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Probiotics in limelight

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Abstract: For centuries, Probiotic and prebiotic foods have been taken, in the form of natural components of food, or as fermented foods. Today probiotics are gaining importance because of the numerous benefits, e.g. treating lactose intolerance, asymptomatic bacterial vaginosis, hypercholesterolemia, irritable bowel disease, cardiac diseases, atherosclerosis and arteriosclerosis. The ability of probiotics to prevent diseases and improve health at all ages is increasing the market potential at a high rate. However, the development of successful probiotic products depends on proof of a probiotic effect as well as on the foods where high numbers of viable organisms survive at the time of consumption as well as at the time it reach to the colon. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host''. Most probiotics lie into the group of organisms' known as lactic acid-producing bacteria. These are normally consumed in the form of fermented milks, yogurt, cereal or other fermented foods. Probiotics beneficially affect the host animal by creating those gut conditions that suppress harmful microorganisms and thus favoring the beneficial ones by improving its intestinal balance. Probiotics have been shown to maintain health by reducing risk diseases, possibly through a reduction in proliferation of pathogenic species. They also help in maintaining microbiota balance in the gut enhancing immune system and increasing resistance to infection.

Keywords: Prebiotics, Cereals, Foods, Probiotics, Biotechnology

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Some commercial examples of probiotics in the Indian market are listed in Table 1. Healthy variants of dairy products like Ice cream, butter, yoghurts/dahi, flavoured milks, market milk, milk powders/infant milk substitutes and fermented drinks are being introduced in the market. Products with fortified calcium, reduced cholesterol, Omega-3 fatty acids, low fat and sugar are being introduced.

Cereals as Prebiotics

Cereal grains are an important source of protein, vitamins, carbohydrates, minerals and fiber, and can be used as sources of non-digestible carbohydrates. In this way they promote several beneficial physiological effects and also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon, by acting as prebiotics. Cereals have been suggested to fulfill the prebiotic concept as these contain water-soluble fibers (such as β-glucan and arabinoxylan), oligosaccharides (Such as galacto- and fructooligosaccharides) and resistant starch (Davis, 2014). Fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial microorganisms as strains of *Lactobacillus* have been recognized to require fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals to grow (Granato et al. 2010).

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Probiotics in food

Probiotic cells are mainly consumed through the food products. It is estimated that the total functional food market of probiotic foods is 60% and 70% (Herbel et al. 2013; Hoover and Steenson 1993).

Probiotic microorganisms are available as culture concentrates in dried or deep-freeze form when added to a food. The most common genera and species are Lactic Acid Bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium*, because they are considered as GRAS (Generally Recognized as Safe). These species are dominant inhabitants in the human intestine (*Lactobacillus* in the small intestine and *Bifidobacterium* in the large intestine) (KrOckel, 2006; Kailasapathy and Chin 2000). However, due to their health-promoting effects, bacterial species belonging to the genera *Lactococcus*, *Enterococcus* and yeasts (e.g. *Saccharomyces cerevisiae* and *Saccharomyces*

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Table 1 Probiotics products marketed in India (Bhadoria and Mahapatra 2011).

S.No	Probiotic Products	Company
1.	Probiotic curd	Heritage Foods (India) Ltd.
2.	'b-Activ' probiotic curd (*L. acidophilus* and *B. lactis* strain BB12)	Mother Dairy
3.	'Nesvita' probiotic yoghurt	Nestle
4.	Probiotic ice creams, 'Amul Prolife' 'Prolite' and 'Amul Sugar free'	Amul (Brand of Gujarat Cooperative Milk Marketing Federation Ltd.)
5.	Yakult, Probiotic curd with *L. casei* strain Shirota	Yakult Danone India (YDI) Private Limited

boulardii) and filamentous fungi (e.g. *Aspergillusoryzae*) are also used as probiotics (Klaenhammer et al. 2005; Luckow and Delahunty 2004; Line et al. 1998). Dairy probiotic products supplement with multispecies are used as they have a more specifically targeted function in the human alimentary tract (Matias et al. 2014).

