



Abstracts

Fingerprints of lactic acid bacteria derived by box-rep-apd

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The identification of lactic acid bacteria (LAB) is very important to verify the presence of added probiotic strains in novel health foods. To this end, traditional differentiation methods relying on morphologic and simple physiologic traits of an organism are not sufficient but must be supplemented by more informative methods like protein or DNA fingerprinting. BOX-rep-APD is a rapid and simple PCR-based fingerprinting method. It is technically easier, faster and cheaper than PFGE and other fingerprinting methods. A single primer derived from the A-subunit of the repetitive BOX element of *Streptococcus pneumoniae*¹ is used to produce species specific amplicon polymorphisms. Separation of these amplicons by agarose gel electrophoresis and successive staining yields typical patterns of DNA fragments which may differ in number, size, and intensity. The method is sensitive enough to allow even for differentiation within one species^{1,2}. PCR amplification of genomic DNA of various strains of *Lactobacillus sakei* and *Lactobacillus curvatus* by BOX-rep-APD resulted in meaningful genomic fingerprints³. Not only could the 2 species be separated clearly from each other, but also within the species polymorphisms could be reproducibly obtained. Fingerprints were also successfully obtained from a variety of other strains belonging to the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Lactococcus*, and *Bifidobacterium*⁴. Each of the LAB species produced its own typical fingerprint. Bacterial strains which are used as probiotics or are related to this field of application, and which served as sources of template DNA in this study included *Bifidobacterium bifidum*, *Lb. acidophilus*, *Lb. casei* subsp. *casei*, *Lb. casei* "Shirota," and *Lb. rhamnosus*. In conclusion, BOX-rep-APD appears to be generally useful to characterize a wide variety of LAB from different origins and because of its simplicity it could find a broad application in the verification of probiotic LAB from "health" foods.

¹Martin et al. *Nucleic Acids Research* 1992; 20:3479; ²Selenska-Pobell et al. *System Appl Microbiol* 1995;18: 425; ³Kröckel. *Mittbl. BAFF Kulmbach* 1997;36(137):286; ⁴Kröckel. *Mittbl. BAFF Kulmbach* 1998;37(139):5.

Shelf life stability data on encapsulated probiotic cultures contained within a prebiotic carrier

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In South Africa the Medicines Control Board classifies probiotics intended for human use as medicines. Applications for registration of medicines must be accompanied by shelf life stability data at three temperatures of which one must be over a 2 y

period. Three products were evaluated for shelf life stability at 4, 25, and 37°C for respective periods of 24, 6, and 3 mo. These products contained *Lactobacillus acidophilus* (Acidoforte); a mixture of *Bifidobacterium bifidum* and *B. longum* (Bifidoforte); and finally a combination of *L. acidophilus*, *B. longum* and *B. bifidum* named Combiforte. All 3 these products contained as carrier lactitol monohydrate. The moisture content of the capsules was between 4% and 5% m/m. With Bifidoforte a very rapid decrease in viability at 37°C of *Bifidobacterium* was observed namely a 6 log decrease within a 3 mo period. At 25°C the decrease was less severe, but even at 4°C a 2 log decrease was observed over the 2 y period. The pure strain of *L. acidophilus* (Acidoforte) was acceptably stable at 25°C over the 6 mo period as well as at 4°C for the 2-y period, since during both instances the decrease in viability was about 0.5 of a log unit. Surprisingly the combination of the *L. acidophilus* and *Bifidobacterium* proved to be the most stable product at 4°C, having a viability count of 1.37×10^9 CFU/capsule after starting at 2.54×10^9 CFU/capsule at the end of the 2 y period. However, at the higher temperatures (25 and 37°C) the decrease in viability was more in line with the Bifidoforte product, showing the instability traits of *Bifidobacterium* at the higher temperatures. It still remains a problem to have probiotic cultures that remain viable to a large degree at room temperature, especially as experienced in countries with high summer temperatures.

Fermented milk products

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Probiotic bacteria are used for production of fermented dairy products. For better taste and consistency of final products also other thermophilic and mesophilic starters are added together with probiotic strains. In this study cultured dairy products were made in pilot scale using probiotic strains of *Lactobacillus acidophilus* and *Bifidobacterium* (Chr. Hansen AS, Denmark). *Streptococcus thermophilus* (ABT-4) and mesophilic culture (ABT-5 + LD-CHN 11) were incorporated to get milder taste and higher viscosity. Pasteurized 2.5% milk was used either with or without addition of skim milk powder. Bacterial growth was followed during fermentation and storage by plating on selective agar media. Consumption of sugars and formation of metabolites was followed by HPLC.

Fermentation time, pH change and bacterial numbers were similar to suggested technology. In ABT-4, sugar consumption and metabolite formation was characteristic to that type of product. Bacterial numbers in 1 d old ABT-4 were: 10^8 – 10^9 for *S. thermophilus*, 10^6 – 10^7 for *Lb. acidophilus* and 10^6 – 10^7 for *Bifidobacterium* depending on the incubation temperature. Remarkable slime production was observed in the product made with ABT + LD cultures. It started already at the second hour of incubation and part

of the polysaccharide was consumed later during fermentation. In parallel, accumulation of unidentified sugar was observed indicating that in complex ecosystems some of the bacteria (obviously *S. thermophilus*) may change its sugar metabolism. The carbohydrate and the mechanism of its formation are currently under investigation. Inhibitory activity against *M. luteus* (probably due to *Lb. acidophilus* bacteriocin) was observed in both products and it was decreasing slowly during the storage. The products had fresh and characteristic taste and flavor and good consistency during 3 wk storage at 4°C. No syneresis was observed. Product made without skim milk addition (ABT-4) had a very thick consistency, similar to those with addition of 1% skim milk powder while texture of ABT-LD product differed significantly. Our study shows that probiotic products with acceptable organoleptic properties and shelf-life can be made from pasteurized milk. Addition of gelling agents is not necessary.

Growth characteristics of probiotic bacteria in milk

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There is an increasing interest in incorporation of selected probiotic bacteria into milk and fermented milk products. However, little is known about their metabolism in milk. The ability to digest milk proteins and lactose varies among the probiotic bacteria. The milk was therefore supplemented with different amounts of tryptone and fructose to improve the growth. Growth of 6 selected probiotic strains with well documented health effects were studied in UHT semi-skimmed milk supplemented with tryptone (0–1%) and fructose (1%). The optimal concentration of additives for growth and metabolism were decided. The growth and metabolism were followed by the following analyses: Organic acids (HPLC); Volatile compounds (headspace GC); Carbon dioxide (infrared CO₂ analyzer); Viable counts (MRS, 37°C, anaerobic); pH. Samples were taken after 0, 20, and 24 h of incubation at 37°C. The amount of tryptone to be added to obtain good growth varied from 0 to 1%. One of the strains needed the addition of fructose to obtain good growth. The final pH varied between 4.2 and 4.6. Maximum CFU/mL was reached after 20 h of incubation and the cell number varied between 8.1 and 9.2 log CFU/mL. The amount of lactic acid varied between 5200 and 9000 ppm and acetic acid varied between 2000 and 7000 ppm. Four of the strains had the ability to metabolize citrate. Significant amounts of differences of volatile compounds were found among the probiotic strains. The amount of carbon dioxide produced varied considerably among the strains, with a *Lactobacillus reuteri* strain producing the highest amount. The probiotic strains tested had different ability to grow in milk. Good growth of the strains was achieved by the addition of tryptone and fructose to the milk. Variations in the amount and type of metabolic products were observed among the tested strains.

Mature cheddar cheese is as effective as fresh yogurt for delivery of viable probiotics to the gastrointestinal tract (GIT)

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Cheddar cheese offers a number of advantages as a food carrier for probiotic strains over more traditional fresh products such as yogurts and fermented milks. These include an increased buffering capacity, a more dense matrix and high fat content, which taken together may offer increased protection to the probiotics during passage through the GIT. This study investigates

the ability of cheddar cheese compared with yogurt to deliver viable microorganisms of the probiotic *Enterococcus faecium* strain Fargo 688 (Quest International) to the porcine gut. This strain was found to survive at high levels in both 12-mo old cheddar cheese (4×10^8 CFU/g) and 21-d old yogurt (4×10^7 CFU/g). Initially, survival of Fargo 688 (from cheddar cheese and yogurt) in gastric juice was compared at low pH values for varying times. Subsequently, these 2 probiotic delivery systems were evaluated in a feeding trial, where 8 pigs/group were fed a rifampicin resistant variant of the probiotic strain at a level of 10^9 – 10^{10} CFU/d for 21 d from either cheddar cheese or yogurt. During the feeding trial the strain was excreted at similar levels (10^6 – 10^8 CFU/g feces) whether ingested from mature cheddar cheese or freshly prepared yogurt. Thus, mature cheddar cheese compares very favorably with fresh yogurt regarding delivery of viable probiotic microorganisms to the GIT, even though the probiotics in cheddar cheese resided there for at least 12 mo.

