Lactobacillus Reuteri: A Newcomer in Dairy Technology

T. Klantschitsch, H. Spillmann and Z. Puhan

Original scientific paper UDC:579.864.1

Summary

Lactobacillus reuteri *is an inhabitant of the gastrointestinal of humans and animals and has been isolated also from food (sausages, cheese, sour dough). It is suggested that* L. reuteri, *a dominant heterofermentative* Lactobacillus *species with unique taits, may interact beneficially in stabilizing the intestinal microflora, thus, having a protective function against pathogenic microorganisms.* L. reuteri *as a newcomer in dairy technology and products are appearing on the market which are supplemented with this microorganism (sweet milk and fermented milk products). It is not quite clear which role* L. reuteri *plays in the intestinal ecosystem and how important it is for health and well-being of the host-organism.*

L. reuteri *is an obligatory heterofermentative* Lactobacillus *and produces under certain conditions reuterin (ß-hydroxypropionaldehyd), a potent broad-spectrum antimicrobial substance acting as inhibitor of a number of undersirable bacteria, yeasts, fungi and protozoa.*

Introduction

Probiotics are specially selected microbic genera chiefly species of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus casei* and recently also *Lactobacillus reuteri*. Thise probiotic microorganisms are supposed to be able to survive in gastrointestinal tract after oral carriage the least passing through small intestine and safely in colon settle and also through supstance exchange activity influence useful and colon flora and eventual unfavourable ecologic conditions. In addition the defence system should be reinforced. All that would have positive influence on human organism. *Lactobacillus (L.) reuteri* is therefore very interesting owing to the fact that certain species could under determined conditions produce "Reuterin", very powerful antimicrobial material having very large spectre of influence, particulary against disease agents. Reuterin is the first indentified ed chemical strong antimicrobial substance produced by a *Lactobacillus*, but it does differentiate from classic products originating from supstance breakdown. The influence of such health improving kinds with probiotic microorganisms enriched food is represented in many scientific publications (1-4), but not discussed (5-6). Sanders (7) is describing advantages of probiotics (especially *Lactobacilli* and *Bifidobacteria*) for health as regards, digestion help for lactose, protection from diarhoea, stimulation of immunosystems, regulation in case of constipation, decline of cholesterin and distrubance when cancer and tumors are concerned.

The inserted piece of probiotic efficacious lactic acid bacteria in dairy products are getting more significance.

A newcomer in dairy technology *L. reuteri* commercially appeared first in Sweden 1991 to enrich one dairy drink and to insert acid milk. In 1995 made appearance on Swiss food market an unusual acid milk product and from its microflora *L. reuteri* was prominent as a more important representative.

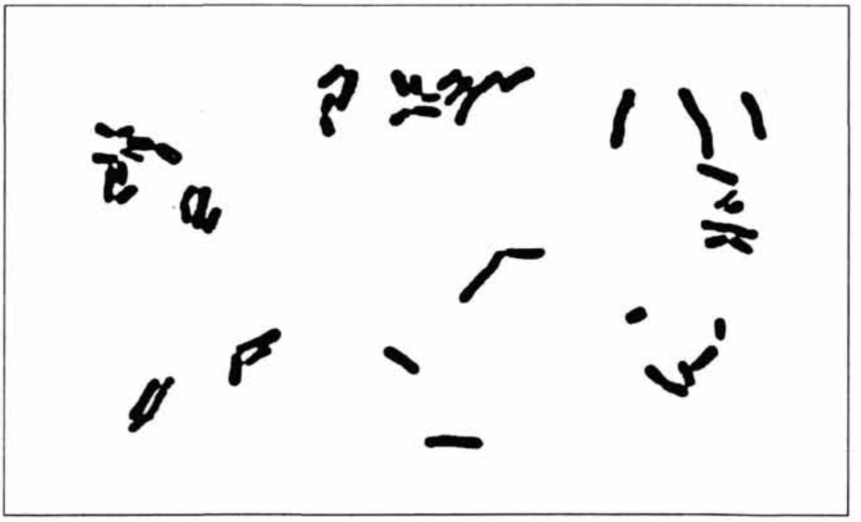
As inhabitant of intestine and mucous membrane *L. reuteri* was isolated from intestine of humans and many animals (porc, hen, cattle, mouse, rat, hamster), from human milk and food as salami, milk, cheese (8), sour dough (9), rice dumplings and fermented molases (10). Laut Mitsouka (11) estimated *L. reuteri* as the most important representative of *Lactobacillus* microflora in humans and numerous animals. Sarra et al. (12) find out *L. reuteri* even as dominant heterofermentative species of *Lactobacilli* in calves' intestine.

