Detection of Plasmid Deoxyribonucleic Acid in an Isolate of *Lactobacillus acidophilus*†

T. R. KLAENHAMMER* and S. M. SUTHERLAND

Department of Food Science, North Carolina State University, Raleigh, North Carolina 27650

Eight strains of *Lactobacillus acidophilus* were examined for the presence of plasmid deoxyribonucleic acid, and one, a pig intestinal isolate, showed the presence of a 13.7- and a 6.3-megadalton plasmid. This is the first reported evidence for plasmid deoxyribonucleic acid in *Lactobacillus acidophilus*. The functions of these plasmids are presently unknown.

The lactic acid bacteria comprise a diverse group of bacteria known primarily for their ability to ferment milk. Plasmid deoxyribonucleic acid (DNA) distribution throughout members of the lactic acid bacteria appears widespread (4, 6, 10, 12, 19) and in some cases correlates directly with milk-fermenting enzyme systems. The dependence of lactose hydrolysis and proteolytic activity on extrachromosomal elements has been well documented in the group N streptococci (1, 13, 14). More recently, evidence suggesting plasmid determinants for lactose metabolism in *Lactobacillus casei* also has been reported (3, 9). However, little attention has been directed toward the presence or roles of plasmid DNA in members of the lactic acid bacteria not typically associated with milk from an ecological or fermentative standpoint.

In recent years, *Lactobacillus acidophilus* has been the subject of numerous studies concerning this organism’s role in the intestinal tract of humans and animals. Although the beneficial effects of *L. acidophilus* have yet to be elucidated, evidence has suggested that resident lactobacilli may facilitate resistance to common intestinal disorders through stabilization of normal intestinal microflora (16, 18, 21). The intestinal association of naturally occurring *L. acidophilus* strains suggest that extrachromosomal determinants for antibiotic resistance, bacteriocin production, or colonization factors, common among the enteric bacteria, may also be important to the intestinal activity of the lactobacilli. However, the sole attempt to isolate plasmid DNA from *L. acidophilus* was unsuccessful (20), and subsequent studies concerning *L. acidophilus* plasmids or plasmid-linked characteristics have not been reported. This investigation was undertaken to establish (i) whether plasmid DNA could be isolated from *L. acidophilus* and (ii) whether extrachromosomal elements are widely distributed among strains of *L. acidophilus*.

The *L. acidophilus* strains surveyed for plasmid DNA have been described previously (8), and include human isolates NCFM, 4962, and 1; chicken isolates C2, C3, and C7; and pig isolates PA3 and PA19. Each isolate was confirmed during this study as *L. acidophilus* by carbohydrate fermentation reactions, growth at 15 and 45°C, catalase activity, and growth on LBS agar (BBL Microbiology Systems, Cockeysville, Md.) (7, 17; S.M. Sutherland, M.S. thesis, North Carolina State University, Raleigh, 1980). Cultures were propagated in MRS broth (Difco Laboratories, Detroit, Mich.) at 37°C and inoculated (1%) into 40 ml of Trypticase soy broth supplemented with 20 mM, DL-threonine (4), 0.1% yeast extract, 0.1% Tween 80, and 0.5% sodium acetate, pH 6.8. For radioisotope labeling 0.05 mCi of [3H]thymidine per ml and 250 μg of deoxyadenosine per ml were added to the supplemented Trypticase soy broth medium. After 5 h at 37°C, cells were harvested and digested with lysozyme (1 h at 37°C; 1 mg/ml; grade A, Sigma Chemical Co., St. Louis, Mo.), and cleared lysates were prepared as previously described (11). We have found that *Lactobacillus* strains propagated in MRS broth supplemented with DL-threonine (4, 19) are highly resistant to lysozyme-sodium dodecyl sulfate lysis and therefore the supplemented Trypticase soy broth was employed.

Figure 1 shows the elution profiles of cesium chloride-ethidium bromide gradients from eight *L. acidophilus* strains examined for plasmid DNA. Satellite DNA peaks, typical of covalently closed circular DNA, were not observed in seven of the eight strains examined. The high specific radioactivity present in the DNA fractions of the gradients would have made possible detection of minute amounts of covalently closed circular DNA. However, satellite peaks were not observed for *L. acidophilus* strains C2, C7, C3, NCFM, 4962, PA19, and 1, indicating that plas-

† Paper no. 6148 of the journal series of the North Carolina Agricultural Research Service, Raleigh, NC 27650.
NOTES
APPL. ENVIRON. MICROBIOL.

