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Food Research International 35 (2002) 109–116

FOOD
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# Introduction to pre- and probiotics

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Received 15 May 2001; accepted 31 July 2001

**Abstract**

During the last 2–3 decades, attempts for improving the human health status, are focusing on ways for modulating the indigenous intestinal flora by live microbial adjuncts, now called ''probiotics''. Comprising ca. 65% of the functional food world market, probiotics represent the major and still growing segment of this huge market, estimated to exceed a total volume of US $ 75 billion. The most typical active components of probiotic products are lactic acid bacteria, including bifidobacteria, lactobacilli and enterococci. Health claims related to probiotics are numerous, but include maintenance of normal/healthy intestinal flora and protection against infections, alleviation of lactose intolerance, and stimulation of the immune system. Strains with proven beneficial effects may be consumed in relatively high numbers in products, and are collectively called *probiotics*. In addition. bifidobacteria and lactobacilli, typical inhabitants of the human GIT, are considered beneficial, and may be stimulated by non-digestible food ingredients such as oligosaccharides, collectively called *prebiotics*. Probably aimed at the two ''target regions'' of the GIT, pre- and probiotics may be combined in a food product, called a *synbiotic*. Confirmed health claims are discussed and reference will be made to open questions and research challenges. 

*Keywords:* Probiotic; Prebiotic; Synbiotic; Gastro-intestinal tract; Gut microbial ecology

## 1. Introduction

Intensified research efforts in recent years confirm the major importance of the microbial population of the gastro-intestinal tract (GIT). Indeed, a beneficial association of ''lactic acid producing'' microorganisms with the human host has been suggested more than 100 years ago by Döderlein (1892) for vaginal bacteria, and, more particularly for lactic acid bacteria (LAB) in gut ecology studies conducted by Moro (1900), Beijerinck (1901), and Cahn (1901). The LAB associated with fermented milk products, were advocated by Metchnikoff (1908) for their health benefits. He suggested the longevity of the Caucasians to be related to the high intake of fermented milk products. Although Metchnikoff viewed gut microbes as detrimental rather than beneficial to human health, he considered substitution of gut microbes by yoghurt bacteria to be beneficial.

It was probably Vergio (1954) who first introduced the term ''probiotic'', when he compared in his manuscript ''Anti- und Probiotika'' the detrimental effects of antibiotics and other antimicrobial substances on the gut microbial population, with factors (''Probiotika'') favourable to the gut microflora. Lilly and Stillwell (1965) referred to probiotics as ''...microorganisms promoting the growth of other microorganisms''. Presently, there is general agreement that a ''probiotic'' refers to viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the GIT (Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001; Holzapfel, Haberer, Snel, Schillinger, & Huis in't Veld, 1998). Such microorganisms may not necessarily be constant inhabitants of the GIT, but they should have a beneficial effect on the general and health status of man and animal (Fuller, 1989; Havenaar, Ten Brink, & Huis in't Veld, 1992; Salminen, Deighton, Benno, & Gorbach, 1998). The prebiotic concept is based on the assumption that particular gut (colon) flora such as bifidobacteria and lactobacilli considered beneficial to human health, may be selectively stimulated by undigestible but fermentable

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0963-9969/02/$ - see front matter 
PII: S0963-9969(01)00171-5

dietary carbohydrates (Cummings, Macfarlane, & Englyst, 2001). In order to understand the function and potential contribution of pre- and probiotics towards health and well-being of the human host, in-depth knowledge of the GIT as ecosystem is required.

## 2. The gastro-intestinal tract (GIT) as an ecosystem

The gastrointestinal tract represents an ecosystem of the highest complexity. The mucosal surface provides a large area for the adherence to and microbial colonisation of the small intestine. When compared to ca. 2 $m^2$ skin surface of our body, the area of our GI system, calculated to be 150–200 $m^2$, is huge (Waldeck, 1990). A three-fold increase in the surface area is accomplished by circular folds, 7–10-fold by folding of the epithelium (intestinal villi) and 15–40-fold by the formation of microvilli in the enterocyte resorptive luminal membrane. Thereby the necessary space for interactions during the digestive process and for adhesion to the mucosal wall and concomitant colonisation is provided.

