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INTRINSIC AND EXTRINSIC FACTORS AFFECTING EFFICACY OF PROBIOTICS—A REVIEW

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Probiotics play important role in human nutrition. Most probiotics fall into the group of organisms known to produce lactic acid and are normally consumed in the form of fermented foods. Probiotic organism is not an ingredient which is added to food but rather these are living cells which quickly changes according to different innate/native and extraneous factors. There is concern that the bacterial colony counts present at the time of manufacture and listed on the probiotic package may not be reflective of the number of viable colonies at the time of purchase and thereby diminishing efficacy. Several extraneous factors such as oxygen and salt concentrations, temperature, pH, acidity, the presence of other microorganisms and nutritional resources influence probiotic growth rates and total cell yields and inherent factors such as genetics, ethnicity, age, and health status might contribute singly or in combination toward efficacy of probiotics. The present review focuses on the effect of various intrinsic and extrinsic factors on viability of probiotics in foods.

Keywords: Viability, Intrinsic, Extrinsic, Genetic, Probiotics

INTRODUCTION

In today's fast pace life there are factors that negatively inûuence the interaction between intestinal microorganisms such as stress and diet, which lead to detrimental eûects in health. Increasing evidence indicates that consumption of 'probiotic' microorganisms can help maintain a favorable microbial proûle which results in several therapeutic beneûts. Probiotics are not an invention but existed in our traditional foods since ages (Amara, 2012). In recent years probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts. Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2002). According to Shah (2001) the functionality of probiotics, is thought that when in order to exert beneûcial effects, they must be viable and available at a high concentration, typically at least 10^8-10^9 cfu per gram of product and should survive the human gastric juice in the stomach and reach the small intestine and the colon.

During the processing and storage of the food, the probiotics have to survive in different physical and chemical conditions. This may alter the functional properties of the probiotic organism present in the food. The influence of all the factors on the probiotic activity in one particular food matrix is very complex. A probiotic strain needs to resist the manufacturing process and secondly they should remain viable during the storage period in the commercial products until the end of the shelf-life to prove its therapeutic effect (Mortazavian *et al.*, 2012). However, low viability of probiotics in market preparations have been reported in many studies (Shah *et al.*, 1995). The combination of various factors affecting viability of probiotic bacteria seems to be rather multifaceted. The factors which influence the survival of a probiotic organism are both intrinsic and extrinsic.

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Figure 1 indicates the factors affecting viability of probiotics in gastrointestinal tract and foods. Several factors such as oxygen and salt concentrations, temperature, pH, acidity, the presence of other microorganisms and nutritional resources influence probiotic growth rates and total cell yields (Shah, 2001; and Mills *et al.*, 2011). Genetics, ethnicity, age, and health status might contribute singly or in combination toward efficacy of probiotics (Huttenhower *et al.*, 2012).

