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LACTIC ACID BACTERIA AND HUMAN HEALTH

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INTRODUCTION

This review will focus on the general considerations of lactic acid bacteria and what is known of their direct effect on human health. Much research, necessarily, has focused on *in vitro* or animal system development. These studies are an important first step in developing a sense of potential outcomes and mechanisms. However, these studies can always be criticised due to their questionable significance to human physiology. Therefore, this review will focus on studies which have been done in humans. These studies take two principle forms: epidemiological studies, which are rare, and direct clinical studies, which are diverse.

PROBIOTICS

A probiotic has been defined by *Havenaar* and *Huis in 't Veld* (1992) as "a mono- or mixed-culture of live microorganisms which, when applied to animal or man, affect the host beneficially by improving the properties of the indigenous microflora". This definition somewhat limits the mechanism of probiotics, since, for example the use of cultures to deliver lactase to aid with lactose digestion would not fit under this definition. In addition, improvement of the properties of indigenous microflora may be more difficult to substantiate than the more empirical benefit of the host. For this reason, simplifying the definition to "a culture of live microorganisms which, when applied to animal or man, affect the host beneficially" may be more suitable for pragmatic purposes.

Bacteria proposed for probiotic uses are usually categorised as lactic acid bacteria. This includes a broad group of Gram-positive, non-sporeforming, catalase-negative rods and cocci that ferment carbohydrates to form lactic acid as their primary metabolic end-product. The primary genera associated with this general grouping are *Lactococcus, Lactobacillus, Pediococcus, Leuconostoc* and *Streptococcus* (usually limited to the *S. thermophilus* species). In discussions on lactic acid bacteria used for probiotic purposes, the genus *Bifidobacterium* is frequently included.

MECHANISMS OF INFLUENCE

Many have advanced the theory that certain lactic acid bacteria, primarily those which normally reside in the human intestine, may exert a positive influence on human health (for reviews, see *Fernandes* et al., 1992; *Hitchins* and *McDonough*, 1989; *Gurr*, 1992; *Fuller*, 1991; *Gorbach*, 1990). The human in-

Table 1: Hypothesis: intestinal functioning of probiotic cultures

Compounds possibly present in the intestine	Action of Intestinal Bacteria	Influence on Health
	Potential negative influence	
bile salts*	→	3oxo-chol-4en-24oic acid
hormones	→	estradiol
peptides*	→	phenolic compounds, amines
steroids	→	polycyclic aromatic hydrocarbons
		Potential beneficial influence
cholesterol	→	cholestenone
lactose	→	glucose and galactose
carbohydrates	→	organic acids

testinal tract is continuously challenged by a variety of bacteria and viruses, some of which are capable of pathogenic activity or producing toxic metabolites. Lactic acid bacteria are not enteropathogenic or enterotoxigenic, and are known for their ability to suppress the growth of species of enteric bacteria *in vitro*. Although physiological conditions *in vivo* are vastly different than these *in vitro* model systems, microbial behaviour in these *in vitro* model systems is still used as evidence of their potential to modulate the intestinal microbial ecosystem. The presence of lactic acid bacteria in high numbers in the intestine is postulated to limit the growth, metabolism or survival of other enteric bacteria, which may in turn limit pathogenic or toxigenic effects of these bacteria. Negative effects of intestinal bacteria have been suggested (Table 1), and include production of toxic metabolites and carcinogens.

Ultimately, the human intestinal system is notorious for its complexity, and the human being reluctant a reluctant subject for certain types of direct experimentation. For these reasons, the mechanisms of probiotic action in humans remains largely unproved.

MEASUREMENT OF PROBIOTIC EFFECTS

Investigation into the effects of probiotics on human health has expanded across a broad range of clinical and *in vitro* systems. *In vitro* systems, including tissue cell culture analysis of adherence potential, identification from probiotic strains of bacteriocins which may play a role in competitive exclusion in the intestine, metabolic studies to identify antagonistic substances produced by probiotic strains, colonisation studies in experimental animals, and other systems have all contributed to a basic understanding of the potential of these microbes. However, the real test of effectiveness is determined by how these *in vitro* activities may affect clinical conditions.