The survival of bifidobacteria in white brined cheese

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This research was conducted to develop applications for bifidobacteria in dairy products other than yogurt, labneh, or ice cream that have already been tested. White brined cheese was selected as it is extensively manufactured in Balkan and Middle eastern countries where both climatic and economic conditions are well suited to this type of product. Batches of cheese were made using full cream pasteurized milk and inoculated with (a) 50/50 mixture of *Streptococcus thermophilus* and *Lactobacillus delbruekii* ssp *bulgaricus* (1%) and *Bifidobacterium bifidum* or *Bifidobacterium adolescentis* (1%); (b) a 50/50 mixture of *Lactococcus lactis* ssp *lactis* and *Lactococcus lactis* ssp *cremoris* (1%) and *B. bifidum* or *B. adolescentis* (1%) to give 4 combinations of organisms. Given that the “therapeutic minimum” for bifidobacteria is $\approx 1.0 \times 10^6$, this strain of *B. adolescentis* showed poor survival in the presence of both yogurt and cheese cultures and did not appear to be suited for incorporation into this product, even though the counts remained stable after the initial decline to 1.0×10^4 and 1.0×10^3 in the presence of cheese and yogurt culture, respectively. However, the cheese made with yogurt culture and *B. bifidum* showed acceptable survival of bifidobacteria, 60 d of manufacture. This level of survival suggests that the strain is fairly tolerant of both salt and acidity and that white brined cheese made with yogurt culture could provide a vehicle for introducing this strain of probiotic culture into diet.

Methods for verification of *Lactobacillus rhamnosus* GG cultivated from human colonic samples

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Lactobacillus rhamnosus GG (L.GG) is one of the most studied probiotic strains. The ability of L.GG to survive and colonize the gastrointestinal tract has been shown for both adults and children. The aim of the present study was to develop specific methods for verification of L.GG findings from human colonic samples. L.GG is distinguished from most other lactobacilli by its relative inability to ferment lactose. Typical



L.GG-like colonies were tested of their ability to ferment lactose. *L. rhamnosus* specific primers capable of discriminating against other lactic acid bacteria by polymerase chain reaction (PCR) were designed on the 16S ribosomal RNA gene. Ribotyping of *L.GG* was performed using the RiboPrinter Microbial Characterization System (Qualicon, USA).

Volunteer colonoscopy patients consumed a commercial product based on lactose-hydrolyzed whey fermented with *L.GG* (Gefilus, Valio Ltd., Finland) twice a day for 12 d. The volunteers were divided into 3 groups that have different pauses between administration and colonoscopy. Results suggest that *L.GG* is able to adhere in vivo to the colon and stay there a quite considerable time after ending the administration of *L.GG* and that for the verification of *L.GG* findings it is sufficient that typical colonies are subjected to PCR with species specific primers.

Detection of yogurt bacteria in the chyme of Göttingen minipigs

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Survival of the gastrointestinal passage is one of the properties claimed to be specific for probiotic in contrast, eg, to yogurt bacteria. However, data on survival of yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) are still a matter of controversy. Problems arise mainly because classical bacteriological methods are prone to errors when applied to the very complex intestinal microflora consisting of high numbers of different kinds of bacteria. To overcome these problems, we applied a combination of modern molecular biological and classical bacteriological techniques to determine the numbers of yogurt bacteria in the chyme.

Yogurt, containing as a quantitative transition marker spores of *Bacillus stearothermophilus*, was fed to the pigs. Samples were taken postprandially between 3 and 8 h through fistulae in the terminal ileum. The samples were immediately diluted and plated onto agar-plates (tM17-medium for *S. thermophilus* and MRS-medium for *L. delbrueckii* ssp. *bulgaricus*). After incubation at 42°C, single colonies were transferred into the respective liquid growth medium, again incubated at 42°C, and then analyzed by PCR. Two recently developed methods—a species-specific, PCR-based detection method for *S. thermophilus*¹ and a subspecies-specific detection method for *L. delbrueckii* ssp. *bulgaricus*, based on nested PCR—were applied. In addition, DNA was extracted from chyme samples and was tested by PCR for the presence of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* DNA, respectively. The PCR method had been calibrated in vitro using serial dilutions of the two bacterial cultures and comparing the number of target DNA molecules yielding amplification products with the numbers of colony forming units obtained by plating and direct cell numbers as counted with the Thoma chamber. Isolates identified as either *S. thermophilus* or *L. delbrueckii* ssp. *bulgaricus* were further confirmed by classical bacteriological methods (carbohydrate fermentation spectra). *S. thermophilus* was even further characterized to the strain level by phage-typing. By comparing to the numbers of *B. stearothermophilus* colony forming units in the chyme contents we could show that 0.1–1% of the *L. delbrueckii* ssp. *bulgaricus* cells and <0.01% of the *S. thermophilus* cells fed to the pigs survived the stomach and ileal passage and entered the large intestine.

¹Lick et al. System Appl Microbiol 1996;19:74–7.

Adherence of *Bifidobacterium* strains to epithelial-like Caco-2 cell line

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Introduction: Bifidobacteria are one of the first to colonize a sterile digestive tract of a new-born, and they represent a dominating large intestine microflora during the lactation period. Colonization of the gastrointestinal tract by these bacteria depends on their ability to adhere to the epithelial cells. Consequently, this ability is an important feature of the probiotic bacteria and constitutes the major criterion for their selection. The objective of the study was to determine adhesive properties of *Bifidobacterium* strains isolated from different sources.

Materials and methods: *Bifidobacterium* strains were freshly isolated from the digestive tract contents of human new-borns, adults, experimental rats, and from commercially fermented milk products. Adhesive properties of the strains were tested in vitro with the microscopic methods described by Bernet et al (1993)¹, using the cell line Caco-2 isolated from an adenoma of human colon. Adhesion was evaluated on the basis of the number of bacteria cells attached to 100 epithelial cells, using the following scheme:

Number of bifidobacteria cells adhering to 100 Caco-2 cells:
> 300—very good adhesion,
100–300—good adhesion,
< 100—weak adhesion.

Results: From among 70 *Bifidobacterium* strains examined, 7 were characterized by very good adhesion, as the number of bacteria cells adhering to 100 epithelial cells ranged from 700 to 1500. Good adhesive properties (100–300 attached cells) were observed for 13 strains. Majority of the strains, however, either showed weak adhesion or did not adhere to Caco-2 cells. No relation was found between the origin of bacteria and their adhesive properties. Strongly adhering strains originated either from the experimental rats, or from the new-borns or adults, and belonged to the following species: *B. longum*, *B. bifidum*, *B. animalis*. Also nonadhering or weakly adhering strains were isolated from all sources.

Conclusions: The results suggest that *Bifidobacterium* strains originating from different sources are able to adhere to human epithelium, thereby to colonize the colon. Origin of bacteria had no significant effect on adhesive properties of particular strains.

Bernet M-F, Brassart D, Nesser J-R, Servin AL. Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. Appl Environ Microbiol 1993; Dec: 4121–41.

Binding of the S-layer protein Slpa of *Lactobacillus brevis* to human intestinal cells

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The S-layer expressing strain *L. brevis* ATCC 8287 efficiently adhered to the small intestinal cell line Intestine 407. Bacterial adhesiveness was completely abolished by removal of

the S-layer protein SlpA from the bacterial surface by guanidine hydrochloride extraction. To confirm the role of SlpA as an adhesin, we expressed various fragments of *slpA*, ranging in size between 315 and 719 base pairs and covering different regions of the gene, as gene fusions in the variable region of the *fljC* flagellin gene of *Escherichia coli*. The resulting chimeric flagella, which carried up to 270 amino-acid-long inserts from the S-layer protein, were assessed by indirect immunofluorescence for binding to the intestinal cells. Chimeric flagella with inserts from the N-terminal part of SlpA bound to epithelial cells, whereas the C-terminal part did not confer binding on the chimeric flagella. The results demonstrate that the S-layer of *L. brevis* ATCC 8287 is an adhesin with affinity to human epithelial cells and that the receptor-binding region is located within a 100-mer nonimmunogenic fragment in the N-terminal part of the molecule.