Characterization and specific traits in substance breakdown

Lerche and Reuter (13) were the first to isolate "*L. reuteri*" but they classified the species as *Lactobacillus fermentum*, biotype II. Kandler and Stetter (14) first suggested this biotype, owing to its characteristics, as finally new species of obligatory heterofermentative Lactobacilli Vescovo et al. (15) assigned using a correct homology investigation of DNA in 98 heterofermentable *Lactobacillus*-kinds and 20 reference-kinds, first elements for exact insertion of *L. reuteri,* the differentiation between *L. reuteri* and *L. fermentum* succeeded trough verification of biochemical, genetic and molecularbiological characteristics. Essential distinction between two kinds lies in GC-content (% GC in DNA basis), in primary structure of a cell and in diversity of electrophoretic mobility of D-lactatedehydrogenase (D-LDH). It is impossible to differentiate *L. reuteri* and *L. fermentum* using only biochemico-physiologic tests (16).

As shown in Fig. 1, *L. reuteri* is microbiologically a little curved stick with round formed ends, from 0.7 to 1.0 x 2.05μm appearing alone or in sets of two or in small groups. *L. reuteri* has no whips and is not mobile. Colonies usually smooth and flat, whitish and without characteristic pigments. *L. reuteri* could be multiplied in aerobic conditions. Decreasing partial oxygen pressure or trough an incubation in strictly anaerobic conditions would accelerate *L. reuteri* multiplicazion. Temperature optimum is at 15°C. Acidity of supstrate relative to multipliction rate is between pH 4.0 and 7.5, optimum being between pH 6.0 and 6.8 (14). Ragout et al. (17) found in their study on the influence of pH-value on *L. reuteri* balance of fermentation in anaerobic conditions at pH 5.0 maximal biomass formation and the highest growth rate.

Fig. 1: L. reuteri DSM 20016, contrast phase photograph taken in Log-phase; MRS-medium; enlargement: 1800x (Kandler et al./14/)
Sl. 1. L. reuteri DSM 20016, slika kontrastne faze, MRS- supstrat, povećanje: 1800x (Kandler et al. /14/)



L. reuteri as *Lactobacillus* belongs to obligatory heterofermentative "low G+C grampositive Bacteria" (mol% G+C in DNA<55%) /18/. *L. reuteri* is obligatory saccharolytic and builds heterofermentative hexoses and owing to medium breaks down to DL-lactate, CO_2, acetate and/or ethanol-glucose, fructose, arabinose, ribose, lactose, melibiose, raffinose and gluconate will be regulary boiled thoroughly, and xylose only seldom. *L. reuteri* would not coagulate milk, what announce, that *L. reuteri* is not multiplicating cheerfuly. *L. reuteri* could supplement arginine to ammonia but during that time indole, H_2S, lipase, lecitinase or urease would not be formed. There would not be reduction of nitrate to nitrite. Gelation of milk would not be hydrolysed, what announce that *L. reuteri* exibitis only very poor or none proteolytic activity. In "Mureintype" of *L. reuteri* the point is in lysine-D-iso-asparagine, and there is none teichonacid in cell wall. Content of GC in DNA lies between 40.0 - 42.3 mol per cent (14).

Formation of antibiotic substance "Reuterin"

Axelsson et al. (19) observed as first antimicrobic activity of *L. reuteri* compared to *Escherchia coli* when succeded cultivation of a mixed culture in presence of glycerin. *E. coli* when prevention is based on a substance eliminated by *L. reuteri* which was isolated using HPLC and could not be identified as main product of fermentation (lactate, acetate, ethanol) or hydrogen superoxide. The explanation on Reuterin synthessis gave experiment with radioactive marked glycerin (14C) in supstance (20). Glycerin transformation in 1,3-propandiol (trimethylglycerin) and hydroxipropionic acid (ß-HPS) ends in two steps: first step will catalyse glycerinhydratase depending upon coenzyme-B_{12}, going round glycerin in ß-hidroxypropion-aldehyde. Second step consists of an ß-hydroxpropionaldehyde reduction using an 1,3-propandiol NAD-oxidoreductase (Fif. 2). Talarico et al. (21) showed also that reuterin appiered in three different forms, namely in one menomer, one hydrated monomer and in one dimer form of ß-hidroxypropionaldehyde, mutually in balance (Fig 3.). *L. reuteri* could not be raised new with glycerin as C- and energy source. In this place glycerin could only serve as alternative hydrogen acceptor in usage of carbohydrates. Besides reuterin formation the addition of glycerin in culture medium for *L. reuteri* enlarged multiplications rate and biomass formation (22). Talarico et al. (23) succeded to clear off and characterized glycerin-hydratase key enizme. How did reuterin synthesis suceded and which one of three forms of ß-hidroxypropionaldehyde was formed as biologically active, is untill today not entirely clear (21). Talarico et al. (21) analysed pure reuterin, using transformed infraredspectroscopy, coreresonance and mass-spectroscopy. Afterwards, reuterin is neutral, in water soluble and not containing inter-mediary product of glycerin metabolisme its molecular weight being <200.

