This review focuses on the science behind the beneficial effects of probiotics on both the intestinal flora and on the host’s condition. These probiotics concern non-pathogenic microorganisms, especially lactic acid bacteria, such as lactococci, lactobacilli and bifidobacteria, as well as yeasts, such as Saccharomyces cerevisiae and Saccharomyces boulardii. For a good understanding we have characterized the site of primary action, its specific flora and the specific properties of the various probiotics. The common property of all living probiotics implies fermentative energy generation from sugars with only a low energy yield and consequently a high fermentation rate. The nature of the probiotics determines the fermentation products and thus the specific probiotic fermentation may involve (i) only 2 moles lactic acid from 1 mole glucose (lactococci and obligate homolactic lactobacilli), (ii) nearly 2 moles lactic acid with a small amount of CO2 from 1 mole glucose (facultative heterolactic lactobacilli), (iii) only 1 mole lactic acid, 1 mole ethanol and 1 mole CO2 from 1 mole glucose (obligate heterolactic lactobacilli), (iv) 2 moles lactic acid and 3 moles acetic acid from 2 moles glucose (bifidobacteria), (v) 2 moles ethanol and 2 moles CO2 from 1 mole glucose (yeasts). A relevant specific fermentation-linked aspect is the stereo-isomeric conformation of lactic acid, i.e., D- or L-lactic acid, especially since in neonates and young children because of too low D-2-hydroxy-acid dehydrogenase activity D-lactic acid may behave as a toxic compound that is comparable with acetaldehyde in case of ethanol intoxication. Another lactic acid-linked factor that may contribute to human health concerns the human hepatic gluconeogenesis since it lowers the intestinal lactic acid load. Other factors that may contribute to human health concern bacterial production of anti-microbial compounds such as nisin and bacteriocines, mechanisms that inactivate microbial toxins, inactivating binding of toxins and viruses, microbial deconjugation of bile acids, and induction of antibodies that cross-react with cell wall components of pathogens and thus contribute to phagocytosis-mediated eradication. Consequences for therapeutic manipulation are discussed as it regards moment of intake, composition of preparation, therapeutic dose, rational choice, clinical limitations and risks.

Because of the presented data we think that probiotics are absolutely not incredible panaceas.

KEY WORDS: Fermentation, Gluconeogenesis, Gut, Health, Host Defense, Lactic Acid Bacteria, Prebiotics, Probiotics, Yeast

INTRODUCTION

Recently, Alison Abbott (Abbott, 2004) questioned whether there is any science behind probiotics. Probiotics are microorganisms that on ingestion exert health benefits beyond inherent general nutrition (Guarner and Schaafsma, 1998). In the last decades especially the interest for a healthy life, but also the increasing problem of antibiotic resistance made probiotics increasingly popular. Nowadays millions of dollars, euros and yens are available for a growing number of studies with probiotics. Still many medical doctors do not belief in a beneficial effect. They have learnt that all bacteria should be destroyed by fire and sword (i.e., with conservatives, disinfectants, antiseptics and antibiotics) and why should bacteria then be used as therapeutic?

In many studies probiotic lactobacilli are seen either as microscopic, magic bullets or micro-bombs, that must anyhow destroy “bad things” and thus promote health, or as magic therapeutics for all kind of intestinal inconveniences (e.g., abdominal discomfort or pain, and diarrhoea) and (infectious) diseases (e.g., irritable bowel disease). The seemingly unlimited application range of the broad variety of fermented acidic milk and even milk-free (sometimes even lyophilized) probiotic preparations does not increase the credibility. Shortly, it seems that this makes probiotics an incredible panacea. What are we
dealing with? This article is a view on probiotics that is based on (i) massive amount of excellent older scientific work on lactic acid bacteria in food and dairy microbiology that has been reviewed in “Berger’s Manual of Systematic Bacteriology” (Hardie, 1986, Kandler and Weiss, 1986, Mundt, 1986, and Scardovi, 1986), (ii) own (medical) microbiological and metabolic work on short small bowel patients, (iii) own (most casuistic) experience with probiotics, and (iv) data on probiotics and prebiotics from modern literature. For a better understanding of probiotics our view on prebiotics is also included.

GENERAL ASPECTS OF PROBIOTICS

From the literature on food and nutrition it is carefully concluded that the use of lactobacilli-fermented dairy products that have been prepared from milk of cows, goats, sheep, reindeers or camels, for health-promoting effects is probably as old as the fermentative production of beer and wine. In many cultures this use has been carried over from mother to daughter till present time. The various commercial preparations contain either one pure strain of lactobacilli, lactococci, bifidobacteria, or yeasts, especially Saccharomyces boulardii or Saccharomyces cerevisiae (McFarland, 1996), as beneficial microorganisms, or mixtures of these microorganisms. Sometimes they include even streptococci and/or an Escherichia coli strain. The significance of streptococci and E. coli for health is not fully clear, since they belong to pathogenic bacterial groups.

