Review Paper

Commercial sources of probiotic strains and their validated and potential health benefits - a review

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ABSTRACT

In the recent past, a number of probiotic strains have been screened and selected for incorporation into commercial functional food products. Some of these strains have been validated for specific health benefits through human clinical trials others have been documented as offering potential health benefits. In the past commercial probiotic strains were selected for their technological properties, however, in the recent past, validated health benefits have assumed much greater importance. This review includes the characteristics of genus Bifidobacteriaand Lactobacillus and other microbes that are currently considered as probiotic organisms, selection criteria employed to screen them as effective probiotic strains, their validated and potential health benefits documented. In the recent screening and selection of strains for probiotic efficacy a change in focus from having technological properties to offering validated health benefits may allow the strains to lose their technological properties including the ability to survive both food processing and gastrointestinal environment. Further, different food matrices may have different interactions with probiotic bacterial cells and hence validated human health benefits cannot be generalised for all food matrices as vehicles for administration and delivery.

Keywords: Probiotics, validated and potential health benefits, commercial probiotic strains

INTRODUCTION

Probiotic functionality depends on the ability of a strain to confer health advantages on the host upon oral consumption of viable cells. In recent times, there has been a growing appreciation for the important role of commensal microbiota in human health, be it through mediation of intestinal development and innate immunity, or digestion of food and protection of the host against disease. This has led to attempts to manipulate or augment the microbiota through the use of probiotics (live microorganisms that when administered in adequate amounts confer a health benefit

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on the host) or prebiotics (non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (FAO/WHO, 2002; Reid *et al.*, 2003).

The concept that specific microbial communities could substantially impact human health was pioneered by Nobel Laureate Elie Metchnikoff in the early 20th century and a century later, is being pursued in the Intestinal Human Microbiome Project (Turnbaugh *et al.*, 2007). Historically, microbes have been studied individually with a lack of knowledge regarding how entire microbial communities work, thrive, exist together in animal hosts. Communities work, thrive, exist together in animal hosts. In fact, medical microbiology historically focussed on human pathogens and infectious diseases, while research has been comparatively limited with regard to human commercial and probiotic organisms. Microbes significantly outnumber human cells in the adult body, and yet the microbial community infrastructure is mostly unknown.

The majority of probiotic organisms studied today are lactic acid bacteria including species of the genera Lactobacillus, Bifidobacterium, and Streptococcus, but this group of beneficial bacteria could be expanded to include a vast array of genera with further explorations of the human microbiome. The abundance of microbiota present in the human gastrointestinal tract encompasses a restricted set of bacterial phyla, suggesting that the autochthonusmicrobiota may be composed of a restricted set of species that possibly form a "core microbiome". The introduction of the core human microbiome concept initiated new ways of thinking about potential clinical application of probiotics.

Initial colonisation patterns of GI tract during infancy may be affected by fundamental dietary issues such as whether infants consume breast or bottled milk and the timing of introduction of solid foods. Exposures to indigenous microbes in breast milk on dairy food products, in addition to antimicrobial agents, may have profound effects on the GI microbiota, especially in early life. The relative plasticity of the intestinal microbiota in infancy may be extended to large-scale shifts occurring at other mucosal surfaces and body sites. Various fluctuations in infantile microbial population occur in the intestine within the first year of human life, indicating that tremendous flux and opportunities for microbial population remodelling occur early in life (Palmer *et al.*, 2007). An adult like complex intestinal microbiotic forms by the end of the first year of life, raising fundamental qualities about the developmental impact of microbes on physiology and immunity.

Microbial population associated with mammalian hosts may have beneficial effects or detrimental effects on immunity and physiology. While important characteristics of probiotics include their abilities to suppress the proliferation and virulence of pathogenic organisms, it is becoming quite clear that these organisms also have direct effect on human physiology and immunity. Studies are beginning to shed light on tangible benefits of probiotics in allergies and acute immune diseases, oral

biology, diseases of the GI tract, and genitourinary tracts, and neurology and psychiatry

Characteristics of Bifidobacteriumspp and L. acidophilus

Genus Bifidobacterium

Bifidobacteria are among the first microorganisms to colonize the intestine of a newborn infant and thereafter rapidly become the dominant flora(Ishibashi and Shimamura, 1993). Bifidobacteria are classified as Gram positive, non-sporing, non-motile and catalase negative obligate anaerobes. They are pleomorphic with shapes including short, curved rods, club shaped rods and bifurcated Y-shaped rods. At present around 30 species are included in the genus Bifidobacterium, 10 of which are from human sources (dental caries, faeces and vagina), 17 from animal intestinal tracts, two from wastewater and one from fermented milk (Gomes and Malcata, 1997). In recent times, the use of DNA probes and pulse-field gel electrophoresis has been applied for strain identification (Tannock *et al.*, 2000).

