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Original scientific paper

Aggregation ability of potential probiotic *Lactobacillus plantarum* strains

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ABSTRACT

Aggregation is the process of reversible gathering of bacterial cells belonging to the same bacterial strain (autoaggregation) or two different bacterial strains (coaggregation). Autoaggregation ability of probiotic bacteria correlates with adhesion, which is a prerequisite for colonization and protection of gastrointestinal tract, while coaggregation provides close interaction with pathogenic bacteria.

In this experiment the aggregation ability of three potential probiotic strains of *Lactobacillus plantarum* were investigated. Coaggregation with different food–borne pathogens: *Salmonella enterica* serotype Typhimurium, *Listeria monocytogenes* EGD strain and enterohaemorrhagic *Escherichia coli* (EHEC) was also studied.

The results showed that all *Lactobacillus* strains when cultivated in broth had better autoaggregation and coaggregation abilities then those cultivated on agar. After 24 hours almost 80 % of *Lactobacillus* aggregated. All lactobacilli coaggregated similarly with selected food-borne pathogens.

All three strains of *L. plantarum* possess the ability to autoaggregate and coaggregate, which is an important feature in the selection of probiotic bacteria.

Key words: aggregation, *Lactobacillus plantarum,* probiotics

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INTRODUCTION

Probiotics are defined as live microorganisms which when administered in adequate quantity confer health benefits to the host [1]. Lactic acid bacteria from the genera *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics [2]. However, probiotic properties are characteristics of each strain, not the genus or even a species. Potential probiotic strain must meet numerous criteria before its commercial usage. Criteria of utmost importance in the selection of probiotic candidates are the ability to aggregate and to adhere to epithelial cells, because they are prerequisite for colonization of probiotic strains.

Aggregation is the process of reversible accumulation of cells, causing them to spontaneously precipitate in the medium in which they are suspended [3,4,5]. There are two different types of aggregation: autoaggregation and coaggregation. Autoaggregation is clumping of bacteria which belong to the same strain, while coaggregation is the result of cell-to-cell recognition between two different bacterial strains. Autoaggregation of probiotic strains has been correlated with adhesion to intestinal epithelial cells, known to be a prerequisite for colonization and enhanced persistence in the gastrointestinal system. Coaggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms [6].

The objective of this experiment was to investigate the autoaggregation abilities of three selected strains of *L. plantarum* as well as their capability to coaggregate with different food-borne bacteria.

It is known that *Lactobacillus* spp. interfere with pathogens by different mechanisms, like production of antimicrobial compounds such as lactic acid, hydrogen peroxide, bacteriocine like substances etc. [7,8,9]. Lactic acid bacteria can prevent adhesion of pathogenic bacteria by competition for bonding places on intestinal epithelial cells, and consequently reduce pathogen colonization and prevent infection [2,3,10]. The objective of this experiment was to investigate the autoaggregation abilities of three selected strains of *L. plantarum* as well as their capability to coaggregate with different food-borne bacteria. Our results indicated the capability of all three *L. plantarum* strains to autoaggregate and coaggregate with selected food-borne pathogens.

METHODS

Bacterial strains and growth conditions

We used three different food derived (isolated from whey – S1, homemade cow cheese – A, and homemade sheep cheese – B) strains of *L. plantarum* obtained from Faculty of Food Technology and Biotechnology, University of Zagreb. Coaggregation abilities of *Lactobacillus* strains were tested with selected food-borne pathogens: *Salmonella enterica* serotype Typhimurium, *Listeria monocytogenes* EGD strain and enterohaemorrhagic *Escherichia coli* (EHEC) from culture collection of the Department of Microbiology and Parasitology, University of Rijeka. All tested bacteria were stored at -80 °C in 10 % glycerol broth. Lactobacilli were grown on de Man, Rogosa and Sharpe (MRS) agar or broth (Biolife, Italy) in microaerophilic atmosphere (5 % CO_2) at 37 °C for 48

h. *S.* Typhimurium, *L.* monocytogenes and EHEC were grown on sheep blood agar and incubated aerobically at 37 °C for 18-20 h. Pathogens were grown on different broths: *S.* Typhimurium and EHEC were grown on Lysogeny broth (LB) and *L.* monocytogenes on Brain heart infusion broth (BHI), aerobically at 37 °C for 18-20 h.