Adhesion of probiotic strains to human intestinal mucus

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The ability to adhere to the intestinal mucosa is one of the major selection criteria for probiotic microorganisms. Adhesion to the mucosa may enhance the possibility of colonization and immune modulation. In this study, we have investigated the adhesion of 13 *Lactobacillus* strains, *Saccharomyces boulardii*, one *Lactococcus*, one *Propionibacterium* and one *Enterococcus* strain to human intestinal mucus. Since the adhesion of enteropathogens to the intestinal mucosa has been shown to be influenced by the age of the host organism. Adhesion was studied in an in vitro assay to mucus isolated from feces of new born, 2 and 6-mo old infants and adults. The adhesion to the intestinal mucus varied substantially between the different micro-organisms tested. Of the micro-organisms applied to the intestinal mucus 40–45% of *Lactobacillus* GG (ATCC 53103) and a *L. rhamnosus* strain (human fecal isolate) adhered, while <10% of the applied *Enterococcus* and 9 of the tested lactobacilli adhered. Of the remaining tested strains, 15–30% of the applied microorganisms adhered to the intestinal mucus. No significant difference could be observed in the adhesion of the microorganisms to mucus from different ages. The microorganisms were found to be bound relatively tight to the immobilized intestinal mucus. Only a small, albeit significant ($P < 0.04$), fraction of the adhered microorganisms was released within 1 h on agitation. Longer agitation did not significantly affect the number of adhered microorganisms ($P > 0.05$). Our results confirm previous findings that probiotic strains vary substantially in their ability to adhere to the intestinal mucosa. This may explain observed differences in the ability to, transiently, colonize the intestinal tract and modulate the immune system by different probiotic microorganisms. The observation that the adhesion of the tested probiotic strains is not significantly different to intestinal mucus from subjects with different age, may suggest that the availability of receptors of the tested microorganisms does not vary with age. This is in contrast to findings for enteropathogens where receptors have often been found to increase with the age of the host. The absence of a difference in adhesion to mucus isolated from subjects at different age and the relative tight binding, may help explain the relative stability of the indigenous microflora throughout life.

Are viable microorganisms essential for the enhancement of intestinal hydrolysis of lactose by the β -galactosidase of fermented milk products?

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To investigate whether improvement by fermented milk products of lactose digestion in lactose maldigesters requires viable bacteria, we fed 1L/day of each of the following experimental diets to 14 Göttingen minipigs for 1 week in the following randomized order: 1) a kefir-based product with viable lactobacilli ($\approx 4 \times 10^8$ CFU/L, β -galactosidase ≈ 800 U/L, o-NPG); 2) the same product, but with lactobacilli killed by γ -irradiation (cell-walls intact, β -galactosidase active); 3) the same product, but with lactobacilli killed by shear-force (partly broken cell-walls) and active enzyme; 4) the same product with lactose, but no lactobacilli added (no β -galactosidase activity; control). Lactose digestion was estimated from postprandial plasma galactose concentrations. When lactobacilli in the kefir were viable or killed by irradiation (diet 1 and 2) plasma galactose peak concentrations and area-under-the-curve values were practically identical and significantly higher than control values. But there was almost no improvement of lactose digestion when lactobacilli cell-walls were damaged (diet 3). In a similar experiment 6 gnotobiotic piglets were fed fermented-and-then-pasteurized milk, fermented milk with viable lactobacilli added, or fermented milk with γ -irradiated lactobacilli, the latter two diets containing active β -galactosidase. Only fermented pasteurized milk led to a significant fecal lactose loss and almost no β -galactosidase activity in the feces. When human maldigesters consumed fermented milk containing active \pm microbial β -galactosidase, but killed lactobacilli with cell-walls partly broken, the H_2 exhalation response was intermediate between that towards a pasteurized milk product (no β -galactosidase activity) and towards a native fermented milk product. It is concluded that lactose digestion in lactose maldigesters can efficiently be improved if the milk product contains active microbial β -galactosidase. The bacteria need not to be alive, but (largely) intact cell-walls are needed as a mechanical protection of the enzyme during gastric passage.

TABLE 1

Effect of differently treated fermented milk products on lactose digestion

Fermented milk product	Pigs (exp.1)	Gnotobiotic pigs (exp. 2)	Humans (exp.3)	Max
	Serum galactose	Fecal lactose	β -galactosidase	breath H_2
	$[\mu\text{mol} \times L^{-1}]$	$[g \times d^{-1}]$	$[U \times d^{-1}]$	$\mu\text{g} \times mL^{-1}$
native	161.2 \pm 37.5	2 \pm 1.2	31.7 \pm 15.0	34 \pm 3.0
γ -irradiated	166.7 \pm 40.5	1.7 \pm 1.0	10.9 \pm 4.0	
+ partly destroyed Ib ¹	62.2 \pm 14.1			51 \pm 4.8
pasteurized	54.5 \pm 10.4	7.5 \pm 1.2	0.9 \pm 0.4	60 \pm 6.1

Effects of fiber-rich rye bread and yogurt with *Lactobacillus* GG on bowel function

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The aim of the study was to investigate the effects of fiber-rich rye bread and yoghurt containing *Lactobacillus* GG (LGG) on bowel function and their interaction in cases of self-reported



constipation. The study was conducted as a 2-by-2 factorial design. The subjects consisted of 59 healthy women with self-reported constipation. After a baseline period the subjects were randomized into 4 diet groups: 1) rye bread + LGG yogurt, 2) LGG yogurt, 3) rye bread, 4) control group. The mean intake of rye fiber were 30–35 g and of LGG, 3×10^{10} CFU. A 3-wk intervention was followed by a 3-wk follow-up period. During each period, total intestinal transit time was measured by means of ingestion of a Sitzmarks capsule and a total fecal collection. The subjects also recorded fecal frequency, gastrointestinal symptoms, consistency of feces, and difficulty of defecation. The rye bread intervention significantly shortened total intestinal transit time ($P = 0.003$), increased fecal frequency ($P = 0.003$), softened feces ($P = 0.002$), and made defecation easier ($P < 0.001$) but it also increased gastrointestinal symptoms ($P < 0.001$) at the beginning of the intervention. LGG yogurt tended to improve the effect of dietary fiber and relieve adverse gastrointestinal effects. The results clearly show that rye bread, rich in dietary fiber, improves bowel function in subjects with self-reported constipation. Yogurt containing LGG seemed to support the effect of rye fiber. LGG also seemed to relieve the gastrointestinal side-effects associated with the increased intake of dietary fiber. Fiber-rich rye bread can be recommended in the treatment of constipation, and a simultaneous consumption of LGG yogurt relieves the adverse gastrointestinal effects possibly associated with increased intake of dietary fiber.

Treatment of irritable bowel syndrome (IBS) with the newer probiotic vsl#3: a multicenter trial

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Introduction: Infectious colitis is an important cause of IBS. There is preliminary evidence that the administration of probiotics could improve the symptom score of subjects with IBS, though controlled trials enrolling a large number of patients are still lacking. We report the results of a trial aimed at evaluating the efficacy of a new preparation (VSL#3, Yovis, Eptavis) highly enriched in lactic acid bacteria that contains 10^{11} living cells/g (*Streptococcus thermophilus*, *Bifidobacteria*, *Lactobacillus acidophilus*, *L. plantarum*, *L. casei*, *L. delbrueckii bulgaricus*, *S. faecium*) for the treatment of postcolitis IBS. A standard preparation containing roughly 2.5×10^7 living cells/capsule (*Enterococcus faecium*) (Bioflorin, Bracco) served as the control treatment.

Methods: 251 outpatients with post-colitis IBS, according to Rome criteria, (146 males, 125 females; mean age 53.6 ± 11.6) were treated with either VSL#3 (3 g/d) or Bioflorin (3 capsules/d) for 10 consecutive days. Clinical efficacy was evaluated using a visual analogue symptom score and safety and tolerability assessments were based on the reported adverse events and the results of standard blood screening.

Results: The results of the study are shown in **Table 1**. Patients treated with VSL#3 had significantly greater improvement compared with Bioflorin-treated subjects in abdominal pain score ($P < 0.002$), flatus score ($P < 0.04$), aerophagia score ($P < 0.014$). No significant differences between the 2 groups were observed with respect to constipation and diarrhoea scores, even though these parameters significantly ($P < 0.001$) improved compared with base line in both groups. VSL#3 and Bioflorin were well tolerated and treatment-related toxicity was not reported by patients in either group and none of them required dose reductions. There

were no significant changes in blood counts and chemistry in either VSL#3 or Bioflorin group at the end of the study period.

Conclusions: Oral bacteriotherapy could be an effective therapeutic strategy for the management of patients with post-colitis IBS. VSL#3 appears to have a better efficacy score compared with standard preparations. We suggest that the presence of high bacterial concentrations ($>10^{11}$ cells/g) as well as of several different strains is crucial to the clinical efficacy of oral bacteriotherapy.

TABLE 1

Changes (%) in symptom scores after treatment with VSL#3 (3 g/d) or Bioflorin (3 capsules/d) for 10 consecutive days

	VSL#3 (n = 130)	Bioflorin (n = 121)
Abdominal pain	54–32	$P < 0.002^1$
Flatus score	46.7–36%	$P < 0.04^1$
Aerophagia	42.5–30	$P < 0.014^1$
Constipation	28.4–25	NS ¹
Diarrhea	46–39	NS ¹

¹VSL#3 compared with Bioflorin. The improvement in each parameter score was strongly significant ($P < 0.0001$) compared with base line.

