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Probiotics: an overview of beneficial effects

Arthur C. Ouwehand[1,*], Seppo Salminen[1] & Erika Isolauri[2]
[1]*Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland;* [2]*Department of Paediatrics, Turku University Central Hospital, FIN-20500 Turku, Finland (*Author for correspondence; Tel.: +358-2-333-6894; Fax: +358-2-333-6860; E-mail: arthur.ouwehand@utu.fi)*

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Abstract

Food products fermented by lactic acid bacteria have long been used for their proposed health promoting properties. In recent years, selected probiotic strains have been thoroughly investigated for specific health effects. Properties like relief of lactose intolerance symptoms and shortening of rotavirus diarrhoea are now widely accepted for selected probiotics. Some areas, such as the treatment and prevention of atopy hold great promise. However, many proposed health effects still need additional investigation. In particular the potential benefits for the healthy consumer, the main market for probiotic products, requires more attention. Also, the potential use of probiotics outside the gastrointestinal tract deserves to be explored further. Results from well conducted clinical studies will expand and increase the acceptance of probiotics for the treatment and prevention of selected diseases.

Abbreviations: IBD – Inflammatory bowel disease; CD – Crohn's disease; UC – Ulcerative colitis

Introduction

The development of probiotics during the past decade has signalled an important advance in the food industry transferring to towards the development of functional foods. The term probiotic, popularised by R. Fuller in 1989, was defined recently by an Expert Committee as 'Living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition' (Guarner & Schaafsma 1998). Such a definition does not require changes in intestinal microflora or so-called 'colonisation' or temporary colonisation of the human gastrointestinal tract as the probiotic organism can exert its effects locally or during transient passage through the gastrointestinal system. This definition, however, still sets the requirements that the micro-organisms must be alive, not pasteurised or otherwise inactivated. Although specific numbers are not mentioned in the definition, generally it is thought that at least 10^9 colony forming units per day need to be ingested. Health benefits must be scientifically established by clinical studies in humans performed by several independent research groups and published in peer-reviewed journals.

The definition may be changing little by little as especially Japanese scientists have shown that also inactivated probiotic micro-organisms or their cell structures may have beneficial effects on human health. This has led to new definitions of probiotics and may change the way we look at probiotics in the future (Lee et al. 1999; Salminen et al. 1999).

Micro-organisms used as probiotics

Microbes from many different genera are being used as probiotics (Table 1). The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; lactobacilli, enterococci and bifidobacteria. In particular lactobacilli are generally used as probiotics. This may have historical reasons since Metchnikoff proposed that the lactobacilli present in yoghurt would have a health promoting effect. Also, the most common means of administration is still a fermented dairy product. However, other microbes and even yeasts have been developed as potential probiotics during recent years (Table 1).

The choice what microbe to use as a probiotic is determined by many different factors (Table 2).

Table 1. Microbes used as probiotics and their documented health benefits in human clinical trials

Genus	Species	Example strains	Health benefit	Reference
Lactobacillus	*acidophilus*	La5	Reduced antibiotic associated diarrhoea	Black et al. 1991
	casei	Shirota	Shortening of rotavirus diarrhoea	Sugita & Togawa 1994
			Reduced recurrence of superficial bladder cancer	Aso et al. 1995
			Immune modulation	Nagao et al. 2000
	crispatus			
	fermentum	KLD		
	johnsonii	La1	Improved oral vaccination	Link-Amster et al. 1994
			Reduced colonisation by *Helicobacter pylori*	Felley et al. 2001
	paracasei	F19		
	plantarum	299v	Relief of irritable bowel syndrome	Niedzielin et al. 2001
			Reduction of LDL-cholesterol	Bukowska et al. 1998
	reuteri	SD2112	Shortening of rotavirus diarrhoea	Shornikova et al. 1997
	rhamnosus	GG	Shortening of rotavirus diarrhoea	Guandalini et al. 2000
			Immune modulation	Kaila et al. 1992
			Relief of inflammatory bowel disease	Gupta et al. 2000
			Treatment and prevention of allergy	Kalliomäki et al. 2001b; Majamaa & Isolauri 1997
	salivarius	UCC118	Reduced symptoms of inflammatory bowel disease	Mattila-Sandholm et al. 1999
Bifidobacterium	*breve*		Reduced symptoms of irritable bowel disease	Brigidi et al. 2001
	longum	BB536		
	lactis	Bb12	Treatment of allergy	Isolauri et al. 2001
			Shortening of rotavirus diarrhoea	Saavedra et al. 1994
			Reduced incidence of travellers diarrhoea	Black et al. 1989
			Improved oral vaccination	Link-Amster et al. 1994
Propionibacterium	*freudenreichii*	JS		
Bacillus	*subtilis*			
	cereus	toyoi		
Escherichia	*coli*	Nissle 1917	Fewer relapses of inflammatory bowel disease	Malchow 1997
Enterococcus	*faecium*	SF68		
Saccharomyces	*cerevisiae*	*boulardii*	Fewer relapses of inflammatory bowel disease	Guslandi et al. 2000

