Sugaring fermentation in probiotic bacteria — an in vitro study


Introduction: Food supplemented with probiotic bacteria is a rapidly growing sector of the market. The aim of the present study was to evaluate and compare the acid production of selected probiotic strains available in commercial products.

Methods: Six Lactobacillus strains (Lactobacillus plantarum 299v and 931; Lactobacillus rhamnosus GG and LB21; Lactobacillus paracasei subsp. paracasei F19, and Lactobacillus reuteri PTA 5289) were cultivated at 37°C in an anaerobic atmosphere on Man, Rogosa, Shape (MRS) agar for 48 h or MRS broth for 16 h. After centrifugation, the cells were washed and resuspended in sterile phosphate-buffered saline and immediately subjected to a fermentation assay with 12 different carbohydrates (nine sugars and three sugar alcohols) in microtiter plates with a pH indicator. The plates were examined for color changes after 24, 48, and 72 h of incubation under aerobic and anaerobic conditions. Three scores were used: negative (pH > 6.8); weak (pH 5.2–6.8), and positive (pH < 5.2). The strains were characterized with the API 50 CH system to confirm their identity.

Results: L. plantarum fermented all the sugars except for melibiose, raffinose, and xylitol. Both L. rhamnosus strains were generally less active although L. rhamnosus GG was slightly more active than strain LB21 in the 5% CO₂ setting. The latter strain exhibited negative reactions for sucrose, maltose, arabinose, and sorbitol under anaerobic conditions. The assays with L. paracasei and L. reuteri had negative or weak reactions for all tested sugars under both aerobic and anaerobic conditions.

Conclusion: The metabolic capacity to form acid from dietary sugars differed significantly between the various probiotic strains.

The production of organic acids from dietary sugars is a key factor in the caries process. Low pH generated from acids challenges the homeostasis in the oral microbial community with a selection towards bacteria that induce caries (4, 15). Lactobacilli, however, may play a role in the maintenance of the microecological balance in the oral cavity (13, 22) and the use of probiotic strains has emerged as an alternative way of combating oral bacterial infections in analogy with other parts of the gastrointestinal tract (17). By definition, probiotics are a live microbial feed supplement that when consumed in adequate amounts beneficially affects the host by improving its intestinal microbial balance as documented in clinical trials (20). Recent clinical studies with Lactobacillus rhamnosus and Lactobacillus reuteri strains have demonstrated that a regular intake can result in a reduction of mutans streptococci numbers in saliva and plaque (1, 6, 18, 19). Similarly, the prevalence of oral candida was reduced in elderly persons in connection with a daily intake of cheese containing L. rhamnosus (10).

To be effective against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as a part of the biofilm and compete with the growth of cariogenic bacteria or periodontal pathogens (7, 11). Bacteria differ in their metabolic capabilities and metabolic end products may serve as growth substrates or inhibit the growth of other species. The Lactobacillus group contains homofermentative and heterofermentative species and all are aciduric. Several strains produce low-molecular-weight antimicrobial substances with an inhibitory activity against a wide range of bacterial species, including oral streptococci (2, 12, 13, 16, 21).

The sector of the market involving live lactobacilli in foods and health products is growing rapidly. When ingested orally...
it is feasible that these bacteria may attach to oral surfaces. It is therefore important to know the capacity of these bacteria to produce acid from dietary sugars to rule out deleterious effects on the teeth. The aim of the present study was to assess the acid production from various sugars and sugar alcohols by six probiotic lactobacillus strains that are available to consumers in over-the-counter products.

Material and methods

Bacterial strains

Six different strains of lactobacilli, Lactobacillus plantarum 299v (ProViva, Skåne Dairy, Malmö, Sweden), L. plantarum 931 (Essum, Umeä, Sweden), L. rhamnosus GG ATCC 53103 (Valio Ltd, Helsinki, Finland), L. reuteri LB21 (Essum), Lactobacillus paracasei subsp. paracasei F19 (Arla Ltd, Stockholm, Sweden), and L. reuteri PTA 5289 (BioGaia, Stockholm, Sweden), that are used in commercial products for oral consumption were selected. The strains were characterized by the API 50 CH system (BioMérieux products for oral consumption were Sweden), that are used in commercial biochemical analysis only. None of the L. rhamnosus strains were fully recognized by the API 50 CH.