Effect of yogurt in reducing *Clostridium difficile* diarrhea

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Introduction: *Clostridium difficile* (CD) is one of the leading causes of nosocomial intestinal infections among patients receiving antibiotics. The antibiotics disrupt normal intestinal flora and facilitate overgrowth of toxigenic CD to produce the intestinal disease. Use of probiotic bacteria such as *Lactobacillus acidophilus* or *Saccharomyces boulardii* has the potential to replenish the natural intestinal flora of the body. These bacteria competitively inhibit the growth and colonization of harmful pathogenic bacteria such toxigenic CD. We evaluated the effectiveness of yogurt with live cultures of *Lactobacillus acidophilus* in reducing the incidence of antibiotic associated CD diarrheal illness.

Methods: We conducted our study at a long-term facility with a resident geriatric population of 350 persons. Preintervention (Group A): Data collection took place from 7/95 to 6/96 on all patients who received antibiotics and developed diarrhea requiring stool culture and therapy. Intervention (Group B): To examine the effect of yogurt with live cultures of *Lactobacillus acidophilus*, we collected data from 7/96 to 2/97 on all patients to whom yogurt was administered along with the antibiotics. Few patients, who could not take yogurt, received Lactinex granules mixed with milk/ice cream which contain viable *Lactobacillus acidophilus* bacteria.

Results: *Clostridium difficile* toxin positive diarrhea

Group A:	52 cases ¹
Group B:	13 cases ¹

¹P value: < 0.0001

Conclusions: Our initial data analysis indicates that yogurt or lactinex supplements to antibiotic therapy is associated with a statistically significant decrease in the incidence of *Clostridium difficile* diarrhea. We are developing a randomized trial of these low cost probiotics to further substantiate these findings.

Does the feeding of probiotics and specific antibodies to newborn calves have synergistic prophylactic effects on infectious diarrhea?

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Introduction: Infectious diarrhea is still one of the leading causes of losses of newborn calves. Therefore the prophylactic use of probiotics, which are supposed to inhibit the propagation of pathogens through repression from colonization on the gut, seems very promising. So the balance of the intestinal flora is affected in a positive manner. Another way to control infectious diarrhea consists of oral or parenteral application of specific antibodies (origin could be serum, milk, colostrum or chicken eggs) against the most common pathogens.

Methods: In a field trial 198 neonate calves (48.5% female; 51.5% male) of mainly the breed "Deutsches Fleckvieh" have been divided according daily treatment into five groups ($n = 39/40$): I [control]; II [5 g probiotic powder with *Bacillus cereus* var. *toyoi* (ToyoCerin) from LAH, Cuxhaven]; III [10 g egg yolk powder containing 200 mg IgY with specific antibodies against rotavirus, coronavirus and *Escherichia coli* K99 (F5) pilus antigen (modified GLOBIGEN 88) from LAH, Cuxhaven]; IV [10 mL colostrum (SERIMMUN 2000) containing 1 g bovine immunoglobulins with specific antibodies against rotavirus, coronavirus and different *Escherichia coli* antigens from LAH, Cuxhaven]; V (egg yolk and probiotic powder according to groups II and III). The prophylactic feed additives were given together with the milk from day 2 to day 14 post-natum. In the case of diarrhea the procedure was continued in addition to therapeutic treatments. The effectiveness of the preparations was estimated through clinical examinations including measurements of the body weight gain and determination of serum immunoglobulin concentrations by means of ELISA. Furthermore the presence of infectious agents in feces was proved by Lactovac (Hoechst, Unterschleißheim) and BioX (Bvd. Edm. Machtens, Bruxelles) ELISA.

Results: 62% (123 animals) of the calves showed diarrhoea; in 80% of these pathogens could be found in feces. Among all calves rotavirus infections (31%) predominated coronavirus (7%), *Escherichia coli* K99 (1%) and cryptosporidia (23%) infections. Relating to frequency of the pathogens and incidence, intensity, beginning and duration of diarrhoea there was no statistical significant difference between the groups. But the combined application of probiotic and egg powder or of the probiotic alone reduced tendentially the occurrence of rota- and coronavirus infections and, in the combined group intensity of diarrhea during infections with rotavirus and such with cryptosporidia was lower. Also, the calves of group II and of group V became ill with diarrhea on average 1 d later than the others and, if pathogen positive, they had a slightly shorter duration of diarrhea. The greatest body weight gain at day 14 showed group V with 7.7 kg (control: 5.8 kg); this parameter was for the pathogen positive animals of group V with diarrhea (6.0 kg) nearly statistically significant in comparison with the corresponding ones in the control group (3.4 kg). Serum concentrations of IgG differed between diarrhea positive (4.4 mg/mL) and negative (5.6 mg/mL) calves without any influence of the group treatments.

Conclusion: To ensure the positive effects seen in this trial it will be necessary to further investigate the dosage and the time schedule of application of these feed additives.

Probiotic administrations of *Lactobacillus reuteri* protect mice from *Salmonella typhimurium* infection: mode of action studies

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Effective probiotics are seen as natural alternatives to excessive use of antibiotics for the prevention and/or treatment of infectious diseases; their use could contribute significantly to curtailing serious public health problems associated with development of antibiotic resistance. Studies have shown *Lactobacillus reuteri* to be a strong candidate for this purpose. It is a naturally occurring symbiot found universally in the GI tract of all vertebrates examined to date, including humans. It has proven to be a safe and effective probiotic for both humans and animals, with demonstrated broad-spectrum efficacy in humans, rats, mice, chickens, turkeys and pigs. In chickens and turkeys, *L. reuteri* colonization of the gut confers dramatic protection against *Salmonella* infection. The objective of the present study was to determine whether the protective effects observed in poultry could be seen in mice as well. If so, the underlying mode(s) of action could be studied using this classic model system. Gnotobiotic BALB/c mice were administered mouse-specific strains of *L. reuteri* orally or water (control) for 2 wk before challenge with 5 LD(50) (2.6×10^6 CFU) of *Salmonella typhimurium*. Mice from the control group remained *L. reuteri*-free during the experimental period. It was shown that *L. reuteri* conferred significant protection from the *Salmonella* infection. Mortalities commenced 7 d post-challenge in both treatment groups, but no deaths were observed in the *L. reuteri*-treated mice after 10 d post-challenge; whereas in the control group, deaths still occurred 17 d post-challenge. Cumulative mortality was higher in the control group (89% compared with 48%), and it was directly correlated with the extent of *Salmonella* translocation. Translocation of *Salmonella* to the MLN, liver, and spleen was significantly reduced in the *L. reuteri*-treated mice until 10 d post *Salmonella*-challenge. *L. reuteri* translocation to the liver and spleen was observed in some cases when *Salmonella* translocation also took place. The results show that *L. reuteri* probiosis in mice elicits effective protection from *Salmonella* infection.

Antagonistic activity of bifidobacteria isolated from piglet anus against *Enterobacteriaceae* rods

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Probiotic bacteria, including *Bifidobacterium* are used for animal feeding in many countries. Colon-settled bifidobacteria were found to produce acetic and lactic acids which lower the pH of the environment, therefore inhibiting the growth of many undesirable bacteria. Low pH also favors the absorption of fat, fat-soluble vitamins, and minerals as well as inhibits the absorption of ammonia and amines by the intestine wall. Antagonistic activity of bifidobacteria against many microorganisms also results, to some degree, from the synthesis of lysozyme-like inhibitors.

Biological material was anus swabs from 5 piglets, aged 3 d to 2 wk. *Bifidobacterium* rods were isolated on a MRS-Agar medium with the addition of a NNL solution as selective factor. Inoculations were incubated anaerobically at 37°C for 72 h. Bifidobacteria were identified using Rapid ID 32A tests from bioMerieux, Warsaw. Antibacterial activity of identified *Bifidobacterium* rods was evaluated against enterotoxic strain of *Escherichia coli* EC 08, and food-isolated rods of *Klebsiella pneumoniae* 499 and *Enterobacter cloacae* 17. The studies were carried out using well method and agar medium. Sixteen strains were isolated from the material, including *Bifidobacterium breve* (4 strains), *Bifidobacterium pseudocatenulatum* (3 strains), *Bifidobacterium adolescentis* and *Bifidobacterium animalis* (each 1 strain). Studies on antibacterial activity against *Enterobacteriaceae* showed that all strains of the *Bifidobacterium* inhibited the growth of the examined Gram-negative bacteria. The isolated *Bifidobacterium* strains had strong, antibacterial activity against the examined *Enterobacteriaceae* rods. They may be therefore considered for use as probiotics in feeding piglets, among others.