Fig. 1. Dye-buoyant density gradient centrifugation of cleared lysates from L. acidophilus strains: (A) C2; (B) C7; (C) 4962; (D) NCFM; (E) C3; (F) PA19; (G) PA3; and (H) 1. CsCl-ethidium bromide gradients were prepared in TES buffer [50 mM NaCl, 5 mM Na₂-ethylenediaminetetraacetic acid, 30 mM tris(hydroxymethyl)aminomethane, pH 8.0] to a refractive index of 1.390x10⁴ (β = 1.595 gm/cm³) as previously described (10). Centrifugation was carried out for 24 h at 47,000 rpm, 20°C, in a Beckman Ti50 rotor. Gradients were fractionated directly onto 25-mm Whatman no. 4 filter disks and washed twice with cold (4°C) 5% trichloroacetic acid followed by two washes in cold 95% ethanol. The filters were placed in 6 ml of Aquasol-2 (New England Nuclear) and counted. For preparative CsCl-ethidium bromide gradients, fractions were collected in small test tubes. Fifty microliters from alternate fractions was then spotted on filter pads and counted. Kcpm = counts per minute x 1,000.

mid DNA could not be detected in these strains under the isolation conditions employed. However, a detectable satellite DNA peak was observed for the pig isolate strain PA3 (Fig. 1G). CsCl-ethidium bromide gradient fractions corresponding with PA3 satellite DNA were pooled, extracted three times with CsCl-saturated isopropanol, dialyzed against TES buffer [N-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid], and concentrated to 100 μl against polyethylene glycol (Carbowax, PEG 6000, Fisher Chemical Co.). Agarose gel electrophoresis of the CsCl-ethidium bromide-purified preparation showed migration of two plasmid DNA species (Fig. 2). Molecular masses for the two molecules were approximated at 6 to 6.5 and 13 to 14 megadaltons by relative mobility determinations (15). Determination of plasmid molec-

rate buffer, pH 8.0 (14), for 3.5 h at 100 V. (A) Escherichia coli electrophoretic migration standards (10, 14) from top to bottom; RP4 (34 megadaltons); SA (23 megadaltons); and RSF1010 (5.5 megadaltons). The light band appearing below RSF1010 is migrating linear DNA fragments. (B) CsCl-ethidium bromide purified satellite DNA from L. acidophilus PA3.

Fig. 2. Agarose gel electrophoresis of CsCl-ethidium bromide satellite DNA from L. acidophilus PA3. Electrophoresis was carried out in a vertical 0.75% agarose gel in tris(hydroxymethyl)aminomethane-bo-
cular weight via mobility in agarose gels has been found accurate to within 10% of the true plasmid molecular weight (15). To obtain an additional estimate of molecular weights, CsCl-ethidium bromide-purified samples were analyzed by neutral sucrose rate zonal sedimentation (Fig. 3). In 5 to 20% neutral sucrose gradients, three peaks were observed at 38.6S, 27.3S, and 21S. Covalently closed circular molecules sedimenting at 38.6S and 27.3S correspond to molecular masses of 13.7 and 6.3 megadaltons, respectively (2). The 21S peak corresponds to the expected sedimentation behavior of an open circular 6.3-mega-
dalton plasmid (5) generated during routine handling of the DNA samples. Appearance of a significant 21S fraction after repeated freezing of the DNA sample at −20°C confirmed the open circular nature of this sedimenting fraction (data not shown).

Data reported here present the first evidence for plasmid DNA in L. acidophilus. In L. acidophilus PA3, two plasmid species were identi-
fied at 13.7 and 6.3 megadaltons and, therefore, may represent two individual molecules. Alternatively, examination of molecular weight estimates also suggests that the larger 13.7-mega-
dalton plasmid may be a dimeric form of the smaller molecule. The functions of the two plasmid species have not yet been identified. How-
ever, this pig isolate harbors substantial anti-
biotic resistance to tetracycline, erythromycin, oleandomycin, kanamycin, neomycin, and gentamycin (Sutherland and Klaenhammer, manu-
script in preparation). The level of antibiotic resistance demonstrated by L. acidophilus PA3 was not observed among plasmidless human or chicken isolates and thus suggests that the two plasmids harbored by this strain may be R-fac-
tors. Identification of the structure and functions of the two L. acidophilus PA3 plasmids is under investigation.

We gratefully acknowledge the excellent technical assistance of Carol L. Lemons. This work was supported in part by the North Carolina Dairy Foundation.

LITERATURE CITED
cromosomal elements in group N streptococci. J. Bac-
8. Gilliland, S. E., M. L. Speck, and C. G. Morgan. 1975. Detection of Lactobacillus acidophilus in feces of hu-
9. Hoffer, F. 1977. Involvement of plasmids in lactose me-
11. Klaenhammer, T. R., L. L. McKay, and K. A. Bald-
win. 1978. Improved lysis of group N streptococci for isolation and rapid characterization of plasmid deoxy-
12. Larsen, L. D., and L. L. McKay. 1978. Isolation and charac-
bution and evidence for a proteinase plasmid in Strep-

![Image](http://aem.asm.org/Downloaded/NOTES_673.png)