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Table 1. Fermentation of sugars in 5% CO₂ and in an anaerobic atmosphere by selected probiotic lactobacillus strains

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<th>Strain</th>
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Symbols in the table indicate: --, negative reaction (pH >6.8); ±, weak reaction (pH 5.2 to 6.8); +, positive reaction (pH <5.2) after 24, 48, and 72 h, respectively.

Discussion

Bacterial fermentation can be either health-promoting or harmful and the present study was performed to evaluate whether probiotic lactobacillus strains would exhibit a pH-lowering capacity that could increase the risk of dental caries. The strains were selected to represent a variety of products readily available for consumers. The L. rhamnosus and L. paracasei species belong to a closely related taxonomic group commonly used in dairy products (8). L. plantarum are found in fruit drinks while L. reuteri is the active bacterium in chewing gums, lozenges, and gruel for children. Promising but inconclusive results have so far been reported for these strains in a number of gastrointestinal conditions (23) and for L. rhamnosus and L. reuteri strains an inhibitory effect on oral pathogens has been reported (25). Neither of the two L. rhamnosus strains was fully identified with the API 50 CH system but as they were obtained from the producers we did not proceed with genotypic classification tools. A standard fermentation assay was used and applied under both aerobic and anaerobic conditions to mimic the newly established biofilm as well as the more complex climax community with a higher degree of gran-negative anaerobic bacteria (4).

It is possible that the present assay, however, did not fully disclose the true capacity of the tested strains because the inducible enzyme systems may not have been adequately reflected. Furthermore, an in vitro assay can never reflect the complexity of the live climax community with its mix of different cells and sugars. With these limitations the present findings displayed substantial differences in the metabolic activity between the tested lactobacilli under given conditions. Sucrose, fructose, and glucose are considered the most important carbohydrates involved in the caries process (15) and they were fermented dramatically differently by the various strains. Our results clearly demonstrated that the L. plantarum strains were most active, the L. paracasei and L. reuteri were almost inactive and the L. rhamnosus strains fell between these two groups. One of the tested strains, L. rhamnosus GG, has previously been described as not capable of fermenting either sucrose (16) or lactose (19) but we were able to demonstrate a slow but clear activity for both of these sugars after 48 and 72 h, respectively. Interestingly, the other L. rhamnosus strain showed no metabolic activity with sucrose while both L. rhamnosus strains were active with fructose. The findings that none of the L. rhamnosus strains, nor L. paracasei F19 or L. reuteri PTA 5289, displayed rapid reactions with sucrose were important because these strains are suggested as probiotic candidates for the prevention of oral infections. The negative reactions on xylitol were also encouraging because it seems unlikely that the probiotic lactobacilli would compromise the suggested antibacterial mechanisms of action of the sugar alcohol (24). The 48–72 h acid production of the L. rhamnosus species may be of limited clinical importance.

Although data obtained in vitro show that the bacteria can survive in saliva and adhere to saliva-coated surfaces (11), there are still very limited clinical data for or against a possible oral colonization with probiotic lactobacilli (5, 26). Furthermore, one should be cautious of drawing any clinical conclusions from an in vitro assay with pure monocultures, which may not be representative for the corresponding events in the oral biofilm. The next natural step would be to investigate the pH-lowering capacity of various probiotic bacteria in the dental plaque with the aid of an in situ micro-touch electrode.

One obstacle for the introduction of probiotics to the oral cavity could be that lactobacilli by tradition are associated with the development of dental caries. Lactobacilli constitute, however, only a small part of the oral microflora and no significant increase of the salivary counts has yet been demonstrated following daily administration of lactobacillus-derived probiotics (25). Secondly, modern molecular analyses have linked lactobacilli to various lesions in the oral cavity and the advancing front of caries lesions rather than to the early enamel demineralization (3, 9). Therefore, as young children with newly erupted teeth constitute the primary target groups for oral probiotic intervention, the present results with generally weak metabolic activity from dietary sugars, except for the two L. plantarum strains, would not present a safety problem.

In conclusion, the present findings clearly suggest that the metabolic activity differs between various probiotic strains.

Acknowledgment

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References