#### **REVIEW**



# Trends in dairy and non-dairy probiotic products - a review

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Abstract Health awareness has grown to a greater extent among consumers and they are looking for healthy probiotic counterparts. Keeping in this view, the present review focuses recent developments in dairy and non-dairy probiotic products. All over the world, dairy probiotics are being commercialized in many different forms. However, the allergy and lactose intolerance are the major set-backs to dairy probiotics. Whereas, flavor and refreshing nature are the major advantages of non-dairy drinks, especially fruit juices. Phenotypic and genotypic similarities between dairy and non-dairy probiotics along with the matrix dependency of cell viability and cell functionality are reviewed. The heterogeneous food matrices of non-dairy food carriers are the major constraints for the survival of the probiotics, while the probiotic strains from non-dairy sources are satisfactory. Technological and functional properties, besides the viability of the probiotics used in fermented products of non-dairy origin are extremely important to get a competitive advantage in the world market. The functional attributes of dairy and non-dairy probiotic products are further enhanced by adding prebiotics such as galacto-oligosaccharide, fructo-oligosaccharide and inulin.

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#### Introduction

Probiotic cultures have been with the mankind ever since people started consuming fermented milks and eating fermented foods. However, their health beneficial effects were uncovered only after Metchnikoff in 1907 suggested that the gut microflora had adverse effects on health and called it "Autointoxication". He further suggested that ingestion of fermented milks ameliorated this condition. Based on the assumption that colonization of the gut is necessary for maximum beneficial effect, he used intestinal strains of Lactobacillus acidophilus for the treatment of constipation (Rettger and Chaplin 1921; Fuller 1991). The word probiotic is coined by Kollath (1953) and is derived from the Greek language, which means "for life". According to Lilly and Stillwell (1965), probiotics are substances produced by microorganisms that promote the growth of other microorganisms. However, the widely adopted definition states probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). There are two more essential terms to know, prebiotics and synbiotics. Prebiotics are defined as the indigestible food ingredients that promote the growth or activity of beneficial bacteria, thereby benefiting the host. Synbiotics are combinations of probiotics and prebiotics that are designed to improve the survival of the ingested microorganisms and their colonization of the intestinal tract (de Vrese and Schrezenmeir 2008). Prebiotics are being added to the food products to stimulate the colonic

microflora to get health benefits to the consumers, besides providing textural attributes to the foods (Saad et al. 2013). An acidophilus milk product added with a prebiotic inulin was standardized using artificial neural network (Amiri et al. 2010). Supplementation of a probiotic-fermented soymilk with the fructo-oligosaccharide (FOS), inulin and pectin increased the angiotensin I-converting enzyme inhibitory activity and enhanced the in vitro antihypertensive effect (Yeo and Liong 2010). The functional and health benefits and recent developments in the production of the galactooligosaccharides (GOS) and its application in fruit juices and beverages have been thoroughly reviewed (Sangwan et al. 2011). Very recently, Rastall and Gibson (2015) reviewed the impact of prebiotics in promoting the growth beneficial microbes and intestinal health. Classification of probiotic foods is shown in Fig. 1.

Several species belonging to the genera of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and some species of *Enterococcus* and *Escherichia coli*, are widely used as probiotics. *Saccharomyces boulardii* is the only nonpathogenic yeast being used as a probiotic. Treatment with probiotics involves modulation of the immune system both

at the local and systemic levels and the beneficial effects include either shortened duration of infections or lowered susceptibility to pathogens (Antoine 2010). As said earlier by Metchnikoff; not all probiotics colonize gut to confer beneficial health effects (e.g., *Bifidobacterium longum*), some act in a transient manner by restoring and maintaining the homeostasis in the microbial gut flora (*Lb. casei*) (Ohland and Macnaughton 2010).