Dairy and non dairy products

The first commercial probiotic product was developed by Yakult Honsha Co. in 1935 (Mills et al. 2011). It is a probiotic drink composed by water, sugar, skim milk powder, glucose, natural and artificial flavors. Today, companies such as Danone and Nestle are the main producers of yogurt probiotic products. Due to the high prevalence of lactose intolerance and vegetarianism it is required to carry out research for the development of new non-dairy probiotic products which could have a big market. Soy can also be used as an alternative for nondairy probiotic carrier. It has some sugars and amino acids in its composition that are used as substrates by lactic acid bacteria to produce aroma compounds. However, due to its undesirable beany flavor and the presence of oligosaccharides (stachyose and raffinose) that often lead to stomach discomfort, soy consumption is limited. Lactic acid fermentation is the way to to improve the sensory quality of soymilk and also to mask undesirable compounds, which can be combined with supplemental sucrose, glucose, and lactose (Mead et al. 2000; Mountzouris et al. 2007; Millette et al. 2013; Naidu et al. 2012; Ozer and Kirmaci 2010).

Fruits and vegetables

Despite potential sensory challenges, there is a genuine interest in the development of fruit-juice based functional beverages, fortified with the probiotic and prebiotic ingredients. The fruit juices have been suggested as an ideal medium for the functional health ingredients because they inherently contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups, and because they are perceived as being healthy and refreshing. The fruits and vegetables are rich in the functional food components such as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population (Ozyurt and Otles 2014).

Viability

Maintenance of probiotic cells throughout gastro-intestinal transit and food-processing is important for the microorganisms to reach the intended site of action in sufficient numbers (10^8cells/gram). Following the consumption of a probiotic there is a considerable loss of viable cells due to passage through the low pH environment of the stomach and high bile salt conditions in the intestine (Rettger and Cheplin1921).

Several factors like Acidity, pH, dissolved oxygen content, redox potential, hydrogen peroxide, starter microbes, potential presence of flavoring compounds and various additives (including preservatives) have been reported to affect the viability of probiotic cultures in fermented milks. These factors not only affect the viability of probiotic bacteria but also have been identified as having an effect during the manufacture and storage of fermented milks (Rask et al. 2013; Rivera-Espinoza and Gallardo-Navarro 2010).

Today, a wide range of dairy beverages that contain probiotic bacteria is available for consumers in the market including: Acidophilus milk, Sweet acidophilus milk, Nu-Trish A/B, Bifidus milk, Acidophilus buttermilk, Yakult, Procult drink, Actimel, Gaio, ProViva, and others (Soccol et al. 2014). Many probiotic bacteria have shown to die in the food products after exposure to low pH after fermentation, oxygen during refrigeration distribution and storage of products, and/or acid in the human stomach (Saarela et al. 2000; Shah, 2000).To support the viability throughout processing, storage, distribution, and gastrointestinal tract to reach the colon probiotic products need to be supplemented with additional ingredients. Several reports have shown that in yogurt survival and viability of probiotic bacteria is often low. The efficiency of added probiotic bacteria depends on dose level. Their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment. The most important factor affecting probiotic viability is probably the pH in beverages. pH of Shelf-stable beverages have values below 4.4 to ensure their microbial stability and this low pH value combined with long storage periods is very demanding for most probiotic strains, especially those representing bifidobacteria. The packaging material should be a good oxygen barrier to promote the survival of especially anaerobic probiotic bacteria (bifido-bacteria). An

important determinant of the shelf-life is transportation and storage temperature; with increasing temperatures viability losses can occur rapidly (Salminen et al. 1999; Schaffer et al. 2009).

Currently used probiotics

The majority of probiotics are bacteria with the species of lactobacillus and bifido bacterium being the most common type of bacteria used. Following shows the list of micro-organisms used as probiotics (Vaughan et al. 2000).