Probiotics are used for preservation of food (milk, dairy products, grains, fish and meat), for prevention and/or treatment of infections such as vaginal infections, antibiotic-associated diarrhoea, traveller’s diarrhoea, and acute diarrhoea in children. Clinical doctors increasingly try to use probiotics for severe diseases, e.g., pancreatitis and liver diseases (Besseling et al., 2004, Solga and Diehl, 2004, Liu et al., 2004), but simultaneously just because of this multipurpose use many other clinical doctors regard probiotics as an incredible panacea.

As long as this image will not change, probiotics will not persuade, unless a crystal clear mechanism is presented. Therefore, first, the mechanism of action of probiotics has to be studied from the view that probiotic bacteria are primarily lactic acid bacteria. The precise mechanism is strongly determined by the specific properties that make these bacteria probiotics: the beneficial effects. Due to less advantageous properties of some lactic acid bacteria the real nature of various probiotic mechanisms will increasingly become clear. This did us especially pay attention to the direct effects of lactobacilli on both the food mass and on the intestinal environment.

NORMAL INTESTINAL FLORA

Our views have arisen from our studies on (the effect of) lactobacilli in the intestines of patients with the short small bowel (SSB) syndrome (Oh et al., 1979 and Vanderhoof et al., 1992), which appeared to constitute a very suitable human model system (Bongaerts et al., 1995 and Bongaerts et al., 1997-b). Our data indicate that faeces of SSB-patients contain 50-99% lactobacilli without anaerobic Bacteroides bacteria (Bongaerts et al., 1995 and Bongaerts et al., 1997-b) and that faecal pH may go down to 3.9 (Bongaerts et al., 2000-a). However, publication was not without problems, since (i) SSB-patients are a minor, rather unknown (“orphan”) group, and (ii) lactobacilli (Kandler and Weiss, 1986) are absolutely not known as pathogenic. In addition, our “very strange” results were initially not believed.

Times have changed and now the interest for our scientific explanations is strongly increasing. In the meanwhile, we have extrapolated SSB-results to new mechanistic concepts of probiotics (Bongaerts et al., 1997-a, Bongaerts and Severijnen, 2001-a and Bongaerts and Severijnen, 2001-b). Several new ideas have already been published as hypotheses (Bongaerts et al., 2005-b, Bongaerts and Severijnen, 2005-a, Bongaerts et al., 2006); many are derived from the host’s responses to basic, metabolic bacterial properties.

Anatomy and function of the gastro-intestinal tract

The main function of the gastro-intestinal tract is to take up important components from food. Therefore it contains three compartments: the stomach, the small bowel and the colon. The stomach is an organ that decontaminates the food mass by gastric juice-mediated low pH that is due to hydrochloric acid, in order to prevent microbial contamination in the small bowel. The most important part for uptake of nutrients from food is the small bowl, since in fact this is a “chemical extractor”. The colon is a container for storage of organic waste that has not been digested in the small bowel.

Appearance of intestinal microorganisms

Intestinal microorganisms do not appear at random in the gastro-intestinal tract. Their appearance is strongly determined by both physical intestinal properties, such as pH and (an) aerobicicity, and physiological properties, such as acid-tolerance, bile acid-resistance, and ‘metabolic pathways’ regarding the digestion of various substrates. The pH of the stomach (normally: pH 1-3) is too low to permit human bacteria to grow. Most bacteria present in the food are killed here. Only some acid-tolerant microorganisms survive this acid treatment and next they arrive in the small bowel as non-resident flora. The effect of the non-resident microorganisms (often <10^3 cfu/g acidic food mass) is often small as compared to the effect of the present, resident flora, unless very high concentrations of the non-resident microorganisms anyhow succeed in passing the stomach.

The first intestinal bacteria that start growing in the duodenum (pH: 2-4) are the acid-tolerant lactobacilli. The second group (at pH 4-5) are the enterococci, such as Enterococcus faecium and Enterococcus faecalis, and the third group (at pH 5-7; i.e., in the distal part) the enterobacteria, such as E. coli. During the passage through the small bowel the food mass that is mixed with the mentioned bacteria becomes strictly anaerobic. In the colon this food mass is mixed with many kinds of strictly anaerobic bacteria, such as bifidobacteria, peptostreptococci, eubacteria, propionibacteria, fusobacteria and bacteroides species.

Intestinal microbial concentrations and metabolic activities

The intestinal microbial metabolic activity depends on intestinal location, bacterial species, bacterial concentrations and diet. In the
acidic stomach with \(<10^3\) cfu/g food mass the microbial activity is absolutely negligible. In the small bowl the total microbial concentration increases from \(10^0\) cfu/g food mass in the proximal duodenum to \(10^2\) cfu/g in the distal ileum. Still the microbial activity in the small bowel is normally restricted. An enormous microbial activity is present in the colon with a total concentration of bacteria that increases from proximally \(10^0\) cfu/g food mass to \(10^{11}-10^{12}\) cfu/g feces. The colonic digestion of the human food waste is a cooperative microbial activity, i.e., with common use of extracellular enzymes that have been excreted in the food mass by the various bacteria. Especially butyric acid, a major waste product of the colonic flora and one of the short chain fatty acids, appears to be a beneficial nutrient for the colonocytes.

