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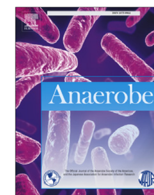

Molecular biology, genetics and biotechnology

Probiotic properties of *Pediococcus* strains isolated from jeotgals, salted and fermented Korean sea-food

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ABSTRACT

Three *Pediococcus pentosaceus* strains were isolated from jeotgals, salted and fermented Korean sea-foods, and their probiotic potentials were examined. After 2 h exposure to pH 3.0, *P. pentosaceus* F66 survived with the survival ratio of 32.6% followed by *P. pentosaceus* D56 (17.2%) and *P. pentosaceus* A24 (7.5%). *P. pentosaceus* F66 also survived better (26.6%) than *P. pentosaceus* A24 (13.7%) and *P. pentosaceus* D56 (5.8%) after 2 h exposure to 0.3% bile salts. Three strains grew slowly on MRS broth with 15% NaCl (w/v), reaching the OD_{600} values of 0.4–0.8 in 36 h. They adhered to Caco-2 cells (10.9–13.9 CFU/cell) with similar degree of adherence of a positive control, *Lactobacillus rhamnosus* GG (12.8 ± 0.5 CFU/cell). Three strains possess some desirable enzyme activities such as β-galactosidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosidase. From these results, *P. pentosaceus* F66 seems qualified as a probiotic and can be utilized for fermented foods including jeotgals.

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1. Introduction

Jeotgals are salted and fermented Korean sea-foods, prepared from various fishes, fish eggs, or shrimps which were salted (10–30%, w/w) and stored for several months at 15–25 °C [1]. Among the various types of jeotgals produced in Korea, the two most important are Myeolchi-jeot and Saeu-jeot, which are prepared from anchovies and small shrimps, respectively [2]. During fermentation of jeotgals, halophilic or halotolerant microorganisms proliferate and contribute to the development of unique flavor and texture of jeotgals by digestion of ingredients. Jeotgals are important side dishes and also additives to various foods to improve the taste of foods [1]. Importantly, jeotgals are one of the main ingredients of Kimchi, a fermented Korean vegetable. Jeotgals are rich sources for proteins, calcium and fat [2]. Jeotgals are also rich sources for microorganisms, especially for halotolerant or halophilic organisms as shown by isolation of 19 species from various jeotgals [3–5].

Lactic acid bacteria (LAB) have been isolated from several kinds of jeotgals [6,7]. Among them, *Pediococcus* species are isolated quite often. Pediococci are Gram-positive, coccus shaped, catalase-negative, non-motile, non-spore forming, and lactic acid producers [8]. Pediococci inhabit on a great variety of plant materials, ripened cheeses, and a variety of processed meats [8]. They also play an important role as a member of some commercial starters for fermented foods [9].

Probiotics are defined as live microorganisms that improve the general health and well-being of the host by maintaining or improving the intestinal microbial balance [10]. If a microorganism is expected to remain viable and exert health-promoting effects inside host, it should possess resistance against low pH and bile salts which a microorganism encounters inside human body. The strain should efficiently adhere to intestinal mucosa for successful colonization [11,12]. In addition, some enzyme activities are regarded as desirable properties for probiotics. For example, bile salt hydrolase (BSH), catalyzing the hydrolysis of glycine- or taurine-conjugated bile salts into amino acid residues and deconjugated bile salts, contributes to the resistance of a strain against the toxic level of bile salts in the gastrointestinal environments [13]. Recently, the bile salt deconjugation ability of LAB is examined for the possibility of lowering the serum cholesterol level in hypercholesterolemic humans [14,15].

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Currently, a few LAB are used as probiotics, mostly *Lactobacillus acidophilus* and closely related lactobacilli. The list needs to be expanded since each species of LAB has unique characteristics and thus can be used for different purposes. Especially, many fermented foods depend on specific species of LAB for the successful production. Pediococci with desirable properties can be used as probiotics in addition to starters for jeotgals. In this report, 3 *Pediococcus* strains isolated from jeotgals were examined for their probiotic properties.

2. Materials and methods

2.1. Isolation and identification of Pediococcus strains from jeotgals

LAB were isolated from jeotgals and potential probiotic strains were screened based on their bile salt hydrolase activities, protease activities and abilities to grow in the presence of NaCl (8%, 15%, w/v). Nine different jeotgal products were purchased from a local market in Jinju, Korea and serially diluted with 0.1% peptone water. Diluted samples were spread on deMan Rogosa Sharpe (MRS, Difco, Becton Dickinson Co., Sparks, MD, USA) agar plates and incubated at 30 °C. Single colonies were picked up. API 50 CHL kit (BioMerieux, Marcy L'Etoile, France) was used to identify isolates first [16]. 16S rDNA sequencing was also used. 16S rRNA genes were amplified by PCR using the universal primer set: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGY-TACCTTGTTACGACTT-3′) [17]. Amplification was done using a thermocycler (MJ Mini personal thermal cycler, BioRad, Hercules, CA, USA) as follows: denaturation at 94 °C for 5 min, 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C. Amplified fragments were purified from agarose gels using a PCR purification kit (FavorPrep PCR purification mini kit, Favorgen, Ping-Tung, Taiwan). DNA sequences were determined at Cosmogenetech (Seoul, Korea) and BLAST program provided by National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) was used to find homologous sequences in the data library (http://www.ncbi.nlm.nih.gov/BLAST).

