Note

A Simple and Rapid Extraction of High Molecular Weight Chromosomal DNA from Bacillus subtilis Protoplasts for Cosmid Cloning and Interspecific Transformation

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After conversion of Bacillus subtilis vegetative cells to protoplasts, a simple and rapid method for extracting high-molecular-weight chromosomal DNA was devised with the inclusion of bovine serum albumin and phenol-chloroform treatments. The DNA sample thus prepared was the size of 100–450 kb and could be used for cosmid cloning and interspecific transformation.

Key words: Bacillus subtilis; DNA; extraction; high-molecular-weight; transformation

B. subtilis AC819 (hisH rpsL smo-1 tet-1)† was grown in three 500-ml flasks containing 50 ml of antibiotic stationary medium 3 (Difco) with shaking at 37°C until early stationary phase (Abs. = 1.8). Cells from 125 ml of these cultures were harvested by centrifugation at 4,000×g for 2 min at 4°C and converted to protoplasts as described previously by incubation at 40°C for 10–15 min in 16 ml of SMM solution containing 0.5 M sucrose, 0.02 M maleate buffer (pH 6.5), 0.02 M MgCl₂, and 4 mg of lysozyme. After adding 4 ml of SMM containing 16 mg of bovine serum albumin (fraction V, Sigma Chemical Co.), the protoplasts (about 10⁹) were pelleted by centrifugation at 3,000×g for 5 min and resuspended in 0.2 ml of SMM containing 0.8 mg of bovine serum albumin. To the homogenous protoplast suspension, 8 ml of TNSB buffer containing 0.1 M Tris-HCl (pH 8.0), 0.1 M NaCl, 0.5% sodium dodecyl sulfate and 0.5% bovine serum albumin was added. The suspension was incubated at 65°C for 5 min with gentle shaking. After 8 ml of phenol-chloroform-isoamylalcohol (25:24:1, v/v) was added, the solution was gently shaken for 5 min at 65°C. The upper, aqueous layer was separated by centrifugation at 2,000×g for 15 min and transferred to a new tube containing 1 ml of TNSB buffer. After mixing, this solution was extracted with phenol-chloroform and centrifuged (2,000×g for 15 min). The aqueous layer (about 8 ml) was mixed with 0.8 ml of 3 M potassium acetate (pH 7.0), cooled on ice-water and added with two volume of ethyl alcohol (−50°C). Precipitated DNA was spun out with a glass microspette, rinsed with 70%, 80%, and 90% ethyl alcohol, dissolved in 5 ml of TE buffer (0.01 M tris(hydroxymethyl)amino methane-hydrochloride, pH 8.0, 0.001 M ethylene diamine tetraacetic acid) containing 100 μg of ribonuclease A, and incubated at 4°C overnight. After 5 ml of phenol-chloroform was added, DNA solution was extracted, centrifuged (2,000×g for 15 min), and precipitated with ethyl alcohol. The precipitated DNA was dissolved in 2 ml of TE buffer (pH 8.0).

When the chromosomal DNA prepared as above was analyzed by pulse-field electrophoresis, 100–450 kb fragments were observed (Figure). The DNA fragments were isolated with a yield of 0.25–0.38 mg and were digestable with several restriction enzymes (BamHI, BglII, CiaI, EcoRI, EcoRV, HindIII, KpnI, PstI, PvuII, SalI, XbaI, and XhoI) without further purification, such as Pronase treatment, CsCl centrifugation, or column chromatography (data not shown). Moreover, 10,000 ampicillin-resistant transformants of Escherichia coli VCS257 (supE44 supF58 hisd3 dapD8 lacY1 glnV44 tonA53 Δ(gal-uvrB)47 tyrT58 gyrA29 Δ(thyA57)) were obtained using B. subtilis AC819 chromosomal DNA (3 μg) partially digested with Sau3AI, BamHI digested pJB8 or pHSG439 DNA (3 μg), and in vitro packaging kit (Gigapackgold, Stratagene). Chromosomal DNA (10 μg) from Bacillus amyloliquefaciens FWS (rpsL) were prepared with this extraction procedure and examined for interspecific transformation of B. subtilis AY1 (cysA metC hisH arg-1) or B. subtilis QB936 (aroG leuB trpC ald-1). Significant Arg+ (eleven), Ara+ (ten), and Leu+ (ten) transformants were found, which have not been detected with purified B. amyloliquefaciens FWS DNA (10 μg) extracted by a conventional procedure.

When the cell wall fraction including peptidoglycan and teichoic acids was removed from vegetative cells by treatment with lysozyme, the resultant pro-

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method was also usable for small scale preparation of chromosomal DNA (4 μg) from 2 ml of overnight culture. Chromosomal DNAs of *Bacillus* species (*B. amylyoliquefaciens, B. halodurans, B. licheniformis, B. megaterium, B. pumilus, and B. stearothermophilus*) could also be extracted like *B. subtilis*, after converting to the protoplasts.

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**References**