Some of the basic mechanisms by which the probiotics confer health benefits to the host include modulating the mucosal barrier function, decreasing the apoptosis of epithelial cells and by increasing mucin production (Mattar et al. 2002; Gaudier et al. 2005; Yan and Polk 2006; Caballero-Franco et al. 2007; Gogineni et al. 2013; Saad et al. 2013), aiding the increased production of antimicrobial peptides like defensins and cathelcidins by host cells (Schlee et al. 2008; Kelsall 2008; Mondel et al. 2009), production of bacteriocins, microcins and other antimicrobial substances that make the intestinal environment less comfortable for other pathogenic microbes (especially by lowering pH) (Alakomi et al. 2000; Penner et al. 2005; Liévin-Le et al. 2006; Duquesne et al. 2007; Venkateshwari et al. 2010; Vijayendra et al. 2010;

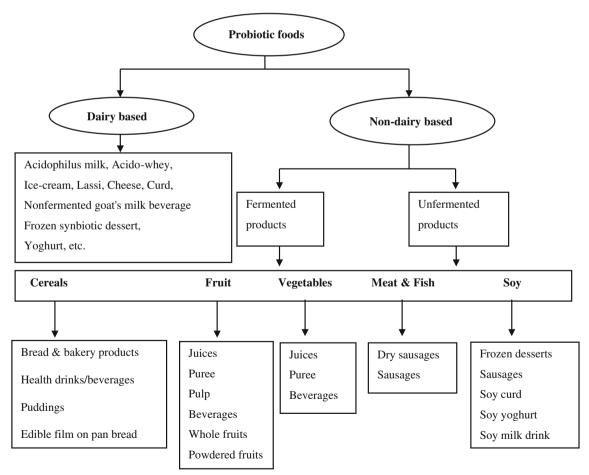
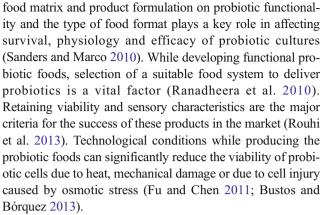


Fig. 1 Classification and types of probiotic foods

Halami et al. 2011; Sharma and Devi 2014), adhering to the epithelial cells in a competitive fashion and by blocking the adherence of pathogens on the epithelial cells either directly or indirectly (Johnson-Henry et al. 2007; Wu et al. 2008), modulating the immune system, by blocking pro-inflammatory molecules and by increasing mucin production (Ogawa et al. 2001; Tien et al. 2006) and interfering with the quorum sensing signaling, process through which the pathogenic microbes communicate with one another (Miller and Bassler 2001; Medellin-Peña et al. 2007).

Many investigations have been carried out to provide the evidence related to the health benefits of probiotics on gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimutagenic properties, anticarcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of Helicobacter pylori infection by addition of selected strains to food products (Gotcheva et al. 2002; Nomoto 2005; Imasse et al. 2007; Shah 2007; Vijayendra and Gupta 2012). Some health benefits of probiotics have been reviewed recently (Sanders et al. 2013). Probiotics are conventionally added to dairy products like yogurt, dahi and other fermented dairy foods (Laroia and Martin 1991; Penna et al. 2007; Vijayendra and Gupta 2013a, b) making them as functional foods. Commercially probiotics are being sold throughout the world mainly in the form of fermented foods and fermented dairy products, which play a predominant role as carriers of probiotics (Heller 2001). However, the increasing health concerns of lactose intolerance, milk protein allergy, high cholesterol content and high amounts of saturated fatty acids of dairy based foods are resulting in a shift towards non-dairy foods such as probiotic fermented cereals, fruits and vegetables juices (Gupta and Abu-Ghannam 2012; Peres et al. 2012; Vijaya Kumar et al. 2013, 2015). However, both the dairy and non-dairy consumers are not forsaking their interest in consuming probiotics for their perceived beneficial health effects (Ranadheera et al. 2010). Hence, the non-dairy based probiotic foods are finding their way into our routine life one by one. However, the non-dairy probiotic preparations are not new and many non-dairy preparations of cereals, soy, etc., are traditionally being made for centuries in all parts of the world. Microorganisms used as probiotics are mostly of human or animal origin; however, some studies show that strains recognized as probiotics are also found in nondairy fermented substrates (Schrezenmeir and de Vrese 2001).