In spite of rapid research advances in gut microbial ecology, our understanding of this complex ecosystem and the microbial interactions is still limited. The GIT of the average human adult is colonised by approximately $10^{14}$ microbial cells (Luckey & Floch, 1972), about 10 times more than all tissue cells of the body taken together. This immense metabolic potential suggests strong regulatory effects on body functions, especially in the colon where the largest concentration of up to $5\times10^{11}$ bacterial cells per g is found. Representing more than 400 species, these ''autochthonous'' microorganisms include diverse bacterial genera, of which the Gram-positive, anaerobic genera *Bacteroides*, *Eubacterium* and *Bifidobacterium*, predominate in the densely populated large intestine. Other groups such as the clostridia, peptostreptococci, ''streptococci'' and lactobacilli also seem to play an important role, e.g. in the maintenance of a stable gut mucosa, and in the generation of short chain fatty acids (SCFA) in a beneficial ratio.

Although the faecal flora appears to be a good qualitative indicator of the distal colonic microflora, it does not reflect the intestinal flora as a whole, and certainly not that of the small intestine. The diversity of the gut flora therefore varies from segment to segment, and, in addition, is determined by factors such as the diet, the genetic background and the physiological state of the host. Stability in species composition may be a feature of the ''normal'' microflora of the host, whilst bacterial strains within the population may be less stable (McCartney, Wang, & Tannock, 1996). Former observations have been based on data generated by cultivation procedures, and have not taken account of non-culturable bacteria. These may comprise important groups of which growth requirements are either not known, or which are in a dormant state. Using genetic fingerprinting techniques, Tannock (1997) showed a ''collection'' of *Bifidobacterium* and *Lactobacillus* strains to be unique of each human. It was also suggested that the composition of these populations may remain relatively constant for some individuals and may fluctuate considerably for others. Application of FISH with group-specific 16S rRNA-targeted oligonucleotide probes enabled Welling et al. (2000) to detect variations in bifidobacterial populations in the faeces of different age groups. Depending on the age group, the percentage of bifidobacteria in the faeces ranged from 0 to 78.9%, whilst also large variations were found within each group. Using denaturing gradient gel electrophoresis (DGGE), banding patterns of humans have been found to differ significantly from those of other mammals. In addition, based on 16S rDNA sequences, three bacterial species, *Ruminococcus obeum*, *Eubacterium halii* and *Fusobacterium prausnitzii* were shown to be probably ubiquitous to humans and were therefore suggested to play an important role in the human GIT (Akkermans, Zoetendal, Favier, Heilig, Akkermans-Van Vliet, & De Vos, 2000).

The key role of gut microorganisms in human health was generally overlooked for a long time, and the main focus was placed on enteric pathogens and factors leading to gastro-intestinal disorders or ''dysbiosis''. A healthy intestinal epithelium, in association with an optimal intestinal flora, provides a vital barrier against the invasion or uptake of pathogenic microorganisms, antigens and harmful compounds from the gut lumen. Also, the intestinal mucosa efficiently assimilates antigens, whilst specific immune responses are evoked by the specialised antigen transport mechanisms in the villus epithelium and Peyer's patches (Heyman, Ducroc, Desjeux, & Morgat, 1982). A stable barrier, typical of healthy individuals, ensures host protection and serves as support for normal intestinal function and immunological resistance. Considered to be the largest ''immune organ'' in the human body, the barriers provided by the gut-associated lymphoid tissues (GALT), serve for intrinsic protection against infective agents. In the small bowel, ca. 80% ($10^{10}$) of all immunoglobulin producing cells are found (Shanahan, 1994), whilst the gut flora as such is essential for mucosal immune stimulation and amplification of immunocompetent cells. The following may be considered as the major physiological functions of the gut microflora (Holzapfel et al., 1998):

- barrier function/restoration
- immune system stimulation
- maintenance of mucosa nutrition and circulation
- production of nutrient/improved bioavailability
- stimulation of bowel motility.