Bifidobacteria produce acetic and lactic acids without generation of CO_2 , except during degradation of gluconate. Fermentation of two moles of hexose results in the formation of 3 moles of acetate and 2 moles of lactate. Other than glucose bifidobacteria can ferment galactose, lactose and fructose (de Vries and Stouthmaer, 1968). Cysteine can be an essential nitrogen source for some bifidobacteria (Shah, 1997). Although considered as obligate anaerobes, some bifidobacteria can tolerate oxygen while some species can tolerate oxygen in the presence of carbon dioxide (Shimamura *et al.*, 1992). The optimum pH for growth is 6-7, with virtually no growth at pH 4.5-5.0 and below or at pH 8 or above. The optimum temperature for growth is 37-41°C with virtually no growth below 25 °C and above 46 °C. Bifidobacteria are predominant in the large intestine contributing to 6-36% of the intestinal microflora in adults. The levels of bifidobacteria than adults (Mitsuoka, 1982).

Genus Lactobacillus

Lactobacilli are distributed in various ecological niches throughout the gastrointestinal and genital tracts and constitute an important part of the indigenous microflora of humans. They are characterised as Gram positive, non-sporing, non-flagellated rods or coccobacilli(Hammes and Vogel, 1995). They are either micro-aerophilic or anaerobic and strictly fermentative. The homofermenters convert glucose to lactic acid predominantly while the heterofermenters produce equimolar amounts of lactic acid, carbon dioxide and ethanol (and/or acetic acid), while currently at least 70 species of lactobacilli have been described (Tannock, 2002), the one most studied for use in dietary purpose is Lactobacillus acidophilus. L. acidophilus belongs to Group A lactobacilli which include obligatory homofermentative lactobacilli (Hammes and Vogel, 1995). L. acidophilus is a Grampositive rod, around 0.6 to 0.9 µm in width and 1.5 to 6.0 µm in length with

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rounded ends. Cells may appear singularly or in pairs as well as in short chains. It is non-motile, non-flagellated and non-sporing. It is micro-aerophilic and an anaerobic environment normally enhances growth on solid media. Most strains of L. acidophilus are homofermentators and can utilise cellobiose, glucose, fructose, galactose, maltose, mannose, salicin, trehalose, and aesculine (Nahaisi, 1986). Hexoses are almost exclusively (>85%) fermented to lactic acid by the Embden-Meyerhof Parnas (EMP) pathway. These organisms lack phosphoketolase and therefore neither gluconate nor pentose is fermented. The optimum growth occurs within 35-40 °C but it can tolerate temperature as high as 45 °C. The optimum pH for growth is between 5.5 - 6.0while the acid tolerance ranges from 0.3 to 1.9% titratable acidity.

Microorganisms that are commonly considered as probiotics are shown in Table 1.

| Lactobacillus spp. | Bifidobacterium spp. | Other species |
|--------------------------------|----------------------|--------------------------------------|
| L. acidophilus | B. adolescentis | Enterococcus faecalis |
| L. brevis | B. animalis | Enterococcus faecium |
| L. casei | B. breve | Escherichia coliNissle |
| L. crispatus | B. bifidum | Saccharomyces boulardii |
| L. curvatus | B. infantis | Streptococcus cremoris |
| L.delbrueckiisubsp. bulgaricus | B. lactis | Streptococcus diacetylactis |
| L. fermentum | B. longum | Streptococcus intermedius |
| L. gasseri | B. thermophilum | Streptococcus thermophilus |
| L. johnsonii | B. essensis | Streptococcus salivarius |
| L. lactis | B. laterosporus | Propionobacteriumfreuden- reichii |
| L. paracasei | | Pediococcusacidilacti |
| L. reuteri | | |
| L. rhamnosus | | |
| L. helveticus | | |

Table 1: Microorganisms commonly considered as probiotics^a

Common sources of probiotic cultures and strains include: ATCC, Danisco, Fonterra Danone, Food Specialites DSM, Yakult, Morinaga Milk and Snow Brand Milk, Japan, Chr Hansen, University College Cork, Rhodia, Nestle, Valio Dairy, Biogaia Institute Rosell, Probi AB, Essum AB

^aAdapted from O'Sullivan *et al.*, 1992; Sanders, 1999; Rolfe, 2000; Isolauri *et al.*, 2003; Shah, 2007; Vasiljevic and Shah, 2008.