It is necessary for the commercial probiotic preparations to be stable during entire storage time and the matrix in which they are present plays a vital role in their stability and interactions among microbes. Not much is known about the effect of



For centuries, the preservation and storage of fermented foods involving cereals, soya, meat, etc., are being practiced. However, fermentation process involves mixed cultures such as yeasts, lactic acid bacteria (LAB) and fungi (Blandino et al. 2003) and traditional fermented foods are the potential source of microorganisms and show probiotic characteristics. The information available on these matrices as raw material for probiotic microorganisms is still significantly less when compared to their dairy counterparts. With respect to non-dairy food matrices, the information regarding the survival of microorganisms against the challenges, the criteria for fermentation, their use as starters and their relationship with other microorganisms is minimal (Schrezenmeir and de Vrese 2001). The type of food matrix, rate of moisture content and cell condition play a major role in the survivability of probiotics during long term storage and processing (Endo et al. 2014). Moisture and cell conditions have a great impact on survival of probiotics under severe heat stress while processing and during long-term storage.

# Health risks associated with fermented dairy foods

Some health risks are associated with milk based probiotic foods. They mainly include lactose intolerance, allergy to milk proteins, high fat and high cholesterol content. These risks are elaborated below.

# Lactose intolerance (LI)

It also known as lactose malabsorption, is the most common type of carbohydrate malabsorption. It is associated with the inability to digest lactose into its constituents, glucose and galactose, due to low levels of lactase enzyme (Hauck et al. 2011). At birth, lactase activity is at the highest and it declines after weaning. The unabsorbed lactose is metabolized by colonic bacteria to produce gases such as hydrogen (H<sub>2</sub>) and methane (CH<sub>4</sub>) and short chain fatty acids (Lee 2015). Symptoms related to LI appear 30 min to 2 h after consumption



of food products containing lactose. Related symptoms include bloating, cramping, flatulence and loose stool and some reports also suggest that they lead to irritability bowel syndrome (Joachim 1999; Vesa et al. 2000). The rate of LI varies in the population and the rate of incidence of LI in different ethnic races worldwide is provided in Table 1 (Scrimshaw and Murray 1988; de Vrese et al. 2001). As seen from the table, highest rates of LI are found in the Asian populations, Native Americans and African Americans (60–100 %), while lowest rates are found in people of northern European origin (including North Americans). Effect of cholesterol in milk as a consummate effect in addition to allergy to milk proteins and lactose intolerance, high cholesterol content in dairy foods and high amounts of saturated fatty acids add to health concerns of probiotic dairy based foods.

# Allergy to milk proteins

Atopic dermatitis (AD) is one disease frequently associated with food allergy in children (Ricci et al. 2006; Johnke et al. 2007) and the rate of occurrence of AD during the first year of life is between 2 and 3 % (Host 2002). Some studies have demonstrated that the usage of probiotics reduces the

Table 1 The rate of incidence of lactose intolerance (LI) in different ethnic races

Ethnicity/ Geographic region	% population with L
East Asian	90–100
Indigenous (North America)	80-100
Central Asian	80
African American (North America)	75
African (Africa)	70–90
Indian (Southern India)	70
French (Southern France)	65
Ashkenazi Jew (North America)	60–80
Balkans Region	55
Latino/Hispanic (North America)	51
Indian (Northern India)	30
Anglo (North America)	21
Italian (Italy)	20–70
French (Northern France)	17
Finnish (Finland)	17
Austrian (Austria)	15–20
German (Germany)	15
British (U.K.)	5–15

The consolidated above table is freely adopted from the link as follows <a href="http://milk.procon.org/view.resource.php?resourceID=000661">http://milk.procon.org/view.resource.php?resourceID=000661</a>. Data compiled from using the references: National Institute of Child Health and Human Development (2006); de Vrese (2001); Scrimshaw and Murray (1988)

occurrence of AD (Reid and Kirjaivanen 2005). However, not all the preparations of probiotics can be used in children who are sensitive to cow's milk. With selective strains of probiotics, some studies have reported a reduction in the severity of signs and symptoms in these patients (Isolauri 2001; Kalliomaki et al. 2001, 2003; Sistek et al. 2006; Moro et al. 2006). However, these studies did attract criticisms regarding their favored design towards desired outcome and interpretation of the data (Matricardi 2002). In addition to this, some studies demonstrated that the probiotic supplementation has no significant impact on the symptoms associated with infantile AD (Brouwer et al. 2006) and hence, increased the risk of allergen sensitization in children with a high-risk of atopic diseases (Taylor et al. 2007). It could be potentially unsafe in people sensitive to cow's milk allergy (Moneret-Vautrin et al. 2006; Lee et al. 2007).