## 3. Probiotics

### 3.1. Importance of the LAB

LAB strains are the major representatives of probiotics, both in the food and pharmaceutical market. The LAB are associated with various habitats, particularly those rich in nutrients such as various food substrates and plant materials, which they are able to ferment or spoil. Other habitats include soil, water, manure, sewage, and silage. Some LAB strains inhabit the human oral cavity, the intestinal tract, and vagina and may beneficially influence these human ecosystems. This explains why they are considered as "ideal" candidates for application as probiotics. In their pioneering work, Reuter and coworkers (Reuter, 1965a, 1965b, 1969) have described the typical lactobacilli associated with the human GIT. Based on their precise documentations, it may be assumed that homofermentative lactobacilli typical of the human host are represented by three groups, i.e. the "*Lactobacillus acidophilus* group" (mainly with strains of *L. acidophilus*, *Lactobacillus gasseri*, *Lactobacillus crispatus* and *Lactobacillus johnsonii*), and the "*Lactobacillus salivarius*", and the "*Lactobacillus casei*" groups. The latter involves strains of *Lactobacillus paracasei*, *Lactobacillus zeae* and *Lactobacillus rhamnosus*. In addition, Reuter and coworkers also identified *Lactobacillus reuteri* and also *Lactobacillus fermentum* as the major heterofermentative lactobacilli associated with the human GIT.

### 3.2. Commercial strains and applications

Viable probiotic strains are supplied in the market either as fermented food commodities or in lyophilised form, both as supplements and as pharmaceutical preparations. The "yoghurt-type" products are prepared primarily with strains of *L. acidophilus*, *L. crispatus*, *L. johnsonii*, *L. casei*/*paracasei* and *Bifidobacterium* spp. (Table 1). The longest history of proved health benefits and "safe-use" may probably be documented for *L. casei* strain "Shirota" (Shirota, Aso, & Iwabuchi, 1966), and some strains of the *L. acidophilus* group. The functional properties and safety of particular strains of *L. casei*, *L. rhamnosus*, *L. acidophilus* and *L. johnsonii* have extensively been studied and well documented (Salminen et al., 1998).

Since at least 40 years in Japan and more than 20 years in Germany, LAB cultures of human origin are applied in the manufacture of fermented milk products. Viable strains of especially "*L. acidophilus*" and *Bifidobacterium bifidum* were introduced in Germany during the late 1960s into dairy products because of their expected adaptation to the intestine and the sensory benefits for producing mildly acidified yoghurts (Schuler-Malyoth, Ruppert, & Müller, 1968). In Germany, such products first became known as mild yoghurts or "bioyoghurts", whilst in the USA, acidophilus milk was developed.

In a survey by Schillinger (1999) on various mild yoghurts and novel-type probiotic yoghurt-type dairy products, 26 *Lactobacillus* strains were isolated and identified by DNA hybridisation methods. The species present were found to be *L. acidophilus*, *L. johnsonii*, *L. crispatus*, *L. casei*, *L. paracasei* and *L. rhamnosus*. These identifications revealed that some strains had been misclassified. Three strains designated as *L. acidophilus* (*L. acidophilus* LA-1, *L. acidophilus* ATCC 43121 and the *Lactobacillus* strain from Biogarde® culture) were found to belong to *L. johnsonii*, and *L. acidophilus* L1 to be *L. crispatus*. Strains designated as *L. casei* were shown to be members of either *L. casei*, *L. paracasei* or *L. rhamnosus*. Viable numbers of lactobacilli in mild and probiotic yoghurts varied greatly, whilst a few products contained only low *Lactobacillus* numbers (Schillinger, 1999). This was followed up by a recent study

Table 1
Microbial species from which strains find application in probiotic products (Holzapfel et al., 1998, modified)

| *Lactobacillus* species | *Bifidobacterium* species | Other LAB | "Non-lactics"[a] |
|---|---|---|---|
| *L. acidophilus* | *B. adolescentis* | *Ent. faecalis*[b] | *Bacillus cereus* (toyoi ≅)[b] |
| *L. amylovorus* | *B. animalis* | *Ent. faecium* | *Escherichia coli* Nissle, 1917 ≅) |
| *(L. casei)* | *B. bifidum* | *Sporolactobacillus inulinus*[b] | *Propionibacterium freudenreichii*[b] |
| *L. crispatus* | *B. breve* | | *Saccharomyces cerevisiae* (boulardii ≅) |
| *L. gallinarum*[b] | *B. infantis* | | |
| *L. gasseri* | *B. lactis*[c] | | |
| *L. johnsonii* | *B. longum* | | |
| *L. paracasei* | | | |
| *L. plantarum* | | | |
| *L. reuteri* | | | |
| *L. rhamnosus* | | | |