Selection criteria for probiotic cultures

Although several probiotic strains have been identified with health benefits (Reid, 2008), for a strain to be beneficial, it must fulfil certain criteria to be considered a valuable dietary component exerting a positive influence. The strain must be a normal inhabitant of the human intestinal tract and be able to survive harsh conditions such as acid in the stomach and bile in the small intestine. Probiotic

strains should also persist in the GI tract to prevent their rapid removal by intestinal peristalsis. In other words, colonisation or at least temporary colonisation is necessary for most probiotic organisms to exert their probiotic effects.

Feeding trials on humans with a number of probiotic strains have been reported where they seem to disappear from the GI tract within a couple of weeks after ingestion is discontinued (Fukushima et al., 1998). However even temporary persistence, which has been observed for several ingested probiotic strains, is said to enhance their chances for gut health effects, and therefore is considered a selection criteria(Mattila-Sandholm et al., 2002). In addition, when incorporated into food, probiotic bacteria should be able to survive the manufacturing process as well as remain viable during the ripening or storage period. Furthermore, the added probiotic bacteria must not negatively affect product quality, and be generally recognised as safe (GRAS). It has been suggested that a potentially successful human probiotic strain will have the following desirable properties : be of human origin, survive the passage of GIT, have certain colonisation abilities, adhere to particular intestinal cells, have sustained health benefits, and lastly but most importantly, be safe for prolonged human consumption. Desirable characteristics of a probiotic strain are shown in Table 2. Technological factors influencing the functionality of probiotics are shown in Figure 1. Several technological aspects need to be considered in probiotic selection. These include good sensory properties, phage resistance, viability during processing and stability in the product during storage. In selecting yoghurt starter culture microorganisms reliable acid forming



Fig. 1. Technological factors influencing the functionality of probiotics (Adapted from Mattila-Sandholm *et al.* (2002).

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| No. | Desired properties | Remarks |
|-------|--|---|
| 1. | Human/food origin | Important for species specific health benefits, human source not "necessary" but may be a factor for colonisation in the GIT |
| 2. | Acid and bile tolerance | Pre-requisite for passage survival or establishment in GIT (oral administration). |
| 3. | Safe for human consumption | With safety demonstration, no risk for opportunistic infections, good tolerance in hosts with abnormal immune responses, genetically stable, no immune reaction against probiotic strain, no pathogenic, toxic, allergic, mutagenic or carcinogenic reaction by probiotic strain itself. |
| 4. | Adhesion to human intestinal mucosal surface | Key factor for immunostimulation, competitive exclusion of pathogen, transient colonisation |
| 5. | Survive the passage of GIT | Basic requirement to exert health benefits, be active in GIT, resistance to degradation by digestive enzymes such as lyozymesresiding in the intestine, tolerant to toxic metabolites and primarily phenols produced during the digestion process |
| 6. | Validated health benefits | Modulation of immune response, production of antimicrobial substances, improvement of lactose intolerance, alleviation of diarrhoeal diseases or other GIT disorders, adjustment of cholesterol metabolism, production of vitamins or beneficial enzymes. Physiological benefits at sites distant from where probiotic and prebiotic products are administered: head/ neck, oral and respiratory tracts, pancreas, liver kidney, bladder and yaging |
| 7.Goo | od technical properties | Culturable on a large scale, survives processing and storage, no negative effect on product quality, good viability in fermented foods, capacity to grow in milk to acidify it. |

Table 2. Selection criteria for probiotic microorganisms

Adapted from Mattila-Sandholm et al., 1999; Ouwehand et al., 1999; Kailasapathy and Chin, 2000; Sarkar, 2008; Reid, 2008.

ability is one of the most important characteristics. However, when selecting probiotics the criterion should be connected to its impact on human health and wellbeing. The viability and activity of probiotic cultures may be affected during all steps involved in processing, storage and delivery as they are exposed to a number of different stress factors (Table 3).