Assays of the activity of probiotics directly in humans has also been varied. In some instances, these assays are not optimally conducted, since insufficient attention has been paid to microbiological factors which may affect probiotic

Table 2: Carcinogens or suspected carcinogens naturally present in food (adapted from *Scheuplein*, 1992)

Food	Carcinogen
garlic	alkyl isothiocyanate
chilies	capsaicin
oranges	d-limonene
mushrooms	hydrazines
cereals, nuts contaminated with mold	aflatoxin
herbal teas	pyrrolizidine alkaloids
spinach, beets, lettuce, radishes	nitrates → nitrosamines
fish and shellfish	polyaromatic hydrocarbons

performance. Studies using human subjects fed probiotics or probiotic-containing foods have measured changes in faecal microbial populations, faecal microbial enzyme activity, intestinal chemistry, survival through the gastrointestinal tract, colonisation (biopsy methods), clinical conditions (gastrointestinal and vaginal infections, intestinal symptoms), breath hydrogen (measure of lactose digestion), and blood chemistry (including blood lipids and immunological factors). Taken together with the *in vitro* research, these studies have begun to paint a picture of the way probiotic cultures may affect human health.

EPIDEMIOLOGICAL EVIDENCE

Diet and cancer

Epidemiologists estimate that from 10 - 70% of all cancer deaths can be attributed to diet, and that the risk stems predominately from the food, not from additives, pesticides, or contaminants (*Scheuplein*, 1992). However, a recent publication from the Council on Scientific Affairs (1993) cautions that a simple solution does not exist to conclusively link specific nutritional factors in cancer progenesis. However, carcinogenic substances in food (Table 2) are suspect for contributing to cancer incidence.

Drasar and *Hill* (1972) advanced the theory that the "internal environment" of the intestinal tract may affect cancer rates, especially colon, breast and stomach. Transformations of foodstuffs, endogenously-produced compounds, or microbial by-products by the plethora of microbes present in the intestinal tract into harmful substances may lead to the progenesis of different types of cancer. Unfortunately, although indirect experimentation on animals or using *in vitro* systems suggests that probiotic cultures can have a positive effect on cancer incidence, epidemiological evidence supporting these claims in humans is sparse. Since direct experimentation on humans and cancer is not possible, these epidemiological studies are critical to gaining an understanding of potential effects.

Breast cancer

One epidemiological study was conducted on breast cancer incidence and fermented milk, Gouda cheese and milk consumption in the Netherlands (*van 't Veer* et al., 1989). This study involved 133 breast cancer patients and 289 con-

trols. Breast cancer subjects were asked to recall dietary consumption patterns for the 12 months prior to diagnosis. The study found that the consumption of fermented milk products was significantly higher among the controls than the breast cancer patients. After correction for dietary fat intake, Gouda cheese consumption also correlated with lower incidence of breast cancer. Milk consumption was not significantly associated with breast cancer risk. The authors speculated that the protective effect of fermented milk products may be due to the interference of lactic acid bacteria with entero-hepatic circulation or their stimulation of the immune system. However, they caution that further observational and experimental research are needed to substantiate any effect. The primary weakness of this study lies with the nature of data collection. Recall studies are inherently less reliable than direct measurement. Furthermore, dietary patterns during 12 months prior to diagnosis are largely insignificant to the progenesis of a disease which may be decades in development. This study is defended by the observation that consumption of fermented milk products in the Netherlands is thought to be a life long pattern. Firm establishment of this fact is essential for proper interpretation of these results.

HUMAN CLINICAL STUDIES

Diarrhoeal illnesses

The assessment of the effect of lactic cultures on diarrhoeal illnesses is a very general topic. Diarrhoea is a single symptom, but with a myriad of progenitors. Disruption of the chemistry, physiology or microbiology of the intestinal system can result in an influx of fluids into the bowel, resulting in clinical diarrhoea. Bacteria, protozoa, viruses, as well as chemical substances (e.g., antibiotics, oligosaccharides, non-digestible fibres) can influence the microbial or chemical balance in the intestine and lead to diarrhoeal illness.