Fig. 2: Proposed scheme of glycerine transformation way in L. reuteri (Talarico et al. /20/)

Sl. 2. Predložena shema transformiranja glicerina u L. reuteri (Talarico et al. /20/)

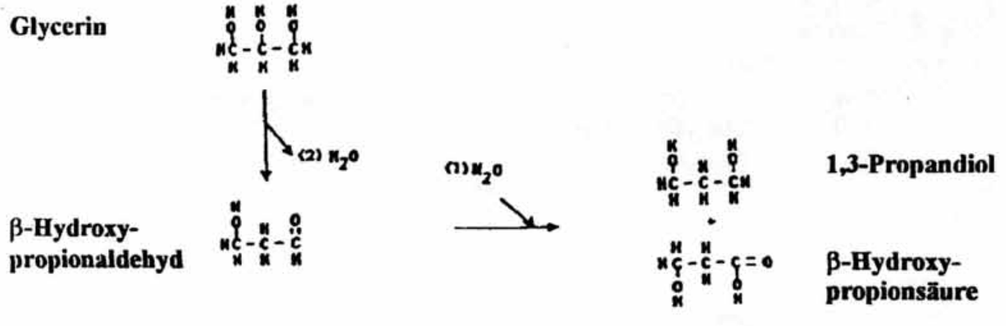
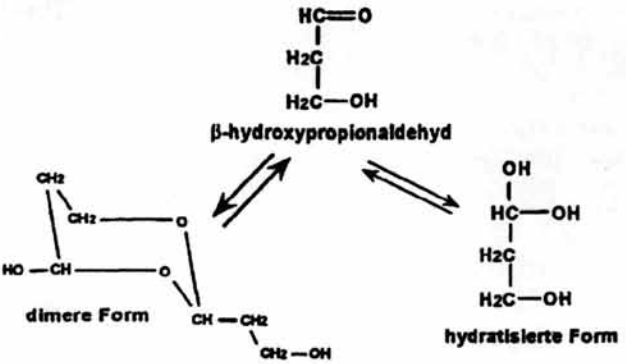


Fig. 3: Three reuterine-forms in water solution (Talarico et al. /21/)

Sl. 3. Tri oblika reuterina u vodenoj otopini (Talarico et al. /21/)



Chung et al. (24) established beyond dispute that *L. reuteri* in an anaerobic mixed culture with *E. coli* forms reuterin at pH 5 to 9 and temperature from 4 to 45°C, optimum being 37°C. According to it, the conditions in alimentary canal could be considered as optimal for reuterin production. Dobrogozs et al. (25) suggested that reuterin in vivo hinders the activity, what would make clear reuterin's large antibiotic activity against bacteria, yeasts, moulds and protozoa. So far discovered reuterin-sensitive microorganisms (gram-positive and gram-negative bacteria, yeasts, moulds and protozoa) are in Table 1. Minimum concentration was determined in "units" reuterin/ml. Generally, 4 to 5 units/ml satisfy to contrain multiplying

(24). So far it is indeed unknown what for lactic bacteria (particularly *Streptococcus lactis, Pediococcus cerevisae, Leuconostoc mesenteroides, L. acidophilus, L. plantarum*) are less sensitive, than the rest of tested bacteria (19). Distinctly reuterin-resistant bacteria until now are not quoted in literature.

Table 1: Reuterin-sensitive microorganisms and obstruction concentrations (units/ml) in paranthesis (Chung et al. /24/)
Tablica 1. Mikroorganizmi osjetljivi na reuterin te koncentracije koje uvjetuju kočenje (jedinice/ml) u zgradama (Chung et al. /24/)

Bacteria Bakterije	Yeasts and molds Kvasci i plijesni	Protozoa Protozoa
Escherichia coli (4)	*Candida albicans (2)*	*Tripanosoma cruzi (5)*
Salmonella typhimurium (4)	*Torulopsis glabrata (4)*	
Pseudomonas fluorescens (5)	*Saccharomyces cerevisae (12))*	
Proteus sp. (4)	*Saccaharmycoides fibuligera (16)*	
Shigella sp. (4)	*Fusarium samfucienum (36)*	
Bacillus megaterium (5)	*Aspergillus flavus (8)*	
Clostridium sporogenes (5)		
Staphylococcus epidermidis (5)		
Lactobacillus bulgaricus (9)		
Lactobacillus plantarum (12)		
Lactobacillus lactis (17)		
Lactobacillus acidophilus (12)		
Leuconostoc mesenteroides (16)		
Pediococcus cerevisae (16)		

