The Effect Of Exopolysaccharide Production on Cholesterol-Reducing Activity of Lactobacillus rhamnosus Strains Isolated from Traditional Turkish Cheeses

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BACKGROUND

Hypercholesterolemia has been reported to be the main cause of cardiovascular diseases and the leading cause of death. Therefore, decreasing serum cholesterol level is very important for preventing the cardiovascular disease. It has been suggested that probiotics in human gastrointestinal tract have the ability to decrease serum cholesterol level by reducing the absorption of cholesterol from the intestinal tract. In the present study, the relationship between exopolysaccharide production and cholesterol removal rates of 12 strains of Lactobacillus rhamnosus isolated from home-made traditional foods made Turkish cheeses was studied. The strains have been identified in species level by 16S rRNA gene sequence analysis. Influence of different bile concentrations on cholesterol removal was investigated.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 11 strains of Lactobacillus rhamnosus were isolated from four different traditional homemade Turkish cheese samples. The strains were identified in species level by 16S rRNA gene sequence analysis. Prior to experiments, all strains were subcultured at least three times every 18 h in DE Man Rogosa Sharpe Medium under anaerobic conditions. The isolates were stored at -80°C in MRS containing 30% glycerol (Difco, Darmstadt, Germany) and subcultured twice before use.

Cholesterol removal. Cholesterol removal was studied according to a modified method of Billard et al. (1985). Freshly prepared MRS broth was supplemented with 3.0, 3.15, and 3.33% serum concentration of goat or as a bile source (Sigma, St Louis, MO, USA). A filter-sterilized cholesterol solution (10 mg/ml in ethanol) was added to the broth to a final concentration of 100 mg/L, incubated with each strain at 37°C and incubated at 42°C for 19 and 48 hour. After the incubation period, cells were removed from the broth by centrifugation at 20 min at 10,000 × g and 1°C. A modified colorimetric method as described by Rosell and Herderich (1973) was used to determine the amount of cholesterol in the reconstituted cells and spent broth. The amount of cholesterol removal was estimated by subtracting the cholesterol amount in the spent broth from that in the unincorporated control broth.

Detection and quantification of EPS. Exopolysaccharides (EPS) was determined according to the method of Ferguson et al. (Ferguson et al., 2000). Total EPS (in mg per liter) was estimated in each sample by the phenol-sulfuric acid method, with glucose as the standard (Dubois and Gilbert, 1956; Turner et al., 2001).

RESULTS

It was confirmed that ACS1, BMD3, EDS4 and BTO4 strains which produce high amounts of exopolysaccharide (253, 290, 278 and 277 mg/L, respectively) were able to remove more cholesterol from the medium compared to those that produce low amounts of exopolysaccharide (MP4). The highest amount of cholesterol precipitation (81%) was performed by ACS1 strain, producing a high amount of exopolysaccharide, in the presence of 0.5% (v/v) bile. The results indicated that (i) there is a correlation between cholesterol removal and EPS production, and (ii) The Lactobacillus rhamnosus ACS1 has an excellent ability on hypcholesterolemic in in vitro conditions.

CONCLUSIONS

To the best of our knowledge, the literature contains no reports on cholesterol removal by Lactobacillus rhamnosus strains of cheese origin. The cholesterol removal mechanism by binding or adhering to the bacteria cells, especially to the EPS produced by the bacteria and surrounding the bacterial cells as a capsule, has potential importance in the control of serum cholesterol concentration in humans. In our study, all of the Lactobacillus strains tested removed cholesterol from media during growth. Among them, Lactobacillus rhamnosus ACS1 strain, which has distinctive features in EPS production and cholesterol removal capacity, removed the highest amount of cholesterol in presence 0.3% bile. Based on these findings, the combination of a probiotic culture that can remove cholesterol and a strain that has high EPS production capacity could be used to manufacture a functional product that would have enhanced anti-cholesterolemic activity.

REFERENCES