Many human clinical studies have been published on the effect of probiotics or probiotic-containing products on diarrhoeal illnesses. These studies are summarised in Table 2, and some of the positive studies will be discussed below.

The onset of diarrhoeal illness during or after the course of antibiotic therapy is a common side effect, in some cases leading to pseudomembranous colitis. Virtually all cases of pseudomembranous colitis resulting from antibiotic therapy can be traced to a toxin produced by *Clostridium difficile* (*George*, 1980). This is a disease of the large bowel, and is unique in that the causative agent, *C. difficile*, is commonly present in the normal bowel, but is not pathogenic until the intestinal microecology is disturbed by antibiotic therapy. Therefore it appears that the normal intestinal flora suppresses growth and toxin formation by *C. difficile*. In most cases, *C. difficile* colitis is cured with focused antibiotic therapy. However, in a small number of cases, the disease relapses, and is refractory to antibiotic treatment. The effect of probiotic therapy on relapsing *C. difficile* pseudomembranous colitis was tested in a limited study by *Gorbach* et al. (1987). Five patients with relapsing *C. difficile* colitis consumed 10^{10} cfu/day dried *Lactobacillus casei* GG suspended in milk for 7-10 days. After treatment, no additional relapses occurred over a follow-up period of 4 months to 4 years.

Additional experiments with the GG strain have been done on diarrhoea

Table 3: Summary of studies to determine the effect of lactic cultures on diarrheal illnesses (ETEC, enterotoxigenic *E. coli*)

Clinical target	Probiotic culture used	Total number of subjects	Effect on diarrhea in treatment	Culture format/ daily culture dose groups	Reference	Placebo controlled?
rotavirus diarrhea 82%	*L. casei* GG	71 children	_ duration by 1 day	yogurt or dried powder 10^{10}-10^{11} cfu/day	Isolauri et al., 1991	yes
rotavirus diarrhea	*L. casei* GG	22 children	_ duration by 1.4 days	10^{10}-10^{11} cfu/day	Kaila et al., 1992	yes
relapsing *C. difficile* colitis	*L. casei* GG	5 adults	follow-up with no relapses: 4 mo - 4 yr	concentrate in 5 ml milk 10^{10} cfu/day	Gorbach et al., 1987	no
erythromycin-associated diarrhea	*L. casei* GG	16 healthy adults	_ incidence	yogurt $2x10^{10}$ cfu/day (est.)	Siitonen et al., 1990	yes
travellers' diarrhea	*L. casei* GG	756 travellers	_ incidence in one of two groups studied	powder $2x10^{9}$ cfu/day	Oksanen et al., 1990	yes
intractable diarrhea	bifidobacteria and/or *L. casei* dried or yogurt	15 children	clinical improvement within 3 to 7 days	10^{10}	Hotta et al., 1987	no
deliberate ETEC infection; neomycin-associated diarrhea	Lactinex®	40 young adults	no effect on ETEC diarrhea; variable effect on neomycin-associated diarrhea	powder $7x10^{7}$	Clements et al., 1983	yes
deliberate ETEC infection	Lactinex®	48 young adults	no effect on ETEC diarrhea	powder $7x10^{8}$	Clements et al., 1981	yes
travellers' diarrhea	Lactinex®	50 healthy adults	no effect	powder $3\text{-}7x10^{9}$	Pozo-Olano et al., 1987	yes
acute-onset diarrhea	dried *L. acidophilus*, *L. bulgaricus* and *S. thermophilus*	94 children	no effect	powder $3\text{-}8x10^{8}$	Pearce and Hamilton, 1974	

Table 4: Comparison of daily dose and overall results from studies on effect of lactic cultures on diarrhoea illnesses