Aggregation assays

Autoaggregation and coaggregation assays were performed for bacteria grown in broth and on agar plates. Bacterial cells from agar plates were harvested and suspension was made in sterile Phosphate Buffer Saline (PBS). Bacteria grown in broth were harvested by centrifugation at 3000 rotation per minute (rpm) for 5 min, then washed and resuspended in PBS to give a final optical density of 1 (about 1×10^9 CFU/mL) at 600 nm, as measured by a spectrophotometer (Eppendorf, Germany).

For autoaggregation assay, suspension of different lactobacilli (4 mL) was divided in glass test tubes and mixed by vortexing. Absorbance was measured immediately, after 5 h and 24 h. Autoaggregation percentage was determined using the equation [6]

$$(1 - A_t/A_0) \times 100,$$

where A_t represents absorbance at different time points (t= 5 h or 24 h) and A_0 represents absorbance at the beginning of the assay (0 h).

On the 24 hour of autoaggregation test, samples of bacterial suspension of all three strains *L. plantarum* were taken from the bottom of the glass test tube. Autoaggregation was monitored by light microscopy at 100 times magnification after Gram staining.

For coaggregation assay, bacterial suspension was prepared in the same way as previously described. Equal volumes (2 mL) of probiotic strain and pathogen suspensions were divided in glass test tubes, and mixed by vortexing. Control tubes contained 2 mL of suspension of each bacterial species. Absorbance was measured immediately, after

Table 1.
Coaggregation ability of *L. plantarum* (S1, A, B) strains after cultivation on agar plates (A) and broth (B), with various pathogenic bacteria after 5 and 24 hours. Experiments were repeated at least two times. The results are expressed as per cent of coaggregated bacteria.

	% coaggregation											
	S. Typhimurium				*L. monocytogenes*				EHEC			
	A		B		A		B		A		B	
	5	24	5	24	5	24	5	24	5	24	5	24
L. plantarum S1	5.8	16.3	13.1	38.1	6.5	37.2	12.8	37.8	4.2	16.7	13.7	41.5
L. plantarum A	5.2	21.4	12	40.5	4.6	36.4	16.2	39.7	3.3	15.8	14.7	37.2
L. plantarum B	8.5	24	9.9	30.5	7.4	32.2	9.5	37.4	6.8	22.8	13.3	31.2

5 h and 24 h. The percentage of coaggregation was determined according to Handley *et al*. [11]:

$$\frac{\{\,[(Ax + Ay)/2] - A(x + y)\,\}}{(Ax + Ay/2)} \times 100$$

where A represents absorbance, x and y represent each of the two strains in the control tubes, and (x + y) their mixture.

RESULTS AND DISCUSSION

Aggregation properties are important characteristics of bacterial strains that are used as probiotics [9,12,13]. *In vitro* evaluation of autoaggregation and ability to coaggregate with potential enteric pathogens can be used for preliminary screening and selection of the best probiotic strain. The aggregation rate was measured for three food-derived strains of *L. plantarum* after 5 and 24 hours, and results show that all have better autoaggregation ability after cultivation in broth. After 24 hours of broth cultivation, the autoaggregation rate was at least 80 % (**Figure 1**). Microscopic analysis further confirmed clustering of cells and the presence of aggregates (**Figure 2**). At the same time, after cultivation on agar plates only 30 % bacteria aggregated (**Figure 1**).

The reason for enhanced autoaggregation rate of MRS broth-grown cells could be explained by better growth conditions in liquid than in solid medium. Our results are in agreement with previous reports of Kos *et al*. that broth increased the autoaggregation behaviour of the *Lactobacillus acidophilus* M92 in comparison with agar-grown cells what could be related to cell surface component, because the autoaggregation capability was not lost after washing and suspending the cells in PBS [6].

Coaggregation assay is a reliable method to evaluate the close interaction between lactobacilli and pathogenic bacteria [12,14] in which lactobacilli could release antimicrobial substances in a very close proximity [15]. Food-associated lactobacilli possessing ability to coaggre-