2.2. Growth of Pediococcus strains

Pediococcus pentosaceus strains were grown on MRS broth without agitation at different temperature (4, 10, 20, 30, 37, and 45 °C), initial pH (3–10), and salt concentration (10, 15, 18, 21, and 24%, w/v). Each strain was first propagated in MRS broth for 18 h at 30 °C and then 1% inoculated into 100 ml MRS broth (v/v) with different conditions. Inoculated cultures were incubated for 240 h and the OD_{600} values of cultures were measured at different time points.

2.3. Enzyme activities of Pediococcus strains

API ZYM system was used to evaluate the enzyme activities of *P. pentosaceus* strains [18]. Bile salt hydrolase (BSH) was assayed by measuring the amount of amino acids liberated from conjugated bile salts [19,20]. Cells were harvested from 24 h culture (MRS broth with 0, 0.1 or 0.3% bile salts) by centrifugation at 8000 × *g* for 10 min at 4 °C. Cell pellets were resuspended in 1 ml of 0.1 M phosphate buffer (pH 6.0). Cells were disrupted by sonication and the resulting suspension was used as the enzyme sample. The reaction mixture consisted of 350 μl of 0.1 M phosphate buffer (pH 6.0), 50 μl of 200 mM taurocholic acid sodium salt hydrate (Sigma, T4009) or 200 mM glycodeoxycholic acid sodium salt (Sigma, G9910), and 200 μl of cell suspension. The mixture was incubated for 20 min at 37 °C and then 10 μl of 6 N HCl was added to terminate the reaction. The mixture was centrifuged at 12,000 × *g* for 5 min and the supernatant was assayed for free amino groups by the ninhydrin reaction [20]. One unit of BSH activity was defined as the amount of enzyme which liberated 1 μmol of amino acids (taurine or glycine) from the substrate per min.

2.4. Acid resistance and bile salt tolerance of Pediococcus strains

Pediococcus isolates were cultivated in MRS broth at 30 °C until the OD_{600} values reached 1.5. One ml was centrifuged at 12,000 × *g* for 5 min at 4 °C and the cell pellet was resuspended in 1 ml of MRS broth where the pH was adjusted to 2.0, 3.0, and 6.5 using 1 N HCl. Cell suspension was incubated for 2 h at 30 °C and then serially diluted in 0.1% peptone-water and plated onto MRS agar. Viable cells were counted after 24 h incubation at 30 °C.

Bile tolerance of a strain was determined by measuring growth on MRS broth with 0.3% bile salts (cholic acid sodium salt 50% and deoxycholic acid sodium salt 50%, Fluka, 48305). *Pediococcus* strains were grown overnight in MRS broth and cells were harvested by centrifugation. Cell pellet was washed with sterile water and resuspended in 1 ml of MRS broth containing 0.3% bile salts. After 2 h incubation at 30 °C, viable cells were counted as described above.

2.5. Antibiotic susceptibilities of Pediococcus strains

Antibiotic susceptibilities of *Pediococcus* strains were examined by agar overlay diffusion method described by Cebeci and Gürakan [21]. *Pediococcus* strains were grown overnight in MRS broth at 30 °C. MRS agar plates were overlaid with 0.7% soft agar with 200 μl of each culture (10^8 CFU/ml) and stood for 1 h at 30 °C. Then paper discs were placed on the plates and antibiotics were applied onto the discs. After 24 h incubation at 30 °C, the diameters of inhibition zones were measured. Three *Pediococcus* strains were tested for their susceptibilities against ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin, tetracycline, and vancomycin. Antibiotics were used at the amount of 0.5–2048 μg.

2.6. Adhesion capacity of Pediococcus strains

P. pentosaceus strains were examined for their adhesion capacities using the human colon adenocarcinoma cell line, Caco-2. Caco-2 cells were cultured in Minimum Essential Medium (Earle's salt, 15 mM Hepes and GlutaMAX™, Life Technologies, Gibco, Rockville, MD, USA) supplemented with 16.5% (v/v) fecal bovine serum (Life Technologies, Gibco) and 50 μg/ml gentamicin (Life Technologies, Gibco). Cells were grown at 37 °C under the atmosphere of 5% CO_2 and 95% air until a confluent monolayer was obtained. Monolayers of Caco-2 cells were seeded at the concentration of 2×10^5 cells/ml and dispensed into each 200 mm^2 well of 6 well tissue culture plates. *Pediococcus* strains were resuspended in the Caco-2 growth medium without gentamicin to the final concentration of 10^8 CFU/ml and 1 ml of this suspension was added to each well of the tissue culture plates. After 1 h incubation, the monolayers were washed four times with phosphate-buffered saline (PBS, pH 7.4) to remove non-adherent bacteria. Caco-2 cells were lysed by the addition of 0.1% (v/v) Triton-X100. The number of viable, adherent bacteria was determined by plating serial dilutions onto MRS agar plates. Colonies were enumerated after anaerobic incubation for 24 h at 37 °C and the adhesion capacities were described as the number of cells adhered to a Caco-2 cell. *Lactobacillus rhamnosus* GG (ATCC53103) was used as a positive control and *Lactococcus lactis* MG1363, a lab strain, as a negative control. Each adhesion assay was conducted three times (three different passages) with duplicate determinations.

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