Micro-organisms used as probiotics

A. Bacteria

i. *Lactobacillus: acidophilus, sporogenes, plantarum, rhamnosum, delbrueckii, reuteri, fermentum, lactus, cellobiosus, brevis*
ii. *Bifidobacterium: bifidum, infantis, longum, thermophilum, animalis*
iii. *Streptococcus: lactis, cremoris, alivarius, intermedius*
iv. *Leuconostoc*
v. *Pediococcus*
vi. *Propionibacterium*
vii. *Bacillus*
viii. *Enterococcus: faecium*

B. Yeast and moulds

A. cerevisiae, *A. niger*, *A. oryzae*, *C. pintolopesii*, *Sacharomyces boulardii.*

Bacteria before being selected as a probiotic, should be non-pathogenic, non-toxigenic, should retain viability during storage and use, should have the capacity to survive and metabolise in the gut, and finally should have documented health effects. *L. rhamnosus* strain GG meets most of these criteria.

Product considerations

Probiotics and prebiotics are marketed as health, or functional foods whereby they are ingested for their advantages in the digestive tract and/or systemic areas such as the liver, vagina or bloodstream. Unlike new drugs or pharmaceuticals, which are screened intensively for safety and effectiveness, probiotics and prebiotics are less rigorously assessed. It is therefore relatively easy to launch a new product, and legislation against such products is loose. Nevertheless, consumers should be provided with an accurate assessment of physiological, microbial and safety aspects. Several criteria for the appropriate use of probiotics and prebiotics exist and may be summarized as follows. They should:

- Exert a proven beneficial effect on the consumer, preferably with a mechanistic explanation of how this occurred;
- Be non-pathogenic, non-toxic and free of adverse side effects;
- Maintain stability in the product;
- Contain a large number of viable cells (for probiotics);
- Survive well in the GI tract (the best products should be resistant to gastric acid, small gut secretions and have a good ability to influence bacteria already in the gut);
- Have good sensory and mouthfeel properties;
- Preferably be isolated from the same species as the intended use; and Have accurate product labelling and content.

Much effort has concentrated on identifying probiotic bacteria and characterizing their beneficial credentials. It is generally considered that probiotic bacteria must possess certain properties. The probiotic must survive passage through the upper regions of the GI tract, and persist in the colon. There must be no adverse host response to the bacterium, its components or metabolic end-products. The probiotic should be antagonistic to pathogenic organisms in the gut, and must be genetically stable. Chosen micro-organisms must be capable to industrial processes and remain viable in the final food product for the successful introduction of the probiotic concept into the food market. Determination of mechanisms involved in probiotic function, such as the production of antimicrobials, mucosal adhesins and organic acids are enabled due to advances in the genetics of probiotic strains usually lactic acid bacteria (LAB) or yeasts. However, this also opens the research area for the possibility of modifying existing strains to increase survival and efficacy in the human GI tract.

Probiotics as commercial products

Probiotic foods are a group of functional foods with growing market shares and large commercial interest (Shah, 2000). Probiotics have been used for centuries in fermented dairy products. However, the potential applications of probiotics in nondairy food products and agriculture have not received formal recognition. Recently, interest to food and agricultural applications of probiotics has increased and the selection of new probiotic strains and the development of new application have gained much importance. The uses of probiotics have been very beneficial to the human health and to play a key role in normal digestive processes and in maintaining the animal's health. However, a number of uncertainties concerning technological, microbiological, and regulatory aspects exist (Saarela et al. 2000). The presence of probiotics in commercial food products has been claimed for certain health benefits. This has led to industries focusing on different applications of probiotics in food products and creating a new generation of 'probiotic health' foods. Among probiotics carrier food products, dairy drinks were the first commercialized products that are still consumed in larger quantities than other probiotic beverages.

Functional dairy beverages can be grouped into two categories: fortified dairy beverages (including probiotics, prebiotics, fibers, polyphenols, peptides, sterol, stanols, minerals, vitamins and fish oil), and whey-based beverages (Saxelin et al. 2010).

Vegetables and fruits are reported to contain a wide variety of antioxidant components, including phyto-chemicals. Phytochemicals, such as phenolic comp-ounds, are considered beneficial for human health,

decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation. There is a genuine interest in the development of non dairy based functional beverages with probiotics because they serve as a healthy alternative for dairy probiotics, are cholesterol free and also favor consumption by lactose intolerant consumers.