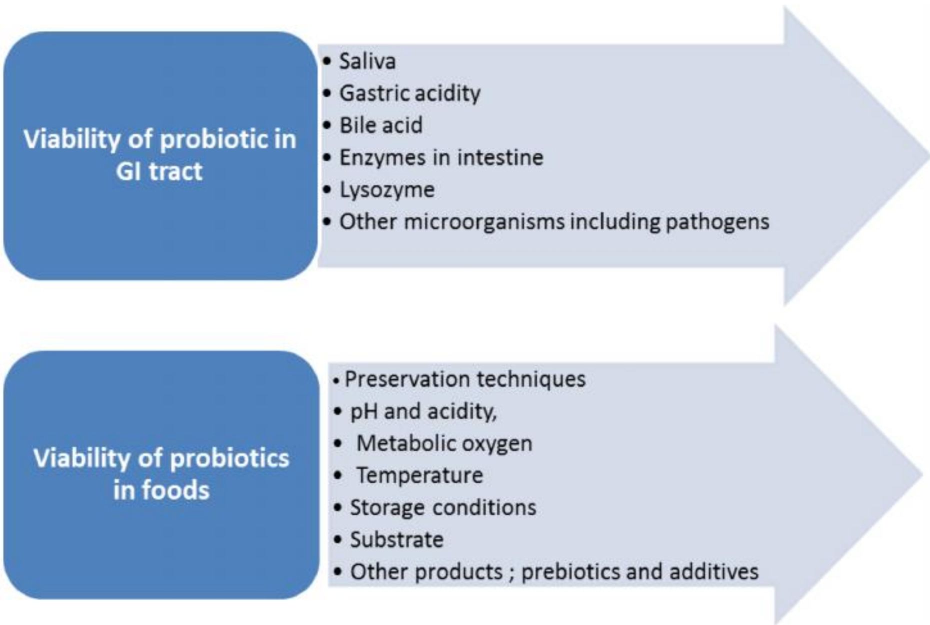
FACTORS AFFECTING VIABILITY OF PROBIOTICS IN GASTROINTESTINAL TRACT

The intestinal tract of adult carries 1-2 kg of microbes (Rhodes, 2007). The microbes that reach our GI tract need to survive through the passage and adhere on GI mucosal surface to colonize, and to establish interaction with the host. The stomach and large intestine are acidic while the small intestine is alkaline. The incoming microbes also need to tolerate the action of digestive enzymes, such as the proteases, amylases and lipases (Lee and Puong, 2002). Approximately 2.5l of gastric juice (Cotter and Hill, 2003) and 1l of bile (Begley *et al.*, 2005) are secreted into the human digestive tract every day. Thus, it is essential for the bacteria to have protection systems to withstand the low pH in the stomach, digestive enzymes and bile in the small intestine.

Viability and activity of the bacteria are important considerations, because the bacteria must survive in the food during shelf life and during transit through the acidic conditions of the stomach, and resist degradation by hydrolytic enzymes and bile salts in the small intestine (Playne, 1994). Clark and Martin (1993) reported that *B. Longum* tolerated bile concentrations of as high as 4.0%, whereas Ibrahim and Bezkorovainy (1993) found *B. longum* to be the least resistant to bile. Clark *et al.* (1994) also studied the survival of *B. infantis*, *B. adolescentis*, *B. longum*, and *B. biûdum*in acidic conditions and reported that *B. Longum* survived the best in bile.

The impact of the resident intestinal microbiota on probiotic function in digestive tract should not be underestimated (Sanders, 2011). Survival of bacterial strains in human gastric juice is a more accurate indication of the ability of strains to survive passage through the stomach. For this reason, an *in-vitro* survival test in simulated gastric juice was conducted. The hydrophobic nature of the outermost surface of microorganisms has been implicated in the attachment of bacteria to host tissue (Kiely and Olson, 2000). This property could confer a competitive advantage, important for bacterial maintenance in the human gastrointestinal tract (Schillinger *et al.*, 2005).

Figure 1: Main Factors Affecting the Viability of Probiotic Food Products and During Delivery Through Gastrointestinal Tract



Various studies have been conducted to analyse the viability of different probiotic organism in our human body. In one of the study by Das and Goyal (2013) proved that the qualities of isolate *L. plantarum* DM5 shows that it can survive in the lower acidic condition of the stomach as well as the basic fluids of the small intestine and thus making it a good contender for the development of probiotic functional foods or nutraceuticals in the food industry. The auto-aggregative activity and the hydrophobic property of the isolate DM5 provide a strong adhesion and colonization ability, an essential attribute of probiotic bacterium. Similarly it was suggested that *L. brevis* FFC199 and *L. Plantarum* CH_3 and *L. plantarum* CH41 (fromcocoa) could be used as probiotic, due to tolerance to low pH and bile salts, which are conditions imposed by the GIT environment (Ramos *et al.*, 2013).

The high bile tolerance was also observed in strain *Enterococcus faecalis* and *E. faecium strains* (Saavedra *et al.*, 2003), because these bacteria are well known to be commensal of the gastrointestinal tract of human and animals, and in this ecological niche, these bacteria come in contact and interact with bile salts (Bhardwaj *et al.*, 2010). During a study it was observed that the highest values of hydrophobicity were found for the *L. acidophilus* and *L. fermentum*, while lower values were obtained for the strains of *L. paracasei* and *L. rhamnosus* (Mathara *et al.*, 2008).