**Amount and character of intestinal waste**

The amount and character of the mainly organic waste depends on the quantitative and qualitative composition of the ingested food (for instance, few or many hardly digestible fibres), the production of human digestive enzymes, the uptake capacity of the small bowel, the peristalsis and the bacterial metabolic activity in the small bowel. The amount of waste products after intestinal digestion of a beefsteak is limited contrasting a bowl of muesli. In case of a rapid intestinal peristalsis non-digested food will prematurely arrive in the colon. A strongly increased bacterial metabolic activity in the small bowel may produce the same effect due to increased gas production. Especially an enormous gas production activates a rapid intestinal peristalsis and consequently only partly digested food, i.e., a nutrient-rich rather than nutrient-depleted food mass, will enter the colon.

**Bacterial products.** The massive bacterial colonic flora is metabolically very active. Important products are (i) short chain fatty acids (SCFAs), such as acetic acid and butyric acid, (ii) various vitamins, such as vitamin B-12, thiamine, riboflavin and pyridoxine, (iii) gases, such as \(\text{H}_2\), \(\text{CO}_2\) and \(\text{CH}_4\) that are often released as flatus, and (iv) stink, the product of bacteria that are able to generate anaerobically energy from \(\text{H}_2\) with \(\text{CO}_2\) as electron acceptor. The profile of the colonic \(\text{CH}_4\)-producers will especially concern reduction of the gaseous volume (see reaction).

\[
\begin{align*}
1\text{CO}_2 + 4\text{H}_2 & \rightarrow 1\text{CH}_4 (\pm 2\text{H}_2\text{O}) \\
5\text{gaseous molecules} & \rightarrow 1\text{gaseous molecule}
\end{align*}
\]

**DISTURBED GUT FLORA**

**Gut surgery as disturbing factor**

Gut surgery may disturb the normal flora. Removal of a major part of the colon will cause a substantial change of faecal flora, but this ought not to imply a strongly disturbed flora in the remaining gut, especially the small bowel. The more of the colon has been resected, the more the faecal flora will resemble the distal small bowel flora. This implies that the bacterial production of SCFAs will be limited.

Resection of a major part of the small bowel creates a quite different situation. Normally in SSB-patients over 70% of the small bowel is resected. Consequently, the gut flora in SSB-patients has changed thus that the faecal flora has even become characteristic (Bongaerts et al., 1997-b). This flora contains both a resident and a transient component. The resident flora is rather stable and contains especially lactobacilli \((10^9-10^{10}\) cfu/g faeces), but also E.coli \((10^8-10^9\) cfu/g faeces); the transient flora (normally rather low, but sometimes up to \(10^{11}\) cfu/g faeces) may contain various bacteria that may both enter the gut and even grow out, but that may not settle and therefore will leave the body within 3-5 days (Bongaerts et al., 1997-b).

**Human constitution as disturbing factor**

At a decreased production of gastric juice the gastrin acid barrier will not adequately function and consequently many bacteria, and even yeasts, will enter the gut. This may lead to disturbed intestinal flora. At decreased production of pancreatic juice the food mass will not be neutralised quickly enough and thus will favour the acidic conditions for lactobacilli. Also in all cases of decreased carbohydrate uptake in the small bowel, such as in celiac disease-patients, lactobacilli will be favoured. Note that similar situations may also be created therapeutically.

**Food as disturbing factor**

A nice example of the development of a food-mediated disturbed flora is the characteristic SSB-flora. In SSB-babies with a carbohydrate-rich diet a faecal flora with \(<1\%\) lactobacilli developed to a flora with about 70% lactobacilli within three weeks (Bongaerts et al., 1997-b).
As soon as oral feeding is stopped, the lactobacilli disappear from the faeces and consequently only a limited amount of enterococci and enterobacteria will reside. These latter bacteria will use intestinal mucus (i.e., glycoproteins) for growth and thus damage this defence mechanism.

Breastfeeding of healthy babies often implies a rather frequent supply of “liquid” lactose. A part of this feeding will soon enter the colon and may especially contribute to the propagation of bifidobacteria. These bacteria ferment the lactose with lactic acid, acetic acid and CO₂ as products and consequently the rapid stool will have an acidic smell.

**Antibiotics as disturbing factor**

At oral antibiotic therapy also many intestinal pathogens other than the target micro-organisms, are removed. Normally this implies killing or at least growth inhibition of various pathogenic bacterial species. Consequently, the nutrients for antibiotic-susceptible bacteria become available for just antibiotic-resistant micro-organisms, both bacteria and yeasts, and so these microorganisms will rapidly propagate.

In cases of antibiotic cocktail therapy, not only the chance of selection and propagation of yeasts is increased, but also of multi-resistant bacteria, such as Clostridium difficile, with as possible consequence antibiotic-associated diarrhoea, antibiotic-associated colitis or even pseudo-membranous colitis (Bongaerts and Lyerly, 1994 and Bongaerts and Lyerly, 1997).