Daily Dose	Probiotic Used	Overall Effect	Reference
7×10^7	Lactinex®	-	Clements, et al., 1983
7×10^8	Lactinex®	-	Clements, et al., 1981
	Sweet Acidophilus Milk	-	Newcomer, et al., 1983
$3\text{-}7 \times 10^9$	Lactinex®	-	Pozo-Olano, et al., 1987
$3\text{-}8 \times 10^8$	Dried *L. acidophilus*, *L. bulgaricus* and *S. thermophilus*	-	Pearce and Hamilton, 1974
10^{10}-10^{11}	*L. casei* GG	+	Isolauri, et al., 1991
10^{10}	*L. casei* GG	+*	Gorbach, et al., 1987
10^{10}-10^{11}	*L. casei* GG	+	Kaila, et al., 1992
2.5×10^{10} (est.)	*L. casei* GG	+	Siitonen, et al., 1990
10^{10}	Bifidobacteria and/or *L. casei* , dried or yogurt	+*	Hotta, et al., 1987
2×10^9	*L. acidophilus* yogurt	+*	Salminen, et al., 1988

*not placebo controlled

caused by rotavirus, *C. difficile*, and erythromycin, as well as the less clearly defined "travellers' diarrhoea". Limitations, especially with the *C. difficile* study, where no controls are conducted and only five subjects are tested, can be readily identified. But overall, these studies suggest that *Lactobacillus* GG can limit the course of some diarrhoeal illnesses. Credit should be given to this focused research program for attempting to build a critical mass of data which can support clinical effects for a single strain. These researchers have, in general, paid attention to issues which are absent in many studies: quantitation of daily dose of a defined strain, maintenance of high daily doses, definition of a clinical system to test, and statistical analysis of results.

When reviewing the literature on anti-diarrhoeal effects, the importance of high daily dose to positive activity becomes apparent. Clinical studies which tested the effect of lactic cultures or fermented dairy products on incidence or duration of different diarrhoeal illnesses were grouped according to extent of effect and daily dose (Table 3). Unfortunately, not all clinical studies could be included in this analysis, since in some, daily dose is not provided or results cannot be determined to be positive or negative, since statistical analysis of the data was not conducted. Considering these limitations, Table 4 suggests that in general, studies which showed a positive effect, used high levels of culture. No studies using daily doses of $<10^9$ showed positive results.

Vaginitis

The lactobacilli are normal inhabitants of the human vaginal tract. Because of this, it is hypothesised that they may be able to exert an effect in the

overall microbial balance of the vagina, discouraging the presence or proliferation of pathogens. Some clinical and microbiological evidence supports this. One line of research has correlated the presence of H_2O_2-producing (but not non-producing) lactobacilli with decreased vaginal infections. *Eschenbach* et al. (1989) demonstrated that 6% and 96%, respectively, of women with or without bacterial vaginosis harboured H_2O_2-producing lactobacilli. 36% of women with bacterial vaginosis harboured H_2O_2-producing lactobacilli. These observations suggest that the antimicrobial effect of the H_2O_2, along with other factors such as vaginal pH, mediates pathogen suppression by vaginal lactobacilli. *Klebanoff* et al. (1991) demonstrated *in vitro* the toxic effects of producing lactobacilli *in vitro* against *Gardnerella vaginalis*. The H_2O_2-producing and non-producing lactobacilli used in the study were unfortunately not isogenic, but catalase-treatment eliminated the positive effect, supporting the role of H_2O_2.

In another line of research, the effect of yoghurt consumption on *Candida* infections in women was studied by *Hilton* et al. (1992). This study was a non-blinded study of crossover design. Nineteen women started the study and served as their own controls. Participants in the test arm of the study were asked to consume 8 oz of yoghurt per day for six months. In the control arm of the study, no yoghurt was consumed. Eight women, however, who began the study in the yoghurt arm, refused to enter the control arm of the study, due to clinical improvement. Of the remaining 11 subjects, results showed that the mean number of infections per six months was 1.66 in the control, and 0.38 in the yoghurt phase of the study, results which were highly statistically significant. Interestingly, these results were obtained with consumption, not direct application, of the lactic culture. This study needs to be repeated as a blinded, placebo-controlled study, but is suggestive of positive influence on yoghurt and the control of vaginal infections. Placebos composed of yoghurt with heat-killed bacteria would substantiate any role viable bacteria have in this clinical effect.