Many leading starter culture manufacturers produce probiotic cultures commercially which may consist of a single strain or a mixture of several strains. Given the many uses of probiotic cultures there is considerable commercial interest in the production of stable probiotic cultures that contain a large number of uninjured viable cells. Due to greater savings in the cost of transport, storage and short shelf life, improvements in culture stability is being made by shifting from liquid and frozen concentrates to freeze-dried and spray-dried preparations. Despite the fact that spray drying is more economical than freeze-drying, especially on a larger scale, many probiotic cultures cannot tolerate the relatively high temperatures that are used during spray-drying (Porubcan and Sellers, 1979). In freeze-drying cryoprotectants (eg. lactose, sucrose) are usually added to the culture to be dried, to prevent, and reduce cell injury during drying and subsequent storage(Champagne et al., 1991). Most commercial probiotic culture preparations are supplied in highly concentrated form and most of them are prepared for direct vat (DVS) applications. The highly concentrated DVS cultures is commonly used in yoghurt manufacture due to difficulties involved in propagating probiotic cultures at the production site. The DVS cultures are supplied either as highly concentrated frozen cultures or freeze-dried cultures. Generally deep-frozen cultures contain >1010 cfu/g, while frozen-dried cultures typically contain more than 10¹¹cfu/g(Obermann and Libudzisz, 1998). The cell concentration per gram of product varies with the culture and the type of organism used (Mattila-Sandholm et al., 2002).

| Table 3. Some stress factors affecting viabi | lity of probiotics during proce | ssing |
|--|---------------------------------|-------|
|--|---------------------------------|-------|

| Processing and delivery | Stress factors |
|---|--|
| Production of probiotic preparations | Presence of organic acids during growth. Cell concentration affected by high osmotic pressure, low aw, higher concentrations of particularly, temperature (freezing, vacuum and spray drying); prolonged storage oxygen exposure, temperature fluctuations. |
| Production of probiotic containing products | Nutrient depletion, strain antagonism, increased acidity, positive redox potential (oxygen), presence of antimicrobial compounds (hydrogen peroxide, bacteriocins), storage temperature. |
| Gastrointestinal transit | Gastric acid and juice(pH, enzymes) Bile salts Microbial antagonismImmunoglobulins |

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Health benefits and therapeutic effects of probiotics

Since Metchnikoff's era, various health and therapeutic effects have been ascribed to products containing probiotic organisms. While some of these benefits have been well documented and established, others have shown a promising potential in animal models, with human clinical trials required to substantiate these claims. Figure 2 shows the health and therapeutic effect of probiotics. More importantly, health benefits imparted by probiotic bacteria are highly strain specific therefore there is no universal strain that would provide all proposed benefits, not even strains of the same species (Vasiljevic and Shah, 2008). Moreover, not all the strains of the same species are effective against defined health conditions. The strains Lb. rhamnosus GG, Saccharomyces cerevisiae, Boulardii, Lb. caseiShirota and B. animalis Bb-12 are reported to be the most investigated probiotic cultures with established human health efficacy data against a number of health disorders. Further, the health and therapeutic benefits differ between probiotic strains and yoghurt strains.

Lactose mal-digestion

Relief of lactose maldigestion symptoms by yoghurt consumption is probably the most widely accepted health benefit. Probiotics are able to do this by surviving acid and bile and producing â-galactosidase activity. For example L. acidophilus LA1 has reduced breath hydrogen excretion (Lin *et al.*, 1991).

Diarrhoea

Traditionally a main application of probiotics, best documented is the shortening of the duration of rotavirus diarrhoea in children. Also the reduction of the incidence of antibiotic associated diarrhoea is also feasible with probiotic administration. Examples of probiotic strains reported to shorten duration of rotavirus diarrhoea, antibiotic associated diarrhoea and traveller's diarrhoea are shown in Table 5.

| Table 4. Clinical effe | cts of selected probioti | c and yoghurt strains (A | Adapted fromMattils | ı - Sandholm et al., | 1999) | |
|--|---|---|--|--|--|---|
| | Probiotic strai | Ë | | | logurt strain | |
| Lb. rhamnosus GG | Lb. johnsonii LJ-1 | B. animalis Bb-12 | Lb. reuteri ATCC 55730 | <i>Lb. casei</i> shirota DSM 9843 | Lb. plantarum | Saccharomyces boulardii |
| Lowers faecal enzyme activities, prevent antibioti c-associated diarrhoea, treat & prevent rotavirus diarrhoea, prevent acute diarrhoea, immunomodulating responses, alleviate chronic inflammation of the intestines | Prevent travellers' diarrhoea, modulate intestinal microbiota, alleviate lactose malabsorption, improve constipation, enhance immune system, adjuvant in <i>Helicobactert</i> <i>pylori</i> treatmen | Prevent travellers' diarrhoea, treatment of viral diarrhoea, including rotavirus diarrhoea, modulate intestinal flora, improve constipation, modulate immune response, alleviate allergy, reduces incidence of travellers diarrhoea | Colonize intestina I tract, shorter duration of rotavirus diarrhoea, treat acute diarrhoea | Modulate intestinal microbiota, lowers faecal enzyme activities, exert positive effects on superficial bladder cancer | Adhere to human intestinal cells, modulate intestinal microbiota | Prevent antibiotic associated diarrhoea, treat <i>Clostridium</i> <i>difficile</i> colitis, reduces relapses from chronic inflammatory diseases No effect on rota virus diarrhoea, no immune enhancing effect during episodes of rotavirus diarrhoea, |
| | | | | | | enzvmes |