Modulation of the colonic bacterial flora affects differently bacterial translocation and liver injury in an acute liver injury model

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Introduction: In liver diseases and liver surgery, infectious complications with enteric bacteria and sepsis are common. Bacterial translocation from the gut has been proposed as one of the underlying mechanisms. We have shown that modulation of the intestinal microflora by the administration of *Lactobacillus plantarum* 299v, reduces bacterial translocation to extraintestinal sites, and the extent of liver injury. In the present study we evaluated the effects of the administration of different bacterial strains on the extent of liver injury and bacterial translocation in an acute liver injury model. The strains represent different predominant species of the intestinal microflora.

Methods: Sprague-Dawley rats were used. Six different bacterial strains (*Bacteroides fragilis* ATCC 25285^T, *Enterococcus faecium*-1, *Enterococcus faecium*-2, *E. coli* F131, *L. plantarum* DSM 6595, and *Bifidobacterium longum* ATCC 15707^T) were administered rectally daily, for 8 d, and liver injury induced on the 8th day by intraperitoneal injection of D-galactosamine (1.1 g/kg BW). Samples were collected 24 h after the liver injury. Liver enzymes and bilirubin, bacterial translocation, and intestinal microflora were evaluated.

Results: The incidence of bacterial translocation to arterial and portal blood decreased significantly in *L. plantarum* and *Bifidobacterium* groups compared with the liver injury. The number of translocated bacteria to portal blood decreased in *Bifidobacterium*, *L. plantarum*, *Enterococcus faecium*-1, *Enterococcus faecium*-2 groups compared with *E. coli* group. In the arterial blood it decreased in *L. plantarum* and *Bifidobacterium* groups compared with *Bacteroides fragilis* and *E. coli* groups. Bacterial translocation to the liver increased significantly in *E. coli* group compared with liver injury, while it decreased in *L. plantarum* and *Bifidobacterium* groups compared with liver injury, *Bacteroides fragilis* and *E. coli* groups. In mesenteric lymph nodes, bacterial translocation decreased in *L. plantarum* group compared with liver injury, *Bacteroides fragilis* and *E. coli* groups. The release of liver enzymes increased in *Bacteroides fragilis* group compared with all

the other groups. It decreased in *L. plantarum* compared with liver injury, *Bacteroides fragilis* and *E. coli* groups.

Conclusion: Modulation of the intestinal microflora by different bacterial types, have different effects on the extent of liver injury and bacterial translocation. Administration of *Bacteroides fragilis* and *E. coli* increased bacterial translocation and the extent of the liver injury, while administration of *L. plantarum* and *Bifidobacterium* decreased them.

Activation of human PBMC by non-pathogenic bacteria in vitro: evidence of NK cells as primary targets

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Introduction: The commensal intestinal microflora contributes to the modulation of immune response. Interaction of bacteria with immunocompetent cells may occur in definite compartments of the mucosal immune system, as limited translocation through the epithelial barrier cannot be excluded. The mechanisms are not yet understood. In this study the stimulation of human peripheral blood mononuclear cells and purified lymphocyte subsets by Gram positive *lactobacilli* (*Lactobacillus johnsonii*, *Lactobacillus sakei*) and non-pathogenic, Gram negative *Escherichia coli* was investigated. **Materials and methods:** Peripheral blood mononuclear cells (PBMC) or leukocyte subsets (T and B cells, monocytes, NK cells) were stimulated with lactic acid bacteria (LAB) of intestinal or fermented food origin and non-pathogenic *E. coli*. Expression of cytokines (IL-12, IFN-g, IL-10, TNF-a) and markers of activation (CD25, CD69) were determined by RT-PCR, ELISA and FACS analysis.

Results: *L. johnsonii* and *L. sakei* strongly induced IFN-g and IL-12, whereas *E. coli* and LPS preferentially induced IL-10 after stimulation of PBMC bulk cultures (16 h). Expression of activation antigens CD69 and CD25 was observed on (CD3-/CD56+) natural killer (NK) cells after stimulation of bulk PBMC cultures. All bacteria mediated proliferation of PBMC and the strongest proliferative response was observed with *L. johnsonii*. Purified CD4+, CD8+ or CD19+ lymphocyte subsets were not activated on bacterial stimulation, but showed normal response to a mitogenic stimulus. In contrast, purified NK cells upregulated IL-2Ra chain (CD25) and underwent proliferation when stimulated by *L. johnsonii*. *E. coli* and LPS were less effective to induce proliferation. Expression of CD25 on purified CD3-/CD56+ NK cells was significantly increased in the presence of bacteria primed macrophages and resulted in secretion of IFN-g.

Conclusion: Whereas LAB stimulated a Th1 type of response in PBMC, *E. coli* induced the production of IL-10. This could be related to the capacity of different micro-organisms to interact with specific cell types and/or to deliver a differential signal. NK cells constitute a primary target for activation by non-pathogenic bacteria. The activation of NK cells required both bacteria and cell contact based signals derived from accessory cells.

Clinical pilot study of probiotic activities in healthy volunteers

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In previous experimental and clinical studies it was shown that the ingestion of specific lactic acid bacteria (LAB) can

enhance the immune function and thereby afford a better protection of the host. Modulatory effects can be detected at the local mucosal immune system and at the systemic level, eg, by increased phagocytotic activity^{1,2}. In a second, randomized double blind placebo controlled study on healthy volunteers, we confirmed the immunomodulatory activities of specific LAB strains and their transient colonization. Healthy volunteers were treated with fermented milk dairy products containing *L. salivarius* UCC-118, *L. johnsonii* La1, *S. thermophilus* alone or a gelified nonfermented milk during three weeks. Blood and feces were collected throughout the study to assess changes in seric markers of inflammation, such as acute phase proteins, soluble (s)IL-2/sIL-6 receptor and changes in bacterial counts following consumption of the fermented products. The immuno-haematological analysis revealed no changes in acute phase proteins, such as pre-albumin, C-reactive protein (CRP) and a-1 acid glycoprotein. No modifications of sIL-2r and sIL-6r were detected, indicating that no inflammatory response was initiated during the treatment. Like in previous studies transient colonization with LAB (*L. salivarius* UCC-118, *L. johnsonii* La1) could be demonstrated. Both strains did not modify *Bacteroides* or *Enterobacteriaceae* of the indigenous microflora. In contrast, both probiotic strains reduced the number of subjects harboring *Clostridium perfringens*. These results clearly demonstrate a transient beneficial modification of the host microflora after consumption of probiotic bacteria. Moreover, the immunomodulatory capacity of these strains is not linked to an inflammatory type of immune response. Thus, functional foods containing probiotic bacteria can preserve health by means of a better protection at mucosal surfaces. These products are important for the general population and could be of further use in specific age population like the elderly, or as supplementation in clinical nutrition.

Schiffirin, EJ et al. J Dairy Sci 1995;78:491-7.

Link-Amster H et al. FEMS Immunol Med Microbiol 1994; 10:55-64.

Effect of ingestion of fermented milk containing *Lactobacillus casei* (Actimel) on the immune response of healthy adults after oral vaccination against *Salmonella typhimurium*

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Endogenous and pathological micro-organism, among them *Lactobacillus casei*, and their products modify some immune responses. The aim of this study was to investigate the effect of a fermented milk containing a yogurt symbiosis and a *Lactobacillus paracasei* on some parameters of the mucosal immune response after oral vaccination with *Salmonella typhimurium*.

Materials and methods: A single center, randomized, double-blind, controlled study with 4 treatment groups was carried. The subjects (59 men and 61 women, aged between 18 and 50 y) were divided at random into 4 groups. A preliminary study with two of the groups is presented here: one was given a fermented milk product by *L. casei* (Danone strain DN-114 001) and the yogurt symbiosis, Actimel, and the other group (control) received semi-skimmed milk. The effects of the fermented milk products on the

immune response were evaluated by giving the subjects an oral vaccination against *Salmonella typhimurium* (simulating a challenge) to stimulate their immune system. The subjects consumed daily the fermented milk products for one week before vaccination (start D-7). They were then vaccinated against *S. typhimurium* (D0) and continued to consume the products for a further 2 wk. Samples of saliva were taken weekly, before and after (on D-7, D0, D + 7, D + 14 and D + 21), total immunoglobulin A concentration and specific anti-*Salmonella* IgA were determined by ELISA. Pair-wise comparison were made between the weekly immune marker concentrations, using normalization (log transformation) of the data and ANOVA (SAS and JUMP programs).

Results: We determined the ratio of specific salivary anti-*Salmonella* IgA (arbitrary units, OD)/total salivary IgA (mg/mL) and tested the significant difference in response kinetics over the study course (5 dates from D-7 to D + 21).