Bifidobacteria helps to stabilize the digestive systems of human beings provided that the carrier food contains not less than 1 million viable cells of *Bifidobacterium*/g of product (Samona and Robinson, 1994). It has been researched that *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* (yoghurt starter cultures) are not bile resistant and do not survive the passage through the intestinal tract. However, *L. acidophilus* and *B. bifidum* incorporated into the yoghurt starter culture have the ability to establish them among the gut flora. Consequently *Lactobacillus acidophilus* and *B. bifidum* grow well at low surface tension and are resistant to lysozyme, which would allow them to survive and grow in the intestinal tract. The adhesion mechanism of *L. acidophilus* is mediated by lectins, synthesized by lactobacilli or by epithelial cells of intestines. A Probiotic organism also needs to survive competitions with other microorganisms including pathogens (Kailasapathy and Chin, 2000).

VIABILITY OF PROBIOTICS IN FOODS

When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product. These factors include: 1) the physiologic state of the probiotic organisms added (preservation techniques used); 2) the physical conditions of product storage (e.g., temperature, packaging material), 3) the chemical composition of the product to which the probiotics are added (e.g., acidity, pH, water activity, and oxygen content), 4) possible interactions of the probiotics with other product components (e.g., food additives, prebiotics, food matrices) (Heller, 2001).

Physiological State

The methods of preparation (e.g., freezing, drying, and ultrasound) of a product might have an impact on probiotic viability and shelf life. It is marked from the numerous studies that probiotic functionality changes in response to culture processing steps. It also highlights that processing and preservation influence has dramatic effects on the stress tolerance levels of probiotics (Mills *et al.*, 2011).

Dehydration impacts the survival and stability of probiotic cell. Dehydration is commonly used as a means to stabilize probiotics for their ease of storage, handling, transport and subsequent use in functional food applications. It is possible to maintain the probiotic viability and retard unfavourable chemical and enzymatic reactions by maintaining cooler temperatures and low water activities (<0.20) (Mäkeläinen *et al.*, 2009). The viability of probiotic organism is enhanced under very low water activity. It has been observed that processing conditions associated with freeze-drying are milder than spray-drying and thus provide higher probiotic survival rates (Wang *et al.*, 2004). It was reported that cell size also has a strong influence on survival of probiotics during freeze-drying, with small spherical cells such as enterococci were more resistant to freezing and freeze-drying than larger rod shaped lactobacilli (Fonseca *et al.*, 2000). Dairy products and juices have a high water activity (>0.90) and a low storage temperature (4-8 °C) and thus have a short storage time of weeks to months at the most as compared to Capsules, tablets and powders have a very low water activity and shelf life of 12-24 months (Mäkeläinen *et al.*, 2009).

Researchers have reported that decline in bacterial counts during freezing is likely due to the freeze injury of cells, leading eventually to the death of cells. However, the mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mix may have resulted in a further decrease in bacterial count (Haynes and Playne, 2002).

Ultrasound is also a type of physical treatment which involves energy generated by high-frequency sound (Yang *et al.*, 2010). Ultrasound increased the growth of L. *casei* which led to increased intracellular and extracellular β-glucosidase activity of cells with subsequent increased bioconversion of isoflavone glucosides to the bioactive glycones in the mannitolesoymilk (Yeo and Liong, 2013). This treatment on lactobacilli and bifidobacteria does not suppress the functional characteristics of these bacteria (Yeo and Liong, 2011) which are indeed a beneficial factor.

Physical Conditions of Product Storage

Storage conditions play an important role in viability of probiotic organism as it influences the oxygen permeability into the product. Several aspects of food packaging materials including the type of the packaging materials (Glass and plastic), their thickness, and the application of active/ intelligent packaging systems could influence survival of probiotic bacteria (Korbekandi *et al.*, 2011). Dave and Shah (1997b) studied the survival of probiotic bacteria in yogurt made in plastic containers and glass bottles. The count was higher in the samples stored in glass bottles than those stored in plastic cups; this was due to low oxygen permeability. Storage temperature also plays a crucial role in viability of probiotic organism. Probiotic food products are usually stored at a refrigerated temperature, preferably between 4-5 ºC. Mortazavian *et al.* (2007) found that storage at 2 °C resulted in the highest viability of *L. acidophilus*, whereas for B. lactis the highest viability was obtained when yogurt was stored at 8 °C. A study also reported that exposure to dissolved oxygen during processing and storage is highly detrimental to *B. bifidum* and *L. acidophilus* (Champagne and Gardner, 2005).

