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Original Article

Anti-pathogenic and probiotic attributes of *Lactobacillus salivarius* and *Lactobacillus plantarum* strains isolated from feces of Algerian infants and adults

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Abstract

Sixty-seven (67) lactic acid bacteria (LAB) isolates belonging to *Lactobacillus* genus were isolated from human feces and tested for their auto-aggregation and cell surface hydrophobicity in order to establish their adhesion capabilities, a prerequisite for probiotic selection. Strains with the upmost auto-aggregation and cell surface hydrophobicity scores were identified by MALDI-TOF spectrometry and 16S rDNA sequencing as *Lactobacillus plantarum* (p25lb1 and p98lb1) and *Lactobacillus salivarius* (p85lb1 and p104lb1). These strains were also able to adhere to human epithelial colorectal adenocarcinoma Caco-2 cells, with percentages ranging from 4.68 to 9.59%. They displayed good survival under conditions mimicking the gastrointestinal environment and remarkably impeded adhesion and invasion of human Caco-2 by *Listeria monocytogenes* and Enteropathogenic *Escherichia coli*. It should also be noted that *Lb. plantarum* p98lb1 was able to reduce *in vitro* cholesterol concentration by about 32%, offering an additional health attribute.
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Keywords: Human feces; *Lactobacillus*; Probiotics; Anti-invasion; Anti-adhesion properties; Cholesterol lowering

1. Introduction

The intestinal microbiota is considered an important source of potentially beneficial microbes usually referred to as probiotics which, according to the FAO/OMS definition, are live microorganisms conferring heath benefits to the host when they are administered in adequate amounts [1]. Probiotic health attributes have been reported in many studies and reviews, arousing great interest in the scientific community [2–4]. The wide applications anticipated for probiotics could explain the increased interest in isolating additional strains from different sources around the world and then characterizing them for their health-promoting issues. Bacterial adhesion to epithelial cells and mucosal surfaces is a key element in selection of probiotics [5,6]. Adhesion to intestinal cells is predicted to have lasting beneficial effects upon human health, i.e. exclusion of pathogens, immunomodulation and production of beneficial bacterial molecules [7]. Bacterial adhesion can be assessed using *in vitro* models such as intestinal epithelial cell lines (e.g., Caco-2), intestinal mucus and human intestinal tissues [8]. Nonetheless, adhesion properties appear to be species- and even strain-dependent [9]. Dimitrov et al. [10] studied adhesion of a set of lactobacilli that included strains of *Lactobacillus gasseri* G7, *Lactobacillus plantarum* F1 and *Lactobacillus helveticus* AC to Caco-2 cell. Those authors [10] elucidated cell wall protein mechanisms and cell-wall-bonded exopolysaccharides for *Lactobacillus delbrueckii* subsp. *bulgaricus* B14. Antikainen et al. [11] categorized these adhesion proteins into five classes, among them anchorless housekeeping proteins, surface layer

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proteins, LPXTG-motif proteins, transporter proteins and 'other' proteins. Bacterial auto-aggregation and cell surface hydrophobicity were shown to be involved in the adhesion and colonization capacities of probiotics [12,13], delineating the importance of these functions as criteria for selecting novel probiotic strains [14,15]. Auto-aggregation influences probiotic adhesion to the intestinal epithelial cells, while their co-aggregation with pathogens could prevent colonization in the gut by these detrimental bacteria [16].

In this study, 67 *Lactobacillus* isolates from human feces collected in Bejaia, a city in the north of Algeria, were screened for their cell surface properties, including auto-aggregation and hydrophobicity. This study led to isolation and identification of *Lb. plantarum* (strains p25lb1 and p98lb1) and *Lactobacillus salivarius* (strains p85lb1 and p104lb1) as strains with the highest auto-aggregation and cell surface hydrophobicity scores. Moreover, these strains were also able to inhibit adhesion and invasion by pathogenic bacteria such as *Listeria monocytogenes* and Enteropathogenic *Escherichia coli* (EPEC). In addition to these functions, the *Lb. plantarum* p98lb1 strain displayed *in vitro* cholesterol lowering capabilities of about 32%.

The data gathered from this study argue for exploitation of novel probiotic sources worldwide and provide insight into the anti-adhesive properties of lactobacilli and their beneficial effects for probiotic applications.