Table 2. Main properties for probiotic bacteria

Property	Benefit
Resistance to pancreatic enzymes, acid and bile	Survival of passage through the intestinal tract
Adhesion to the intestinal mucosa	Immune modulation
	Pathogen exclusion
	Enhanced healing of damaged mucosa
	Prolonged transient colonisation (?)
Human origin	Species specific interactions with the host
Documented health effects	Proposed health effects are 'true'
Safe	No health risk to consumer
Good technological properties	Strain stability
	Production at large scale
	Oxygen tolerance

In order to survive passage through the gastrointestinal tract, resistance to low pH, bile and pancreatic enzymes are important. Acid and bile tolerance can be easily monitored and they are considered intrinsic properties of lactic acid bacteria. Thus, in fermented milks acid stability is already required during the fermentation. Adhesion to the intestinal mucosa is considered important for immune modulation (the intestine is the largest immune organ of the body), pathogen exclusion, enhanced healing of damaged mucosa and prolonged transient colonisation. To obtain reasonable assurance on adherence, the use of at least two different test systems is required to describe both mucus and epithelial adhesion which represent the early and late stages of adherence to the mucosa. Human origin is thought to be important for host specific interactions by the probiotic, although e.g. *S. cerevisiae* (*boulardii*) is not of human origin. The microbes administered should obviously be safe. This is, however, often not specifically assessed. Lactobacilli and bifidobacteria are simply considered safe based on their taxonomic position. Although this may seem improper, it is difficult to assess the safety of generally non-pathogenic species. In practice, the first human feeding trial will also be the first safety trial, although this is often not recognised as such. Finally, potential probiotics need to have good technological properties so that they can be cultured on large scale, have an acceptable shelf life and, in case of application in fermented products, contribute to a good taste.

Lactobacilli have often good resistance to the *in vivo* stresses, as described in the next section, and several strains have good technological properties. This may, in addition to the historical reasons, explain their frequent use as probiotics. Bifidobacteria are also commonly used, though less then lactobacilli. They are sensitive to oxygen and have more strict growth requirements. This makes them technologically more difficult to use. The other probiotic species are, with the exception of propionibacteria and enterococci, usually not used in fermented products but as dietary supplements, in capsules, powders, etc.

Gut mucosal barrier: first line in host defence

The gastrointestinal tract is a complex microenvironment where the cells of the largest lymphoid organ of the human body interface with a myriad of endogenous and exogenous stimuli. The intestinal mucosa provides protective host defence to the constant presence in the gut lumen of antigens from food and the normal microflora.

Protection against potentially harmful agents is ensured by a number of factors including saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, intestinal flora, and epithelial cell membranes with the intercellular junctional complexes (Sanderson & Walker 1993). Together with the well-functioning immunological defence, these processes provide antigen exclusion in the gut. However, there are specialised antigen transport mechanisms in the villous epithelium (Ducroc et al. 1983). Antigens are absorbed across the epithelial layer by transcytosis, and here the main degradative pathway entails lysosomal processing of the antigen. This second line of defence, immune elimination, is directed towards removal of antigens that have penetrated the mucosa. A minor pathway allows the

transport of unprocessed antigens (Isolauri 1999; Heyman & Desjeux 2000). The immunological regulation takes place in several compartments: aggregations of lymphoid cells in follicles and the Peyerþs patches, distributed within the mucosa and in the intestinal epithelium, as well as in secretory sites (Brandtzaeg 1995). The intraepithelial T-lymphocytes have mainly a suppressor/cytotoxic phenotype, while the lamina propria cells show the helper/inducer phenotype. Peyerþs patches, crucial in determining the subsequent immune responses to the antigen, are covered by the M-cells. In general, antigen transport across this epithelium is characterised by rapid uptake and reduced degradation (Ducroc et al. 1983).