[a] Mainly as pharmaceutical preparations.
[b] Mainly applied for animals.
[c] Probably synonymous with *B. animalis*.

which, once more, showed the identity given for strains in some products to differ from that found by DNA homology studies (Table 2). Moreover, in two products the viable numbers of lactobacilli were $<10^5$/ml. This again reflects on a major issue regarding the "minimal effective dose" of viable bacteria by which scientifically confirmed beneficial effects may be expected.

### 3.3. Functional aspects

Several beneficial functions have been suggested for probiotic bacteria (Holzapfel et al., 1998) , e.g.:

- nutritional benefits:
- vitamin production, availability of minerals and trace elements
- production of important digestive enzymes (e.g. β-galactosidase)
- barrier/restoration effects:
  infectious diarrhoea (traveller's diarrhoea, children's acute viral diarrhoea)
  antibiotic-associated diarrhoea, irradiation-associated diarrhoea
- cholesterol lowering effects
- stimulation of the immune system
- enhancement of bowel motility/relief from constipation
- adherence and colonisation resistance, and
- maintenance of mucosal integrity.

Some of these effects (e.g. cholesterol-lowering effects) are yet to be substantiated by well-controlled clinical trials. On the other hand, strain specific effects of probiotic lactic cultures on the human immune system and on diarrhoea are well documented, e.g. for counteracting rotavirus or antibiotic associated diarrhoea (Table 3) using strains such as the LGG strain of *L. rhamnosus* and the Shirota strain of *L. casei* (*L. paracasei*; Marteau, De Vrese, Cellier, & Schrezenmeir, 2001; Salminen, 1996; Salminen, Isolauri, & Salminen, 1996). In attempts to clarify some of the underlying mechanisms, research is focusing, amongst others, on adhesive and immunomodulating properties of effective strains (Salminen & Tuomola, 1998).

It appears that adhering probiotic strains transiently may colonise the GIT, and thereby cause an increase in IgA levels (Schiffrin, Rochat, Link-Amster, Aeschlimann, & Donnet-Hughes, 1995; Tanaka, 1996). This has been shown to result in enhancement of serum IgA response to pathogens such as attentuated *Salmonella typhi* Ty21a (Collins, Thornton, & Sullivan, 1998). It appears that many probiotic effects are mediated through immune regulation, and especially through balance control of pro-inflammatory and anti-inflammatory cytokines, thereby suggesting the use of probiotics as innovative tools to alleviate intestinal inflammation, normalise gut mucosal dysfunction, and down-regulate hypersensitivity (Isolauri, Sütas, Kankaanpää, Arvillomi, & Salminen, 2001). Ideally,

Table 2
Information on various probiotic yoghurts or yoghurt-like products in the German market, and identification of *Lactobacillus* strains isolated from retail products before the expiry date