Faecal Metabolites

Repeated experiments have been conducted on enzymatic activity in faeces. Table 5 summarises these results. Certain enzymes produced by microbes present in human faeces are thought to play a role in the production of carcinogens, and therefore, overall cancer rates. Enzymes which have been assayed include ß-glucuronidase, nitroreductase, glycocholic acid hydrolase, urease, ß-glucosidase and steroid 7-α-dehydroxylase. The most consistent results of these studies indicate that ß-glucuronidase activity can be influenced by high daily doses of *L. acidophilus* or bifidobacteria strains. The results have been achieved with several different *L. acidophilus* strains. Choice of enzymes for these studies has been criticised by *Marteau* and *Rambaud* (1993). However, experiments with rats (*Goldin* and *Gorbach*, 1984) showed that *L. acidophilus* supplements reduce DMH-induced colon tumours. Although the mechanism of effect may not be proven as yet, the results suggest an overall positive effect. These results suggest that probiotic bacteria in adequate numbers can influence intestinal activity which may stimulate tumour formation.

Although information has been generated which suggests that *L. acidophilus* feeding can influence some metabolites of the faecal microflora, a recent study suggests that not all probiotic dietary intervention will necessarily have the same result. *Bartram* et al. (1994) studied changes in the faecal mi-

Table 5: Summary of the effects of oral consumption of lactic cultures on faecal enzyme activity in humans

Reference	Bacteria and daily dose	Reduction of faecal enzyme activity
Goldin, et al., 1992	*L. casei* GG frozen concentrate-10^{10} (8 subjects)	+ ß-glucuronidase
Ling, et al., 1994	*L. casei* GG (3 x 1010/day, in yogurt or yogurt + fiber) (64 female subjects)	+ ß-glucuronidase + nitroreductase + glycocholic acid hydrolase - urease - ß-glucosidase
Marteau, et al., 1990	galactooligosaccharides 2.5 g/day (which increase bifidobacteria)	+ ß-glucuronidase - nitroreductase
Goldin and Gorbach, 1984a	milk fermented with *L. acidophilus* (10^9), *B. bifidum* (10^{10}), and mesophilic cultures (10^{10}) (9 subjects)	+ nitroreductase - azoreductase - ß-glucuronidase + ß-glucosidase
Goldin and Gorbach, 1984b	*L. acidophilus* NCFM (10^{10}) (7 subjects)	+ nitroreductase - azoreductase + ß-glucuronidase
Ayebo, Angelo, and Shahani, 1980	*L. acidophilus* NCFM (10^9) *L. acidophilus* N-2 (10^9) (22 subjects)	+ nitroreductase + azoreductase + ß-glucuronidase
Goldin, et al., 1980	*L. acidophilus* DDS1 in milk ($1.4x10^9$) (12 subjects)	? ß-glucuronidase ? ß-glucosidase (no statistical analysis)
Ito, et al. (1993)	*L. acidophilus* ($4x10^{10}$) (7 subjects)	+ nitroreductase - azoreductase + ß-glucuronidase - steroid 7-α-dehydroxylase