#### High fat and cholesterol

Milk contains fat and its amount depends on the source of milk. Cow milk has 4-5 % fat, whereas, it's content in buffalo milk is up to 7–8 %. It has a polyunsaturated to saturated fatty acid ratio of 0.05. Consuming large volumes of milk would increase the total cholesterol and LDL-cholesterol contents in the blood (Levy and Feinleib 1980) and the dietary saturated fat is responsible for the increase of plasma cholesterol levels, which is a major risk factor for coronary heart disease. This risk can be reduced by lowering of low-density lipoproteins (LDL) cholesterol by reducing the saturated fats in the diet (Hill et al. 2009). However, very recently Ebel et al. (2014) have reviewed the impact of probiotics on the risk factors of cardiovascular diseases including its impact on hypercholesterolemia. A significant reduction of 2.63, 4.1 and 4.68 mg/100 ml of serum cholesterol level at the end of 30 days in rats fed with yoghurt, probiotic dahi and probiotic yoghurt, respectively, containing Lb. acidophilus and Bifidobacterium bifidum indicating the hypocholesterolaemic effect of the probiotic cultures was reported recently (Vijayendra and Gupta 2012).

# Non-dairy probiotic products

To alleviate the disadvantageous of diary based fermented foods several non-traditional non-dairy based fermented foods have been developed (Table 2). Rivera-Espinoza and Gallardo-Navarro (2010) have reviewed various non-dairy probiotic foods developed worldwide. Among the non-dairy based fermented foods, fruit and vegetable based, cereals based and soy based foods are gaining importance (Prado et al. 2008; Gupta and Abu-Ghannam 2012; Gawkowski and Chikindas 2013; Martins et al. 2013). The major differences between dairy and non-dairy based fermented foods are summarized in Table 3. The following sections highlight some



 Table 2
 List of some non-dairy probiotic products developed recently

Category	Product	Reference
Fruit and vegetable based	Vegetable-based drinks	Lambo et al. (2005)
	Fermented banana pulp	Tsen et al. (2004)
	Fermented banana	Tsen et al. (2009)
	Beets-based drink	Yoon et al. (2005)
	Tomato-based drink	Yoon et al. (2004)
	Many dried fruits	Betoret et al. (2003)
	Green coconut water	Prado et al. (2008a)
	Peanut milk	Mustafa et al. (2009)
	Cranberry, pineapple, and orange juices	Sheehan et al. (2007)
	Ginger juice	Chen et al. (2008)
	Grape and passion fruit juices	Saarela et al. (2006)
	Cabbage juice	Yoon et al. (2006)
	Carrot juice	Nazzaro et al. (2008)
	Noni juice	Wang et al. (2009b)
	Onion	Roberts and Kidd (2005)
	Probiotic banana puree	Tsen et al. (2009)
	Non fermented fruit juice beverages	Renuka et al. (2009)
	Blackcurrant juice	Luckow and Delahunty (2004)
	Plum juice	Sheela and Suganya (2012)
	Cashew apple juice	Pereira et al. (2011)
	Table olives	De Bellis et al. (2010)
	Fruit juices (mango, sapota, grape)	Vijaya Kumar et al. (2013)
Soy based	Non fermented soy-based frozen desserts	Heenan et al. (2004)
,	Fermented soymilk drink	Donkor et al. (2007)
	Soy-based stirred yogurt-like drinks	Saris et al. (2003)
	Soy based products	Bedani et al. (2013)
	Soyghurt	Bedani et al. (2014)
	Soy curd	Roopashri and Varadaraj (2014)
	Soy product fermented with Kefir	Baú et al. (2014)
Cereal based	Cereal-based puddings	Helland et al. (2005)
	Rice-based yogurt	Boonyaratanakornkit and Wongkhalaung (2000)
	Oat-based drink	Angelov et al. (2006)
	Oat-based products	Martensson et al. (2002)
	Oat milk	Bernat et al. (2014)
	Oat, barley, and malt based	Salmerón et al. (2014)
	Yosa (oat-bran pudding)	Blandino et al. (2003)
	Mahewu (fermented maize beverage) Maize-based beverage	McMaste et al. (2005)
	Wallewii (Territeffied filalize beverage) Walize-based beverage	Wacher et al. (2000)
	Wheat, rye, millet, maize, and other cereals fermented probiotic beverages	Blandino et al. (2003)
	Malt-based drink	Kedia et al. (2007)
	Boza (fermented cereals)	Moncheva et al. (2003)
	Maize, sorghum, and millet malt fermented probiotic beverages	Blandino et al. (2003)
	Millet or sorghum flour fermented probiotic beverage	Muyanja et al. (2003)
	Mixed cereal beverage	Rathore et al. (2012)
	Bread and baked products	Côté et al. (2013)