Hisiao *et al.* (2004) have earlier studied the effect of the packaging material and the storage temperature on the viability of microencapsulated bifidobacteria. The samples filled in: (1) glass bottles; (2) polyester bottles with dessicant and oxygen absorber and (3) polyester bottles without desiccant and oxygen absorber were evaluated. It was reported that the product in glass bottles stored at 4 °C, with a reduction of only 0.15-0.20 log CFU/ g after 42 days storage gave best results. Kasýmog¢lu, Go¨ncu¨og¢lu, and Akgu¨n (2004) also investigated the effect of a packaging system using vacuum and brine on Turkish white cheese ripening. The authors observed that vacuum packaged cheese presented best performance in the sensorial evaluation (good flavour and texture) besides a high level of proteolysis.

Chemical Composition

The chemical composition of food such as pH and titratable acidity affects the survival of probiotics during storage (Mortazavian *et al.*, 2010). A very low pH value increases the concentration of undissociated organic acids in fermented products, thereby enhancing the bactericidal effect of these acids. Beverages such as fruit juices with low pH values possess a significant challenge to probiotics. Hydrogen ions damage probiotic cells via disrupting mass transfer through the cell membranes and acidic starvation of the cells (Mortazavian and Sohrabvandi, 2006). Results show that pH is the most crucial factor for the survival of *L. acidophilus* culture. It was noted during a study that the optimum pH for growth of *L. acidophilus* is 5.5-6.0, but for bifidobacteria this range is 6.0-7.0 (De Vuyst, 2000). It was observed that if the pH in yogurt dropped below 4.4 at the time of fermentation, it resulted in a 3 to 4 log cycle decrease in *L. acidophilus* numbers (Dave and Shah, 1997a). Lactobacilli are aerotolerant or anaerobic, and strictly fermentative, while bifidobacteria are strictly anaerobic and saccharoclastic (Holzapfel *et al.*, 2001). To overcome this issue several techniques are being used such as vacuum packaging, using packaging materials with low permeability to oxygen, adding antioxidants and oxygen scavengers to milk (such as ascorbic acid), and controlling the production process in such a way that minimum dissolved oxygen entered into product (Dave and Shah, 1997b; Shah, 2000; Talwalkar *et al.*, 2004; and Korbekandi *et al.*, 2011).

Effect of Substrate

Food substrate/diet is considered as one of the major factors in regulating colonization of micro-organisms in gastrointestinal tract. Food helps to buffer the bacteria through the stomach and may contain other functional ingredients that could interact with probiotics to alter their functionality. Dairy is the most common food matrix used for delivery of probiotics, although other matrices are increasingly used (e.g., fruit juice). The functional traits of bacterial species are expressed differently in different food matrix. These matrix effects differ among bacterial species in same food matrix as well (Saxelin *et al.*, 2010).

Curd (fermented milk), freshly made by inoculation with starter curd, is a good source of bioavailable vitamin, minerals and folate, and has less lactose and galactose than milk (Cano *et al.*, 2002). It is likely that the daily ingestion of curd in certain cultures and communities confers health benefits beyond nutrient intake in curd. (McNulty, 2011). It is well documented that probiotic bacteria grow slowly in milk because they are devoid of proteolytic enzymes, and contribute poor sensory and rheological characteristics to the product; hence, the practice is to blend these organisms with yoghurt starter culture (Damin *et al.*, 2008). Yogurt has long been recognised as a product with many desirable eûects for consumers, and it is also important that most consumers consider yogurt to be 'healthy'. In recent years, there has been a signiûcant increase in the popularity of yogurt as a food product, accentuating the relevance of incorporating *L. acidophilus* and *B. biûdum* into yogurt to add extra nutritional-physiological value.

Cheese provides a valuable alternative to fermented milks and yogurts as a food vehicle for probiotic delivery, due to certain potential advantages. It creates a more favourable environment for probiotic survival throughout the gastric transit as it acts as a buffer against the high acidic environment in the gastrointestinal tract. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach (Ross *et al.*, 2002; and Bergamini *et al.*, 2005).