The expectations of probiotic bacteria have become the most demanding for any bacterial group. Probiotics have become a very important element to everyday health food products, and their global market is estimated above US$28.8 billion by 2015 (Tilley et al. 2014). Nowadays, consumers are very concerned of chemical preservatives and processed foods, even though it provides a grade of safety and food diversity never seen before. However, consumers accept easily LAB as a natural way to preserve food and promote their health. In the last decade the interest in bacteriocins produced by LAB has increased dramatically (Tripathi and Giri 2014).

FUTURE ADVANCES

Biotechnology and probiotics

With the revolution in sequencing and bioinformatic technologies well under way it is timely and realistic to launch genome sequencing projects for representative probiotic microorganisms. The rapidly increasing number of published lactic acid bacterial genome sequences will enable utilizing this sequence information in the studies related to probiotic technology. If genome sequence information is available for the probiotic species of interest, this can be utilized, e.g. to study the gene expression (transcription) profile of the strain during fermenter growth. This will enable better control and optimization of the growth than is currently possible. Transcription profiling during various production steps will allow following important genes for probiotic survival during processing (e.g., stress and acid tolerance genes) and identifying novel genes important for the technological functionality of probiotics (Matias et al. 2014). Increasing knowledge of genes important for the technological functionality and rapid development of the toolboxes for the genetic manipulation of *Lactobacillus* and *Bifidobacterium* species will in the future enable tailoring the technological properties of probiotic strains. However, before wide application of tailored strains in probiotic food products, safety issues are of utmost importance and have to be seriously considered for each modified strain (Mridula and Sharma 2015).

In addition to dairy products, fruit juices have been shown to be suitable carriers for probiotics. The limiting factor for many of the probiotic strains is the low pH of the juices. There is growing interest in consumers towards healthier foods and probiotic fruit, vegetable juices and cereal based beverages can serve as a good option. But, the application of probiotic cultures in non-dairy products and environments represents a great challenge and needs to be researched at the industrial level for commercial production of these healthy products.

Development of novel, economical and technological matrices is a dire necessity to bring the non-dairy probiotic foods on par with the demand they have to their nature of healthy alternatives to dairy probiotic foods. Although there is a great potential for the use of fruit juices as probiotic products, very few reports on their preparation and production are available. Hence, there is a scope for further research in this area. While developing, functional properties, stability, sensory acceptance, especially related to taste, appeal and price are to be kept in mind, as these factors play a major role in their successful commercialization. Care should be taken while selecting the probiotics to avoid removal of micronutrients from the product or to produce biogenic amines. As all cultures or strains may not have probiotic properties, selection of strain(s) with potential probiotic properties plays a major role in the success of the non-milk probiotic products. Technological issues that can affect the survival of probiotic cultures throughout the production process and during storage should also be addressed while formulating new probiotic products. Functional properties are extremely important to get a competitive advantage in the world market. Hence, care should be taken while confirming the functional attributes of starters before incorporating in the product. In conclusion, research non-dairy probiotic products can be widened to better understanding and exploiting the benefits of non dairy probiotic products for the mankind. Use of probiotics in combination with non-dairy probiotic products can also be attempted to produce synbiotic products.

Both traditional cell culture methods, as well as the alternative techniques (direct imaging and visual enumeration, nucleic acid-based enumeration methods, and flow cytometry and cell sorting), offer advantages and limitations for enumerating probiotic microorganisms. The new methods and techniques show considerable promise for quantifying live microorganisms in different metabolic states. But the probiotic efficacy cannot be predicted solely on the basis of viable cells. Very few microorganisms have been subjected to thorough *in vitro* studies confirming their specific health promoting activity, and even fewer have been subsequently subjected to and passed the appropriate human trials. Additionally, probiotics can be dangerous, as they have been linked to an increase in mortality rate if administered to severely immune compromised patients. Subsequent studies are needed to evaluate the health-promoting activity of probiotic bacteria (Céspedes et al.2013).

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