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Characterization of *Lactobacillus* spp. isolated from the feces of breast-feeding piglets

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***Lactobacillus* spp., referred to as IJ-1 and IJ-2, were isolated from the feces of breast-feeding piglets and analyzed for probiotic properties. According to the analyses of 16S rDNA sequence, *Lactobacillus* sp. IJ-1 showed greater than 99% homology with *Lactobacillus reuteri* DSM 20016^T, and *Lactobacillus* sp. IJ-2 had greater than 99% homology with the *L. gasseri* ATCC 33323^T and *L. johnsonii* ATCC 33200^T. The pH changes in the culture media of *Lactobacillus* sp. IJ-1 and *Lactobacillus* sp. IJ-2 were from 6.5 to 4.2 and 4.6, respectively. Their respective resistance against artificial gastric acid and artificial bile acid led to survival rates of nearly 186 ± 44% and 13 ± 5%. Neither strain produced the carcinogenic enzyme β-glucuronidase. Both strains inhibited the growth of pathogenic microorganisms, such as *Listeria monocytogenes* ATCC 19111, *Salmonella enterica* KCTC 12401, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* KCTC 3881, and *Bacillus cereus* 3711, within 24 h of growth.**

[**Key words:** Probiotics; *Lactobacillus* spp.; Breast-feeding piglet; Feces; Pathogenic microorganisms]

Weaning time is a very stressful period in a piglet's life. Piglets suffer from and are sensitive to many environmental factors, such as an abrupt separation from their mother and a change in diet. Additionally, the animal's digestive immune system is in an immature, incomplete state until five to six weeks of age. If the microflora composition in the piglet's intestines is unbalanced, an allergic reaction and the growth of pathogenic microorganisms can occur, leading to a severe digestive disorder and even death. Monitoring the stable digestive microflora of a piglet is therefore important during its two- to three-week weaning diet (1–4). A balanced digestive environment with a proper population of commensal microflora protects the young animal from hazardous pathogens (5). Post-weaning diarrhea can be caused by pathogenic microorganisms such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Clostridium* spp. (6). The use of antibiotics and other chemicals has generally decreased in farm yards (7, 8); instead, probiotics have been used. The major role of probiotics is to keep intestinal microorganisms in balance, prevent the proliferation of pathogenic microorganisms, and increase animal growth rate by improving their feed conversion rate (9–11). Probiotics for piglets should have the following properties: (i) be a natural inhabitant of the intestine, (ii) adhere to the intestinal mucosa, (iii) exhibit tolerance to bile salts and acidic conditions, and (iv) demonstrate activity against pathogenic microorganisms (12, 13).

There are many species of bacteria that can be used as probiotic material, such as *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Streptococcus*, *Bacillus*, and *Escherichia coli*. Among them, *Lactobacillus* and *Bacillus subtilis* have the most potential (14, 15). Lactic acid bacteria (LAB), when used as probiotics, modulate the infant immune system and prevent pathogenic bacteria from multiplying in the intestine (16, 17). In piglets, lactobacilli ingestion decreases the coliform population in intestinal feces (18, 19). Lactobacilli and bifidobacteria have each been isolated from the intestinal digesta or feces of infants and piglets; *Lactobacillus sobrius* was the most dominant species of lactobacilli in piglets, while *L. rhamnosus*, *L. gasseri*, *L. paracasei*, and *L. fermentum* were commonly isolated from human infants (20–23). Microorganisms used in hog-raising probiotics had powerful acid-producing capacities and excellent pathogen-inhibiting abilities. Lactobacilli have been considered an ideal species for probiotic use; they include *L. acidophilus*, *L. lactis*, *L. reuteri*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. delbrueckii*, *L. bulgaricus* and *L. brevis* (1).

This study investigated which probiotics are appropriate for use in weaning piglets. LAB, considered an ideal species for use in probiotics, were isolated from the feces of breast-feeding piglets. The probiotic properties of these LAB were analyzed, including their tolerance to gastric and bile acids, enzymatic properties, and antimicrobial activity against pathogenic microorganisms.

MATERIALS AND METHODS

Bacterial strains and culture media *Listeria monocytogenes* ATCC 19111, *Salmonella enterica* KCTC 12401, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* KCTC 3881, and *Bacillus cereus* 3711 were obtained from the Korean Collection for Type Cultures (KCTC, Daejon, South Korea) and used as pathogenic strains. MRS broth medium (Difco, Detroit, MI, USA) was used for the isolated LAB.

Isolation and identification of LAB from feces of breast-feeding piglets For the isolation of LAB, feces of breast-feeding piglets (*Landrace white*) were obtained from the Keumho Hog Farm in the Asan area, South Korea. They were diluted with 0.85% of saline solution and incubated with MRS broth adjusted to pH 2.5 with 0.1 N HCl for 2 h.

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After acid treatment, they were inoculated on MRS media containing 0.1% bile salts (Sigma, USA) and incubated at 37 °C for 3 days in a 2.5 L jar with an anaerobic pack.

The isolated LAB were identified by using 16S rDNA analysis. To analyze the 16S rDNA gene sequence, polymerase chain reaction (PCR) was conducted using a PCR System 2700 (Applied Biosystems, Foster, California, USA) with forward primer 5′ AGAGTTTGATCMTGGCTCAG-3′ and reverse primer 5′ GGYTACCTTGTTACGACTT-3′ (25). PCR was carried out over 30 cycles (initial denaturation at 97 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and polymerization at 72 °C for 1.5 min) with a final 4-min polymerization step at 72 °C. PCR products were purified with a Gel Extraction Kit (AtmanBio, Uiwang, South Korea) and analyzed with amplified ribosomal DNA restriction analysis (ARDRA). The PCR products were digested with restriction enzymes HaeIII and HpaII. DNA band patterns of the resulting PCR products were analyzed by 2% agarose gel electrophoresis and sequenced in the Macrogen Co. (Seoul, South Korea). The sequences of the 16S rDNA genes were analyzed with the EzTaxon server (26). A phylogenetic tree was constructed by using the neighbor-joining method, which produced a unique final tree under the principle of minimum evolution using the MEGA4 programs (27, 28).