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| Table 5. Effect of selected probiotic strains on three types of diarrhoe | a |
|--|---|
|--|---|

| Disease | Strain | Effect | Reference |
|--|----------------------|---|-----------|
| Rotavirus diarrhoea | L. rhamnosusGG | Shorten duration (1day) | 1 |
| | L reuteri | " | 2 |
| | L.caseiShirota | ** | 3 |
| | B. animalisBb 12 | Reduced diarrhoea | 4 |
| Acute diarrhoea Antibiotic-associated | L. rhamnosusGG | Reduced incidence & duratio | n 5 |
| diarrhoea | L. rhamnosusGG | Reduced incidence & reduction in frequency of | |
| | a | stools | 6 |
| | S. cereviseaboulardi | Reduced incidence | 7 |
| Travellers' diarrhoea | L. rhamnosusGG | Reduced incidence | 8 |
| | B. animalisBb 12 | " | 9 |
| | S. cereviseaboulardi | " | 10 |

1. Guandalini et al. (2000), 2. Shornikova et al. (1997), 3. Sugita & Togawa (1994), 4. Saavedra et al (1994), 5. Szajewska et al (2001), 6. Arvoia, et al. (1999), 7. McFarland (1998), 8. Okasanen et al.(1990), 9. Black et al (1989), 10.Kollaritsch (1993).

Immune modulation

Probiotics may directly or indirectly (by changing the composition or activity of the intestinal microflora) influence the body's immune function. Many probiotic strains have been observed to modulate the immune system, in particular IgA levels and the non-specific immunity.

Examples of immune modulation by selected probiotic bacteria are shown in Table 6.

| Table 6. Selected examples of minunomodulatory effect by problotic bacteria | | | |
|---|--|--------------------------|------------|
| Strain | Effect | Comment | References |
| L rhamnosusGG | Increased | Subjects were children | 1 |
| L. mainosusee | non-specific response & rotavirus specific Ig. | with rotavirus diarrhoea | 1 |
| B. animalisDR10 | Increased IFN-á, increased total, helper | Elderly subjects | 2 |
| | & activated T lymphoe increased phagocytic activity in elderly | cytes, | |
| L. caseiShirota | Stimulation of natural killer cell Activity | Subjects were adulta | 3 |

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References: 1. Kaila et al (1995), 2. Gill et al (2001), 3. Nagao et al (2000)

Inflammatory bowel disease

IBD (Ulcerative colitis &Chron's disease) is related to the intestinal microflora, though it appears to be connected to genetic predisposition. Probiotics can prolong the remission of the disease (after treating with steroids and/or surgery, thus help to reduce relapses).

Examples: When L. salivariusUCC 118 was administered, it reduced the use of steroids for treating IBD (Mattila-Sandholm *et al* (Mattila-Sandholm *et al.*, 1999). When a mixture of probiotic strains, VSL # 3 (L. plantarum+ L. casei+ L. acidophilus + L. delbrueckiisspbulgaricus+ S. thermophiles + B. longum+ B. breve+ B. infantis) was administered to patients with chronic pouchitis, there were fewer relapses and reduction in pro-inflammatory cytokines and increase in anti-inflammatory cytokines (Gionchetti *et al.*, 2000).

Necrotising enterocolitis

Necrotising enterocolitis accounts for a significant morbidity and moratlity among premature infants. When L. acidophilus and B. infantiswere administered to patients with NEC, the results showed reduced incidence and reduced mortality (Hoyos, 1999).

Irritable bowel syndrome

IBS is characterised by abdominal discomfort or pain and an altered bowel function. Colonic fermentation results in the generation of variable gas volumes in the intestine. Symptoms of abdominal pain, bloating and flatulence are commonly seen in patients with IBS. When VSL # 3 probiotic mixture was administered to patients with IBS, the results showed positive reduction of IBS symptoms(Brigidi *et al.*, 2001).