Table 2 (continued)

Category	Product	Reference
	Sorghum based 'Sorghurt'	Sanni et al. (2013)
	Pseudo cereals (amaranth, buckwheat)	Monika et al. (2013)
	As an edible film on pan bread	Soukoulis et al. (2014)
Other non-dairy foods	Starch-saccharified probiotic drink	Oi and Klitabatake (2003)
	Probiotic cassava-flour product	Molin (2001)
	Dosa (rice and Bengal gram)	Soni et al. (1986)
	Meat products	Krockel (2006)
	Meat based products	Amor and Mayo (2007)
	Dry-fermented sausages	Sidira et al. (2014)

of the recent developments in the area of non-dairy based probiotic fermented foods.

# Fruit and vegetable based products

Research is being continued in developing alternate solutions to dairy based probiotic products and preference for non-dairy based probiotic products especially using fruit and/or vegetable juice as a major ingredient is a choice. Fruit juices offer several advantages: they are a rich source of nutrients and unlike in dairy products, it obviates the necessity of using starter cultures and hence no competition for nutrients with probiotic cultures. Non-dairy sources are fortified with acidulants which could increase the shelf-life by creating an anaerobic environment that is more optimal for probiotic cultures, which is attained by scavenging the oxygen available. Fruit juices also contain sugars to support the growth of probiotics (Ding and Shah 2008). Besides the advantages said above, they also have a good refreshing taste profile and a

 Table 3
 Comparative account of dairy and non-dairy probiotic foods

Parameter	Dairy probiotic foods	Non-dairy probiotic foods
Lactose intolerance	Negative effect	No issue
Calcium availability	Positive effect	No issue
High fat	Negative effect	No issue
Cholesterol content	Negative effect	No Issue
Dietary fiber	No issue	Positive effect
Digestibility	Not easy	Easy to digest
Survival rate of probiotics	High	Low
Flavour (diacetyl/acetaldehyde)	Positive effect	No issue
Phyto-chemicals	No issue	Negative effect
Isoflavons	No issue	Positive effect

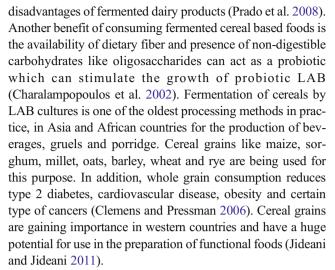
choice for people of all age groups. One more advantage is that these juices stay very less time in the stomach and thus the probiotic species spend very less time to the harsh acidic environment of the stomach. Several fruits and vegetables such as apples, oranges, blackcurrant, banana, blueberry, pineapple, cashew apple (Anacardium occidentale L.), cantaloupe melon, raspberry, pomegranate juice, carrot, beetroot, etc. (Savard et al. 2003; Yoon et al. 2005; Pereira et al. 2011; Nualkaekul et al. 2012; Fonteles et al. 2013; Anekella and Orsat 2013) and mixed vegetable juice (Nosrati et al. 2014) are being exploited for this purpose. The viable cell counts of Lb. casei in cashew apple juice even after storage for 6 weeks were found to be more than 8.00 log cfu/mL and, hence, proved to be as efficient as dairy products for its growth (Pereira et al. 2011) and similar trends are seen in pineapple juice (Sheehan et al. 2007) and melon juice (Fonteles et al. 2013). Also, stability and sensory acceptance are considered while developing a probiotic fermented fruit juices (Granato et al. 2010). Novel probiotic Lb. plantarum 299v culture based blackcurrant juices were found to have good aroma and flavour in comparison to non-probiotic blackcurrant juices (Luckow and Delahunty 2004). The encapsulation of probiotic cells is one more advancement to protect them from the acidic environment of the juices by encapsulating with readily available and non-toxic alginates, which further extend the cell viability during shelving (Ding and Shah 2008; Anekella and Orsat 2013; Kailasapathy 2014). These alginate beads are also coated by chitosans to offer extended protection to probiotic cells (Nualkaekul et al. 2012). Microencapsulation of probiotics and their effect on usage in food applications has been reviewed by Heidebach et al. (2012). The cell viability of the probiotic cultures Lb. acidophilus and Lb. casei increased with the incorporation of encapsulating matrices with galactooligosaccharides (Krasaekoopt and Watcharapoka 2014). Recently, fermented fruits and vegetables of Asian region have been indicated as a potential source of probiotic cultures (Swain et al. 2014) and its health benefits have been reviewed (Vijayendra and Halami 2015).