The lamina propria is also endowed with lymphocytes belonging to the B-cell lineage. IgA antibody production is abundant at mucosal surfaces, where secretory IgA is present in dimeric or polymeric form. Secretory IgA is relatively resistant to intra-luminal proteolysis and does not activate complement or inflammatory responses. There are differences between the upper and lower parts of the human gut-associated immune system in the isotype distribution of immunoglobulin-producing cells (Brandtzaeg 1995; Salminen et al. 1998). IgA1 immunocytes predominate in the small intestine while IgA2-producing cells are most frequent in the colon, the latter being more resistant to bacterial proteases. The secretory IgA antibodies in the gut are part of the common mucosal immune system including respiratory tract and lacrimal, salivary and mammary glands. Consequently, an immune response initiated in the gut-associated lymphoid tissue can affect immune responses at other mucosal surfaces.

The intestine's mucosal surface provides a defence barrier against antigens encountered by the enteric route. As a result of the barrier function, systemic hyporesponsiveness to antigens such as food proteins, oral tolerance, is a hallmark of the intestinal immune system. In this system also a balance is generated and maintained between the host and the normal resident microflora. In addition to antigen degradation and thereby participating in tolerance induction, intestinal colonisation acts as an important endogenous stimulus for the maturation of the gut-associated lymphoid tissue (Helgeland et al. 1996). So far, the human gut microflora is still an unexplored organ of host defence and its impact in health and disease may be stronger than currently known. As stated by MacDonald (2001): "It is likely that the normal flora also produces immunoregulatory molecules and it is not entirely unfeasible that the disease-free state of the gut in normal individuals is caused by the flora and not by sophisticated immunoregulatory circuits".

Health effects of probiotics

Probiotic therapy and modulation of the intestinal microflora

The original idea with probiotics has always been to change the composition of the normal intestinal microflora from a potentially harmful composition towards a microflora that would be beneficial for the host. In general this would mean a reduction in the number of, e.g. coliforms and clostridia and an increase in lactobacilli and/or bifidobacteria. Probiotics that survive gastrointestinal transit are likely to cause an increase in faecal levels of that particular genus, especially when initial levels were low. Due to competition for adhesion sites and nutrients, and possibly the production of antimicrobial substances, levels of certain less desirable genera can decrease. A concomitant increase in faecal levels of genera other than the probiotic consumed has also been observed for certain probiotics. E.g. consumption of *L. rhamnosus* GG has been observed to be associated with an increase in faecal bifidobacteria (Benno et al. 1996) and consumption of *L. salivarius* UCC118 caused an increase in faecal *Enterococcus* levels (Mattila-Sandholm et al. 1999).

It is obvious that avoiding colonisation by pathogens and reducing the risk for over growth of potential pathogenic bacteria is beneficial to the host. However, in some cases too much emphasis is placed on this change in microflora composition without considering the actual health benefit. A mere change in intestinal microflora composition is not a sufficient biomarker for a potential health benefit of a given probiotic strain. Moreover, for some health effects, like immune modulation, it may not be necessary to obtain a measurable modification of the intestinal microflora composition.

Immune modulation by probiotics

The demonstration that in the absence of the intestinal microflora antigen transport is increased indicates that the gut microflora is an important constituent in the intestines defence barrier. In affecting the development of gut-associated lymphoid tissue at an early age the gut microflora directs the regulation of systemic and local immune responsiveness, including hyporesponsiveness to antigens derived from micro-organisms and

food. Experimental animals lacking interleukin-10 or transforming growth factor-β generate a mucosal inflammatory response to the resident gut microflora (Groux et al. 1999). The role of the intestinal microflora in oral tolerance induction has been investigated in germ-free mice (Sudo et al. 1997). In contrast to control mice, germ-free animals were seen to maintain their tendency to a systemic immune response, for example production of IgE antibodies, upon oral antigen administration. Abrogation of oral tolerance was due to the absence of intestinal flora. The aberrant IgE response could be corrected by reconstitution of the microflora at the neonatal stage, but not at a later age. In human infants, colonisation has been associated with the maturation of humoral immune mechanisms, particularly of circulating IgA- and IgM-secreting cells (Grönlund et al. 2000), reflecting the dependency of the regulation of the mucosal immune response on the normal gut microflora.