L. reutei is not the only kind of genus *Lactobacillus* able to produce reuterin. Dobrogosz et al (25) could not prove reuterin's production in kinds as *L. acidophilus, L. bulgaricus, L. helveticus, L. cellobiosus, L. fermentum* and *L. plantarum*. According to Schütz et al. (26) hetero-fermentative *Lactobacilli L. brevis* and *L. buchneri* have also the coenzyme-B_{12}-dependent glycerindehydrase as well as NAD^+-1,3-propandiol-dehidrogenase that makes possible to use glycerine as hydrogen acceptor in anaerobic glucose fermentation. For all that however, ß-hydroxy-propionaldehyde will be directly and completely turned into 1,3-propandiole. *L. reuteri* seems to be the only *Lactobacillus* not reducing completely ß-hydroxypropionaldehyde to 1,3 propandiole, but as antimicrobic efficacious substance delivers reuterin in environs. Eliminations of ß-hydroxypropion-aldehyde by *L. reuteri* would cause selfhindering. It is not clear (22) how *L. reuteri's* cells are produced, eliminated and reduced. One has to remaind that not all but only specific *Lactobacillus* genera of *L. reuteri* form reuterin under mentioned conditions. Literature (4) gives no instructions on posible reuterin's toxycity for suckling animals. It is important in case of insertion of genera producing reuterin in medicine and food- and fodder- industry. Reuterin producing genera were used experimentally in preservation of food and feed. Lindgren et al. (27) soaked herrings' filets in a suspension containing glycerin and 10^9/ml of a reuterinproducing genus of *L. reuteri* enabling the improving of storing capacity. These procedures influenced in such a manner that gramnegative fish microflora staying 6 days at 5°C and 100 per cent N-atmosphere multiplied only about 10 par cent whereas nontreated gramnegative fish microflora respectively, treated with a genus of reuterin-nonproducing *L. reuteri* in a control experiment incraesed about 3 par cent.

Lactobacillus reuteri as intestinal germ of suckling animals

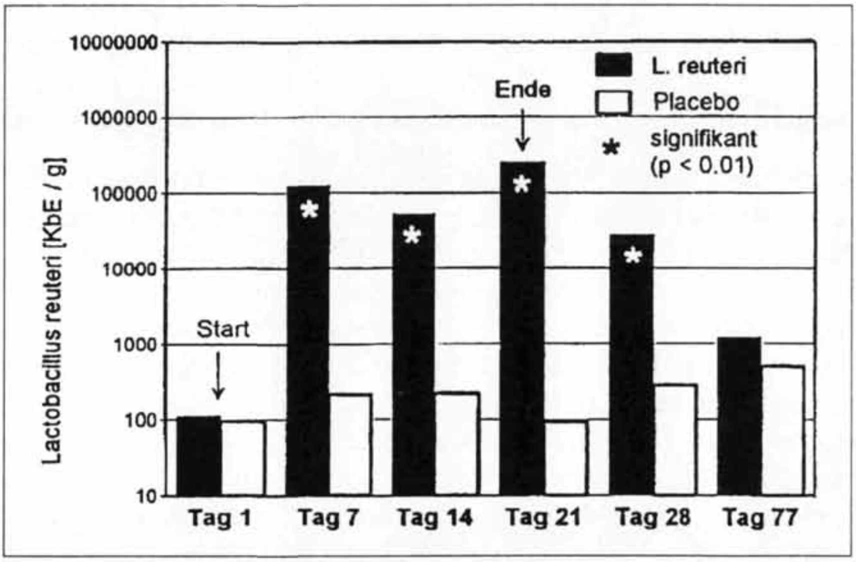
Bifidobacteria will dominate in the intestinal microflora, particularly that of large intestine of healthy sucklings, in adults would predominate kinds of *Bacteroides* and *Bifidobacteria* (about 10^{11} KBE/g). *Lactobacilli* are not represented in great numbers. In this ecosystem they are important owing to their activity in substance exchange balancing between useful and conditionally harmful, as in case of *Enterobacteria* and *Clostridia* species and in such a manner stabilize that the last could not overcome (28). In this context can reuteri-positive *L. reuteri* species contribute an important chare.