**Significance of “disturbed flora”**

In spite of the foregoing the conception “disturbed flora” needs a critical reflection. The composition of intestinal flora depends on several factors that may vary, such as intestinal conditions, e.g., pH and (an)aerobicity, intestinal functions, e.g., production of gastric and pancreatic juice, and diet. As soon as one of these factors is strongly disturbed, the flora will adapt and the new flora is characterized as “disturbed flora”. In fact, this only means that the composition differs from the standard flora in healthy people and thus also that the quality of the disturbed flora, e.g., the capacity to produce various metabolites, may strongly vary. This ought absolutely not involve that the disturbed flora is a bad one! It has only adapted to the situation and may even be very advantageous for the patient, e.g., the capacity to decrease the pH value of the faeces. This is especially important in de novo born SSB-patients, where the flora is highly disturbed and has to adapt to new conditions. This has been demonstrated by us (Bongaerts et al., 2005-a).

**Definitions and preparations**

For a good understanding at least the conceptions “probiotics” and “prebiotics” should be defined. Probiotics are living non-pathogenic microorganisms that as food ingredients (supplements) beneficially affect host’s health. Prebiotics are food ingredients (supplements) that are non-digestible in the small bowel, and that beneficially affect host’s health due to its fermentation by bacteria in the colon. Sometimes probiotic preparations contain sugars, such as glucose or lactose, or polysaccharides, such as the fructose derivatives inulin and fructose oligosaccharides (FOS), that are all suitable to permit the probiotics to start their work immediately after intake. The combination is often called “symbiotic” (or “synbiotic”). However, others define synbiotics as combinations of probiotics and prebiotics; in this combination the prebiotic should be (fermentable) substrate for the probiotic. Since most probiotics are metabolically active in the small bowel, whereas prebiotics are metabolized in the colon, the definitions of synbiotics may be misleading. Therefore, we only use the conceptions “probiotic” and “prebiotic”.

Probiotic preparations can be supplied in fluid or semi-solid condition, mostly as yogurt-like preparation ready for use, or (after lyophilisation) as powder that must be revitalised by suspending in watery solution before use.

**Prebiotics**

Lactulose is a synthetic disaccharide that is not absorbable in the small bowel; it is an example of a prebiotic with effects on gut flora. The effects of lactulose may be explained as the result of bacterial fermentation in the colon, especially by the massively present anaerobic *Bacteroides* spp (normally about 10¹¹ - 10¹² cfu/g faeces). Here, lactulose is as easily fermented as glucose, sucrose and lactose in the small bowel. Fermentation of lactulose by bacteroides bacteria yields lactic acid, SCFAs, such as acetic and butyric acid, and gas, especially molecular hydrogen (H₂). These weak acids are excellent substrates for the colonocytes. Since a total daily dose of 10-20 g is small compared to 500 - 1000 g faeces/day, the impact on the faecal flora is limited. The production of H₂ in the colon is most important. Especially as a lot of faeces is distally present in the colon, the abdomen may become painfully extended due to gas that cannot escape. Because of the molar gas volume (22.4 L), a gas volume of about 1 L in the colon is created by only 0.04 mole or 0.08 g of the gaseous fermentation product H₂. If one H₂ molecule is produced from one monosaccharide moiety (i.e., two H₂ molecules from one lactulose molecule), this means that 1 L H₂ gas is produced from 7.0 g lactulose. Such massive volumes will cause flatulence and intestinal hurry and this removes massive amounts of colonic bacteria (Roth et al., 1957). During colonic stasis increased amounts of several toxic bacterial metabolites, e.g., ammonia from amino-acid degradation, are taken up from the intestine (Bongaerts et al., 2005-b). As soon as the stasis ends, the uptake of toxic
compounds immediately drops. Thus, the main effect of lactulose is a rapid stool due to the bacterial gas-mediated activation of intestinal peristalsis. The same applies to other low-molecular sugar-like compounds like lactitol and even to high-molecular polymeric carbohydrates, e.g., various fibres, as resistant starch, that are indigestible in the small bowel, whereas they are excellent substrates for the large amounts of colonic bacteria. A fundamental difference between low-molecular and high-molecular prebiotics concerns the moment of fermentation. The low-molecular prebiotics, e.g., lactulose and lactitol, are easily fermented as soon they enter the colon just like glucose should be, whereas the high-molecular prebiotics, e.g., polysaccharides like resistant starch and various fibres, are only fermented after they have been depolymerised, i.e., during the whole transit through the colon (Bongaerts et al., 2005-b).

The active probiotic agent

The active probiotic agent is a micro-organism that just as human beings, needs energy for life. All known probiotics belong to the groups of fermenting micro-organisms, i.e., bacteria and yeasts that need sugars for energy generation. Inside the strictly anaerobic gut even the probiotic yeasts with the capacity to generate very efficiently energy by oxidative phosphorylation, are only capable to generate energy by very inefficient fermentation. A very important common effect seems to be the fermentation of just non-absorbed sugars. With lactobacilli, lactococci or yeasts this occurs in the small bowel and with bifidobacteria in the colon. Under adequate conditions both lactic acid bacteria, such as lactobacilli, lactococci and bifidobacteria, and yeasts, such as S. cerevisiae and S. boulardii, are able to convert at a high rate each free glucose molecule to either two molecules of lactic acid (homolactic lactobacilli), or one molecule of lactic acid, one molecule ethanol and one molecule CO₂ (heterolactic lactobacilli), or two molecules ethanol and two molecules CO₂ (yeasts). This makes that the choice of the probiotic may contribute to the results of therapeutic courses.

