Isolation of Specific DNA Probe for 
*Lactobacillus acidophilus* Group A 1 
(Johnson et al. 1980)

Keizo ARIHARA, Makoto ITOH and Yo KONDO
School of Veterinary Medicine and Animal Sciences, 
Kitasato University, Towada-shi 034

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Abstract Specific DNA probe for *Lactobacillus acidophilus* group A 1 (Johnson et al. 1980) was isolated in this study. A genomic library of *L. acidophilus* type strain JCM 1132T DNA fragments from a *Pst I* digestion was constructed in *Escherichia coli* JM109 utilizing plasmid pUC18. Of 14 tested clones carrying random pieces of chromosomal DNA, a clone designated as pSP1, pUC18 with a 2.8 kilobase chromosomal DNA insert, established to be a probe specific for the *L. acidophilus* species (group A 1) strains. This specific DNA probe would lead to the rapid and exact method for identification and detection of *L. acidophilus*.


Key words: *Lactobacillus acidophilus*, lactobacilli, DNA probe, DNA-DNA hybridization, species identification

*Lactobacillus acidophilus* group lactic acid bacteria have been believed that they are predominant in intestinal flora of mammals and are beneficial to the host. For their therapeutic activity, this group bacteria have been used for dairy products (i.e. acidophilus milk) and probiotics for man and animals. Despite their industrial importance, the systematics of this species have been confused for a long time. As delineated by Johnson et al. in 1980 and refined by others, within the *L. acidophilus* group there are 6 genome clusters: *L. acidophilus* (group A 1), *L. crispatus* (group A 2), *L. amylovorus* (group A 3), *L. gallinarum* (group A 4), *L. gasseri* (group B 1), *L. johnsonii* (group B 2). Although confusion on *L. acidophilus* systematics are put on end, these species are difficult to differentiate solely on phenotypic characteristics. Many strains besides *L. acidophilus* (group A 1), such as *L. gasseri* (B 1), are still catalogued in culture repositories as *L. acidophilus* strains. Therefore, it is required to pay special attention for the name “*Lactobacillus acidophilus*”.

Specific nucleic acid probes have been identified from several lactobacilli (*L. curvatus*), *L. helveticus*, *L. plantarum*, *L. delbrueckii*). Such probes have proven highly sensitive and specific for detection and characterization of *Lactobacillus* species. In this report, we describe the isolation of a specific DNA probe which can be used in hybridization.
zation procedures to specifically identify and detect strains belonging to the L. acidophilus (group A1) species.

**Materials and Methods**

**Bacterial strains**

Lactobacillus strains used in this study were obtained from 2 culture repositories, JCM (Japan Collection of Microorganisms, Wako) and NCFB (National Collection of Food Bacteria, AFRC Institute of Food Research, Reading, England). Cultures were maintained as frozen stocks kept at −55°C in MRS broth (Difco Laboratories, Detroit, MI) plus 10% glycerol. Prior to use, all strains were passed at least twice at 37°C in screw—capped test tubes containing MRS broth.

**Preparation of chromosomal DNA**

Chromosomal DNA from lactobacilli was prepared as described by Luchansky et al. and checked on 0.7% agarose gel for quality. DNA concentration was determined using a Hoefer (San Francisco, CA) DNA mini-fluorometer TKO 100.

**Genomic library construction**

Standard procedure for genomic library construction by using plasmid vector pUC18 was employed. Chromosomal DNA of the type strain L. acidophilus JCM 1132T (other designation, ATCC 4356) was digested to completion with Pst I and ligated into pUC18 which was linearized at its unique Pst I site and phosphatase treated. The ligation mixture was transformed into Escherichia coli JM109, plated onto LB plates supplemented with ampicillin sodium (50 μg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (100 μg/ml) and isopropyl-β-D-thiogalactopyranoside (60 μg/ml), and grown at 37°C overnight. Transformants that gave to rise white colonies were picked to LB broth with ampicillin (50 μg/ml). Their plasmids were extracted, digested with Pst I and analyzed by agarose gel electrophoresis.

DNA samples (vector pUC18 with inserts of chromosomal DNA or chromosomal DNA of Lactobacillus strains) were digested with Pst I and fractionated electrophoretically on a 0.7% agarose gel. The gels were blotted overnight in 2 × SSC (0.6 M NaCl plus 0.06 M sodium citrate) onto Nytran membranes (0.2 μm pore size, Schleicher & Schuell, Dassel, Germany). Hybridization was performed by random—primer DNA labeling with digoxigenin—dUTP, and hybrids were detected by enzyme immunoassay as specified by the manufacturer (Boehringer Manheim GmbH, Manheim, Germany).