In several gut-related inflammatory conditions the healthy host-microbe interaction is disturbed and inflammation is accompanied by imbalance in the intestinal microflora in such a way that an immune response may be induced by resident bacteria (Isolauri 1999). Normalisation of the properties of unbalanced indigenous microflora by specific strains of the healthy gut microflora constitutes the rationale in probiotic therapy. The success of probiotic therapy manifests itself in normalisation of the increased intestinal permeability and altered gut microecology, improvement of the intestine's immunological barrier functions and alleviation of the intestinal inflammatory response. The targets for probiotic therapy are identified as clinical conditions involving impaired mucosal barrier function, particularly infectious and inflammatory diseases (Isolauri 2001).

Probiotics and allergic disease

The prevalence of atopic diseases has been progressively increasing in Western societies. The hygiene hypothesis conceives the rapid increase in atopy to be related to reduced exposure to microbes at an early age and subsequent lower number of infections in early life (Strachan 1989). This is related to smaller family size, vaccinations, consumption of almost sterile food and over hygienic practices in Western societies, which may cause the infants immune system to develop an inflammatory response. The earliest and most massive source of such exposure is associated with the establishment of the gut microflora. Indeed, differences in the neonatal gut microecology were recently documented as being associated with the development of atopic diseases (Kalliomäki et al. 2001a).

The T helper (TH) 2 responder phenotype is associated with enhanced production of IgE antibodies against ubiquitous environmental antigens, eosinophilia, and consequently constitutes a hallmark of atopic diseases. Specific strains of the gut microflora have been shown to contribute to the generation of counter-regulatory TH1- and TH3-type immune responses (Isolauri et al. 2001). In addition, these contribute to the processing of food antigens in the gut and reduce their immunogenicity *in vitro* and *in vivo*, together with a potential to dampen inflammatory responses to these antigens (Sütas et al. 1996; Majamaa et al. 1997; Isolauri et al. 2000; Pessi et al. 2000a).

The regulatory role of probiotics in allergic disease was first emphasised in a demonstration of a suppressive effect on lymphocyte proliferation and interleukin-4 generation *in vitro* (Sütas et al. 1996). Subsequently, the immunoinflammatory responses to dietary antigens in allergic individuals were shown to be alleviated by probiotics, this being partly attributable to enhanced production of anti-inflammatory cytokines, e.g. interleukin-10 (Pessi et al. 2000b) and transforming growth factor-β (Haller et al. 2000), and partly due to control of allergic inflammation in the gut (Majamaa & Isolauri 1997). The mucosal dysfunction caused by inflammation, characterised by the altered rate, route and mode of antigen presentation, is stabilised by probiotics (Isolauri 2001). So far, clinical effects have been seen as a significant improvement in the course of atopic eczema in infants given probiotic-supplemented elimination diets (Majamaa & Isolauri 1997; Isolauri et al. 2000). The preventive potential of probiotics in atopic disease has recently been demonstrated in a double-blind, placebo-controlled study (Kalliomäki et al. 2001b). Probiotics administered pre- and postnatally for 6 months to children at high risk of atopic diseases succeeded in reducing the prevalence of atopic eczema to half as compared with that in infants receiving placebo.

Probiotics in diseases of the gut

Probiotics have traditionally been used to treat disease related to the gastrointestinal tract, although other diseases have also been suggested to be relieved by the use of probiotics.

Lactose intolerance

Lactose intolerance, or more correctly lactose maldigestion, is caused by a reduced production of β-galactosidase. This is a normal condition in all adult mammals, with the exception of people from north-west European decent, and should therefore not be considered a disease as such. In these subjects, consumption of lactose leads to an increased osmotic load in the small intestine with subsequent secretion of fluids which leads to loose stools (Launiala 1968). The origin of the abdominal pain that is associated with the consumption of lactose by lactose maldigesting subjects is not well understood though it does not appear to relate to the production of gasses from the fermentation of lactose by the intestinal microflora (Lasser et al. 1975). Fermented milk products have been observed to be tolerated well by lactose maldigesters as compared to milk. This can be explained by the presence of β-galactosidase in the bacteria fermenting the milk. Upon ingestion, the bacteria are lysed by bile in the small intestine, the enzyme is released and degrades lactose. In addition to this, the more viscous properties of fermented milks, compared to plain milk, gives them a longer gastro-caecal transit time, thus further aiding digestion of lactose (Vesa et al. 2000). This beneficial effect is usually more associated with products fermented with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. To what extend probiotics contribute to relief of lactose intolerance symptoms is uncertain, some probiotics like, e.g. *L. rhamnosus* GG are not able to ferment lactose.