TABLE 1

Effects of products on the production of specific salivary IgA against *Salmonella typhimurium*

Milk	Actimel	P value
Saliva anti- <i>Salmonella</i> IgA/Saliva whole IgA	0.14	0.02

Discussion: Within the study course no statistical significance difference has been observed on salivary whole IgA concentration (Milk $P = 0,71$ and Actimel $P = 0,99$ respectively) which is suitable considering Actimel and *Lactobacillus casei* are not immunostimulators. Salivary IgA immune response against *Salmonella typhimurium* is usually weak: no statistical significant difference during the course of the study has been observed with milk. Conversely a statistical significant difference during the course of the study has been observed with Actimel. Although the P values are closed, these results indicated that the 2 products have slightly different effect on the specific salivary IgA immune response: specific IgA against *Salmonella typhimurium* are increased. This single center, randomized, double-bind study in human ($n = 60$) with 'mass market products' agree with the probiotic and immune adjuvant effects yet observed in animals.

Yogurt as an immunomodulator for adolescents and anorexia nervosa patients

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Introduction: Anorexia nervosa patients (ANP) show a depleted immune function. Besides, yogurt has been shown to act as a probiotic, in addition to its accepted high nutritional value. Both features (immunomodulatory effect and high nutrient density) could be of great relevance in the nutritional therapy of ANP, who are concerned about dietary intake.

Objective: to find out possible benefits of yogurt (*L. bulgaricus*, *S. thermophilus*) intake on immunocompetence in healthy adolescents and in ANP.

Subjects and Methods: 35 healthy female controls (C) and 27 ANP, aged between 14-19, were evaluated. Both groups were divided into two subgroups to carry out a 20 wk cross-over study (Yogurt/Milk or Milk/Yogurt intakes): C1 ($n = 16$) and AN1 ($n = 16$) consumed yogurt (375g/d) up to the 10th wk and milk (475 ml/d) up to the 20th wk; C2 ($n = 19$) and AN2 ($n = 11$)

consumed milk (475 ml/d) up to the 10th wk and yogurt (375g/d) up to the 20th wk. Immunocompetence was assessed in five different periods in each subgroup: P1) initial values, P2) 6th wk; P3) 10th wk; P4) 16th wk; P5) 20th wk. The effects of dairy product intake were studied in each period by measuring the CD4 lymphocyte subset (by flow cytometry, interleukin 2 (IL-2) and tumor necrosis factor (TNF- α) secretion (ELISA) after in vitro lymphocyte stimulation, in vitro interferon- γ (IFN- γ) secretion (by ELISA) and the response to a delayed hypersensitivity skin test (DHT) (Multitest IMC).

Results: C1 showed a decrease in CD4 lymphocyte subset and IFN- γ secretion after milk consumption (P5), with no changes in DHT. Yogurt did not produce any alteration in C1. However, an increase in CD4 cells, IFN- γ secretion and DHT response was found in AN1 after yogurt consumption (P3), which remained unmodified during milk intake period (P4 and P5), except for DHT, which decreased after milk consumption. Both C2 and AN2 showed similar results regarding IFN- γ secretion, showing a decrease after milk consumption (P3) and an increase after yogurt intake (P5). No relevant modifications were found in DHT response and CD4 cells in C2 and AN2.

Conclusions: Yogurt may be considered to exert an immunomodulatory effect both in healthy young females and in ANP. Among the parameters tested, IFN- γ could be the most reliable marker of this effect. After the results obtained, ANP should be encouraged to consume yogurt to improve their immunocompetence.

Production of nitric oxide by propionibacteria: a new criterion for their utilization as probiotics?

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Nitric oxide (NO) is produced in humans and animals by constitutive and inducible NO-synthases from arginine. This gas has fundamental roles in many vital functions such as neurotransmission, vasodilatation, or intestinal motility. Constipation could be due to a deficient production of NO by intestinal nerve fibers. Bacterial production of NO by intestinal flora is not known. We have hypothesized that ingestion of dietary bacteria surviving in the colon and producing NO could influence intestinal transit in a beneficial way. For the first time, we demonstrated the production of NO and its origin in propionibacteria using a *P. acidipropionici* strain (*Pa1*), known for nitrate-reducing activity. *Pa1* was grown in a YEL medium containing $^{14}\text{N-NO}_3$, $^{15}\text{N-NO}_3$ or $^{14}\text{N-NO}_2$. Dissolved and gaseous NO was measured by GC-IRMS in anaerobic conditions. We observed that concentrations of NO regularly increased during the exponential growth phase and maintained a constant concentration at the beginning of the stationary growth phase. Although the NO/NO $_3$ ratio decreased with initial concentrations of nitrate or nitrite (100% with 50 $\mu\text{mol/L}$ NO $_3$ compared with 20% with 1 mmol/L NO $_3$), final concentrations of NO linearly increased. Addition of arginine to the medium did not lead to the production of NO. In the second part of our study, we compared the ability of 13 strains of propionibacteria (*P. acidipropionici* and *P. freudenreichii*) to produce and accumulate NO in YEL medium containing 500 $\mu\text{mol/L}$ NO $_3$. The results showed that the strains could be separated into 3 distinct categories: one group (3 strains) unable to reduce nitrate and produce NO, a second group (6 strains) slowly reducing nitrate and producing low levels of NO and a third group (4 strains including *Pa1*) quickly reducing nitrate and nitrite and producing the highest concentrations of

NO. Recently, we have selected some NO-producing strains (*Propiovitum*) particularly resistant in vitro to digestive stress (acidity and bile). Further studies are currently in progress to verify, in animals and humans, their potential influence on intestinal motility, constipation, composition and activity of intestinal flora.

Antagonistic and antioxidative activity of lactobacilli and survival in oxidative milieu

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Recently it has been shown that some lactobacilli possess remarkable antioxidative capacity (AOC) that may lower the risk of peroxidants damaging the host. However, it is not known whether AOC possessing intestinal lactobacilli are resistant to toxic oxidative compounds and at the same time display antagonistic activity against pathogenic micro-organisms. The aim was to test the antioxidative capacity of intestinal lactobacilli, and survival of antagonistically active lactobacilli in the highly oxidative milieu.

Materials and methods: Three strains of lactobacilli isolated from faeces of healthy 1 y old children and one strain from an adult person were identified by API CHL. The antagonistic activity (score values 0–12) of isolated lactobacilli strains against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Shigella newcastle* was estimated by the streak line procedure. The AOC of lactobacilli was tested by linolenic acid (LA) method based on inhibition (%) of lipid peroxidation of LA standard by lactobacilli cells. The resistance of isolated lactobacilli to 400 $\mu\text{mol/L}$ hydrogen peroxide, to 1 mmol/L paraquat as source of superoxide radical and to hydroxyl radical, generated via Fenton reaction using 10 mmol/L terephthalic acid as a dosimeter, was bacteriologically estimated.

Results: One strain of *Lactobacillus acidophilus* 821–3 and 2 strains of *L. fermentum* E-3 and E-18 obtained from children expressed noticeable AOC values (15, 29, 21%, respectively). The adult's *L. plantarum* 8-RA-3 strain showed the lowest (10%) AOC. None of the strains of lactobacilli was inhibited by paraquat in a disk diffusion assay, confirming superoxide dismutase production. The strain E-3 with highest AOC (29%) survived after 420 min exposure to H $_2$ O $_2$, after a 60 min exposure to hydroxyl radical and expressed also very high antagonistic activity (score 12). The survival time of strain 8-RA-3 with the lowest AOC value was also the shortest by H $_2$ O $_2$ (300 min) and hydroxyl radical (45 min) yet its antagonistic activity was high (score 12).

Conclusions: The bacteriologically revealed resistance to toxic oxidative compounds, the high antagonistic activity against opportunistic pathogens together with biochemically estimated noticeable antioxidative capacity may help lactobacilli to serve as defensive components in intestinal microbial ecosystem.

Cholesterol reduction in culture fluids by lactobacilli

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One of the best known effects of probiotic products is the reduction 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. Cholesterol reduction is not caused by a chemical modification. The results confirm the BSH (bile salt hydrolase) theory, but there does not seem to be responsible only a single process for

the cholesterol reduction. The part of cholesterol, that is bound to the microorganisms at pH<9 may be removed from the gastrointestinal tract together with the microorganisms.

