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PROBIOTICS AND THEIR INDIAN AND GLOBAL VALUE: A REVIEW

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ABSTRACT

Increased influx of probiotic products have been occurred in the Indian market during the last decade. However, there has been no systematic approach for evaluation of probiotics in food to ensure their safety and efficacy. Probiotics are known to confer several health benefits to human. In India, probiotic market has also been flourished in the form of dairy based probiotic products, fortified foods, soy based products etc. Attempts have also been made towards enhancement of shelf life of probiotic products by encapsulation and other technical approaches. Probiotics have found application as both food as well as medicine. There is need to isolate the strains very carefully screened for the probiotic characteristics. Regular consumption of probiotics could reduce the dependence on drugs and medical expenses. This review keeping in mind the Indian scenario aims to evaluate the health benefits of probiotics in food and pharmaceutical sectors, the existing knowledge in rural population and the current Indian market for probiotics.

KEYWORDS: Probiotics, Fortified, Market, Health benefits, Food, Pharmaceuticals, Drugs.

1. INTRODUCTION

Over the past decades, probiotic foods gained so much of popularity. Now a days probiotics cultures are incorporated in variety of foods. Strains are selected on the basis of their specific health-promoting effects, but the safety aspects are also carefully considered to make sure that they probiotics enriched should not cause health risk to the consumer. The safety evaluation of probiotic strains includes screening for (transferable) antibiotic resistance genes, virulence or pathogenic properties, adverse metabolic activities, and collection of evidence to reveal a "history of safe use", etc.[1]

Once a bacterial strain has been standardize for probiotic characteristics, it must still be determined whether the analytical methods that are commonly used to authenticate the microbiological safety of the ingredients and the finished products can also be used for the product with the probiotic strains.

At the beginning of 19th century, role of lactobacilli for improvement of human health and longevity was first hypothesized by Metchnikoff. He described the gut microbes for their detrimental effect rather than beneficial and suggested that desirable effects might only be expected from their substitution by yogurt bacteria. Since then probiotics have been employed to improve the health status by modulating the indigenous intestinal microflora by live microbial adjunct.[2]

The word "probiotic" was originated from the Greek word which means "on behalf of ". The concept was introduced by researcher involves production of substances by one microorganism which enhances the growth of another.[3] Thus probiotics are the exact opposite of antibiotic. Later this word probiotic was used to refer to animal feed supplements and defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance.[4]

According to the report of the joint "Food and Agriculture Organization/World Health Organization (FAO/WHO) expert consultation on assessment of health and nutritional qualities of probiotics in food including powder milk with live Lactic Acid Bacteria", probiotics were redefined for the reason of the meeting as: "Live human friendly microorganisms which when orally administered in adequate amounts confer a health benefit on the host".[5] Following the FAO/WHO definition, the International Life Sciences Institute[6] and the European Food and Feed Cultures Association (http://effca.drupalgardens.com) have defined the probiotics similarly, namely "a live microbial food ingredient that, when orally administrated in sufficient amounts, confers health benefits on the consumers" and "live microorganisms ingested or locally applied in sufficient numbers, provide the consumer with one or more proven health benefits", respectively.

Probiotics are also added with starter culture in non dairy foods to obtain maximum health benefits.[7] For each type of non-dairy product strains that can flourish in the food environment and take part to the formation of the sensory profile are selected. So in starter cultures for raw dried meat products probiotic bacteria that are capable to flourish in the meat environment are involved; in soy fermented foods as components of the starter cultures lactobacilli and bifidobacteria strains which can grow and multiply in soy milk are included while in fruit and vegetables and fruit and vegetable juices microorganisms with probiotic qualities suitable for this type of food are applied.[8] Some strains of lactobacilli with potent probiotic potential are used as ingredient of sourdough in bread-making which take part in extending the shelf life, improve the quality and also improve the technological properties of the final product.[9,10,11,12]

Now a day's probiotic foods are available in grocery stores and supermarkets, whereas food supplements are sold mainly in pharmacies and health food shops. Probiotics enriched fresh and fermented dairy products (e.g., milk, fermented milk, and yogurt) are easily available in market. The packages are traditional single or multipack cups or tubs, rather than bigger sizes. Yogurt eaten with a spoon is prepared with probiotic bacterial strains in the fermentation technology, together with a yogurt culture. 1 billion Euros market is observed for probiotics. The most popular format, however, is the "daily-dose" drink (i.e., a single serving of 65-125 ml, which is hypothetically contain an effective dose of bacteria), which was introduced to Europe in 1994 by the Japanese company Yakult, when it established a production plant in the Netherlands. Me-too products followed. Today's market leaders include Danone (Actimel [DanActive in the United States]) and Yakult (both of which use a strain of *Lactobacillus casei*), with a combined market value of 1.2 billion Euros in 2003.[13]

The commonly used species of bacteria in probiotic foods are *Lactobacillus acidophilus*/johnsonii/gasseri, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Bifidobacterium animalis*/lactis *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, The most common species in probiotic food supplements are Lactobacillus and Bifidobacterium species, a *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus* sp, *Enterococcus faecalis*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus clausiic*, *Escherichia coli* strain *Nissle*, *Saccharomyces boulardii* and other yeasts.