The use of a probiotic implies that the effect depends on the amount of both the ingested probiotic organisms and the fermentable substrates, and on a suitable environment. Normally, the advised therapeutic dose of probiotic lactic acid bacteria will be about 10⁸ to 10¹⁰ viable micro-organisms. Lactobacilli are most acid-tolerant and therefore they thrive in an acidic environment unlike other intestinal micro-organisms. This implies that the acidic barrier of the stomach should be intact (i.e., no inhibition of gastric acid production or neutralization of gastric acid). Otherwise, they don’t need special precautions to pass the acidic gastric juice barrier. In contrast, yeasts must pass the stomach within acid-resistant capsules. In order to survive they should leave the capsules in the small bowel at a rather high pH. The complete intestinal passage of probiotics is retarded by the microvilli in the small bowel. To maintain intestinal presence of probiotic microorganisms rather frequent ingestion is necessary.

IMPORTANCE OF PROBIOTIC FERMENTATION

Fermentation-related effects

Sugar fermentation is a very important aspect of the probiotic mechanism of action. It concerns all probiotics, either lactic acid bacteria or yeasts. The primary effects concern the direct intestinal consequences of sugar fermentation and the secondary effect the indirect metabolic consequence as effected in the human body by hepatic gluconeogenesis.

Direct fermentation-mediated effects

The first two primary effects (Bongaerts et al., 2001-a) concern the massive fermentation of sugars in the acidic food mass by about 10⁻¹⁰ (or even more) ingested probiotic lactobacilli in the nearly sterile gut that normally contains only about 10¹ living bacteria/g wet food mass at the entrance of the duodenum. By this competitive action the concentration of small energy-rich substrate molecules remains small and consequently growth of other, less acid-tolerant, but more pathogenic, intestinal bacteria, e.g., enterococci and enterobacteria, will be retarded (first primary metabolic effect). The products of the fermentation process constitute the second (very closely related) primary metabolic effect. All lactobacilli produce lactic acid (Kandler and Weiss, 1986), and due to the massive fermentation with either 1 or 2 lactic acid molecules from each fermentable monosaccharide (e.g., glucose) a lower pH may arise that will contribute to growth inhibition of many micro-organisms.

Heterolactic lactobacilli (Kandler and Weiss, 1986) produce 1 mole of CO₂ from each fermentable monosaccharide. This implies a massive gas production that will soon cause an extended abdomen and next a severe expulsion of the food mass (Severijnen et al., 2005). The most severe effect will be seen with probiotic yeasts that produce even 2 moles CO₂ from each fermentable monosaccharide. Since homolactic lactobacilli (Kandler and Weiss, 1986) will produce only 2 moles of lactic acid (stronger pH effect) and no gas, they may contribute to intestinal stasis. The group of facultatively heterolactic lactobacilli (Kandler and Weiss, 1986) strongly resembles the homolactic lactobacilli, but produces also some CO₂ and thus permits a better intestinal movement.

The mentioned fermentative probiotic mechanisms of action can be used against many microbial inconveniences and diseases that manifest from the bowel. The therapeutic aim is to remove or to inactivate the causative intestinal flora. At oral therapy the probiotic flora is normally thus prominently present in the proximal part of the small bowel, that it controls the flow of the food mass till the pH becomes too high, i.e., thus high that enterococci will grow well. Because of availability of fermentable sugars in the small bowel and less in the colon it is concluded that most probiotics are primarily meant for interference in the small bowel. For the same reason use of probiotic bifidobacteria is thought very fruitful just in the colon of neonates with breastfeeding. The mechanism of action is essentially the same.

Hepatic gluconeogenesis and metabolic bypass

An important effect of probiotics is that through lactic acid production they activate hepatic gluconeogenesis. Consequently, they keep the systemic lactic acid load low. This is also the basis for a metabolic bypass (Bongaerts et al., 2006 and Bongaerts and Severijnen, 2005-b).

As the intestinal capacity for uptake of sugars is insufficient, too much sugar may reside in the bowel. Non-lactic acid bacteria may
consume this surplus and consequently cause several unwanted effects, such as extended abdomen, flatus and/or diarrhoea. In these patients probiotics may be advantageous by rapidly fermenting the intestinal glucose. However, to prevent acidification of cells, tissues and organs after intestinal uptake, the body needs an active hepatic gluconeogenesis. The price is that about 20% of the initial energy content of glucose is lost as heat in the gluconeogenesis, but the inconvenience is strongly reduced (Bongaerts et al., 2006). Note well that in this case for optimal energy intake probiotic gas production must be low.

**Lactose-intolerance**

In patients with lactase-intolerance lactose cannot be split by the intestinal enzyme lactase. Therefore, in these patients lactose has become a prebiotic, just as lactulose and lactitol. The profit of probiotics is that they can ferment lactose in the small bowel and thus prevent unwanted non-probiotic fermentation with enormous gas production. Therefore, especially in babies with lactase-insufficiency homolactic probiotics may be very healthy.