Mentioned balance of mixed flora could be influenced and dependent on microflora brought in food and nutritive substances. Aggravating disturbance could stipulate further antibiotics and chemotherapeutics. Oral supply desired intestinal *Bacteria*, especially in view of enriched milk products, could after cancelling medicaments again operate corecting (4).

Implantation and probiotic activity

Wolf et al. (4) gave daily 10^{11} *L. reuteri* (human isolate) during 21 one after the other day and already after seven days attained significantly higher quality of *L reuteri* in faeces of test persons, as at taking one placebo preparation (Fig. 4). It was possible to establish significant difference even after one week following the end of experiment. First eight weeks following the end of experiment were *L. reuteri* contents in faeces, *L. reuteri* and placebo-receiver again comparable. Except some detached light swellings set in despite of very high daily doses of *L. reuteri* reaching 10^{11} microorganismes there were no health's difficulties. These experiments suggest that taken of very big daily doses of *L. reuteri* (at least short-term) and undoubtedly sanitary and that orally taken germs passing through alimentary canal could colonize. However, for one long lasting settlement of *L. reuteri* in alimentary canal one needs regular oral supply of that microorganism.

Fig. 4: Number of L. reuteri in faeces of a healthy, grown man after taking L. reuteri during 21 days (W o l f et al. /4/, modified)
Sl. 4. Broj L. reuteri u fecesu zdravog, odraslog muškarca poslije uzimanja L. reuteri tijekom 21 dana (W o l f et al. /4/, modificirano)



M o l i n et al. (3) examined in rat feeding-experiments (nine days) with fermented oats grits freeze dried soup the influence on blood-cholesterin picture and on microbial composition in rat's bowel wall. Daily administering of 23 g lyophilisat per animal contained six different *Lactobacillus* species ($1{,}7 \times 10^6$ KBE/g, between two kinds of *L. reuteri* and indeed one human isolate (H_J108) and one isolate from rat (R2LC). Feeding experiment caused not significant difference between blood's cholesterin picture during the experiment or during the control phase. Biopsy samples on the contrary showed that *L. reuteri* rat-isolate opposite to human isolate could colonize intestines mucous membrane and still yet 24 days after feeding experiment represent about 30% of *Lactobacilli* population. It seems that bowel colonizing could be realized only with specific *L. reuteri* species.

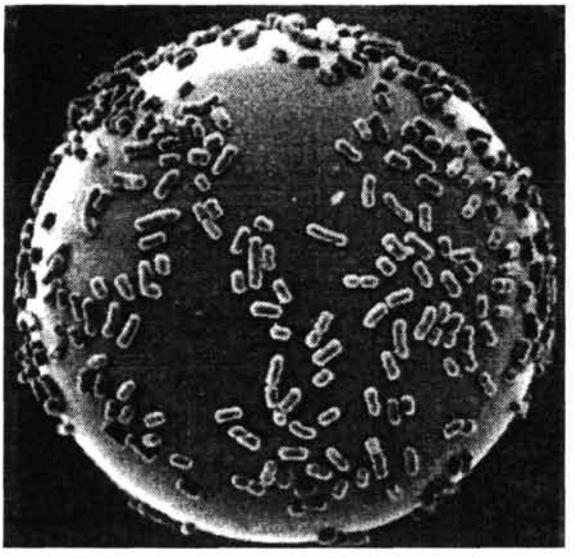
J o h a n s s o n et al. (2) affirmed that hypothesis of oral colonizing of alimentary canal by *L. reuteri* species in a in vivo study of colonizing human mucous membrane. Healthy test persons were supplied with freese dried and agaín reconstituted fermented oats grits soup (100 ml) containing 19 different *Lactobacilli* kinds (per $5x10^6$KBE/ml). Among them were two *L. reuteri* species, i. e. one human-isolate (H_j108) and one rat-isolate (R2LC). From biopsiesamples taken from large animals small intestine, the 11th and 21st day day of experiment isolation was possible of five brought about *Lactobacillus* species and between them human-isolate of *L. reuteri* but not that of rat-isolate. Even 11 days after the end of feeding experiment it was possible to identify five test-species.