1.1 Probioics – An Indian Scenario (http://probioticindia.com/)

Probiotics is the rapidly expanding active arena in the field of functional foods,. India can play key role in probiotic revolution, as it is largest producer of milk and having world's highest cattle population. Only initialisation of probiotic industries have occurred in India and presently accounts for only a small fraction i.e. less than 1% of the total world market turnover in the probiotic industry. This probiotic industry is growing at steady pace with conditions set for tremendous growth in near future. India is rising as a major probiotic market of the future with annual growth rate of 22.6% until 2015. Most common brands involve in Indian probiotic industry are Amul, Mother Dairy, Yakult Danone and Nestle along with other minor brands operating in different areas in their own capacities. With their advent, the Indian probiotic market turnover has reached $8 million by the year 2015. In India, probiotics comes in two forms, milk and fermented milk products with the former occupying 62% of the market share and the latter having 38% market share (Indian consumer survey, 2010).

Amul has launched probiotic ice creams at National level with its prolife in February, 2007. Amul get success in launching probiotic ice cream as well as probiotic lassi. Probiotic products contribute to 10% to its ice-cream sales and 25 per cent of its Dahi (Indian yoghurt) sales. In Asia, mother Dairy has the largest milk (liquid/unprocessed) plants, selling more than 25 lakh liters of milk per day. Some of the probiotic product like b-Activ Probiotic Dahi, b-Activ Probiotic Lassi, b- Activ Curd and Nutrifit (Strawberry and Mango) are the company's probiotic products. Probiotic products are accounts for 15% of the turnover of their fresh dairy products. India's first Dahi with Probiotics was Nestle NESVITA which used for Healthy Digestion. Yakult Danone India Pvt Ltd (YDIPL) is a 50:50 joint venture between Japan's Yakult Honsha and The French-Danone Group and is offering Yakult, a probiotic drink made from fermented milk, *Lactobacillus* and some sugar. The entry of Yakult in India is expected to increase the visibility and growth of probiotic category. Major brands of the probiotics drug market in India involve companies like Ranbaxy (Binifit), Dr. Reddy's Laboratories, which has four probiotic brands, Zydus Cadila, Unichem, JB Chem, and Glaxo SmithKline. Drug forms of probiotics are widely accepted while probiotic foods are still viewed with scepticism. Acceptance of probiotic products is growing slowly, but it has to travel long journey while before changing the mindset of Indian consumers.

In current scenario, the Indian market's contribution to the world's demand for probiotics, however, is less than one per cent in terms of turnover. The Indian probiotic industry is miniscule and at a very burgeoning stage. Currently, it stands at about Rs 50 crore and is growing at 20 per cent." Major pharmaceuticals companies have become active and are trying to formulate newer drugs and products, and packaged products like probiotic-based nutritional supplements with special needs such as lactation, pregnancy, immunodeficiency etc and products especially for pediatric and geriatric patients. In this aspect, some probiotic based pharmaceutical

formulations are Sporolac, ViBact, Darolac, Biglac, Bifilac etc. Currently, probiotics are often used as animal feed supplements for cattle, poultry and piggery. This requirement is also met by importing probiotics from other countries. The most commonly found commercial probiotic drinks for human consumption are found in the form of probiotic drinks, ice creams and frozen desserts. The latest and recent addition to the list of probiotics in India is ViBact (which is made up of genetically modified Bacillus mesentricus), which acts as an alternate to B-complex capsules.[14]

1.2 Global Scenario of Probiotics

There is continuous increase of knowledge observed in probiotic and continues to expand on daily basis. Due to their protective role in the gut to keep our gut healthy and fit, progressive interest in 'these magic bugs' has grown enormously during the last few years. Probiotics have gained tremendous popularity as it is safest "natural" means to promote intestinal health amongst individuals.