**Metabolic differentiation between probiotics**

With regard to the probiotic fermentation products, i.e., lactic acid, ethanol, and CO₂, theoretically five major groups of probiotics may be distinguished. (Bongaerts et al., 2005b) An essential differentiation within each of the three groups of lactobacilli is based on the production of D- and/or L-lactic acid (indicated with [D], [D+L], or [L], respectively). These groups are:

a. lactococci (Lactococcus lactis [L]) and the obligately homolactic lactobacilli (e.g., Lactobacillus salivarius [L] and Lactobacillus acidophilus [D+L]) (Kandler and Weiss, 1986) these bacteria produce only lactic acid (2 moles from 1 mole glucose) and no CO₂;

b. the facultatively heterolactic lactobacilli (e.g., Lactobacillus casei [L], Lactobacillus rhamnosus [L] and Lactobacillus plantarum [D+L]) (Kandler and Weiss, 1986); these bacteria produce mainly lactic acid (nearly 2 moles from 1 mole glucose) and only a small amount of CO₂;

c. the obligate heterolactic lactobacilli (e.g., Lactobacillus fermentum [D+L] and Lactobacillus bifermentans [D+L]) (Kandler and Weiss, 1986); these bacteria produce 1 mole lactic acid, 1 mole ethanol and 1 mole CO₂ from 1 mole glucose;

d. yeasts (e.g., S. boulardii): these microorganisms produce 2 moles ethanol and 2 moles CO₂ from 1 mole glucose;

e. the strictly anaerobic bifidobacteria, which are all obligate heterolactic (e.g., Bifidobacterium bifidum [L], Bifidobacterium longum [L] and Bifidobacterium infantis [L]) (Scardovi, 1986); these bacteria produce 2 moles L-lactic acid, 3 moles acetic acid and no CO₂ from 2 moles glucose.

The preferred intestinal niche of a. to d. is the small bowel. In the order from a. to d. the amount of lactic acid produced from 1 mole glucose decreases from 2 moles via 1 to 0 mole per fermented glucose molecule and the amount of CO₂ produced from 1 glucose molecule increases from 0 via 1 to 2. This implies that with highly active probiotics the acidity in the small bowel lumen will be highest with a. and the expulsive force (seen as diarrhoea) highest with d. The last group, the bifidobacteria (e.), is not mentioned in the comparison with a. to d., since these bacteria are known as colonic bacteria, but they still mostly resemble a. as regards the absence of ethanol and CO₂ production.

A closer differentiation of the groups a. to e. with regard to the production of lactic acid (D- and/or L-lactic acid) is important with regard to neonates and young children because of the toxic effects of D-lactic acid (Bongaerts et al., 1995). For these children the D-lactic acid-producing obligate homolactic lactobacilli, Lactobacillus bulgaricus and Lactobacillus lactis, should not be used. In adults this toxic effect of D-lactic acid is not observed because of the presence of the enzyme D-2-hydroxy-acid dehydrogenase, that converts D-lactic acid to pyruvic acid (Oh et al., 1979 and Yasuda et al., 1993).

**Therapeutic aspects**

What is required for a lactobacillus to be a good probiotic? Since ethanol and D-lactic acid are at least toxic substrates for neonates and young children, heterolactic lactobacilli and even the probiotic yeasts are not most favourable as universal probiotics. Homolactic lactobacilli may cause stasis since they produce only lactic acid. Therefore, they are also not most favourable. Normally, probiotic yeasts seem to be advisable only to remove the content of a diseased bowel (Berg et al., 1993 and Rodrigues et al., 1996). All together, from this approach we conclude that best universal probiotic lactobacilli should be facultatively heterolactic lactobacilli, that do not produce ethanol and/or D-lactic acid, i.e., L. casei and L. rhamnosus. However, since L. rhamnosus is most frequently, but still rather rarely, encountered in bacteriemias (Salminen et al., 2004), it is not the preferred number one. Similar L-lactic acid-producing homolactic lactobacilli (Kandler and Weiss, 1986), i.e., L. salivarius, we regard as second best, since they do not produce the CO₂ needed for intestinal motility. With the same argument regarding CO₂ production we qualify D- and L-lactic acid-producing facultatively heterolactics, like L. plantarum, as third best and homolactics, like L. acidophilus, as fourth best.

It is clear that the human requirements regarding probiotics are not always the same in various cases. For example, contrasting neonates and young children adults and older children are able to convert D-lactic acid just like L-lactic acid.

**NON-FERMENTATIVE MECHANISMS**

Beside the presented fermentation-related mechanisms also other non-fermentative mechanisms are known to occur. These are often probiotic-specific mechanisms that concern or might concern:

i. killing of other microorganisms by antibiotic-like compounds, such as the lantibiotic, nisin, i.e., an antimicrobial peptide compound that contains the unusual aminocacid lanthionine (Cheigh and Pyun, 2005);

ii. killing of microorganisms by bacteriocins, i.e., high-molecular antimicrobial proteins (Collado et al., 2005);
iii. (enzymatic) mechanisms that inactivate toxins, e.g., enzymatic inactivation of *C. difficile* toxins by *S. bouardii* (Castagliuolo, 1999);

iv. inactivation of microbial toxins by binding of the toxin (Gratz et al., 2004, Meriluoto et al., 2005 and Paton et al., 2006);

v. similar inactivation of viruses by binding of the virus;

vi. cross-reactive antibodies that are induced by cell wall components of probiotics, but that are also able in favour of phagocytosis to opsonise pathogenic microorganisms with identical cell wall epitopes (Mennink-Kersten et al., 2005);

vii. probiotic-mediated enzymatic deconjugation of human bile acids (De Smet et al., 1994 and Bongaerts et al., 2000-b).