+ : statistically significant positive results
- : negative results
? : results not definitive

croflora and chemistry in 12 healthy volunteers fed yoghurt made with normal yoghurt cultures (*S. thermophilus* and *L. bulgaricus*), serving as a control, or that same yoghurt supplemented with *Bifidobacterium longum* (daily dose > 5 x 10^8) and lactulose (daily dose 2.5 g). Faecal levels of *Bifidobacterium* increased when yoghurt with or without *Bifidobacterium* and lactulose was fed. However, faecal analysis revealed that no statistically significant differences in oro-anal transit time, wet or dry weight of stools, total, aerobic or anaerobic bacteria, concentrations of acetate, propionate, n-butyrate, isobutyrate, i-valerate, neutral sterols, or faecal pH could be attributed to bifidobacteria/lactulose consumption. The low level of daily dose of bifidobacteria may be

partially responsible for the lack of significant effects seen. Similar experiments with lactobacilli have fed in excess of 10^{10} lactobacilli per day. Significant differences were seen in the breath hydrogen levels in subjects consuming the yoghurt with bifidobacteria and lactulose, likely due to the faecal flora fermentation of the lactulose, and in the mouth-to-caecum transit time. The authors conclude that human faecal flora are refractory to probiotic and lactulose dietary intervention.

In interpreting these results in general, it should be remembered that the relationship between a statistically significant drop in microbial enzyme activity and improved health or decreased risk of cancer in humans, has not been determined. Until epidemiological studies confirm these results, the ultimate effect on human health must be considered speculative.

Toxic Metabolites

The effect of *Lactobacillus acidophilus* on the health of chronic kidney failure patients is currently being studied. These patients demonstrate bacterial overgrowth of the normally sparsely-populated small bowel. This bacterial growth results in the production of toxic metabolites, including dimethylamine (DMA), which when nitrosated in the gastrointestinal tract, forms the potent carcinogen, nitrosodimethyamine (NDMA). *Simenhoff* et al. (1994) have tested the effect of feeding 2×10^{10} freeze-dried, enteric-coated viable cells of *L. acidophilus* per day on levels of DMA in the blood of six chronic kidney failure patients. The study was placebo-controlled. Statistically significant ($p<0.01$) drops in the levels of blood DMA were seen for all patients during *L. acidophilus* feeding. The levels of DMA rebounded during placebo-feeding. Furthermore, NDMA levels decreased during *L. acidophilus* feeding. These effects were correlated with improved appetite and nutritional status of all patients. These results indicate that in these patients, feeding of *L. acidophilus* significantly altered the course of small bowel bacterial overgrowth, lending credence to the hypothesis that probiotic cultures can influence the microbiology and biochemistry of the gastrointestinal tract. These studies need to be expanded to include more, and perhaps less critically-ill, patients. If this type of result could be extended to less extreme cases of small bowel bacterial overgrowth, which can occur in people with atrophic gastritis, high blood pressure, diabetes, or heart disease, then the results could suggest a large impact on controlling toxic metabolites formed in the gastrointestinal system.

Lactose intolerance

It has long been believed that yoghurt is more readily digested than milk by lactose-intolerant people. There is now a significant body of scientific evidence which substantiates this effect (for reviews, see *Shah*, 1993; *Schaafsma*, 1993; *Savaiano* and *Levitt*, 1987; *Savaiano* and *Kotz*, 1988). It has been found that yoghurt containing viable yoghurt cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) consumed by lactose intolerant subjects resulted in lower breath hydrogen production and fewer symptoms than consumption of milk. It is thought that these lactase-containing yoghurt cultures deliver lactase to the intestine mediating the *in vivo* digestion of lactose to glucose and galactose before intestinal bacteria can degrade the lactose into more troublesome byproducts. Also contributing to better digestion of lactose in yoghurt as compared to fluid milk is the slower oral-caecal transit time of yoghurt. *Marteau* et al. (1990) used an intestinal perfusion technique to determine that only 20% of the lactase

activity in yoghurt reached the terminal ileum, and that 90% of lactose from yoghurt is digested in the small intestine of lactose intolerant subjects.