As mentioned earlier, current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content (Heenan *et al.*, 2004). Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way (Betoret *et al.*, 2003). This could make them ideal substrates for the culture of probiotics, since they already contain nutrients such as minerals, vitamins, dietary ûbers, and antioxidants (Yoon *et al.*, 2004), while lacking the dairy allergens that might prevent consumption by certain segments of the population.

It has been found that some probiotic strains have the capability to grow in fruit matrices (Shah, 2007). In general, according to the probiotic studies reported, the growth and the viability of cells in fruits and vegetables depends on the strains used, final acidity and the concentration of lactic acid and acetic acid of the product Lactic acid bacteria are capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment (Yoon *et al.*, 2004). Kyung *et al.* (2005) found that *L. acidophilus* and *L. plantarum* can use the beet juice to grow and *L. casei* required some essential growth nutrients which were deficient in cabbage juice (Yoon *et al.*, 2006).

The probiotic cashew apple juice provides good matrix for *L.casei* growth and at the end of the storage period, the viable cell counts of *L. casei* was higher than 8.00 Log CFU/ml, which is considered a great value for fermented products containing probiotics (Shah, 2007). Pure carrot juice proved suitable as growth medium for *B. lactis* strain and *B. bifidum* strains. The bacterial addition led to a more intensive lactic acid production but a moderate degradation of carotenoids (e.g., α and β carotene), thus promoting the nutritional quality of said juice (Kun *et al.*, 2008).

The fruit juices have been suggested as an ideal medium for the functional health ingredients because they inherently contain beneficial nutrients (Tuorila and Cardello, 2002). 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 which will promote its consumption among larger segments of the population (Luckow and Delahunty, 2004).

In cereals fermentations endogenous grain amylases generate fermentable sugars that serve as a source of energy for the lactic acid bacteria. Other operations such as size reduction, salting or heating also affect the final product properties (Nout and Motarjemi, 1997). Non-digestible carbohydrates in cereal grains also stimulate the growth of Lactobacilli and Bifidobacteria (Andersson *et al.*, 2001). Charalampopoulos *et al.* (2002) have done experiments with different cereals to determine the main parameters that need to be considered in the growth of probiotic microorganisms, such as; the composition and processing of cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the ûnal product. Further Charalampopoulos *et al.* (2003) reported that many cereals supported the growth of probiotics with some differences. Malt medium supported the growth of all examined strains (*L. plantarum*, *L. fermentum*, *L. acidophilus* and *L. reuteri*) better than barley and wheat media due to its chemical composition, while *L. plantarum* and *L. fermentum* appeared to be less fastidious and more resistant to acidic conditions than *L. Acidophilus* and *L. reuteri*. Also, wheat and barley extracts were found to exhibit a significant protective effect on the viability of *L. plantarum*, *L. acidophilis* and *L. reuteri* under acidic conditions (pH 2.5). The lactobacilli strains exhibited good adaption to the cereal substrates and the cell populations of the functional beverages were of satisfactory probiotic values (Salmerón *et al.*, 2015).

Experiments studying the survival of probiotics indicate that soymilk is a good substrate for bacteria such as the Lactobacillus species (Garro *et al.*, 1999). Probiotic bacteria generally do not grow rapidly in cows' milk. Thus in yogurt manufacture, they do not attain as high numbers as the starter cultures (Champagne *et al.*, 2005). Studies indicating that soymilk is a good substrate for probiotic bacteria, suggest that some probiotics could grow better with yogurt cultures in a soybased substrate. Rice, the major cereal in Asia, and its products could be an economical and beneficial medium to develop probiotic foods (Trachoo *et al.*, 2006). Furthermore, meat has also been found to protect LAB against the lethal action of bile and act as a good food matrix (Ganzle *et al.*, 1999).

The lactic acid *bacteria Leuconostoc mesenteroides, Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lactobacillus fermenti*, *Lactobacillus lactis* and *Pediococcus cerevisiae* have been found to be responsible for the fermentation process, although *L. mesenteroides* and *S. faecalis* are considered to be the microorganism essential for leavening of the batter and for acid production in idli (Purushothaman *et al.*,1993; and Ramakrishnan, 1993). Fermentation of idli batter appears to have a significant effect on the increase of all essential amino acids and in the reduction of antinutrients (such as phytic acid), enzyme inhibitors and flatus sugars (Steinkraus *et al.*, 1993).