There is a specific possibility for colonization of microorganisms to fasten on intestine mucous. Wadström et al. (29) demonstrated that owing to *L. reuteri* 1063 and its hydrophobic surface to cling to cells isolated from pigs intestine epithelium. Lindgren et al. (1) studied this mechanism to cling using immobilized human fibronectine on glass pearls, one glycoprotein that appears in blood plasma and in extracelullar matrix (i. e. intestine mucous). From tested *L. reuteri* species (1063, 1068, DSM, 20016) and *L. acidophilus* VPI 1754 were only to show *L. reuteri species* 1063 (isolate from pig's intestine) at pH values from 3.0 to 9.0 a strong and *L. acidophilus* a weak binding on immobilized fibronectine (Fig. 5). Probably is responsible, for fibronectinbinding, a surface protein, that was not perceptible after the treatement of test with proteolytic enzymes. Such a reduction was done treating Bacteria with urine, NDS (Natrium-dodeccl-sulphate), fibronectine and warmt (80°C). These experiments proved farther that the posibility of clinging to fibronectine is not the characteristic of all *L. reuteri* species, but absolutely necessary for the intestine colonizing. The ability to bind fibronectine by means of hydrophobic proteins from surface is with this important hypothesis for a probiotic efficacious species. In the experiment it was not possible to find out the relation between the possibility to bind reuterin and to form it. However, it has to be recognized that for obseved specific colonization in other experiments besides the ability to bind fibronectin probably also further unknown factors should be competent.

Suitably to mentioned experiments *L. reuteri* species taken orally can survive passing through its intestine and if there is possibility to fasten on intestine mucous and colonize it and by this also the part of *L. reuteri* between bowel *Lactobacilli*. It is yet not clear (2) if *L. reuteri* species are in vitro reuterin positive, and also in vivo produce reuterin and which sources of glycerine are at their disposal.

Fig. 5: Electronmicroscopy of glass pearls (Ø > 35µm) insured by immobilized human-fibronectine and covered with L. reuteri 1063 (L i n d g r e n et al. /1/), with publishers permission

Sl. 5. Elektronska mikroskopija staklenih perli (Ø >35µm) osigurana imobiliziranim humanim - fibronektinom i prekrivena s L. reuteri 1063 (L i n d g r e n et al. /1/) uz dozvolu izdavača



Some research-workers stated in view to reinforce natural repulsive strenght, at least in experiment with animals giving *L. reuteri* orally. E d e n s et al. (30) could in such a way drastically solve the mortality of young hens caused with *Salmonella typhimurinum*. In three days old pigs diarhoea could decrease owing to *Cryptosposporidium parvum*. A study in Sweden indicated that rat colitis could be prevented when their bowel was before housed with *L. reuteri* species rat's specific (31). Young hens reacted increasing proportionally CD4 in CD8 T-cells in own lamina of small intestine (32).

Lactobacillus reuteri as supplement for foodstuff

Not long ago *L. reuteri* was inserted in food industry, particularly in dairy industry, as probiotic in fermented and nonfermented products. W o l f et al. (4) recommend *L. reuteri* as supplemet in acid milk products. The international dairy federation FIL/IDF in the most recent revision-draft of IDF standard 149 (33) mention also *L. reuteri* between thermophilic lactic acid Bacteria as "starter" microorganisms.

In Sweden started in 1991 the production of a special drink - and fermented milk using *L. reuteri* (ATCC SD2112, isolate from human milk) together with *L. acidophilus* and *Bifidobacterium infantis*. These products are commercialized under the name "BRA"-milk. The insertion of this probiotic efficacious *L. reuteri*-species are patented and protected (34). On Swiss market there is since 1995 an acid milk product named "Sym Balance^{R}" having in its permission under application of Swedish protected patent of *L. reuteri* species along with other probiotics as *L. acidophilus*, *L. casei* and two *Bifidobacterium* species. This acid milk will be supplemented at the same time with inuline, an oligosaccharide having characteristics in feeding stages completed as bifidogenous factor. The inuline in stomach is only insignificantly hydrolysed, not being taken to pieces by body enzymes, but nevertheless, the activity in substance exchange and multiplyng of body *Bifidobacteria* are stimulated in large intestine. The consumption of "SymBalance®" should, owing to enrichement with probiotic *Bifidobacteria* species and inuline following effect attain: positive influence of microflora-balance of microflora in large intestine (eubiose), promotion autochthon of *Bifidobacteria* microflora in large intestine, the digestion normalization, strengthening of natural repulsive strenght and checking potential pathogenic microorganisms (35).

As an important hypothesis for these specific microorganisms is attributed to its helthpromoting activity when regularly taken in high doses of about 10^9 KBE/day. It means again a high content of at least 10^7 units of different probiotic species per gram of product. It is very difficult task for dairy tecnologists when producing acid milk products if a four weeks shelf life is wanted. The multiplying of *L. reuteri* in milk particularly when mixed with the other lactic acid Bacteria is rather not researched, as well as microorganisms distribution in product depending upon pH, temperature, redoxpotential, a_w-value, O_2-partial pressure etc. and their possibility to survive. Besides technologically relevant researches some information was given on toxicologic doutlessness of reuterin and the influence of *L. reuteri*-taking on composition of human intestine microflora as well as comprehension of different probiotic effects desirable for men.