Helicobacter pylori infection

H. pylori has been shown to be the causative agent of gastric ulcer although it appears to be present in the stomach of a large part of the western population without causing symptoms and is therefore likely to be an opportunistic pathogen. Probiotics do not appear to eradicate H. pylori, but may reduce associated inflammation. For example, the strain L. johnsoniiLa1 when administered reduced H. pylori colonisation and inflammation (Felley *et al.*, 2001).

Colorectal cancer

The causes of colorectal cancer are multifactorial. Some epidemiological studies suggest an inverse relationship between the consumption of fermented dairy products (yoghurt). In humans, it has been observed that many probiotic strains reduce the faecal enzyme activity that converts procarcinogens to carcinogen. For example, when L. rhamnosusGG was administered it reduced the faecal enzyme activity in patients with colorectal cancer (Ling *et al.*, 1992).



Fig. 2. Health and therapeutic effects of probiotics [Adapted from Saarela *et al* (2002)].

Atopic dermatitis

AD is traditionally treated with anti-inflammatory agents, however, studies have shown that treatment with probiotics can ameliorate symptoms as well as inhibit production of inflammatory cytokines. For example, in a study, a group of children with AD was administered L. rhamnosus19070-2 and L. reuteriDSM 122460. The results were positive with reduced AD severity. Also observed was a reduction in the level of serum eosinophil cationic protein-a cytotoxic protein used to monitor AD disease activity (Rosenfeldt, 2003).

Probiotics can act directly or they can act through changes brought about to the existing intestinal microbiota. Probiotic bacteria may compete with resident intestinal microbiota to influence the intestinal environment, the activity of glucuronidase, glucosidase and urease enzymes, immune system, inflammation and tolerance or intestinal lumen permeability (Figure 3)



Fig.3. Mechanisms by which probiotic bacteria may effectively prevent and treat gastrointestinal disorders [Santosa *et al.*, 2006].

CONCLUSION

In the past probiotic bacterial strains were selected for their technological properties including culturability on a large scale, survival during food processing and storage, no negative effects on product quality, good viability in fermented foods and the capacity to grow in milk to acidify it. Thus the strains are simply selected based on their technological capabilities and utilised in fermented dairy foods. In the recent past, however, validated health benefits have assumed a much greater importance and the probiotic strains need to be clinically (human) evaluated and documented for their specified health benefits.

When bacterial strains are screened and selected for health benefits, for example, modulation of immune responses, there may be a possibility that they lose their inherent technological properties. Commercial culture manufacturers will need to make sure that when they select strains on the basis of validated health benefits that these strains do not lose their technical properties. This may be difficult to

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accomplish in practice. Microencapsulation may be able to protect strains that have been selected providing validated health benefits but lost their inherent technological properties. It may be cheaper to protect a strain that has been selected through time consuming and expensive human clinical trials. In addition, a strain that is good in providing a health benefit may not necessarily have good technological properties.

In the recent past, the development of probiotic based foods has been extended to include non-fermented products. For example, probiotic chocolates, breakfast cereals, sports energy bars and chewing gums. There is no guarantee that a strain which exhibits good technological properties in a food matrix such as cheese will exhibit the same in a different food matrix such as chocolate. It will be futile to assume that a strain which survived in adequate cell numbers in one food matrix will do the same in a different food matrix. The pH, structure, consistency, oxygen presence etc. can affect the viability of the probiotic bacterial strains. Thus, the commercial strain suppliers and the food matrices before embarking on a new probiotic-based product development. Further, validated health benefits offered by a probiotic strain in a particular food matrix such as cheese cannot be assumed to be the same in a different food matrix.

Many countries are now imposing regulations on the type of probiotic strains used for developing probiotic products. For example, specific minimum cell numbers of probiotic bacteria at the time of consumption and specific validated health benefits through double blind human clinical trials. A probiotic strain selected for a health benefit in a food matrix may not be able to do the same in a different food matrix. This may be a difficult issue to comply with regulatory agencies and can be very expensive and time consuming to repeat clinical trials with different food matrices.

Many countries do not accept genetic engineering of microbial cells due to the concern with food safety. In this context, any attempt to genetically engineer probiotic bacterial strains to improve either technological or health imparting properties will not be able to obtain the acceptance of regulatory authorities.

In the future, however, development of "super and smart" probiotic strains offering multiple benefits will assume importance in the light of the rapid development and increasing global market in probiotic-based functional food products.

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