As mentioned earlier in this review, the lactose intolerance and cholesterol content of dairy based probiotics is a setback for its commercialization (Heenan et al. 2004). For our benefit, technological advances have helped in changing the matrix components of the foods in a controlled way to improve the cell viability and cell functionality (Betoret et al. 2003). The modification of the matrix makes them ideal substrates for the culture of probiotics as they readily have beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants (Yoon et al. 2004). They lack the dairy allergens, which are avoided by certain segments of the population (Luckow and Delahunty 2004). The appealing tastes and the refreshing profiles offered by the fruit juices have instilled in us a genuine interest for the development of probiotic based fruit juices (Tuorila and Cardello 2002; Yoon et al. 2004; Sheehan et al. 2007). However, unfavorable aromas (perfumery, dairy) and flavors (sour, savory) were observed with the addition of Lb. plantarum to fruit juices (Luckow and Delahunty 2004). The sensory impact study by Luckow and Delahunty (2004) have shown that consumers are interested in conventional orange juices over the probiotic-based ones; however, the awareness of health benefits due to probiotic-based juices might alter their interests. Luckow et al. (2006) proposed that the perceptible off-flavors in juices resulting with the addition of probiotics that contribute to consumer dissatisfaction could be overcome by adding 10 % (v/v) of tropical fruit juices. LAB are the organisms that require essential amino acids and vitamins for growth (Salminen and Von Wrigh 1993). However, some probiotic strains were found to have the capability to grow in fruit matrices. It has been proposed that cell viability is a factor which dependents on the substrate, the oxygen content and the final acidity of the matrix used (Shah 2001). Sheehan et al. (2007) reported extensive differences with respect to the acid resistance property of Lactobacillus and Bifidobacterium in orange, pineapple and cranberry juices. They have observed longer and higher survival rates in orange and pineapple juices which are in contrast to that of cranberry. Lb. casei, Lb. rhamnosus, Lb. paracasei strains have shown higher resistance, surviving over 7.0 log cfu/ml in orange juice and above 6.0 log cfu/ml in pineapple juice for at least 12 weeks. Very recently, a probiotic beverage using coconut water was developed by fermenting it with Lb. plantarum (Prado et al. 2015).

#### Other non-dairy based products

Cereals have complex nutrient composition and are being consumed on a daily basis all over the world as one of the staple foods. Cereals are considered as healthy non-diary carriers to prepare probiotic foods since they can overcome the



Fermentation of cereals increase the bioavailability of minerals such as phosphorous, iron and zinc (Sankara and Deosthale 1983), due to the action of microbial enzymes such as phytases, or due to the organic acids produced during fermentation of cereals (Teucher et al. 2004; Hotz and Gibson 2007). Application of cereals and cereal components in functional foods development was reviewed (Charalampopoulos et al. 2002; Blandino et al. 2003). The health benefits of using cereals (whole grains) and cereal components (brans) in the preparation of probiotic foods have been reviewed (Lamsal and Faubion 2009). Kalui et al. (2010) recently reviewed the probiotic potential of spontaneously fermented cereal based foods. As an alternate to dairy based probiotic foods, single and mixed cereals (barley and malt) based probiotic beverages containing Lb. plantarum and Lb. acidophilus in the range of 7.9 and 8.5 log cfu/mL have been developed (Rathore et al. 2012). Sorghum flour based yoghurt like product 'sorghurt' was prepared recently and it had a viable count of >8 log10 cfu/mL of the product, which is higher than the minimum desirable count of 10<sup>6</sup> cfu/g, with acceptable sensory scores (Sanni et al. 2013). Waters et al. (2015) consolidated the findings of various research workers in determining the role of LAB in the fermentation of cereals used for the preparation of beverages.