LACTOBACILLUS REUTERI: NOVAJLIJA U TEHNOLOGIJI MLIJEKA

Sažetak

Lactobacillus reuteri je stanovnik gastrointestinalnog trakta ljudi i životinja, a izoliran je i iz hrane (kobasice, sir, kiselo tijesto). Navodi se da je L. reuteri dominantna heterofermentativna vrsta Lactobacillus jedinstvenih svojstava. Može povoljno djelovati na stabiliziranje intestinalne mikroflore, prema tome, štiti od patogenih mikroorganizama. L. reuteri je novajlija u tehnologiji mlijeka i proizvodima koji se pojavljuju na tržištu, a taj se mikroorganizam dodaje (slatko mlijeko i fermentirani mliječni proizvodi). Nije posve jasna uloga L. reuteri u intestinalnom ekosustavu i koliko je važan za zdravlje i dobrobit domaćina.

L. reuteri je obligatni heterofermentativni Lactobacillus i proizvodi u određenim uvjetima reuterin (ß-hidroksipropionaldehid), antimikrobnu tvar širokog spektra, koja priječi razvoj niza nepoželjnih bakterija, kvasaca, gljiva i protozoa.

Literature

1. Lindgren, S.E., Swaisgood, H.E., Janolini, V.G., Axelsson L.T., Richter C.S., Mackenzie, J.M., Dobrogosz, W. (1992), Binding of *Lactobacillus reuteri* to fibronectin immobilized on glass beads, Zbl. Bakt. (Naturwissenschaft) 277, 519-528
2. Johansson, M.L., Molin, G., Jeppsson, B., Noback, S., Ahrné, S., Bengmark, S. (1993), Administration of different *Lactobacillus* strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora, Applied and Environmental Microbiology 59 (1), 15-20
3. Molin, G., Andersson, R., Ahrné, S., Lönner, C., Marklinder, I., Johansson, M.L., Jeppsson, B., Bengmark, S. (1992), Effect of fermented oatmeal soup on cholesterol level and the *Lactobacillus* colonization of rat intestinal mucosa, Antonie van Leeuwenhoek 61 (3), 167-173
4. Wolf, B., Garleb, K., Ataya, D., Casas, I. (1995), Safety and tolerance of *Lactobacillus reuteri* in health adult male subjects, Society for microbial ecology in health and disease, Microbial Ecology in Health and Disease 8 (1), 41-50
5. Marteau, P., Rambaud, J.C. (1993), Potential of using lactic acid bacteria for therapy and immunomodulation in man, FEMS Microbiol. Rev. 12, 207-220
6. Teuber, M. (1995), The influence of fermentation on the nutritional quality of dairy products, The World of Ingredients (1), 43-46
7. Sanders, M. (1993), Effect of consumption of lactic cultures on human health, Advaces in Food and Nutrition Research 37 (1), 67-130
8. Dellaglio, F., Severino, A., Ledda, A. (1981), Classification of citrate fermenting Lactobacilli isolated from lamb stomach, sheep milk and Pecorino Romano cheese, Zbl. Bakt. Hyg., I. Abt. Orig. C2, 349-356

