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Aggregation and adhesion properties of 22 *Lactobacillus* strains

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ABSTRACT

In this paper, the autoaggregating, coaggregating, hydrophobicity, and adhering abilities of 22 *Lactobacillus* strains belonging to different species were assessed. No correlation existed between autoaggregation and adhesion of the strains belonging to different species, whereas a positive correlation existed between autoaggregation and adhesion of the strains belonging to the same species. After treating with guanidine HCl, the autoaggregating and adhering abilities of some *Lactobacillus* strains decreased, indicating that surface-bound proteins and other macromolecules played a role in the adhering and autoaggregating abilities. The strains *Lactobacillus plantarum* 20 and 66 had higher adhesion and coaggregation abilities and should be further studied for their probable probiotic properties. Aggregating, coaggregating, and adhering abilities of *Lactobacillus* strains could be used as the preliminary criteria for selecting strains having probiotic potential.
Key words: autoaggregating, coaggregating, hydrophobicity, adhesion

INTRODUCTION

A probiotic is generally defined as "a live microorganism which, when administered in adequate amounts, confers health benefit on the host" (Araya et al., 2002). *Lactobacillus* could exert beneficial effect on humans by inhibiting invasion of pathogens, improving the epithelial barrier function, modulating host immune system, and so on (Saxelin et al., 2005). Many strains of lactobacilli have a variety of health-promoting effects in humans. Adherence and colonization of *Lactobacillus* strains in the gastrointestinal tract is the prerequisite for the strains to exhibit beneficial effect on humans (von Ossowski et al., 2010). The assessment of autoaggregation, coaggregation, hydrophobicity, and adherence to intestinal epithelial cell lines (Caco-2 and HT-29 cells) of *Lactobacillus* strains has been used as an in vitro method to screen strains having probiotic potential (Kos et al., 2003; Vlková et al., 2008; Tamang et al., 2009; Kirtzalidou et al., 2011).

Human enterocyte-like Caco-2 and HT-29 cell cultures have been used as models to assess the adherence ability of *Lactobacillus* strains to intestinal epithelial cells (Gopal et al., 2001; Servin and Coconnier, 2003), but the methods are time-consuming and expensive. The autoaggregation abilities and hydrophobicity, rather than adherence ability, of *Lactobacillus* strains were assessed for preliminary identification of potentially adherent bacteria (Collado et al., 2007b; Collado et al., 2008; Bao et al., 2010; Chen et al., 2010). It was reported that adherence and autoaggregation of *Lactobacillus* and *Bifidobacteria* strains were strongly related (Del Re et al., 2000). Some probiotic strains can inhibit adherence of pathogenic bacteria to intestinal mucosa either by forming a barrier via autoaggregation or by direct coaggregation with the pathogens (Collado et al., 2007a; Vlková et al., 2008; Ferreira et al., 2011). Lactobacilli that had high autoaggregation ability showed high hydrophobicity (Chen et al., 2010; Nikolic et al., 2010). It was reported that the proteins, glycoproteins, and teichoic and lipoteichoic acids on the cell wall surface of bacteria play important roles in the autoaggregation and hydrophobicity of the strains (Lahtinen et al., 2009; Goh and Klaenhammer, 2010).

Adhesion ability is regarded as an important property of probiotics. When probiotics adhere to the epithelium, they can function stably in the intestine. A correlation between adhesion ability and hydrophobicity has been observed in some lactobacilli (Kos et al., 2003). In this study, we examined the autoaggregation, hydrophobicity, coaggregation, and adhesion ability of 22 lactobacilli strains to preliminary screen some strains having probiotic potential and determined the correlation between cell surface characteristics and adhesion.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Lactobacillus strains and *Escherichia coli* O157 were used from our stock culture collection. *Lactobacillus rhamnosus* GG (**LGG**) was presented as a gift from

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L. Zhang from the Harbin Institute of Technology (Harbin, China). Lactobacilli were cultured anaerobically in de Man, Rogosa, and Sharpe (**MRS**) broth (Merck KGaA, Darmstadt, Germany) at 37°C for 20 h. *Escherichia coli* O157 was cultured aerobically in brain heart infusion (BHI) broth (Difco Laboratories Inc., Detroit, MI) at 37°C for 20 h.