Acute gastro-enteritis

Acute gastro-enteritis may have bacterial or viral origin. Rotavirus is one of the most common causes of acute childhood diarrhoea in industrial countries (Claeson & Merson 1990). Rotavirus invades and replicate in the differentiated absorptive columnar cells of the small intestinal epithelium. This results in partial disruption of the intestinal mucosa with loss of microvilli, a decrease in the villus/crypt ratio and an increased intestinal permeability (Salim et al. 1990). Several studies have shown that selected probiotics, such as *L. rhamnosus* GG, *L. reuteri*, *L. casei* Shirota and *B. lactis* Bb12, can shorten the duration of rotavirus diarrhoea by approximately 1 day (Kaila et al. 1992; Saavedra et al. 1994; Sugita & Togawa 1994; Shornikova et al. 1997). Several mechanisms maybe behind this favourable out come. The production of rotavirus specific IgA has been observed to be enhanced in response to treatment with certain probiotics (Kaila et al. 1992), the permeability of the intestinal mucosa has been observed to be reduced (Isolauri et al. 1993) and the composition of the intestinal microflora normalised (Salminen et al. 1996).

Antibiotic associate diarrhoea (AAD) is mainly due to an overgrowth of *Clostridium dificille*. In particular, *Saccharomyces cerevisiae* (*boulardii*) has been observed to reduce the risk for AAD (Surawicz et al. 1989). The incidence of AAD was less than half or a third in the *S. cerevisiae* (*boulardii*) group compared to the control group. Also other probiotics like *Lactobacillus rhamnosus* GG, *L. acidophilus* and *Enterococcus faecium* SF68 have been observed to prevent or treat AAD (Gismondo et al. 1999).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is clinically characterised by two overlapping phenotypes, Crohn's disease (CD) and ulcerative colitis (UC), which predominantly affect the colon (UC and CD) and/or the distal small intestine (CD). The aetiology of the disease is not completely understood, but a genetic predisposition and the normal intestinal microflora are thought to play an important role. Modifying the composition and activity of the normal microflora may thus improve the disease. Indeed selected probiotics have been observed to reduce the number of relapses and prolong the period of remission. Interestingly, not only lactic acid bacteria, *L. salivarius* UCC118 and *L. rhamnosus* GG, but also *S. cerevisiae* (*boulardii*) and a strain of *E. coli* (Nissle) have been observed to be effective in alleviating the symptoms of IBD (Mattila-Sandholm et al. 1999; Gupta et al. 2000; Guslandi et al. 2000; Hamilton-Miller 2001).

Colorectal cancer

The aetiology of colorectal cancer is diverse and diet has clearly been indicated to be involved (Greenwald et al. 2001). Diets, especially high in meat and fat or low in fibre, have been observed to cause changes in the composition of the intestinal microflora, with increasing levels of *Bacteroides* and*Clostridium* and decreased levels of *Bifidobacterium* (Benno et al. 1991). This change in microflora composition is associated with an increase in faecal enzyme activity, β-glucuronidase, azoreductase, urease, nitroreductase and glycocholic acid reductase. These enzymes con-

vert procarcinogens into carcinogens and may thus contribute to an increased risk for colorectal cancer. The consumption of selected lactobacilli have been observed to reduce this faecal enzyme activity. Whether this also reduces the actual risk for colorectal cancer remains to be proven. However, most, but not all, epidemiological studies suggest that regular consumption of fermented dairy products are related to lower risk for certain types of cancer (Hirayama & Rafter 2000). Some positive effect of probiotic lactic acid bacteria on the risk for colorectal cancer can therefore be anticipated although definite proof remains to be presented.

Constipation

Constipation is a major digestive complaint among the elderly, in particular the institutionalised. Although also, otherwise healthy, adults and hospitalised subjects may experience constipation. Constipated subjects have been observed to have a modified faecal microflora with reduced levels of bifidobacteria, *Bacteroides* and, in particular, reduced levels of clostridia (Shimoyma et al. 1984). Probiotics have been suggested to relieve constipation (Goldin 1998; Lee et al. 1999). However, review of the literature does not substantiate this claim. This may relate to the causes of constipation; physical inactivity, low-fibre diets, insufficient liquid intake and some drugs. The altered microflora composition is more likely to be a consequence than the cause of constipation, correcting the microflora composition may therefore not be of help.