Physicochemical properties of the LAB Morphological properties of the isolated strains were analyzed according to Bergey's Manual (24). To study cell growth pattern, the isolated LAB were incubated in 100 ml of MRS broth media at 37 °C for 24 h with shaking (150 rpm) and then cell densities were measured at 600 nm at 2 h intervals, using a spectrophotometer (Beckman Du 650, Fullerton, CA, USA). Changes in pH value during cell growth were analyzed with a pH meter (Orion420A, Orion research Inc, USA).

The fermentation efficacy of sugars and the enzyme activities were analyzed with the API 50 CHL System Kit and API ZYM Kit according to the procedures described by the manufacturer (BioMérieux, Lyon, France). For enzyme activity analysis, the LAB strains were cultured in MRS broth at 37 °C for 24 h and then adjusted to a concentration of 1×10^5 cells/ml. A 65-μl sample of diluted cells was transferred to the strip of the API

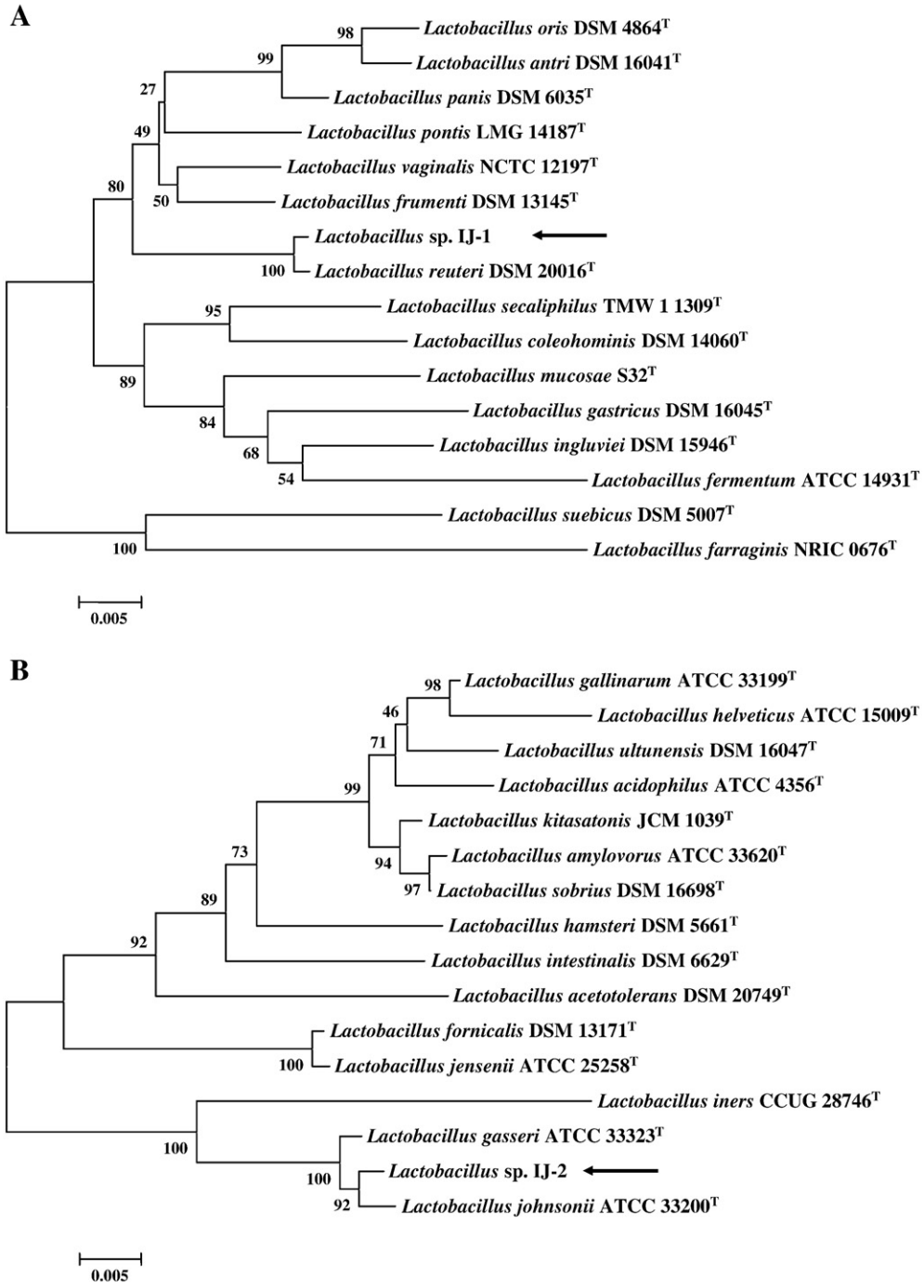

FIG. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strains isolated from feces of breast-feeding piglets. (A) *Lactobacillus* sp. IJ-1; (B) *Lactobacillus* sp. IJ-2. Numbers of nodes of levels of bootstrap support (%) from a 1,000-record resample dataset. Bar: 0.1, nt substitution per position.