The improved digestion of lactose by lactase-deficient subjects has been demonstrated with a variety of commercial and laboratory-prepared yoghurts. *Wytock* and *DiPalma* (1988) showed that two of three commercial yoghurts resulted in significantly lower breath hydrogen levels than a liquid lactose control. Results with one of the commercial yoghurts were not statistically different from the control, suggesting that some commercial yoghurts are more effective than others. Since no lactase activities or starter culture population levels were conducted on the commercial yoghurts, it is not possible to determine the cause of the differences. In general, little attention has been paid to the strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* used in yoghurts tested, and no reports of strain optimisation for this application have been published. This may partly be because the relationship of internal lactase levels of different strains and their effectiveness at aiding yoghurt digestion is unclear. *Martini* et al., (1991b) tested four laboratory yoghurts prepared with different *S. thermophilus* and *L. bulgaricus* strains for their lactase levels and their ability to aid lactose digestion in lactase-deficient subjects. Although lactase levels differed substantially, the effect on breath hydrogen levels were not statistically different and were similar. Intuitively it would seem that selection of strains with high lactase levels would provide for optimal effectiveness, but other parameters may also be involved in delivery of lactase to the intestine. Important parameters may include: (1) the inherent resistance of lactase to stomach acid or intestinal bile, (2) the ability of specific strains to shield intracellular lactase from adverse conditions but allow contact with lactose when needed in the intestinal tract, or (3) inherent lactase activity under physiological conditions.

Testing of commercial unfermented milk products containing *L. acidophilus* cultures suggests that these products are not as effective as yoghurt in facilitating lactose digestion. Recent research suggests that this may be due, at least in part, to the lower level of culture added to these fluid products. In the USA, no fluid products currently are supplemented with yoghurt bacteria. Fluid products containing *L. acidophilus* or *Bifidobacterium* strains are available, but these bacteria do not appear to be as effective as yoghurt cultures and levels of these bacteria in fluid milk are much lower than yoghurt cultures in yoghurt (approximately 2 x 10^6/ml). Only two states, Oregon and California, regulate these levels; therefore no means of enforcement of minimal levels in other states exists. In contrast, high quality yoghurt generally has in excess of 10^8/ml each of *S. thermophilus* and *L. bulgaricus*. If adequate levels of yoghurt bacteria are provided in a fluid milk, this product becomes an effective aid for lactose digestion (*Lin* et al., 1991).

Specific differences in lactase delivery potential between yoghurt cultures and *L. acidophilus* exist. One hypothesis suggests that yoghurt cultures, permeabilised by bile in the intestine, more readily enable contact between ingested lactose and lactase. Bile-resistant *L. acidophilus* are not permeabilised in the intestine and lactose digestion is more regulated. Regardless of the mechanism, it appears that even at equivalent bacterial levels, *L. acidophilus* strains are not as effective as yoghurt strains in aiding lactose digestion *in vivo*, and among *L. acidophilus* strains, some are better than others (*Lin* et al., 1991).

Research also suggests that the role of yoghurt in facilitating the digestion of

lactose in lactose-intolerant people is more complex than simple lactase delivery. The evidence for this is the lack of linearity between lactase delivery and breath hydrogen results (*Martini* et al., 1991b). Total lactase levels in the yoghurt differed up to three-fold and specific lactase activities up to 2-fold, but no differences in *in vivo* lactose digestion were seen. The authors conclude that "All yoghurts dramatically and similarly improved lactose digestion, regardless of their total or specific ß-gal (lactase) activity". The delivery of lactase to the intestine is likely a function of several factors, possibly including survival of lactase through the stomach, level of contact between lactase and lactose in the intestine, and lactase levels in the yoghurt.

Many different aspects of yoghurt-mediated improvement of lactose digestion have been tested. Research suggests that bacteria delivered in yoghurt are not able to aid the digestion of lactose beyond that normally present in yoghurt (*Martini* et al., 1991a), that both *S. thermophilus* and *L. bulgaricus* strains individually can suitably mediate the effect, i.e. a mixed culture is not essential (*Lin* et al., 1991), and yoghurt with heat killed bacteria is not as effective as yoghurt containing viable lactic acid bacteria (*Savaiano* et al., 1984), but some improvement is seen (*Marteau* et al., 1990).

