Cholesterol reduction in culture fluids by lactobacilli

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Introduction

One of the best known effects of probiotic products is the reduction of the concentration of plasma cholesterol. By radiotracer methods a lactobacillus salivarius strain has been examined regarding its cholesterol reducing and metabolizing effects. This strain before in tests at the Federal Research Centre for Nutrition in Karlsruhe had proved effective in reducing the cholesterol level in oxgall containing culture fluids.

Methods

The lactobacillus salivarius NCFB 1555 strain was subcultured twice in MRS broth before incubation in MRS with 0,6% oxgall and radiolabelled cholesterol(4-^{14}C) for 24h at 37^{o}C.

The lactobacilli protoplasts have been prepared by the action of lysozyme and mutanolysin.

For HPLC analysis on cholesterol and metabolites the culture fluid and the microorganisms were extracted with diethyl ether. HPLC has been performed with an ALLTECH Alltima Silicia column (250x4,6mm), hexane/isopropanol (97/3) as eluent and a liquid flow cell as radioactivity detector.

Results

Investigation on chemical reactions: HPLC analysis showed no cholesterol metabolites. Also in the polar residues of the extraction and in the extracted microorganisms no relevant parts of the added radioactivity could be found. Thus the possibility remained, that cholesterol was chemically unmodified coprecipitated, bound or incorporated into the microorganisms.

Dissolving the cell wall: In case of an attachment of the chemically unchanged cholesterol to the surface of the lactobacilli it should be possible to remove the cell walls without a loss a viability. After the isolation of the protoplasts the activity was localized in the protoplasts (92-94%) and especially in the plasma membranes (96-98%). The addition of cholesterol during the enzymatic dissolution had the same result. Obviously cholesterol is attached to the lipophilic surface of the protoplasts instantly. The enrichment in this fraction seems to be caused primarily by the affinity of cholesterol to the lipid membrane of the protoplasts since the found mechanism of enrichment contrary to the experiments with intact lactobacilli works also in the absence of oxgall (fig.1). Without added oxgall the bound cholesterol activity is relatively small (about 10%). For that reason it is not probable that the binding to the plasma membrane is the main reason for the enrichment found with the intact lactobacilli.

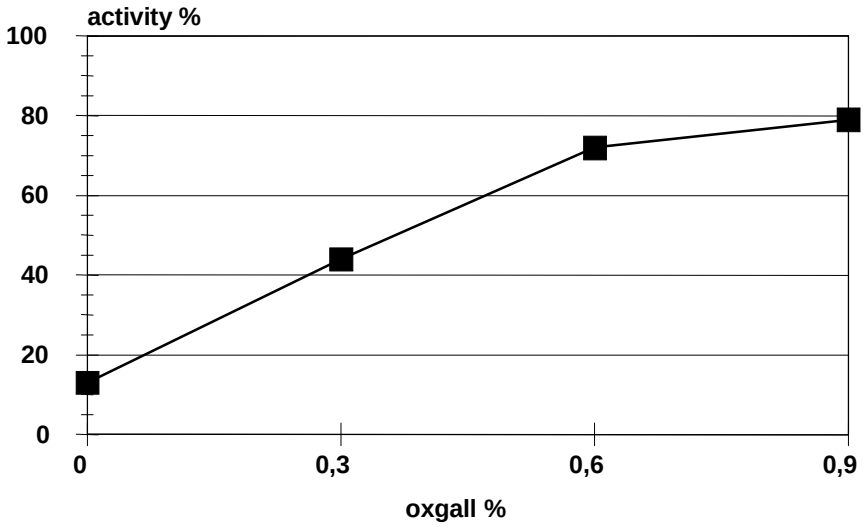

Fig. 1: Amount of bound cholesterol as a function of bile concentration

Experiments for dissolving the precipitated deconjugated bile acids and cholesterol respectively: Some strains of lactobacilli are able to cleave (deconjugate) the bile acids (which are conjugated with glycine or taurine) to free amino acids and free bile acids (BSH theory, Klaver et al. 1993). The latter coprecipitate in acid solution (as it is prepared by lactobacilli) together with cholesterol and it is possible, to redissolve them again in alkaline solution, as it could be confirmed in an experiment with deconjugated bile acids. In a corresponding experiment with microorganisms loaded with cholesterol even after 24h in a buffer solution of pH 9 not the whole amount of cholesterol could be dissolved (about 10-20% remain in the fraction of the microorganisms). For that reason a weak, surfacial fixation of cholesterol is not probable. There is the possibility too, that there are coexisting at the same time different possibilities of precipitation or fixation, what is suggested also by a microscopically perceptible inhomogenity (microorganisms, crystals, oily masses) of the precipitate. This solution experiment also points to a part of cholesterol being excreted together with the microorganisms, since there are similar conditions in the small intestine at pH 9.

Conclusion

A lactobacillus salivarius strain has been found to reduce the cholesterol concentration in culture fluids without chemical modification. The results confirm the hypothesis of the coprecitiation together with bile acids, but cannot be explained by it exclusively. The bound cholesterol dissolves in alkaline media incompletely (under conditions as in the small intestine). Thus it partially can be excreted and withdrawn from the organism respectively.

Reference

Klaver, F.A., van der Meer, R.: The assumed assimilation of cholesterol by Lactobacilli and Bifidobacterium bifidum is due to their bile salt-deconjugating activity. Appl.Environ.Microbiol. **59**, 1120-1124 (1993)