**Microdilution plate hybridization method**

Microdilution plate hybridization method for the identification of bacteria developed by Ezaki et al. was also employed in this study. The procedure applied for lactobacilli described by Hayashiya and Ogawa was carried out as below in brief. Chromosomal DNA isolated from 6 L. acidophilus group type strains was single—stranded and immobilized on microplate wells. Then single—stranded DNA (from 15 test strains) biotinylated by photoprobe biotin (Vector Laboratories Inc., Burlingame, CA) was reacted with immobilized DNA on plate wells. Following the reaction with peroxidase—labeled streptavidin (Kirkegaard & Perry Laboratories Inc., Gathersburg, MD), substrates (TEMD and H2O2) were added and color—developed. After stop the reaction with H2SO4, by measuring color of the solution in using a microplate reader at 450 nm, DNA homology was calculated, and test strains were identified.

**Results and Discussion**

Clones carrying random pieces of L. acidophilus JCM1132T chromosomal DNA were constructed in Escherichia coli JM109 utilizing pUC18. Fourteen different clones, pUC18 with chromosomal DNA inserts of between 0.1 and 3.0 kilobase pairs in length, were chosen at random to be used as DNA samples in South-
ERN hybridization experiments. The hybridization results revealed that 3 of 14 tested clones hybridized only with labeled chromosomal DNA of *L. acidophilus* (group A1) type strain (JCM 1132T) but not with that of other type strains (*L. crispatus* (group A2), *L. amylovorus* (group A3), *L. gallinarum* (group A4), *L. gasseri* (group B1), *L. johnsonii* (group B2)). Of 3 chosen clones, a clone hybridized with group A1 chromosome greater than other 2 clones, and therefore, only this clone was retained for further experiments. This clone, pUC18 with a 2.8 kilobase chromosomal DNA insert, was designated pSP1 in this study. Of 11 clones, besides 3 chosen clones, 5, 7, 4, 4, and 1 clones hybridized with chromosomal DNA of group A2, A3, A4, B1, and B2, respectively.

The specificity of DNA probe, recombinant plasmid pSP1 containing genomic fragment of *L. acidophilus* DNA, was tested against 14 *L. acidophilus* group strains by Southern hybridizations (Fig. 1). These 14 strains were identified as each species by DNA-DNA hybridization experiments in the past. The hybridization results demonstrated that labeled pSP1 specifically recognized chromosomal DNA from the *L. acidophilus* (group A1) strains alone. Also, all other strains tested from different species of lactic acid bacteria, such as *L. helveticus* and *L. delbrueckii*, were negative (data not shown). Therefore, pSP1 proved to be a genetic probe specific for the *L. acidophilus* (group A1) species.

By using labeled pSP1, 15 strains (JCM 1021, 1023, 1026, 1028, 1030, 1032, 1033, 1034, 1035, 1036, 1038, 1039, 1132, 1229, 5807) of lactobacilli registered as *Lactobacillus acidophilus* in JCM Catalogue of Strains Fifth Edition (1992) were tested for their hybridization reactivity. Only 5 (JCM 1021, 1023, 1028, 1132, 1229) of 15 strains were hybridized with pSP1. Since these results agreed well with the results by microplate hybridization technique (data not shown), the experimental procedure with labeled pSP1 would be reliable for the identification of *L. acidophilus* (group A1) strains.

In this study, we isolated a DNA probe specific for *L. acidophilus* (group A1) strains from the genomic library. This specific DNA probe would establish the rapid and exact method for identification and detection of *L. acidophilus* (group A1). Utilization of rRNA sequencing is another desirable method for the bacterial identification. However, complete identification of bacteria to the species level requires the back-up of a large database of rRNA sequences and for very closely related species even rRNA sequence analysis may not be sufficient. Actually, a similarity of 99.4% was found in the 16S rRNA sequence of *L. gasseri* (group B1) and *L. johnsonii* (group B2).

Recently, Uemura et al. reported that *L. acidophilus* group species could be differentiated between by comparing the elec-
trophoretic mobilities of L- and D-lactate dehydrogenases on polyacrylamide gel. However, these 6 species have been classified based on the DNA-DNA homology and designated as the respective species. Also, stability of phenotypic characteristics are not guaranteed. For reliable complete identification of L. acidophilus group strains, techniques based on the DNA-DNA homology are still required. Today, if species-specific probes are given, techniques by DNA-DNA homology (i.e. dot hybridization) are not complicated and time-consuming in the laboratory. Recent progress of regents for molecular biology produced the hybridization techniques without radioisotope method for labeling DNA. Further efforts for the isolation of specific DNA probes for other each species of L. acidophilus group are now in progress in our laboratory. In near future, the complete identification system for L. acidophilus group species would be established by using these specific DNA probes.

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References


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