Interaction with Other Products Present in Food Matrix

Food additives used in the food industry could significantly affect the growth and viability of probiotic bacteria. Ingredients in the food can be protective, neutral, or detrimental to probiotic stability (Mattila-Sandholm *et al.*, 2002), These additives include salts (NaCl and KCl), sugars (sucrose and lactose), sweeteners (acesulfame and aspartame), aroma compounds (diacetyl, acetaldehyde and acetoin), natural colorings for fermented milks (red, yellow and orange colorings), flavoring agents (strawberry, vanilla, peach and banana essences), flavoring-coloring agents (strawberry, vanilla and peach), nisin (a polypeptide-type antibiotic produced by *L. lactis* which is active against spore- forming bacteria and could be used as a natural preservative in addition to lactic acid), natamycin, lysozyme and nitrate. Elevated levels of ingredients can inhibit probiotics during storage and drastically affect the growth and viability of probiotic bacteria (Vinderola *et al.*, 2002; and Lee and Salminen, 2009). Hence the compatibility of probiotics with different food ingredients plays a major role in their survival.

Cheese whey and oligofructose contribute in functional properties of lactic acid beverages (Castro *et al.*, 2009). It is further reported that lactulose also improves the quality of fermented skim milk by typical co-cultures of the yoghurt and probiotics cultures of *L. acidophilus, L. rhamnosus* and *B. lactis* in combination with *S. thermophilus* (Oliveira *et al.*, 2011). FOS was also found to be the most effective of the probiotic amongst Hi maize, FOS and inulin helps in retaining the viability of probiotic organisms in yoghurt (Capela *et al.*, 2006).

During cold storage of probiotic strains tested for the production of fiber-enriched skim yoghurts it was found that Apple and banana fiber also helped to preserve the viability of the strain (Espírito Santo *et al.*, 2012). Silva *et al.* (2014) evaluated the effect of processing conditions on the probiotic content of fermented ice cream. The results revealed that the formulation, whipping and air incorporation did not affect the survival of *B. animalis*. The same tendency was observed for the survival of *L. delbrueckii* in ice cream with different fat levels (Leandro *et al.*, 2013).

Different growth promoters such as glucose, vitamins, minerals, casein, whey protein hydrolysates, yeast extract, and antioxidant are fortified in dairy products to increase the growth rate of probiotic species (Korbekandi *et al.*, 2011). These supplements have significant effects on the survival of probiotic microorganisms during storage (Mohammadi *et al.*, 2011). Certain protein derivatives promote growth of the probiotic organism by providing nutrition for the cells. They reduce the redox potential of the medium and increase the buffering capacity of the medium which results in a minor decrease in pH (Dave and Shah, 1998; and Mortazavian *et al.*, 2010).

CONCLUSION

Probiotic act as functional foods and exhibit numerous health benefits but still it is unexplored in market scenario. This could be due to lack of studies regarding the efficacy of probiotic products available in human system and also during the shelf life of the product. Therefore, the process of producing and manufacturing probiotic functional foods should have standardized protocols and quality control procedures. To provide health benefits related to probiotic organisms and maintaining viable counts of each probiotic strain in gram or milliliter of probiotic products above a minimum standard level is very important. There are various challenges for survival of probiotic organism such as bio relationships among the starter bacteria, preservation techniques used, pH and acidity, metabolic oxygen, temperature, storage conditions, substrate, interaction with other products like prebiotics and additives. Therefore, a wide range of research has been focused on optimization of formulation and processing conditions of probiotic food products in order to increase the viability of probiotic cells in them until the time of consumption. The biggest hurdle is survival rate after the arrival of the cells in the intestine. Therefore to prove the probiotic health benefits to the host further human studies needs to be carried out to establish the scientific fact and different strains of microorganisms growing can be identified. Although traditional fermented foods have been part of our diet since ages but we still lack information about the identity and the source of some probiotic strains present. Thus further studies are required to identify various factors affecting viability of probiotic in a one single product so that the probiotic quality is maintained throughout the supply chain.

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