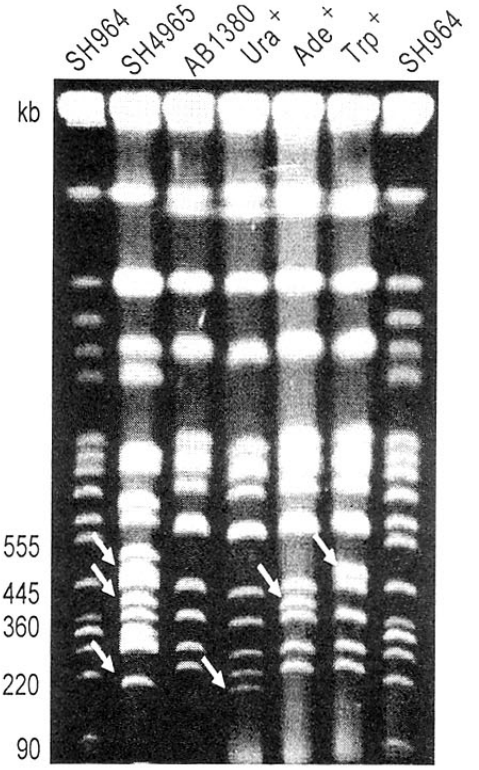

Fig. 2. PFG band patterns showing transformation of the yeast chromosomes to recipient yeast cells.

Transfection of HeLa cells by bio-active beads immobilizing plasmid DNAs (pEGFP-C1) was also examined. After treatment with bio-active beads and PEG, transient expression of EGFP was observed after 24 hours as shown in Figure 3. The expression efficiency reaches the maximum when the concentration of sodium alginate was 1% and the amount of plasmid DNA was 100 μg. The expression efficiency of the method using bio-active beads was 2-10 times higher than the conventional PEG method (Higashi et al. submitted).

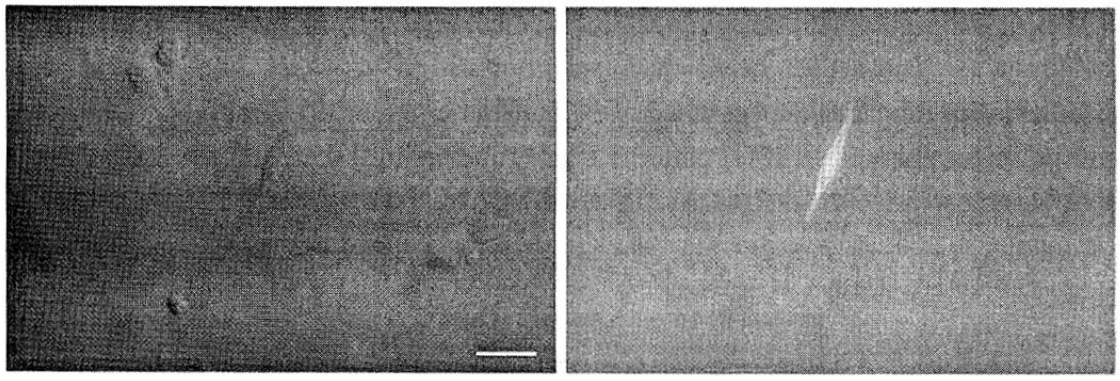

Fig. 3. Expression of EGFP gene in a HeLa cell.
Left: Phase contrast image. Right: A fluorescent image using a GFP fluorescent filter. Scale bar represents 20 μm.

Transformation of plant cells with bio-active beads

Transformation ability of bio-active beads with entrapped DNAs was examined using protoplasts from several plants, such as tobacco, carrot, eggplant, etc. In this case, we also employed the simple mixing method of bio-active beads with protoplasts in the presence of PEG.

Figure 4 shows the transient expression of sGFP in eggplant, tobacco SR-1, and carrot protoplasts indicating that the method has little preference in the plant species for its application. Then tobacco SR-1 was transformed to obtain stable transgenic plants with a reporter gene (sGFP gene) and a selection marker (NPT2 gene). Five days after the transformation, the protoplasts began to divide and formed calli in the selection medium. Then the seedlings were regenerated and flowering plants were obtained (Fig. 5).

Integration of sGFP and NPT genes in the regenerated plants was confirmed by both PCR detection of the genes and genomic Southern experiments using the genes as the probes (Liu et al. accepted). Furthermore, delivery of YACs with 100 kb into plant cells was detected using sGFP gene included in the YACs. Thus the method using bio-active beads is quite promising to transform plant cell with high efficiency and ability to introduction of the larger DNA molecules.