| Manufacturer | Lactobacilli cfu's/g of yoghurt | Identification by DNA Homology | Information of manufacturer |
|---|---|---|---|
| 1 | $4\times10^6$ | *L. acidophilus* | No indication |
| 2 | $1.5\times10^8$ | *L. acidophilus* | *L. acidophilus* LA7 |
| 3 | $7\times10^2$ | *L. johnsonii* | BIOGARDE cultures |
| | $1.5\times10^3$ | *L. paracasei* | *L. casei* |
| 4 | $7\times10^4$ | *L. paracasei* | *L. casei* |
| | $3.5\times10^4$ | *L. delbrueckii* subsp. *bulgaricus* | *L. acidophilus* |
| 5 | $1\times10^7$ | *L. acidophilus* | No indication |
| 6 | $4\times10^8$ | *L. paracasei* | *L. casei* |
| 7 | $4\times10^7$ | *L. johnsonii* | *L. johnsonii* |
| | $4\times10^6$ | *L. helveticus* | *L. helveticus* |
| 8 | $4\times10^8$ | *L. paracasei* | *L. casei* |
| 9 | $1.5\times10^7$ | *L. paracasei* | No indication on the lactobacilli |
| 10 | $4.5\times10^6$ | *L. paracasei* | LGG |
| | $2\times10^4$ | *L. delbrueckii* subsp. *bulgaricus* | |
| 11 | $5\times10^7$ | *L. paracasei* | *L. casei* |
| | $1.5\times10^4$ | *L. johnsonii* | |
| 12 | $1.5\times10^8$ | *L. acidophilus* | *L. acidophilus* LA5 |
| 13 | $3\times10^6$ | *L. paracasei* | *L. casei* |
| | $1\times10^7$ | *L. acidophilus* | *L. acidophilus* |
| 14 | $4\times10^7$ | *L. acidophilus* | LA 5-cultures |
| 15 | $2\times10^7$ | *L. acidophilus* | *Acidophilus* "group" |

immune stimulation by probiotic strains would appear to be based on transient or longer term colonisation through adhesion and aggregation without invasion (Collins et al., 1998).

In practice, direct health-related claims for foods are not allowed in the EU. A major step towards clarification of this controversial issue has been achieved through a decision by the high court of Hamburg (''Hanseatisches Oberlandesgericht'') which issued approval of claims on the ''stimulation'' of the immune system by a food (Hans. OLG Hamburg, Urteil vom 30.11.200—3 U 86/00; ZLR I/2001, p. 147).

Focus on cancer prevention by probiotic cultures coincides to some extent on immune stimulation and modulatory effects. Antiproliferative effects, also in relation to antigenotoxic and antimutagenic activities, seem to be strain specific (Rafter, 1995), and deserve attention.

### 3.4. Selection of suitable strains

A continued increase is observed in the range even of non-dairy probiotic food products such as fermented meats and vegetable and fruit juices. When taking into account the wide range of potential (fermentable) substrates, and the different conditions under which LAB stains may be challenged for ''functional performance'', it is clear that the selection of new strains provides an exciting challenge both to science and industry. However, even considering that probiotic microorganisms are claimed to promote health, the mechanisms involved have not been fully elucidated yet. Approaches for selection of an ''ideal'' strain are therefore still difficult and indeed require considerable resources. Desirable technical features, and factors related to health promotion or health sustaining, serve as important criteria for strain selection (Holzapfel et al., 1998). It is generally considered now that three major categories should be taken into account as key criteria for selection of an appropriate strain:

- general aspects, including origin, identity, safety, and resistance (e.g. to mutations and environmental stress, and to the antimicrobial factors prevailing in the upper GIT),
- technical aspects (growth properties in vitro and during processing, survival and viability during transport and storage), and
- functional aspects, and beneficial features.

The safety and non-pathogenicity of a new strain is considered of major importance. The assessment and proof of a ''safe'' or ''GRAS'' strain, without a previous ''history of safe use,'' has been the topic of controversial discussions in recent years. Approaches for assessing the safety of probiotic and starter strains have been recommended by Salminen, Von Wright, Ouwehand, and Holzapfel (2000), and imply the following:

- characterisation of the genus, species and strain and its origin which will provide an initial indi-

Table 3
Randomised controlled trials showing a significant therapeutic effect of probiotics to shorten the duration of acute gastroenteritis