Caco-2 Cell Culture

The human colonic cell line Caco-2 was provided kindly by X. Chen (Ruijin Hospital, Shanghai, China). The Caco-2 cells were grown routinely in Dulbecco's Modified Eagle Medium (Gibco by Life Technologies Corp., Grand Island, NY), supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (Sijiqing; Zhejiang Tianhang Biological Technology Co. Ltd., Hangzhou, China) and penicillin (100 U/mL) and streptomycin (100 μg/mL; Pen Strep; Gibco by Life Technologies Corp.) at 37°C in 5% CO_2 atmosphere. The culture medium was replaced every 48 h to maintain the cells.

Autoaggregation and Coaggregation Assays

Autoaggregation and coaggregation assays were carried out according to Collado et al. (2008) with little modification. *Lactobacillus* strains were grown at 37°C for 20 h in MRS broth. The bacteria cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C and washed twice with PBS (pH 7.2), and then resuspended in the PBS. Absorbance at a wavelength of 600 nm ($\mathbf{A_{600nm}}$) was adjusted to 0.25 ± 0.05 to standardize the number of bacteria (10^7–10^8 cfu/mL). Then, the bacterial suspensions were incubated in 1-mL aliquots at 37°C, which were monitored at different times (0 or 5 h). Autoaggregation percentage was expressed as $[1 - A_t/A_0] \times 100$, where A_t represents the absorbance at time t = 5 h and A_0 the absorbance at t = 0.

Bacterial suspensions were prepared as described for autoaggregation analysis. Equal volumes of cells (1 mL) of the different probiotic and pathogen strains were mixed and incubated at 37°C without agitation. The A_{600nm} of the mixtures described above were monitored at different times (0 or 5 h). Absorbance was determined for the mixture and for the bacterial suspensions alone. Coaggregation was calculated as follows: $\{[(A_{pat} + A_{probio})/2 - A_{mix}]/(A_{pat} + A_{probio})/2\} \times 100$, where A_{pat} and A_{probio} represent A_{600nm} of the separate bacterial suspensions in control tubes and A_{mix} represents the absorbance of the mixed bacterial suspension at 5 h.

Bacterial Adhesion to Hydrocarbons

The hydrophobicity of the strains was determined by xylene extraction according to Collado et al. (2008). Bacterial suspensions were prepared as described above. Absorbance (A_{600nm}) was adjusted to 0.25 ± 0.05 and then an equal volume of xylene was added. The 2-phase system was thoroughly mixed by vortexing for 3 min. The aqueous phase was removed after 1 h of incubation at room temperature and its absorbance at 600 nm was measured. Affinity to hydrocarbons (hydrophobicity) was reported as adhesion percentage according to the formula $[(A_0 - A)/A_0] \times 100$, where A_0 and A are the absorbance before and after extraction with organic solvents respectively.

Adhesion of Bacteria to Caco-2 Cells

The adhesive activity of lactobacilli strains was assessed by using Caco-2 cells as an intestinal epithelial cell model according to Nueno-Palop and Narbad (2011). Caco-2 cells were seeded on 12-well cell culture plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a concentration of 1×10^5 cells per well. The plates were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air until a confluent monolayer were obtained.

Caco-2 cell monolayers on the 12-well plates were washed twice with PBS (pH 7.2) before the adhesion assay. Bacteria were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C and washed twice with PBS (pH 7.2), and then resuspended in Dulbecco's Modified Eagle Medium (antibiotic-free, fetal bovine serum-free). One milliliter of the bacterial suspension was added to the 12-well plates and incubated for 1 h at 37°C in 5% CO_2 atmosphere. After incubation, each well of the plates was washed 4 times with PBS (pH 7.2) to remove free, nonattached bacterial cells. Then 1 mL of 1% (vol/vol) Triton X-100 was added to each well. Then suspension was stirred to detach the bacterial cells from Caco-2 cell monolayers. Serial dilutions of the suspension were plated onto MRS plates and incubated under anaerobic conditions at 37°C to determine the viable bacterial cell number.

The Effect of Treatment by Guanidine-HCl on the Autoaggregation and Adhesion of the Lactobacilli

All tested strains were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C and washed twice with PBS (pH 7.2). Each of the tested strains was divided into 2 equal parts, one resuspended in 0.5 mL of PBS as control and the other resuspended in 0.5 mL of 4 *M* guanidine-HCl, and incubated at 37°C for 1 h. Then, the autoaggregation and adhesion of the guanidine-HCl-treated lactobacilli were assessed as described above.