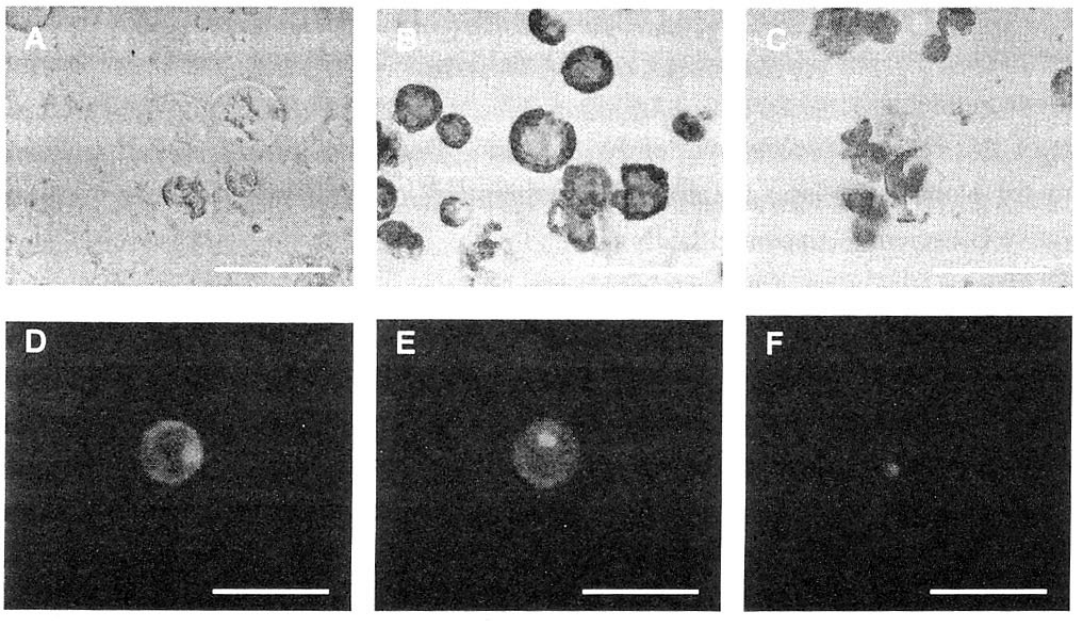

Fig. 4. Transient expression of GFP in the protoplasts from different three plants including the eggplant (*Solanum integrifolium*), tobacco (*Nicotiana tabacum* SR-1) and carrot (*Daucus carota*). Upper and lower panels show the phase-contrast and fluorescent images, respectively. Bars indicate 5 μm.

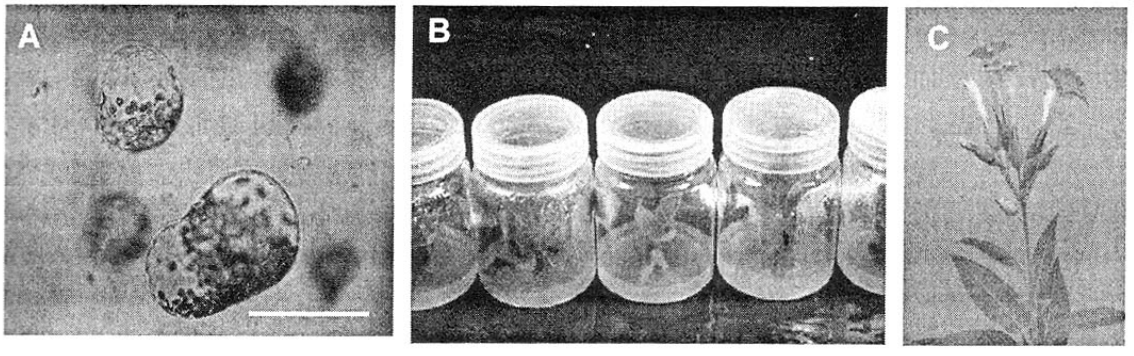

Fig. 5. Regeneration of tobacco plants after the transformation.

Conclusion

All the results that were obtained using yeast, mammal, and plants clearly indicate that the method is simple and efficient. This method may be especially useful to deliver the large sized DNA molecules into the mammalian and plant cells as already shown in yeast cells. Although the upper limitation of the DNA size to be trapped on the bio-beads is not known to date, the fact that we already trapped a barley chromosome and a human nucleus in the bio-active

beads indicates the high feasibility of trapping even the largest YAC into a bio-active bead.

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