2. Materials and methods

2.1. Lactobacillus *strains isolation from human feces*

Feces from Algerian infants and adults were collected at Bejaia Hospital (Algeria) and serially diluted in saline water (8.5 $g \cdot L^{-1}$ NaCl), inoculated into MRS agar (Sigma–Aldrich, Steinheim, Germany) containing L-cysteine hydrochloride (0.05% [w/v], Sigma–Aldrich), and then incubated at 37 °C for 48 h. The obtained colonies were subcultured in MRS agar and analyzed for Gram staining and catalase activity. Bacterial isolates that were Gram-positive and devoid of catalase activity were stored at −20 °C in MRS broth containing 30% (v/v) of glycerol (Sigma–Aldrich) until further characterization.

In this study, *E. coli* ATCC 25922, enteropathogenic *E. coli* (EPEC) kindly provided by Dr. Stéphanie Blanquet (Université d'Auvergne, France), *Staphylococcus aureus* ATCC 25923, *Listeria innocua* (*L. innocua*) CIP 74915 and *L. monocytogenes* 162 of food origin, kindly provided by Dr. Marie France Pilet (Oniris, Nantes, France) were used as indicator strains. These strains were grown in brain-heart infusion broth (BHIB, Sigma–Aldrich) at 37 °C, and kept under appropriate conditions until further use.

2.2. Screening of lactobacilli for auto-aggregation and cell surface hydrophobicity properties

Aggregation assays were performed as described by Kos et al. [17]. Briefly, *Lactobacillus* strains were grown for 18 h at 37 °C in MRS broth. After centrifugation (8000 × *g*, 10 min), the pellets were recovered, washed twice with sterile 0.01 M phosphate-buffered saline (PBS, pH 7.2) and re-suspended at 10^8 CFU·mL^{-1} in the same buffer. Then, cell suspensions were mixed by vortexing and auto-aggregation was determined after 2 h of incubation at 37 °C. Subsequently, an aliquot (1 mL) of these suspensions was carefully removed from the upper suspension and its absorbance was read at 600_{nm} on a spectrophotometer (Specord®, Shimadzu, Germany) [17]. The auto-aggregation percentage was calculated using the following formula: auto-aggregation (%) = $1 - (A_t/A_0) \times 100$, where A_t represented the absorbance at time t = 2 or 4 h and A_0 the absorbance at t = 0 h.

Cell surface hydrophobicity was determined using the microbial adhesion to hydrocarbon method (MATH) described by Rosenberg et al. [18]. Bacteria from overnight culture were harvested by centrifugation (8000 × *g*, 10 min), washed twice with PBS (pH 7.2) and re-suspended in the same buffer at 10^8 CFU·mL^{-1}. Absorbance of the cell suspension was measured at 600_{nm} (A_0). One milliliter of xylene was added to 3 mL of cell suspension and mixed by vortexing for 2 min. The suspension was incubated at room temperature to allow phase separation. The aqueous phase was removed and its absorbance at 600_{nm} (A_1) was read. The percentage of bacterial adhesion to solvent was calculated using the following formula: hydrophobicity (%) = $1 - (A_1/A_0) \times 100$, where A_1 represented the absorbance of the aqueous phase after two-phase system separation and A0 the absorbance of the initial bacterial suspension [18].

2.3. Molecular identification by MALDI-TOF spectrometry and 16S rDNA sequencing of the most relevant Lactobacillus *strains*

The selected strains were identified using MALDI-TOF spectrometry. To this end, strains were grown in MRS agar for 48 h and pure colonies were deposited on a ground steel Maldi target. The spots (three spots for each isolate) were overlaid with 1 µl of 70% formic acid solution (Sigma–Aldrich), dried at room temperature, then overlaid again with 1 µl of matrix solution (α-cyano-4-hydroxycinnamic acid [HCCA]; Bruker Daltonics) dissolved in 50% (v/v) acetonitrile (Sigma), 47.5% (v/v) water, and 2.5% (v/v) trifluoroacetic acid (Sigma) and allowed to dry prior to analysis using the Maldi Biotyper. The target was analyzed using the Maldi-Tof MS spectrometer Autoflex speed TM (Bruker Daltonics, Bremen, Germany) in a linear positive mode. Mass spectra were analyzed in *m/z* range of 2000 to 20,000 and a bacterial test standard (BTS, Bruker Daltonics) was used for instrument calibration according to Bruker's recommendations. The determination of *m/z* ratios of detected ions in each Maldi-MS profile was performed under Flex analysis 3.4 for comparison with data base. The manufacturer-recommended identification scores used were: 2.00–3.00, high-confidence identification; 1.70–1.99, low-confidence identification; 0.00–1.69, no organism identification possible.

Total DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). For 16S

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