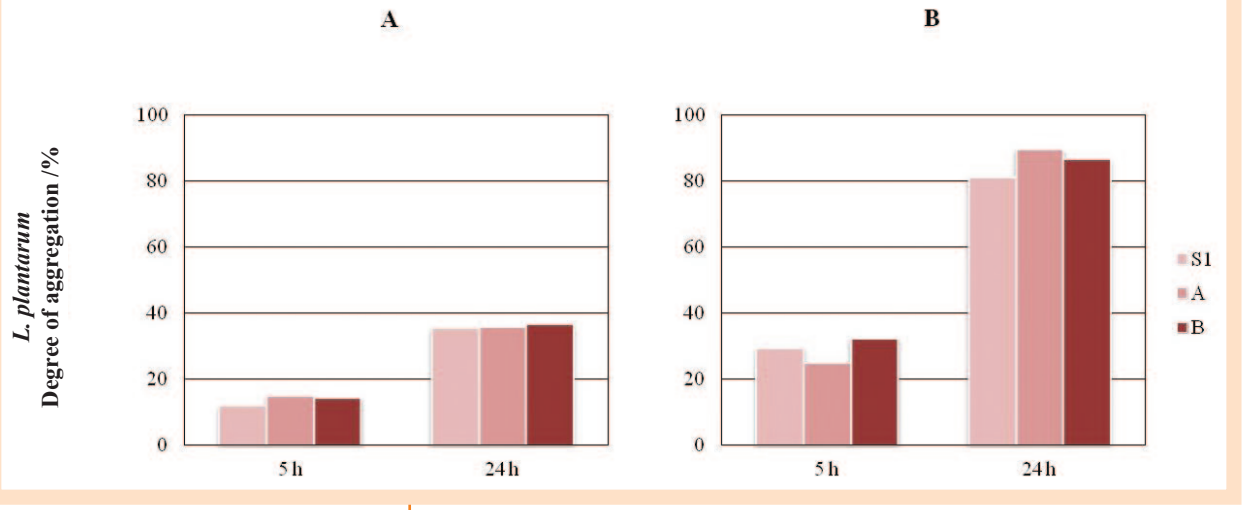

Figure 1.
Autoaggregation ability of *L. plantarum* (S1, A, B) strains after cultivation on agar plates (A) and in broth (B). Experiments were repeated at least two times. The results are expressed as per cent of aggregated bacteria.

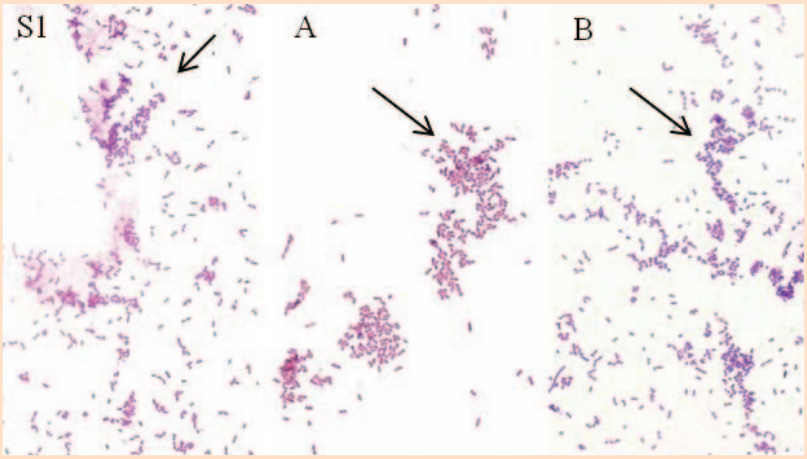

Figure 2.
Autoaggregation of *L. plantarum* (S1, A, B) after 24 hours cultivation in broth. Preparations were stained by Gram. Magnification x 1000.

gate with numerous pathogens are of special interest with regard to potential applications. We have tested coaggregation of three dairy *L. plantarum* probiotic candidates with three food-borne pathogens: *S.* Typhimurium, *L. monocytogenes* and EHEC.

Our results show that all three strains of *L. plantarum* also had better coaggregation ability after cultivation in broth.

L. plantarum S1 showed the best coaggregation result with EHEC, where 41,5 % of bacteria coaggregated, for *L. plantarum* A the best result of coaggregation was 40,5 % with *S.* Typhimurium, and *L. plantarum* B had the best result with *L. mononocytogenes* with 37,4 % of coaggregated bacteria.

In this study, all three *L. plantarum* strains (S1, A and B) showed a high autoaggregation percentage (≥ 80 %) and microscopic clustering of cells which may increase adhesion to intestinal epithelial cells. Also, all three tested strains showed similar degrees of coaggregation with selected food-borne pathogens, and that could allow them to release antimicrobial substances in a very close proximity of pathogenic bacteria.

To conclude, *L. plantarum* strains S1, A and B exhibited desirable autoaggregation and coaggregation abilities as potential probiotic strains.

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