Note

Isolation and Characterization of a Plasmid from *Brevibacterium lactofermentum*Harumi KANEKO,* Teruo TANAKA
and Kenji SAKAGUCHIMitsubishi-Kasei Institute of Life Sciences,
11 Minamiooya, Machida-shi, Tokyo, Japan

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Brevibacterium lactofermentum is a gram positive, non spore-forming bacterium, and used widely as an amino acid producer. In the interest of constructing the molecular breeding system in *B. lactofermentum* and in its relative bacteria, we isolated a plasmid from *B. lactofermentum* 2256 (ATCC 14067), and its molecular size was determined.

B. lactofermentum 2256 was cultured at 30°C in the following medium (per liter: $(\text{NH}_4)_2\text{SO}_4$, 10 g; urea, 3 g; K_2HPO_4 , 1 g; NaCl, 50 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 2 mg; $\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$, 2 mg; glucose 20 g; biotin, 50 μg ; thiamine 200 μg ; yeast extract (Difco), 1 g; pH 7.2). At the mid-exponential phase, penicillin G was added (0.3 units/ml). At this concentration, no growth inhibition was observed. The cells were harvested 1 hr after penicillin addition. The plasmid isolation procedure and the preparation of radio isotope labeled deoxyribonucleic acid (DNA) were the same as those described by Tanaka *et al.*,¹⁾ except that DNA was labeled with [³H]adenosine which was added to the growth medium at the concentration of 100 μCi per ml, because this strain did not incorporate [³H]thymidine. Figure 1 shows the presence of a satellite peak in CsCl-EtBr equilibrium centrifugation

FIG. 1. CsCl-EtBr Equilibrium Centrifugation of [³H]adenosine Labeled DNA from *B. lactofermentum* 2256.

The CsCl density decreased from left to right.

* Present address: Japan Grain Inspection Association, 22-2-1 Arai, Ichikawa-shi, Chiba, Japan.

FIG. 2. Neutral Sucrose Density Gradient Centrifugation (5 to 20%) of [³H]adenosine-labeled Plasmid DNA.

The arrow indicate the position of [¹⁴C]thymidine-labeled λ DNA cosedimented as a molecular weight marker. Density decreases from left to right.

FIG. 3. An Electron Micrograph of Circular Plasmid DNA.

The molecular weight of the plasmid was determined by use of the *ColEI* DNA as internal standard (4.2×10^6 daltons). Bar corresponds to a molecular weight of 1×10^6 .

of [³H]adenosine labeled DNA of *B. lactofermentum*. This satellite peak on the left side of the main peak was collected and centrifuged in a sucrose density gradient (5~20%). As an internal standard, ¹⁴C-labeled λ phage DNA was added (Fig. 2). The main peak has a *S* value of 67 corresponding to a molecular weight of 2.7×10^7 dalton, as calculated by the equation of Hudson *et al.*²⁾ The presence of the open circular form (minor peak) of the plasmid is also seen in Fig. 2. The minimal number of copies of the plasmid per chromosome (molecular weight was assumed 2×10^9 daltons) was estimated to be 1 to 2. This plasmid was examined by electron microscopy as described by Tanaka *et al.*,¹⁾ with *ColEI* DNA (MW 4.2×10^6 dalton) added as an internal standard (Fig. 2). The molecular weight was determined to be 3.7×10^7 daltons. This plasmid was cleaved at several sites by *EcoRI* (prepared as previously described³⁾) and *BamNI* (prepared according to Shibata and Ando⁴⁾). But clear-cut digestion patterns by agarose gel electrophoresis were not obtained.

This plasmid belongs to a big size class of vector for constructing recombinant molecules. Its specific functions and its transformation system in *B. lactofermentum* are not known at present. But this plasmid would be a candidate to construct a mini-plasmid through cleavage by various restriction enzymes and ligation.

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