All these mechanisms are not really new. Most of them (i-vi) may essentially contribute to the body’s defence. The mechanisms (i)-(v) concern immediate ecological mechanisms, but the mechanism (vi) concerns an immunological mechanism that may be long-lasting. The mechanisms (iv)-(vi) may explain why also killed probiotics may be beneficial. The mechanism (vii) is thought to have potentials for slim-lining (manuscript in preparation).

As explained above, lactic acid intestinally produced by probiotics may negatively affect the conditions for other microorganisms and induce the hepatic gluconeogenesis. Especially these mechanism together with the mechanisms (i)-(iii), and to a lesser extend also the mechanisms (iv) and (v) may explain why culture fluids of probiotics may also be beneficial shortly after consumption. It is not excluded that consumption of adequate culture fluids of probiotics may contribute to beneficial long term effects regarding the mechanisms (vi) and (vii).

**PROBIOTIC CONTRIBUTION TO HOST DEFENCE**

As explained, probiotics are able to remove bacteria. However, as they also inhibit growth of bacteria, at prolonged therapy they may also remove bacterial species, pathogens included, from the small bowel. Consequently, the diversity of the small bowel flora decreases and thus only a “microbial noise” of many bacteria resides. Therefore a pathogen-specific stimulation of the Peyer’s patches also decreases, but the body may be protected by the cross-reactivity of the anti-probiotic antibodies. This implies that the range of human antibodies will be less broad. In the case of an intestinal infection the variety of antibodies is thought to be rather small and therefore the specific production of adequate antibodies strongly increased.

Most probiotics hardly produce proteolytic and lipolytic activity and consequently the mucins that protect the bowel wall, are not damaged and thus even not the intestinal epithelial barrier (Russeeu-Van Embden et al., 1995). In addition, probiotics are less polluting than intestinal micro-organisms, since they do not produce exo- and endo-toxins and even not mutagenic compounds (Hardie, 1986, Kandler and Weiss, 1986, Mundt, 1986, and Scardovi, 1986).

To be short: due to fermentative activities and specific properties probiotics may both prevent inconvenience or disease, and let the diseased body recover.

**THERAPEUTIC ASPECTS OF PROBIOTIC MANIPULATION**

**General remarks**

Probiotic therapy requires enteral nutrition with a sufficient amount of fermentable carbohydrates. Excessive consumption of probiotic lactobacilli and a simultaneous extensive intestinal presence of (various) sugars, such as glucose, fructose, galactose, sucrose and lactose may lead to similar symptoms as in SSB-patients, *e.g.*, lactic acidosis with hyperventilation. Another consequence may be a decreased effectiveness of bile acids due to enzymatic deconjugation of bile acids by probiotic lactobacilli, and consequently a lower uptake of fat and other lipophilic compounds from food. This may cause a deficit of the lipophilic vitamins A, D, E and K in the body (Bongaerts et al., 2000-b).

**Non-clinical daily use**

Non-clinical daily use may be useful, especially for people with a lower intestinal sugar uptake rate. In this people the probiotics are thought to take away the energy-containing substrates before other more pathogenic bacteria can do so. In people with a normal or even rapid sugar uptake the fermentation rate will be (very) low and without any effect. Due to the mentioned growth inhibition of enterococci and enterobacteria, probiotics may also lower the production of carcinogenic compounds.

**Moment of probiotic intake**

Just as people, bacteria, and thus also probiotics, need energy for life and therefore also food. This makes that the best moment for probiotic intake is during the various meals, especially the main meals. This implies that the probiotics must be mixed thus with the food that they will optimally ferment.

Probiotics are often used during and after oral antibiotic therapy to permit the intestinal flora to recover. The probiotic effect during such an antibiotic therapy may be minimal, unless adequate antibiotic-resistant lactobacilli or yeasts are used. In order to confine the antibiotic damage of the intestinal flora it seems wise to take the probiotics during the meals and the antibiotics between successive meals.

**Monostrain probiotic or multispecies preparation**

Just as other bacteria probiotics may be either antibiotic-susceptible or -resistant. Probiotics with specific resistance regarding the antibiotic of treatment are seen as most ideal. If several different probiotics are present, the chance increases that not all probiotics are susceptible and thus that not all probiotics are killed. In other words: at this moment probiotic cocktail therapy is favoured as blind first therapy.

**Therapeutic dose**

Since intestinal fermentation is a kinetic process, not only the amount of suitable substrates is important, but also the number of active, fermenting cells, and even the condition of the cells. For a real clinical therapy very high amounts (about 10^{12} cfu/day) are...
recommended, whereas for non-clinical daily use a lower dosage (about $10^7$-$10^{10}$ cfu/day) will suffice. It should be kept in mind that probiotics need sugar for both activity and effect. In patients with completely parenteral feeding probiotic therapy is useless, and this applies also to intake many hours after a meal, e.g., at night just before going to sleep.