Influence of probiotic yogurt on serum lipids in women

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One of the specific properties of probiotics is the influence on lipid metabolism, although the underlying mechanism is not yet fully understood. Probiotics are able to deconjugate bile acids and to assimilate cholesterol. Therefore a human experiment was carried out to measure the interrelationship between the intake of probiotic yogurt and the concentration of cholesterol fractions. Young healthy women ($n = 29$) daily consumed 300 g probiotic yogurt (*Lactobacillus acidophilus*; *Bifidobacterium longum*) after a period of eating a standard yogurt (*Streptococcus thermophilus*, *Lactobacillus lactis*). The volunteers were divided in a normocholesterolaemic ($n = 15$, total cholesterol <250 mg/dL) and a hypercholesterolaemic group ($n = 14$, total cholesterol >250 mg/dL). The experiment consisted of 3 periods. Each period lasted 51 d. During these 3 periods, from day 43 to 51, all food consumed was offered and weighed. From day 45 to 51 the total urine and feces were collected. At the beginning of the experiment and at the end of each defined diet period blood samples were taken. The concentration of total cholesterol, LDL cholesterol and triacylglycerides decreased after consuming the standard and probiotic yogurt (Table 1). This reduction did not depend on the cholesterol status. Inversely, the concentration of the HDL cholesterol increased. Therefore the atherogenic ratio (LDL/HDL cholesterol) improved during these three periods significantly. There was no significant difference in fat excretion between normo- and hypercholesterolaemic women. The fecal fat and cholesterol were not influenced by probiotic bacteria.

TABLE 1

Serum lipids at the beginning and after consuming standard or probiotic yogurt ($n = 29$)

Serum lipids in mg/dL	Normocholesterolaemic			Hypercholesterolaemic		
	At the beginning	Standard yogurt	Probiotic yogurt	At the beginning	Standard yogurt	Probiotic yogurt
Total cholesterol	220 ± 23	187 ± 28	197 ± 23	293 ± 62	245 ± 40	255 ± 41
HDL cholesterol	46 ± 8	45 ± 89	60 ± 8	50 ± 8	54 ± 21	64 ± 14
LDL cholesterol	158 ± 15	129 ± 26	123 ± 23	220 ± 62	171 ± 431	73 ± 46
Triacylglycerides	79 ± 36	66 ± 28	70 ± 21	114 ± 53	97 ± 37	91 ± 28
LDL/HDL chol.	3.4 ± 0.8	2.9 ± 0.9	2.1 ± 0.5	4.4 ± 1.9	3.2 ± 1.3	2.7 ± 1.8
Fecal output						
Fat (g/d)		4.1 ± 1.1	3.6 ± 1.1		4.0 ± 1.1	3.7 ± 1.1
Cholesterol (g/d)		136 ± 42	116 ± 56		117 ± 57	113 ± 46

Growth promoting effects of galactooligosaccharides on *B. lactis* hn019 (dr10) and *L. rhamnosus* hn001 (dr20)

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Main site of microbial colonisation in human GI tract is colon. It is estimated that the total bacterial count in this region of human gut is between 10^{11} and 10^{12} CFU/g (dry weight). More than 400 bacterial species have been detected in human feces. It is believed that a maintenance of a balance between the putrefac-

tive bacteria and beneficial bacteria plays a crucial role in general health of the host. Members of genera *Bifidobacterium* and *Lactobacillus* are known to produce health promoting effect in this ecosystem and are often the probiotic strains belong to these two genera. The compounds that in turn promote growth of probiotic bacteria in the human colon are known as *prebiotics*. There is considerable evidence in the literature that suggests that galactooligosaccharides selectively stimulate the growth of such beneficial bacteria in the human gastrointestinal tract. However, little is known about the underlying mechanism(s) for such observations. The aim of present study was to investigate whether probiotic bacteria have mechanisms for selective utilization of galactooligosaccharides generated from enzymatic hydrolysis of lactose. Fifty strains of lactic acid bacteria, including members from genera *Bifidobacterium* and *Lactobacillus* were studied for their ability to utilize lactose derived oligosaccharides. A perfect correlation was observed between the ability of a strain to uptake oligosaccharide and presence of enzyme β -galactosidase.

A milk powder containing mixture of galactooligosaccharides was used to identify the preferential use of different classes of carbohydrates by two potentially probiotic strains. *Bifidobacterium lactis* HN019 also known as DR10 and *Lactobacillus rhamnosus* HN001 also referred to as DR20, were selected for their probiotic properties from a large culture collection of dairy lactic acid bacteria held at NZDRI. The growth promoting effects of individual galactooligosaccharides were followed using HPAE chromatography and FACE analysis. The results clearly demonstrated that *B. lactis* HN019 preferentially utilizes tri- and tetrasaccharides whereas *L. rhamnosus* HN001 grows better on disaccharides. This in vitro data strongly suggested that galactooligosaccharides in milk-powders may exert a prebiotic effects.

An in vitro method for investigation of the effects of novel oligosaccharides on the colonic microbiota of humans

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Interest in functional foods that modulate the colonic microflora of humans has increased in recent years, largely due to the health benefits attributed to lactic acid producing bacteria. Much of the research on prebiotics has focused on fructo-oligosaccharides (FOS). However, current technology enables the synthesis of novel or 'designer' oligosaccharides which may be biologically superior to those naturally occurring. To identify the potential prebiotic of synthetic oligosaccharides an in vitro screening method was developed. The optimum prebiotic dose and appropriate sampling times were investigated using FOS in batch culture fermentation during separate experiments. Selective media were used to monitor the population dynamics of the predominant bacterial groups during 24h batch culture fermentations. Overall, the bacterial population dynamics demonstrated that a FOS dosage equivalent to a 4 g daily dose afforded a prebiotic effect in vitro. The greater proportion of fermentation was shown to occur within the first 8–10 h. Sampling times of either 0, 4, 8, and 24 or 0, 5, 10, and 24 h post inoculation were thus proposed as ideal for monitoring the effects of novel prebiotics on the microflora in batch culture fermentations. This technique was used to study the effects of enzymatically manufactured mannobiose on the colonic flora, with FOS used as a prebiotic control. An initial increase was observed in the total anaerobe population,

followed by a reduction in numbers between 4 and 8h incubation. In particular, the bifidobacterial population was maintained throughout the 24h incubation. However, the lactobacillus population increased significantly between 0 and 4h incubation using mannobiose, and between 4 and 8h using FOS, with a return to baseline levels for both substrates between 8 and 24h incubation. *Clostridia* numbers decreased significantly between both 4 and 8, and 8 and 24h incubation, for both FOS and mannobiose. Bacterial population dynamics, therefore, demonstrated that at a concentration equivalent to a 3 g daily dose, synthetic mannobiose elicited a prebiotic effect consistent to that shown using FOS.

D-tagatose changes the composition of the microbiota and enhances butyrate production by human fecal samples

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Introduction: D-tagatose is a stereo-isomer of D-fructose that in experiments with pigs has been shown only to be poorly absorbed from the small intestine, but to be metabolised by the microbiota in the large intestine resulting in high productions of butyrate. The aim of the present study was to investigate how adaptation to D-tagatose affects the composition of the microbiota and microbial fermentation in fecal samples from human volunteers.

Methods: The study was carried out with 16 healthy human volunteers aged between 21 and 36 y. The experimental period lasted 18 d, from day 4 to day 0 and from day 10 to day 14 the volunteers were administered a controlled diet and from day 0 to 14 they received $3 \times 10\text{g}$ D-tagatose/d. Stool samples were obtained from each volunteer at day -1 (non-adapted) and day 14 (adapted). The fecal samples were analyzed for the composition of the microbiota and for in vitro fermentation of D-tagatose and sucrose.

Results: Adaptation to D-tagatose significantly increases the population density of lactic acid bacteria in fecal samples (8.39 compared 7.39 log CFU/g, $P = 0.002$), while the population density of coliforme bacteria was reduced (5.58 compared with 6.43 log CFU/g, $P = 0.02$). The major fermentation products from D-tagatose were acetic acid (34–41% on a molar basis), butyric acid (38–49%) and caproic acid (14–15%). The rate of fermentation of D-tagatose was higher with fecal samples of adapted than non-adapted volunteers (1.67 compared with 0.63 g/kg feces/h, $P < 0.001$), and the resulting SCFA produced had a higher proportion of butyric acid (49.0 compared with 37.7%, $P = 0.01$). Adaptation to D-tagatose also increases the proportion of sucrose converted to butyric acid. Based on the production and composition of SCFA, it was calculated that 56.7 and 49.6% of the energy in D-tagatose were recovered in SCFA after microbial fermentation with adapted or non-adapted fecal samples, respectively.

Conclusion: Our data strongly indicate that D-tagatose may function as a prebiotic in human nutrition as it is poorly absorbed in the small intestine but selectively stimulates the growth and activity of beneficial bacteria in the colon.

