The impact of exopolysaccharide production on hydrophobicity and aggregation properties of *Enterococcus faecium* strains isolated from different regions of Iran and Turkey

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BACKROUND

Exopolysaccharides (EPSs) are exocellular polymers present in the surface of many lactic acid bacteria. Exopolysaccharides (EPSs) are exocellular polymers present in the surface of many lactic acid bacteria. EPS is thought to protect microorganisms against bacteriophages, antibiotics, and toxic compounds. Another physiological benefit is that EPS is retained longer in the gastrointestinal tract, so that colonization by probiotic bacteria can be enhanced (1,2). Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (3). Adhesion ability is regarded as an important property when probiotic microorganisms are selected. Bacterial adhesion is initially determined by nonspecific and reversible interactions that involve physicochemical interactions (including hydrophobicity and charges) followed by specific and irreversible interactions mediated by adhesins of the bacterial surface and complementary receptors of the host cell (4). Properties generally associated with the initial stage are hydrophobicity of the cell surface and aggregation. Collado et al. (5) reported that 'in order to manifest beneficial effects, probiotic bacteria need to achieve an adequate mass through aggregation. The ability of probiotics to aggregate is a desirable property. Organisms with the ability to coaggregate with other bacteria such as pathogens may have an advantage over non-coaggregating organisms, which are more easily removed from the intestinal environment'.

OBJECTIVES

The objective of the present study was to determine whether the aggregation and hydrophobicity of *E. faecium* strains would be affected by the production of EPS. This is necessary to test future adhesion properties and to find the best combinations to be included in fermented milk products.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. faecium* RT 81, RI 41, RI 56 strains isolated from traditional naturally fermented cheese produced in different regions of Iran and Turkey. Prior to experimental use, organisms were subcultured at least three times every 18 h in De Man Rogosa Sharpe Medium under aerobic conditions. The isolates were stored at -80 ºC in MRS containing 30% glycerol (Merck, Darmstadt, Germany) and subcultured twice before use.

Isolation and quantification of EPS. Exopolysaccharides (EPSs) was determined according to the method of Frengova et al. (6). Total EPSs (expressed as milligrams per liter) were estimated in each sample by the phenolsulfuric acid method, with glucose as the standard (7).

Aggregation experiments. Autoaggregation ability was determined as described by Del Re et al. (8).

Hydrophobicity assay. Microbial adhesions to solvents were measured by the method of Zárate et al. (9) with some modifications. Briefly, bacterial suspensions resuspended in 0.1 M KNO_3 (low ionic strength) (pH 6.2). Two milliliters of bacterial suspension (A_0= 0.6±0.02) was put in contact with 0.5 ml of each of the following test solvents: p-xylene (nonpolar neutral solvent), chloroform (mono polar acidic solvent), toulene and ethyl acetate (mono polar basic solvent) after 10 min of preincubation at room temperature, the two phase system was mixed on a vortex for 2 min. After allowing the hydrocarbon phase to rise completely (for 4 h at room temperature), the aqueous phase was removed and the absorbance at 600 nm was determined again.

RESULTS

Table 1. EPS production of *Enterococcus faecium* strains

	Strains	Final Culture pH	EPS (mg/L)
1	*E. faecium* RT 81	4.69	129±1.0
2	*E. faecium* RI 41	4.83	185±0.0
3	*E. faecium* RI 56	4.64	156±0.6

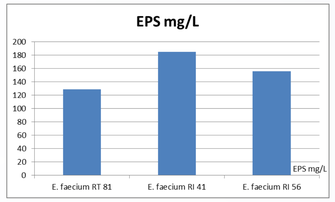


Table 2. Autoaggregation ability of *E. faecium* strains

No	Strains	Autoaggregation %
1.	*E. faecium* RT 81	43.35±0.21
2.	*E. faecium* RI 41	53.50±0.14
3.	*E. faecium* RI 56	38.80±0.14

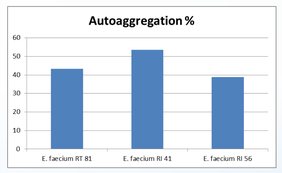


Table 3. Coaggregation ability of *E. faecium* strains

Coaggregation with *Escherichia coli* ATCC 11230		
No	Strains	*E.coli*
1.	*E. faecium* RT 81	35.25±0.21
2.	*E. faecium* RI 41	33.55±0.07
3.	*E. faecium* RI 56	18.55±0.07

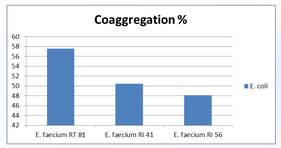
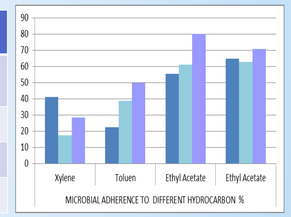


Table 4. Hydrophobicity of *E. faecium* strains

	MICROBIAL ADHERENCE TO DIFFERENT HYDROCARBON %			
Strains	Chloroform	Xylene	Toluen	Ethyl Acetate
E. faecium RT 81	59.72±0.12	41.20±0.23	22.59±0.23	55.48±0.00
E. faecium RI 41	64.55±0.12	17.60±0.12	38.68±0.23	61.24±0.12
E. faecium RI 56	84.92±0.36	28.59±0.12	49.67±0.23	80.17±0.23



CONCLUSİONS

To our knowledge, the interaction between the aggregation ability and the EPS-producing capacity of *E. faecium* strains has not been examined until now. In this study, high EPS-producing strains showed a high autoaggregation and coaggregation ability, with hydrophobicity also affecting autoaggregation. A better understanding of the mechanisms related to EPS production and aggregation can be used for preliminary screening in order to determine potentially probiotic bacteria applications for human or animal use. Further studies are needed to identify and characterize bacterial cell wall compositions and properties to understand their role in adhesion to hydrocarbons, autoaggregation and their relation with coaggregation mechanisms. Of course, these strains do require further *in vitro* and *in vivo* investigations in areas such as adhesion to cultured human intestinal epithelial cells, characterization of bacterial cell wall compositions and properties to understand their role in adhesion to hydrocarbons, autoaggregation and their relation with coaggregation mechanisms.

SUMMARY

Enterococci belong to the lactic acid bacteria (LAB) and they are of importance in foods due to their involvement in food spoilage and fermentations, as well as their utilisation as probiotics in humans and slaughter animals. The purpose of this study was to evaluate the probiotic properties of *Enterococcus faecium* RI 41, RI 56, RT 81 strains isolated from traditional naturally fermented cheese produced in different regions of Iran and Turkey. The ability to autoaggregation and coaggregation are desirable properties for probiotics in health-promoting foods. Therefore, in the current study, we assessed the effect of exopolysaccharides (EPSs) produced by *E. faecium* strains on the aggregation and hydrophobicity properties. All *E. faecium* strains tested showed auto aggregation (38.80-53.50%) and coaggregation ability (18.55-35.25%) with *Echerichia coli* ATCC 11230, but the results were strain specific and dependent on exopolysaccharides production (129-185 mg/L). In addition, enterococci strains tested were showed affinity to all solvents (chloroform, p-xylene, toluen and ethyl-acetate), suggesting a high complexity of the cell surface. Our results indicate that the ability to autoaggregation, together with cell-surface hydrophobicity and coaggregation abilities with *E. coli* strain can be used for preliminary screening in order to identify potentially probiotic bacteria suitable for human or animal use.

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