CONCLUSIONS ON CLINICAL STUDIES

Some studies focused on specific clinical conditions suggest that probiotics can have a positive influence on health. Perhaps the most conclusive evidence is for aiding the digestion of lactose. Also, good evidence exists for some anti-diarrhoeal effects of some probiotics given in high enough doses. The ability of probiotic cultures to alter metabolic activity of faecal flora has been supported by several lines of research. However, it is not clear to what extent these results relate to improving health of healthy adults.

The clinical results generated so far also suggest that adherence is not required for the observed effects. Any effects seen appear to require high numbers of viable bacteria fed on a regular basis. Cessation of feeding correlates with an absence of effect.

SAFETY CONCERNS

Efforts to substantiate clinical effectiveness of probiotic lactobacilli and bifidobacteria in humans have resulted in exploration of clinical situations where their benefits may be revealed. Many clinical trials have been conducted, some with healthy subjects and some with clinically-compromised patients. The safety of this approach has been justified since lactic acid bacteria are considered rarely pathogenic to man. Although the safety of fermented food products containing large numbers of traditional lactic acid bacteria is unquestioned, supported by centuries of safe consumption, human consumption of large numbers of lactic cultures of intestinal origin is a more recent development (*Driessen* and *de Boer*, 1989). Intestinal lactobacilli and bifidobacteria have been included in food products in more recent years. Consumption patterns of these products are geographically distinct, but Japan and some

countries in Europe have shown high per capita levels of consumption for at least a decade, with no ill effects.

From the backdrop of a long history of safety, however, comes isolated medical reports of association of some lactic acid bacteria with human infection. *Aguirre* and *Collins* (1993) have recently reviewed this area. They cite publications documenting clinical infection in humans associated with *Lactobacillus* (68 cases), *Lueconostoc* (27 cases), and *Pediococcus* (18 cases) strains. *Torre* et al. (1990) reported necrotising pneumonitis caused by *Lactococcus cremoris* in a HIV-infected intravenous drug addict. Antibiotic treatment was successful and the patient recovered. The authors speculated that the organism infected the patient through the oropharynx, and originated from unpasteurised milk and cheese ingested by the patient. *Giraud* et al. (1993) report a fatal case of *Leuconostoc* infection in an adult bone marrow transplant recipient treated with vancomycin. The natural vancomycin-resistance of *Leuconostoc* species makes them a threat for these patients.

In evaluating the reported cases, it is clear that most of the patients involved with these infections are in a compromised health state, which leads some of these bacteria to express as opportunistic pathogens. No indications have suggested a potential problem with administration of high levels of these bacteria in clinical studies or in commercial products. However, it is also important to keep in mind that clinical trials using probiotics have also turned more recently to their effect on clinically-compromised patients. These experiments should be considered with caution. Furthermore, it should be recognised that food products containing these bacteria will be considered safe for general use, and therefore must be judged safe for all consumers, healthy or not. Although not all reported cases have definitively established the causative role of these lactic acid bacteria in human infection, *Aguirre* and *Collins* (1993) recommend that "the view that lactic acid bacteria are non-pathogenic merits reassessment in view of the increasing numbers of reports of their association with human clinical infection".

THE FUTURE

The future of probiotics in the diet of humans is largely tied to (1) successful research programs focused in this area and (2) responsible, thoughtful formulation of commercial probiotic products. Although many products are currently being marketed, and are promoted directly or indirectly with health claims, many of them deliver low levels of strains of questionable benefit to humans. Marketing products based on testimonial evidence alone does not impose reasonable scrutiny of product quality and efficacy. Since no quality standards exist for probiotic efficacy beyond, perhaps, viable population levels and purity, efficacy of commercial products is difficult to substantiate as a consumer. In the long run, products which do not meet with customers' expectations will not endure, and an overall negative impact will surely be seen for probiotic-containing products in general.

In the United States, it is now required that health claims made on foods comply with specific Food and Drug Administration (FDA) regulations, published as part of the newly enacted Nutrition Labelling and Education Act. Any health claims will be required to be approved by the FDA, a process which will require substantial scientific support for claims.

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