Very recently pan bread slices were coated with sodium alginate film impregnated with probiotic cells of *Lb. rhamnosus* GG and the viability was found to be 6.55–6.91 log cfu/30–40 g portion of bread after in vitro digestion under simulated gastro-intestinal conditions and no impact of the bread crust matrix on cell inactivation was noticed (Soukoulis et al. 2014). Meat mainly in the form of sausages is also being used as an alternate to dairy based probiotic foods. Use of alginate microencapsulated *Lb. reuteri* and *B. longum* in the preparation of meat based sausages was reported (Muthukumarasamy and Holley 2006). Rivera-Espinoza and Gallardo-Navarro (2010) have reviewed the cultures used in meat based probiotic products.



# Phenotypic and genotypic similarities between dairy and non-dairy probiotics

Both the dairy and non-dairy species show more similarities than discrepancies in their phenotypic and genotypic natures. The Lactococcus lactis is one of the major probiotics used in fermentation of dairy products. However, Lc. lactis is not limited to dairy foods; they are also found on plant surfaces and in other sources (Salama et al. 1995; Ulrich and Müller 1999). Green plant material is a natural source for Lc. lactis subsp. lactis and Lc. lactis subsp. lactis biovar diacetylactis (Salama et al. 1995). Lc. lactis has also been isolated from different sources like soil (Klijn et al. 1995) and termite hindguts (Bauer et al. 2000). Based on the 16 s RNA analyses of nearly 106 isolates of LAB, phenotypic and genotypic characteristics of both dairy isolates and non-dairy isolates were analyzed in order to compare one another in their efficiency of fermenting food sources (Nomura et al. 2006). These isolates were investigated by cluster analysis based on randomly amplified polymorphic DNA profiles. There were no significant differences between isolates from milk and those from plant. The reports were very satisfactory and plant-derived strains showed tolerance for high salt concentration and high pH value, and more kinds of carbohydrates were fermented by non-dairy strains than the milk-derived strains. Reports also suggested no notable differences in the profiles of enzymes, such as lipases, peptidases and phosphatases. One more reliable observation made was that the fermented milks manufactured using the plant-derived strains had the same flavour as that produced by milk-derived strains (Nomura et al. 2006).

# Matrix dependence of cell viability and functionality

In the dairy based probiotic foods, the physico-chemical composition of milk, which is rich in protein and lipids (fats), acts as a protective matrix for the probiotics and these factors help the survival of probiotics from adverse conditions of the stomach and small intestine (Saxelin et al. 2003). In addition, milk proteins, as a carrier matrix, can act effectively in protecting the probiotic cells till they reach the site of action in small intestines (Ritter et al. 2009). However, non-dairy food matrices are very different from dairy based; they are more versatile and less understood. Fermented dairy products, such as fermented milks and fresh cheeses, have been the food vehicles with the biggest technological and commercial success for the incorporation of probiotic bacteria (Saxelin 2008; Figueroa-González et al. 2011). Despite the commercial success of dairy probiotics, consumers have a genuine interest in fruit juice based functional beverages prepared with probiotics because they offer varied taste profiles that are appealing to all age groups and also they are perceived as healthy and refreshing in contrast to dairy foods (Rivera-Espinoza and GallardoNavarro 2010; do Espirito Santo et al. 2011). However, cell viability is an important attribute to cell functionality (Ouwehand and Salminen 1998) and cell functionality is mainly influenced by the food matrix components (Ranadheera et al. 2010). To assess the viability of *Lb. casei* cells in commercially available non-dairy drinks, the growth inhibitory capacity of the drinks was studied using well-diffusion agar assay. Out of 13 non-dairy drinks, only citric orange juice affected the growth of both strains with an inhibition zone of 6 to 7 mm for both strains, measured from the edge of the well (Céspedes et al. 2013).

