



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 diseases. It has been supposed 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 home 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

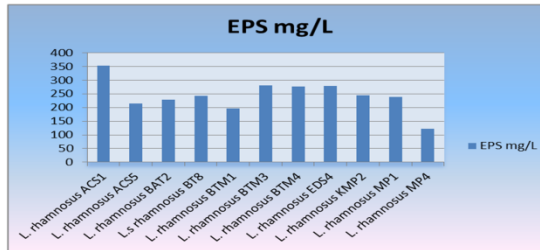
Bacterial strains and growth conditions. A total of 11 strains of *Lactobacillus rhamnosus* were isolated from four different traditional home-made Turkish cheese samples. The strains were classified in species level by 16S rRNA gene sequence analysis. Prior to experimental use, organisms were subcultured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

Cholesterol removal. Cholesterol removal was studied according to a modified method of Gilliland *et al.* (1985). Freshly prepared MRS broth was supplemented with 0.01, 0.15 and 0.03 % w/v concentration of oxgall 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 µg/mL, inoculated with each strain (at 2%), and incubated at 42°C for 19 and 48 hour. After the incubation period, cells were removed from the broth by centrifugation for 20 min at 10 000 × g and 1°C. A modified colorimetric method as described by Rudel and Morris (1973) was used to determine the amount of cholesterol in the resuspended cells and spent broth. The amount of cholesterol removed was estimated by subtracting the cholesterol amount in the spent broth from that in the uninoculated control broth.

Isolation and quantification of EPS. Exopolysaccharides (EPSs) was determined according to the method of Frengova *et al.* (Frengova, *et al.*, 2000). Total EPSs (expressed as milligrams per liter) were estimated in each sample by the phenolsulfuric acid method, with glucose as the standard (Dubois and Gilles, 1956; Torino *et al.* 2001).

Table. Cholesterol removal in different bile concentrations (CFE: Cell Free Extract, (-): not detected).

	EPS mg/L	0.01 % bile		0.15 % bile		0.30 % bile	
		CFE %	Pellet %	CFE %	Pellet %	CFE %	Pellet %
<i>Lactobacillus rhamnosus</i> ACS1	353±5	74±4	-	78±3	-	81±2	5±0
<i>Lactobacillus rhamnosus</i> ACS5	215±0	41±6	-	65±4	8±0	47±1	8±3
<i>Lactobacillus rhamnosus</i> BAT2	228±7	50±0	17±0	54±0	-	57±4	19±6
<i>Lactobacillus rhamnosus</i> BT8	243±2	56±1	-	59±4	13±1	63±2	7±1
<i>Lactobacillus rhamnosus</i> BTM1	197±2	24±0	11±0	30±2	-	40±4	3±0
<i>Lactobacillus rhamnosus</i> BTM3	280±5	65±0	-	73±0	7±0	77±2	21±3
<i>Lactobacillus rhamnosus</i> BTM4	277±2	62±5	21±0	67±3	-	76±3	14±0
<i>Lactobacillus rhamnosus</i> EDS4	278±1	63±3	-	71±2	-	74±1	2±0
<i>Lactobacillus rhamnosus</i> KMP2	244±0	55±4	-	58±0	15±1	60±1	10±1
<i>Lactobacillus rhamnosus</i> MP1	239±3	52±2	9±1	56±1	-	66±1	-
<i>Lactobacillus rhamnosus</i> MP4	123±0	16±6	-	45±1	7±0	51±1	13±2



REFERENCES

- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Pebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
- Frengova, G. I., E. D. Simova, D. M. Beshkova, and Z. I. Simov. 2000. Production and monomer composition of exopolysaccharides by yogurt starter cultures. Can. J. Microbiol. 46:1123-1127.
- Gilliland S.E., Nelson C.R., Maxwell C. (1985) Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl Environ Microb 49(2): 377-81.
- Rudel L.L., Morris M.D. (1973) Determination of cholesterol using o-phthalaldehyde. J Lipid Res 14: 364-6.
- Torino, M. I., M. P. Taranto, F. Sesma, and G. F. De Valde. 2001. Heterofermentative pattern and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in response to environmental pH. J. Appl. Microbiol. 91:846-852.

RESULTS

It was confirmed that ACS1, BTM3, EDS4 and BTM4 strains which produce high amounts of exopolysaccharide (353, 280, 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 0.3% (w/v) bile. The results indicated that: (i) there is a correlation between cholesterol removal and EPS production; and (ii) The *Lactobacillus rhamnosus* ACS1 had an excellent ability on hypocholesterolemia 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 fermented dairy product that would have enhanced anti-cholesterolemic activity.