Benefits for healthy subjects

Determining the potential health effects of probiotics for healthy subjects is difficult although this is of major importance since probiotics are mainly marketed for healthy subjects. The health effects of probiotics on healthy subjects are likely to be limited to risk reduction. As mentioned above, consumption of fermented dairy products maybe related to a reduced risk for colorectal cancer. However, that evidence is rather circumstantial. More direct evidence suggests that, at least in children, long term consumption of probiotics in non-fermented milk may reduce the risk for infections, absence from day care due to illness and the use of antibiotics (Hatakka et al. 2001). This study indeed indicates that probiotics can also be of benefit to the healthy consumer. Probiotics are often marketed as 'boosting the immune system'. For healthy individuals this may not be the case, since the immune system is likely to be working optimally (Spanhaak et al. 1998). However, in combination with oral vaccination, improved antibody titres have been observed with probiotics (Link-Amster et al. 1994).

Developing future probiotic strains

Quality of probiotic strains

Probiotics are special ingredients that are used in both foods and pharmaceutical or special dietary applications. They have been selected to express strain specific properties which are important for their proposed health effects. Such characteristics should be retained through food and pharmaceutical processes and storage to be of benefit to the consumer.

The most important factor is to retain the strain characteristics and the purity of the preparation. It has been reported that especially dried probiotic preparations may have contaminants. This sets the requirements for hygienic preparation of the products and careful identification of the strains used. All commercial strains should be placed in an international type culture collection for future comparison of the properties and identity. Some probiotic preparations may also mislabel the strains they contain, using old or non-existing nomenclature.

Unlike pharmaceuticals or food chemicals such as additives, the quality criteria for probiotics are largely undefined. This is a key factor for health effects as long term transfer of probiotic lactic acid bacteria or bifidobacteria in food processing along with the storage may result in changes in their characteristics and health properties. To control these properties, criteria for assessing such changes should be included in functional food regulations. The criteria currently used for selecting new probiotics have been suggested as the optimal quality control measures to be used in industrial practise (Tuomola et al. 2001).

In recent studies the necessity of testing the stability of strain characteristics was established for model bacteria and common probiotics used in foods (Tuomola et al. 2000, 2001). Adherence properties can be considered as the main selection criterion for current probiotics and adherence is important for both local colonisation and immune modulation through contact with the gut associated lymphoid tissue. Adherence varies greatly among the current probiotic strains and

adherence characteristics can vary in two different *in vitro* models. Processing and gastric secretions also influence adhesion and they should be taken into consideration (Tuomola et al. 2000; Ouwehand et al. 2001).

Early reports have documented that adherence properties depend on culture conditions, the number of transfers in industrial scale fermentation and use of cryoprotectants in freeze-drying (Elo et al. 1991). Transfer of cultures in processing over a period of 3 years decreased adhesion and changing the culture medium could also result in diminished adhesion properties (Elo et al. 1991; Tuomola et al. 2001). We have also shown that specific probiotics isolated from a similar product from different countries show very different adhesion properties. Viability is crucial for lactic acid bacteria used as starter cultures or probiotics.

Viability may be important for health effects as currently most of the clinical evidence has been reported for viable strains and relatively few effects have been documented for non-viable strains (Ouwehand & Salminen 1998). There are recent reports on the viability of probiotic formulations in Britain and the United States and they demonstrate a lack of quality control in this respect. In the US, of 30 supplements tested 11 contained no viable bacteria and in Britain only six out of 13 formulations were satisfactory in terms of viability (Temmerman et al. 2001; Hamilton-Miller 2001). Viability can relatively easily be assessed by the culture method or by flow cytometry (Virta et al. 1998; Bunthof et al. 2001). It is important to guarantee the viability of probiotics in the final product especially when viability has been documented as one of prerequisites for immune effects (Gill & Rutherfurd 2001).