| Objective | Probiotic | Study population | *n* |
|---|---|---|---|
| *Curative treatment* | | | |
| Rotavirus-associated diarrhoea | *Lactobacillus rhamnosus* strain GG | Infants | 71 |
| | *L. rhamnosus* strain GG | Infants | 39 |
| | *L. rhamnosus* strain GG | Infants | 49 |
| | *L. rhamnosus* strain GG | Infants | 42 |
| | *Lactobacillus casei* strain Shirota | Infants | 32 |
| Gastroenteritis | *L. rhamnosus* strain GG | Infants | 32 |
| | *L. rhamnosus* strain GG | Infants | 26 |
| | *L. rhamnosus* strain GG | Infants | 100 |
| | *L. rhamnosus* strain GG | Infants | 123 |
| | *L. rhamnosus* strain GG | Infants | 287 |
| | *Enterococcus faecium* SF68 | | 104 |
| | *E. faecium* SF68 | Adults | 56 |
| | *E. faecium* SF68 | Adults | 78 |
| | *E. faecium* SF68 | Adults | 211 |
| | *Saccharomyces boulardii* | Infants | 38 |
| | *Lactobacillus reuteri* | Infants | 66 |
| *Prevention* | | | |
| Acute diarrhoea or rotavirus | *Bifidobacterium bifidum* and *Streptococcus thermophilus* | Infants | 55 |

Cited by Marteau et al. (2001) from various papers (modified).

cation of the presumed safety in relation to known probiotic and starter strains,
- studies on the intrinsic properties of each specific strain and its potential virulence factors,
- studies on adherence, invasion potential and the pharmacokinetics of the strain, and
- studies into interactions between the strain, intestinal and mucosal microflora, and the host.

Still, there appears to be no indication that the general public is at risk from the consumption of lactobacilli or bifidobacteria used as probiotics or starters (Salminen et al., 2000).

## 4. Pre- and synbiotics

Some undigestible but fermentable dietary carbohydrates may selectively stimulate certain bacterial groups resident in the colon such as bifidobacteria, lactobacilli and eubacteria, considered beneficial for the human host, and are collectively called *prebiotics*. Such resistant short-chain carbohydrates (SCC) are also referred to as nondigestible oligosaccharides (Cummings et al., 2001), or low-digestible carbohydrates (LDCs; Marteau & Flourié, 2001). These SCC or LDCs provide interesting possibilities for inclusion into conventional food products for their "bifidogenic" effects. Several such preparations are presently under consideration by the industry for application (Table 4). Inulin and fructo-oligosaccharides are probably the most commonly used prebiotics; several typical probiotics contain either of these oligosaccharides, thereby comprising a "synbiotic" (see later). It is important that these substances reach the caecum where they should be fermented and be well tolerated. However, some dose-related undesirable effects, due to osmotic potential and/or excessive fermentation, may occur, e.g. excessive flatus, bloating, abdominal cramps and even diarrhoea. Although dose-related intolerance symptoms may occur after ingestion of LDCs, the dose of intolerance generally appears to be high, thereby allowing a relatively broad "therapeutic window", i.e. the dose above the minimal effective level (Collins et al., 1998).

There is, however, general agreement on the major beneficial effects of prebiotics, also related to the small bowel with regard to (e.g.) desired influence on sugar digestion and absorption, glucose and lipid metabolism and protection against known risk factors of cardiovascular disease (Scheppach, Luehrs, & Menzel, 2001). In the colon, the fermentative production of short-chain fatty acids is considered a major beneficial feature related to the primary prevention of colorectal cancer (Scheppach et al., 2001).

Confirmed effects/aspects with regard to prebiotics are:

- non-digestible and low energy value ($<9$ kJ/g)
- increase in stool volume
- modulation of the colonic flora by:
  stimulation of beneficial bacteria (*Bifidobacterium*, *Lactobacillus* and *Eubacterium* spp.)
  inhibition of "undesirable" bacteria (*Clostridium* and *Bacteroides*)

Postulated effects that have not been finally confirmed, include the following:

- prevention of intestinal infections
- modulation of the immune response
- prevention of colorectal cancer
- reduction of the serum cholesterol level
- improved bio-availability.

Sufficient strong indications suggest that LDCs may play a role in the maintenance of the human GIT (Scheppach et al., 2001); however, this issue as a whole deserves further attention.

A *synbiotic* refers to a product in which a probiotic and a prebiotic are combined. The synbiotic effect may be directed towards two different "target regions" of the GIT: i.e. both the small and the large intestines. In addition, if the prebiotic carbohydrate is utilised by a probiotic strain, its growth and proliferation in the gut will be selectively promoted.