Food matrices and cell conditions influence survival of Lb. rhamnosus GG under heat stresses and during storage (Endo et al. 2014). They have noticed 7.0 to 7.7 log cfu/ml of freezedried cells Lb. rhamnosus GG after 20 min heating in oils at 80 °C, whereas, the viable count of the cells suspended in phosphate buffer saline dropped to below one log by 10 min itself indicating the importance of food matrix in the survival of the probiotic bacterial cells. Processed fruits and vegetables have good matrices and are considered as ideal substrates for probiotics due to the presence of minerals, vitamins, antioxidants and fibers (Soccol et al. 2010). It has been observed that the cell wall of the autochthonous LAB is more resistant and thus allows the bacterial adaptation to the environmental conditions like low moisture and antimicrobial compounds, etc., surrounding to the food matrices, like olives (Masuda et al. 2010). Fruits, such as apple, guava, banana and melon, have been found to be potential carriers of probiotic bacteria and strong adherence of these bacteria on fruit tissue was found (Martins et al. 2013).

The efficacy of the bacteria employed in non-dairy drinks or even dairy drinks for that matter must first be tested for their resistance to gastric digestion in stimulatory in vitro studies. But reports suggest that when studying the gastric resistance of probiotic strains, incongruent results were obtained like strains with a well-documented ability to perform beneficially in the human gut didn't look so much positive in in vitro assays of gastric acid resistance. Hence, these results suggest the necessity of much more refined tests to estimate in vitro and the in vivo resistance to gastric digestion (Morelli 2007). Nonetheless, in vitro tests cannot be ruled out to study the impact of some inherent factors, such as effect of storage or food matrix, on the gastric resistance of probiotic bacteria, as previous reports suggest (Vinderola et al. 2011, 2012).

Cell viability and cell functionality has been found to work according to the phenomena called "cross adaptation", which states that pre-exposure to sublethal levels of the stress factor will allow cells to adapt to subsequent exposure to higher levels of the same stress factor or to different stresses (Bunning et al. 1990; O'Driscoll et al. 1996). Such results were obtained in a study, where lactobacilli gained higher resistance to simulated gastric digestion over storage that could be due to the exposure to the acidic conditions of juices



during refrigeration (Céspedes et al. 2013). Several other reports are also found standing in this line of phenomena, where enhanced resistance to bile salts by nonintestinal lactobacilli due to the prior exposure to gradually increased levels of bile (Burns et al. 2008) and an enhanced resistance to simulated gastric digestion in probiotics in commercial fermented milks during storage (Vinderola et al. 2011) are reported. Similar observation was noticed in bifidobacteria that are grown at low pH values (Vinderola et al. 2012) and in spray-dried lactobacilli due to preliminary heat-treatment and spraydrying (Páez et al. 2012). However, few reports suggested contrasting evidence considering the food matrix, where reduction of gastric resistance in lactobacilli along storage was observed for Lb. casei (Wang et al. 2009a). In another study, bifidobacteria maintained at 4 to 20 °C for 6 weeks has shown reduced resistance to gastric digestion (Saarela et al. 2006). Lactobacillus and Bifidobacterium have shown extensive differences in their resistance to the acid in the orange, pineapple and cranberry juice (Sheehan et al. 2007). Fruit juice being a more heterogeneous food source with different physicochemical properties compared to fermented milks, such erroneous results sometimes are expected and the cell viability and cell functionality are variable and product-dependent.

#### **Future perspectives**

The necessity of non-dairy probiotic drinks, the feasibility and development of adaptable technologies for their production are not going together compared to dairy probiotics at least for now. The current research is occurring at a brisk pace and scope looks pretty healthy but not satisfactorily enough when compared to their dairy counterparts. The heterogeneous food matrices of non-dairy food carriers are the major constraints, while the probiotic strains from non-dairy sources are satisfactory. 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 prebiotics in combination with non-dairy probiotic products can also be attempted to produce synbiotic products.

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