**Rational choices**

The nature of the micro-organism determines the fermentation products that are intestinally generated, and thus also the therapeutic course. These products are L-lactic acid, D-lactic acid, ethanol and CO$_2$. In patients with flatulence as complaint homolactic lactobacilli are preferred, since they produce only lactic acid and no CO$_2$. In young children and neonates with complaints due to D-lactic acid (such as acidosis with hyperventilation) especially homolactic L-lactic acid producing lactobacilli are preferred, and in patients with an alcohol-intolerance (especially people from South-East Asia) (Higuchi et al., 1994) homolactic lactobacilli. Patients with liver disease, e.g., liver cirrhosis, should especially take care with regard to the use of probiotic yeast and obligate heterolactic lactobacilli. From each mole glucose these probiotics produce 2 moles and 1 mole ethanol, respectively, with an adverse effect on liver function. By withholding fermentable substrates effects of probiotic gut flora can be stopped rather quickly. As the intestinal content must be removed, and this applies also to intake many hours after a meal, e.g., at night just before going to sleep.

**Clinical limitations**

Patients with insufficient neutralising capacity of the pancreatic juice or with celiac disease resemble SSB-patients. In patients with insufficient neutralising capacity of the pancreatic juice the conditions are optimal for growth of lactobacilli. Patients with celiac disease suffer from malabsorption due to the fact that the absorbing surface of the small bowel has strongly been reduced because of flattening erosion of the microvilli. In these patients conditions are optimal for lactobacilli as long as the gut has not yet sufficiently been recovered, and therefore the intestinal lactic acid production will strongly be increased. Especially in patients with a late-stage cancer the internal lactic acid load is permanently increased (Van Halteren et al., 2004). In conclusion, at least in these three categories every therapeutic use of probiotic lactobacilli may unnecessarily increase the acid load of the body.

**Lactic acid-intolerance**

For most people lactic acid bacteria, either lactobacilli, lactococci or bifidobacteria, are not a problem. Still some people cannot bear lactic acid and consequently also not lactic acid bacteria. In cancer patients with a high tumour-mediated lactic acid production additional lactic acid from probiotics may cause that patient will feel very miserable. A quite different situation may exist in a few people that are lactic acid-intolerant (Hommes et al., 1985, Tontonoz et al., 1995 and el-Maghrabi et al., 1995). They cannot bear sauerkraut and yogurt, i.e., fermented food products with high concentrations of lactic acid. Due to a mutation in the hepatic gluconeogenetic pathway the conversion of lactic acid to glucose is very slow and consequently also these people become miserable from probiotics.

**Risks**

Till now worldwide a low number of serious infections with lactobacilli or probiotic lactobacilli in the blood has been reported (Salminen et al., 2004). Still therapeutic consumption of probiotics seems to be less dangerous than use of rather innocent medicines as paracetamol and aspirin.

Probiotics are partly self-regulating since they can only ferment if a sufficiently high amount of sugar is present in the bowel. In a small bowel with a high sugar uptake capacity the intestinal concentration of sugars available for probiotics is normally low and consequently the probiotics cannot function. Therefore, regarding probiotics it may be said: try it, it does not harm.

**DISCUSSION**

Because of the probiotic mechanisms of action it is clear that probiotic bacteria and yeasts are absolutely not incredible panaceas. They are studied in gastroenterological inconveniences and disease, such as diarrhoea, IBS and IBD, acute pancreatitis, and further regarding food allergy, hepatic encephalopathy and antimicrobial activity. Use of probiotics means manipulation of intestinal ecology without dangerous resistances. It is important to recognize that probiotics do not replace antibiotics, but rather postpone antibiotic therapy. Individual probiotic therapy is thought to be a new clinical perspective. From this view probiotic mixtures are thought to be suitable for the first treatment. For not fully healthy people probiotics may behave as "microbial vitamins", since they may broaden the health range. Safety of probiotics seems not to be a real problem. Many advantages seem to overwhelm the sporadic disadvantages.

We think that the presented mechanisms and their consequences are the first steps to recognition regarding the credibility of probiotics as new clinical therapeutics. However, we do not pretend that the foregoing is the whole explanation, especially as it regards indirect immuno-modulation.

It is not excluded that in the future new strategies will be developed. In the past we have already presented ideas that had been derived from the first fermentative mechanisms (Bongaerts and Severijnen, 2001-a). By extrapolation we deduced therapeutic possibilities for both acidic solutions with lactic acid or even acetic acid that are free of micro-organisms, and even for their salts, the lactate and acetate salts, since these compounds can not be metabolized further by anaerobic bacteria. In addition, the non-fermentative mechanisms may be separated from the probiotic organisms, and thus they offer quite new, non-toxic therapeutic perspectives. Future development will imply that probiotics become genetically modified in order to harbour new enzymatic machineries for therapeutic aims (Paton et al., 2005 and Paton et al., 2006).

**REFERENCES**