Effects of a synbiotic milk product on fecal microflora and bowel habits in healthy volunteers

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The objectives of the study were to investigate if oral supplementation with a synbiotic milk product showed any effects on the

composition of the colon microflora and on bowel habits in healthy volunteers. The randomized, double-blinded study included 22 healthy volunteers. Eleven volunteers received fermented milk containing the probiotic strain *Lactococcus lactis* L1A and 1,5% inulin (inulin group). The other 11 volunteers received fermented milk containing the same probiotic strain but without inulin (placebo group). In both groups each person consumed 150 mL twice a day of the inulin product and the placebo product, respectively. The consumption continued for 11 d. Fecal samples were collected before and at the end of the feeding period. The fecal samples were analyzed for the total counts of lactobacilli, lactococci and bifidobacteria. Furthermore, the volunteers recorded their bowel habits during the study. The results show that the total fecal counts of lactococci increased significantly during the study in the inulin group. In 8 out of 11 volunteers the counts had increased more than one log¹⁰ at the end of the feeding period. However, the results also show that 7 out of 11 volunteers in the inulin group experienced more flatulence than normal. In the placebo group the total fecal counts of lactococci increased in 2 out of 11 volunteers and 3 out of 11 volunteers experienced more flatulence than normal. The total counts of lactobacilli and bifidobacteria did not increase significantly in the inulin group compared with the placebo group. To conclude the supplementation with 4,5 g of inulin/d stimulates the growth of lactococci in the colon of healthy volunteers. However, supplementation with inulin also increases the frequency of flatulence.

Inulin alters bile acid profile and increases fecal bile acid concentration in hamsters

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Introduction: Inulin is a non-digestible fructo-oligosaccharide which is readily fermented in the cecum and colon resulting in the production of short chain fatty acids. As a result of acidic pH conditions, intestinal bile acid reabsorption may be impaired resulting in an enhanced fecal excretion and a subsequent up-regulating of hepatic bile acid synthesis. Therefore, the main focus of this study was to evaluate the impact of dietary inulin on bile acid metabolism specifically on the composition of the bile acid pool and on fecal sterol excretion.

Methods: Male golden Syrian hamsters were fed semipurified diets containing 20% fat, 0.12% cholesterol and 0 (control), 8%, 12% or 16% inulin (Raftiline HP, Orafit, Tienen, Belgium) for 5 wk. Plasma lipoproteins, biliary bile acid profile (glycine and taurine conjugates determined by HPLC) and fecal excretion of bile acids and neutral sterols (determined by GC) were analyzed.

Results: Plasma total cholesterol was significantly lowered by 18%, 15%, and 29% and triacylglycerol by 34%, 40%, and 63% with 8%, 12%, and 16% of inulin, respectively. All 3 inulin doses caused distinct changes in the bile acid profile of gallbladder bile. Taurochenodeoxycholate and taurocholate were significantly lower with inulin, whereas glycocholic and glycodeoxycholic acid were increased. As a result, the glycine:taurine conjugation ratio was 1.7- to 2.0-times higher with inulin, and the cholate:cheno ratio was increased by 24% with 16% inulin. Fecal concentration and daily excretion of neutral sterols was not altered by dietary inulin suggesting that cholesterol absorption was not affected. Fecal cholesterol tended to increase whereas coprostanol, the main break-down product, was substantially lower indicating a reduced bacterial degradation of cholesterol as a result of inulin digestion. Fecal total bile acid concentrations



were significantly increased while daily bile acid excretion tended to be higher with all levels of dietary inulin. Analysis of the fecal bile acid composition revealed that lithocholic and deoxycholic acid were the major bile acids excreted. Fecal bile acid composition was in % of total bile acids:

	Control	8% inulin	12% inulin	16% inulin
Lithocholic acid:	38.2 ± 3.8	32.8 ± 7.2	31.6 ± 6.9	33.5 ± 5.0
Deoxycholic acid:	27.2 ± 3.7	22.5 ± 4.8	31.4 ± 3.9	29.5 ± 4.2
Hyo-deoxycholic acid:	11.8 ± 2.4	12.2 ± 5.5	11.1 ± 1.9	10.6 ± 1.5
12-Keto-lithocholic acid:	4.3 ± 3.4 ^a	12.3 ± 2.1 ^b	11.9 ± 4.0 ^b	7.3 ± 4.7 ^{ab}
Chenodeoxycholic acid:	9.7 ± 3.3 ^a	5.7 ± 5.3 ^{ab}	1.1 ± 1.7 ^b	4.6 ± 4.6 ^{ab}
Ursodeoxycholic acid nd:	4.1 ± 5.8	4.5 ± 4.0	5.7 ± 4.6	
5b-Cholanic-acid-3b-ol:	5.3 ± 2.8	4.6 ± 2.7	5.8 ± 1.6	4.2 ± 3.3

Different superscripts indicate significant ($P < 0.05$) differences

Conclusions: These data indicate that inulin altered the circulating bile acid pool by selectively “entrapping” taurine-conjugated bile acids (eg, taurochenodeoxycholic acid). Therefore, quantitative and qualitative changes of the bile acid pool appear after inulin ingestion. This study was supported by a grant from ORAFIT, Tienen, Belgium.

Long term effect of oligofructose on bone trabecular structure in ovariectomized rats

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We have shown that oligofructose (OF) improved bone mineral content (BMC) in ovariectomized (OVX) rats. However, fragility of bone rather depends on structure of trabecular network than on BMC. We therefore analyzed the effect of OF on tibia trabecular structure in OVX rats after 8 wk and 16 wk on diets.

Methods: Ninety-five 5 mo old Fisher-344 rats were allocated to 7 groups. Rats were fed semipurified diets for 16 wk containing 5g/kg Ca without OF (1) and (2), or 5g/kg Ca plus 2.5% (3), 5% (4) and 10% (5) OF, or 10g/kg Ca either without (6) or with (7) 5% OF. Group (1) was sham operated, groups 2–7 had undergone ovariectomy. Fifty percent of animals were killed after 8 and 16 wk each. Trabecular network of distal tibiae was analyzed on microradiographs followed by computer supported picture analysis. This method allows quantification of bone structure.

Results: (mean ± pooled SEM, $n = 6-7$): After 16 wk OVX had caused a significant decrease in trabecular bone area (Tb.B.Ar.) with $0.77 \pm 0.05 \text{ mm}^2$ (2) versus $1.01 \pm 0.05 \text{ mm}^2$ (1) ($P < 0.01$). Trabecular perimeter was significantly lower with 27.0 ± 0.19 compared with $34.2 \pm 0.18 \text{ mm}$. Trabecular number was not dif-

ferent. When diets contained 5g/kg Ca, OF tended to prevent OVX-induced bone loss: Tb.B.Ar. was 0.98 (2.5% OF), 0.96 (5% OF), and 1.04 (10% OF) $\pm 0.12 \text{ mm}^2$ (groups 3–5). When diets contained 10g/kg Ca, Tb.B.Ar. was higher with 1.70 ± 0.14 (5% OF) compared with $1.24 \pm 0.14 \text{ mm}^2$ (0% OF) after 8 wk ($P < 0.05$). After 16 wk Tb.B.Ar. tended to be higher with 1.33 ± 0.12 (5% OF) compared with $1.04 \pm 0.12 \text{ mm}^2$ (0% OF), while trabecular number was significantly higher when 5% OF was given with 47.4 ± 3.7 compared with 34.1 ± 3.7 ($P < 0.03$) in the corresponding rats without OF.

Conclusion: We conclude that oligofructose effectively prevented OVX induced loss of trabecular structure in rats, especially when dietary Ca was high.

Effect of oligofructose on bone mineralization in ovariectomized rats is affected by dietary calcium

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It has been reported that in intact young rats calcium balance was significantly increased within 4 wk, when a diet containing 7g/kg Ca was supplemented with 10% oligofructose. We investigated the effect of 3 concentrations of oligofructose and the interaction between different concentrations of dietary Ca and oligofructose on femur mineralization in ovariectomized (OVX) rats.

Methods: 42 five mo old Fisher-344 rats underwent bilateral OVX and were allocated to 6 semipurified diets containing 5g/kg Ca without oligofructose (1), or 5g/kg Ca plus 2.5% (2), 5% (3) and 10% (4) oligofructose, or 10g/kg Ca either without (5) or with (6) 5% oligofructose. Finally, after 8 wk, femora were removed and ashed. Ca was analyzed by atomic absorption spectroscopy.

Results: (mean ± SEM): Femur Ca (mg) was 1) 87.7 ± 1.5 , 2) 87.4 ± 2.4 , 3) 89.1 ± 1.8 , 4) 90.2 ± 1.9 , 5) 88.2 ± 2.4 , and 6) 97.2 ± 1.4 . There was a positive trend for linear relation between dosage of oligofructose and bone mineralization, when diets contained 5g/kg Ca (diets 2–4). Based on a diet containing 1% Ca, the supplementation of 5% oligofructose significantly increased femur Ca ($P < 0.01$) (diet 5 compared with 6). Without supplementation of oligofructose the rise of dietary Ca from 5g/kg to 10g/kg did not increase femur Ca (diet 1 versus 5). However, when 5% oligofructose was added, rats utilized the higher supply of dietary Ca more efficiently (diet 3 compared with 6) ($P < 0.01$).

Conclusion: We conclude that the stimulating effect of oligofructose on bone mineralization is dose dependent. Moreover, oligofructose is more effective at a high calcium intake, ie when depression of mineral solubility may occur.