9. Okada, S., Ishikawa, M., Yoshida, I. (1992), Identification and characteristics of lactic acid bacteria isolated from sour dough sponges, Bioscience, Biotechnology, Biochemistry 56 (4), 572-575
10. Kaneuchi, C., Seki, M., Komagata, K. (1988), Produktion of succinic acid from citric acid and related acids by Lactobacillus strains, Applied and Environmental Microbiology 54 (12), 3053-3056
11. Mitsouka, T. (1992), The human gastrointestinal tract, in "The lactic acid bacteria in health and disease", Editor Wood, B., Elsevier Applied Science, Vol. 1, 69-144
12. Sarra, P.G., Magri, M., Bottazzi, V., Dellaglio, F., Bosi, E. (1979), Frequenza di bacilli lattici eterofermentanti nelle feci di vitelli lattanti, Arch. Vet. Ital. 30, 16-21
13. Lerche, M., Reuter, G. (1962), Das Vorkommen aerob wachsender grampositiver Stäbchen des Genus *Lactobacillus Beijerinck* im Darminhalt erwachsener Menschen, Zbl. Bakt. Hyg., I. Abt. Orig. 185 (1), 446-481
14. Kandler, O., Stetter, K., Köhl, R. (1980), *Lactobacillus reuteri* sp. nov., a new spezies of heterofermentative Lactobacilli, Zbl. Bakt. Hyg., I. Abt. Orig. C1, 624-269
15. Vescovo, M., Dellaglio, F., Bottazzi, V., Sarra, P.G. (1979), Desoxyribonucleic acid homology among *Lactobacillus* species of the subgenus *Betabacterium Orla-Jensen*, Microbiologica 2, 317-330
16. Kandler, O., Stetter, K. (1973), Der Beitrag neuerer biochemischer Merkmale für die Systematik der Laktobazillen, 3. Symposium Technische Mikrobilogie, Berlin 501-504
17. Ragout, A., Sineriz, F., Diekmann, H., Devaldez, G.F. (1994), Effect of environmental pH on the fermentation balance of *Lactobacillus reuteri*, Journal of Applied Bacteriology 77 (4), 388-391
18. Olsen, G.J., Woese, C.R., Overbeek, R. (1994), The winds of (evolutionary) change: Breathing new life into Microbilogy, Journal of Bacterilogy 176 (1), 1-6
19. Axelsson, L., Chung, T., Dobrogosz, W., Lindgren, S. (1989), Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*, Microbial Ecology in Health and Disease 2 (2), 131-136
20. Talarico, T., Casas, I., Chung, T., Dobrogosz, W. (1988), Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*, Antimicrobial agents and chemotherapy (32) (12), 1854-1858
21. Talarico, T., Dobrogosz, V. (1989), Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*, Antimicrobial agents and chemotherapy 33 (5), 674-679
22. Talarico, T., Dobrogosz, W. (1990b), Purification and characterization of glycerol dehydratase from *Lactobacillus reuteri*, Applied and Environmental Microbiology 56 (4), 1195-1197
23. Talarico, T., Dobrogosz, W., Axelsson, L., Novotny, J., Finzat, M. (1990a), Utilization of glycerol as a hydrogen acceptor by *Lactobacillus reuteri*: Purification of 1,2-Propanediol: NAD^+ Oxidoreductase, Applied and Environmental Microbiology 56 (4), 943-948
24. Chung, T., Axelsson, L., Dobrogosz, W., Lindgren, S. (1989), In vitro studies on reuterin synthesis by *Lactobacillus reuteri*, Microbial Ecology in Health and Disease, 2 (2), 137-144

25. Dobrogosz, W., Casas, I., Pagano, G., Talarico, T., Sjöberg, B., Karlson, M. (1989), *Lactobacillus reuteri* and the Enteric Microbiota, in "Regulatory and protective role of the normal microflora" Editors Grubb, R., Mitved, T., Noren, E., Mc Millan Ltd. London UK, 283-292
26. Schütz, H., Radler, F. (1984), Aerobic reduction of glycerol to propandiol-1-3 by Lactobacillus brevis and Lactobacillus buchneri, Systematic and Applied Microbiology 5, 169-178
27. Lindgren, S., Dobrogosz, W. (1990), Antogonistic activities of lactic acid bacteria in food and feed fermentation, FEMS Microbiol. Rev. 87, 149
28. Klupsch, H.J. (1992), Saure Milcherzeugnisse, Milchmischgetränke und Desserts, Verlag Th. Mann Gelsenkirchen, 2. Auflage, 47
29. Wadström, T., Andersson, K., Sydow, M., Axelsson, L., Lindgren, S.E., Guillmar, B. (1987), Surface properties of lactobacilli isolated from the small intestines of pigs, Journal of Applied Bacteroiology 62, 513-520
30. Edens, W., Parkhurst, C., Pagano, G., Talarico, T., Sjödberg, B., Karlsson, M (1989), *Lactobacillus reuteri* and whey reduce Salmonella colonization in the ceca of turkey poults, Vortrag am Southern Poultry Science Society Annual Meeting, Atlanta GA
31. Fabia, R., Arrajab, A., Johansson, M., Willen, R., Andersson, R., Molin, G., Benmark, S. (1993), The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fiber on acetic acid induced colitis in the rat, Scand. J. Gastroenterol. 28, 156-162
32. Rothschild, P. (1995), Internal defences, Dairy industries international 60 (2), 24-25
33. IDF D Doc 276 (1995), Standard of identity for lactic acid starters, Report of group D38, Vorschlag für die annual sessions in Wien, Sept. 95
34. Dobrogosz, W., Lindgren, S. (1988), Antibiotic reuterin, International patent, Int. application number PCT/US88/01423
35. Henck, M., Schluep, K. (1995), SymBalance: Grundlegen und Produktinformation, ToniLait AG, Bern, Schweiz, 8

Author's addresses:
T. Klantschitsch
H. Spillman
Z. Puhan
Labor für Milchwissenschaft,
Institut für Lebensmittelwissenschaft,
ETH - Zentrum, 8092 Zürich

Received:
15. 10. 1996.