The combination of a pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own. An enhanced reduction was, for example, shown for the number of colonic aberrant crypt foci (ACF; Rowland, Rumney, Coutts, & Lievense, 1998) and for colon carcinogenesis in rats (Gallaher & Khil, 1999).

## 5. Conclusions

The important role of the intestinal flora in the maintenance of health and in the prevention of disease is well recognised. Its continuous interaction or "communication" with the environment, the central nervous system, the endocrine system and the immune system (Shanahan, 1997; Umesaki, Okada, Imaoka, Setoyama, & Matsumoto, 1997; Wang, Whetsell, & Klein, 1997) indicates the complex underlying mechanisms. Disturbance of this delicate balance may lead to other disorders and thus facilitate establishing a state of disease (Holzapfel et al., 1998). Moreover, this explains the tremendous challenges with regard to the presentation of

Table 4
Composition (given in % by weight) of some candidate prebiotics available for human consumption (Cummings et al., 2001; modified)[a]

| Product name | Commercialisation envisaged by | Dry Matter | Ara | Xyl | Man | Gal | Glc | Total |
|---|---|---|---|---|---|---|---|---|
| Fructooligosaccharide | Suntory, Japan | 94.0 | – | – | 34.0 | 0.2 | 53.3 | 87.4±1.3 |
| Isomaltooligosaccharide | Showa Sangyo, Japan | 77.8 | – | – | – | – | 29.8 | 29.8±0.5 |
| Oligomate | Yakult, Japan | 74.9 | 0.1 | – | 0.8 | 18.6 | 22.7 | 42.2±2.5 |
| Palatinose | Südzucker, Germany | 92.6 | 0.1 | – | 10.5 | – | 35.7 | 46.3±2.8 |
| Polydextrose | – | 89.8 | – | – | 0.3 | 1.9 | 36.5 | 38.7±2.6 |
| Pyrodextrin | Matsutani, Japan | 94.3 | – | – | – | 0.2 | 18.8 | 20.0±0.5 |
| Raftiline | Orafti, Belgium | 93.3 | – | 0.1 | 34.7 | 0.8 | 50.2 | 85.8±3.4 |
| Soybean oligosaccharide | Calpis, Japan | 76.5 | – | 0.1 | 7.5 | 8.4 | 15.6 | 32.3±3.9 |
| Xylooligosaccharide | Suntory, Japan | 94.9 | 0.8 | 25.9 | 0.6 | – | 1.6 | 29.4±1.3 |

[a] Recovery of fructose was measured as mannose (man) and glucose (Glc). Total values are the mean±S.D. of three samples. Ara, arabinose; Xyl, xylose; Gal, galactose.

scientifically confirmed evidence of "functional" effects related to pre- and probiotics. It has particularly been suggested that microbiologists should play a major role in isolating strains and testing mechanisms of action, and in "packaging these into reliable products for human use" (Potera, 1999).

Impressing progress has been made in recent years in the development and validation of in vivo and in vitro test models, and in the conduction of placebo-controlled, double-blind clinical studies, by which a substantial number of functional effects could be verified. Yet, both for pre- and probiotics, a number of postulated effects still need to be confirmed.

For prebiotics, studies may particularly be directed towards open questions regarding:

- the influence on blood serum cholesterol values,
- the role of some dominant but hardly studied bacterial groups in the colon (*Fusobacterium*, *Eubacterium*, *Veillonella*, *Peptostreptococcus*, etc.),
- the influence of composition of the colonic microbial population on a "favourable" ratio of SCFAs, and
- mechanisms for the prevention of intestinal functional disturbances.

Pre- and probiotics most probably have different "target" regions. With the focus mainly on the small intestines, in vivo studies on the colonisation and interactions of probiotics with the gut mucosa, constitute both a challenging and complex area for investigations. For a "safety and acceptability record" of a probiotic strain, experience and history are still important. In spite of an explosion in recent years of publications dealing with probiotic organisms, both by clinicians, microbiologists, food scientists and nutritionists, vital information is still needed. More novel methods should be developed to monitor changes in the composition of the intestinal flora and their mutual interaction with the host's immunological functions and metabolism.

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