Future probiotics

Probiotics for specific target groups

Current probiotics have mainly been selected based on the common criteria as outlined in Table 2. To refine the selection criteria, understanding of the mechanisms of probiotic action is necessary. This will make it possible to select future strains with more specific characteristics, to suit the needs of specific age and patient groups. This need is clearly indicated by the difference in mucosal adhesion of probiotic bifidobacteria to mucus from different age groups (Ouwehand et al. 1999) and the influence of disease on mucosal adhesion of selected probiotics (Ouwehand et al. 2002). The use of specially selected probiotics for particular subject groups may provide more specific health effects.

Non-viable probiotics

Most definitions of probiotic bacteria stress the importance of the viability of the microbes. However, very little research has been done on non-viable probiotics. Non-viable probiotics would have several advantages over viable ones: longer shelf life, improved safety and no need for refrigerated storage or transport. Review of the literature suggests that non-viable probiotics may have positive health effects as well (Ouwehand & Salminen 1998). This has been shown for shortening of rotavirus diarrhoea (Kaila et al. 1995) and alleviation of lactose intolerance (Vesa et al. 2000). Although viable probiotics appear to have more health effects than non-viable ones, the latter are not always without health effects. This also implicates that heat-inactivated products can not be used as controls without verifying their lack of activity.

Alternative applications

Probiotics are mainly used to influence the composition or activity of the intestinal microflora. However, in principle any part of the body which harbours a normal microflora can be a potential target for specific probiotics.

The oral cavity has a microflora that equals the intestinal microflora in complexity. Here too, some of the members of the normal microflora have a detrimental effect on the host, causing, e.g. dental caries or periodontal disease. Probiotics could have potential applications in the oral cavity. Yoghurt have been observed to reduce the colonisation by mutans streptococci, which are responsible for dental caries (Petti et al. 2001). While a specific probiotic *Lactobacillus* strain has been detected in saliva samples (Meurman et al. 1994). Although there is a considerable potential for probiotic use in the oral cavity, very little work has been done in this area.

The normal microflora of the urogenital tract is less complex than the microflora of the intestine and the oral cavity. However, more than 50 species are thought to colonise the urogenital tract and in health, H_2O_2-producing lactobacilli predominate (Redondo-Lopez et al. 1990). Disturbances in the *Lactobacillus*

flora are thought to be related to the risk for urinary tract infections. Some work has therefore been done on the use of probiotics for urogenital tract infections. Selected *Lactobacillus* strains have been observed to reduce the recurrence of urinary tract infections (Reid et al. 1992) and reduce the risk for vaginitis (Hilton et al. 1992; Reid et al. 2001). Much work has also been done on the mechanisms of probiotic lactobacilli on urinary tract infections; production of hydrogen peroxide and of biosurfactants appear to be important factors contributing to the efficacy of the probiotic strains for use in the urogenital tract (Reid 2001). The probiotic *L. casei* Shirota has been observed to reduce the recurrence of superficial bladder cancer (Aso et al. 1995). These findings indicate that use of probiotics for the urogenital tract is a promising future area.

The skin has a normal microflora which is different depending on the site of the body. The most common genera found in the microflora of the skin are propionibacteria, *Staphylococcus, Micrococcus, Corynebacterium* and the yeast *Malassezia*. Several species within these genera can be opportunistic pathogens. However, the potential use of probiotics for the skin has been considered little to non (Barefoot & Ratnam 1998).

Also, the nasopharynx has a normal microflora, *Streptococcus pneumoniae* being frequently one of its normal members. Even lactobacilli have been isolated from the upper respiratory tract. Their potential use as probiotics in there has only recently been considered and may have interesting applications (Cangemi de Gutierrez et al. 2001).

Thus, there are many potential applications for probiotics which have received little attention but which may provide significant health effects.

Conclusion

The specific health effects of selected probiotic strains are becoming increasingly accepted thanks to an expanding volume of documentation from double-blind, placebo-controlled, clinical studies. In particular, relief of lactose intolerance symptoms, by yoghurt cultures, shortening of rotavirus diarrhoea and treatment of allergies are now well established. Also, the mechanisms behind these health effects are being elucidated through *in vitro* and animal studies, this can be expected to lead to more carefully formulated selection criteria for probiotics. However, many proposed beneficial health effects of probiotics still need further investigation, in particular the potential benefits for healthy consumers. For this, it is important to use well defined strains, since each strain has to be judged on its own merits, and that appropriate biomarkers are used for the evaluation of the effects. In addition to this, well selected target groups are needed. Such studies may indicate additional areas for probiotic use and further consolidate the acceptance of probiotics.

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