In the developed world, growth of probiotic products has been quite amazing. According to a new market research report, 'Probiotics Market' (2009-2014), published by Markets and Markets, the global probiotics market had increased by US $ 31.1 in 2015 with the Europe and Asia accounting for nearly 42 and 30% of the total revenues respectively. The largest market of probiotics found in Europe with an estimated $13.5 billion by 2014. Asia is the second largest segment, developing at with an estimated CAGR of 11.2% and has reached $9.0 billion in 2014. As given by Euromonitor International's packaged food data, in 2000, pro/prebiotic yogurt (both drinking and spoonable combined) accounted for one-quarter of global yogurt sales by cost. A decade later, in 2010, it accounted for one-third global value for sales of probiotic dietary supplements almost three times, amounting to US$2.2 billion in 2010, thereby, further suggesting that probiotics are liked by most of the peoples around the globe. **(**http://probioticindia.com/**).**

According to one of the market report source from Transparency Market Research (2013) titled "Probiotics Market (Dietary Supplements, Animal Feed, Foods & Beverages) - Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast, 2012 - 2018, which mentions that the global probiotic demand was $27.9 billion (2011) and is expected to reach $44.9 billion in 2018 at a CAGR of 6.8%. The global demand for probiotics is dominated by Asia-Pacific and Europe, while, Asia-pacific is expected to be the prominent player in near future with an expected CAGR of 7.0% from 2013 to 2018. In Asia-Pacific, China and Japan dominates the market revenue for probiotics with India and other regions also showing significant growth. While, in Europe, Germany and U.K. are the most attractive markets, with an expected CAGR of over 6% each from 2013 to 2018.

1.3 Health Benefits of Probiotics

There are several reasons that people are interested in probiotics for health purposes. Firstly, microorganisms have omnipresence (including bacteria), and so are people's bodies in and on the skin, in the gut, and in other orifices. Health friendly bacteria are vital to proper development of the immune system, to protection against bad microorganisms that could cause disease, and also add to the digestion and absorption of food and nutrients. Each person's mix of bacteria varies. Interactions between a person and the microorganisms in his body, as well as among the microorganisms themselves, can be crucial to the person's health and well-being. This bacterial "balancing act" can be understood in two major ways:

First: the consumption of antibiotics kills the friendly bacteria in the gut along with unfriendly bacteria. Some people use probiotics to try to reduce the side effects from antibiotics like gas, cramping, or diarrhea. Similarly, some lactose intolerant use the probiotics as their gut lacks the enzyme needed to digest significant amounts of the major sugar in milk, and which also causes gastrointestinal symptoms.

Secondly by "Unfriendly" microorganisms such as disease-causing bacteria, yeasts, fungi, and parasites can also upset the balance. Researchers are exploring their mode of action whether probiotics could impede these unfriendly agents in the first place and/or suppress their growth and activity in conditions like:

- Infectious diarrhea
- Irritable bowel syndrome
- Inflammatory bowel disease (e.g., ulcerative colitis and Crohn's disease)
- Infection with Helicobacter pylori (H. pylori), a bacterium that causes most
- ulcers and many types of chronic stomach inflammation
- Tooth decay and periodontal disease
- Vaginal infections
- Stomach and respiratory infections that children acquire in day-care
- Skin infections.

Another part of the interest in probiotics stems from the fact there are cells in the digestive tract connected with the immune system. One theory is that if made alteration in the microorganisms found in a person's intestinal tract (as by introducing probiotic bacteria), you can affect the immune system's defences.

1.4 Probiotic foods

The significance of probiotics is well-known across civilizations and strata of societies over centuries in the form of practice of consuming preserved foods obtained throughout the course of fermentation. Since ages, traditional fermentation processes, by means of in the neighbourhood existing ingredients, which may be of plant or animal source, are renewed into edible

commodities by the physiological actions of microorganisms.[15]

The conventional fermented foods are principally obtained from dairy stuffs viz. yoghurt, dahi, kefir, cheese (after long storage), and fermented vegetable or from vegetable juices and from non fermented fruit and berry juices. Native fermented foods have been formed and consumed for thousands of years, and are powerfully correlated to culture and custom.

In today's modern world, due to urbanization and alter in nutritional practice and lifestyle outline has itself required the health aware consumers to investigate for an alternative treatment system for various ailments caused by way of life linked diseases. In this way, probiotics and probiotic based food supplementation have drawn attention of clients. The expenditure of probiotics exerts numberless of beneficial effects which is evidenced by exact scientific evaluation. Promotes suitable digestion, a well-built immune system, restores gut microbiota, improves barrier role of gut epithelium, modifies inflammatory response, aids in treating diarrhea originated due to traveling or during antibiotic treatment or upsetness of gastrointestinal tract, prevents ulceration caused due to helicobacter pylori[16,17] and aids in assimilation of nutrients from our food and supplements, in particular B vitamins and omega-3 fatty acids.

These health benefits encouraged the research to maintain the concept that there are clinical health benefits to ingest these micro-organisms[18] It is a main focus of notice of scientists across the world due to their promising health benefits and their applications offers a novel approach to formulate the innovative probiotic formulations.

1.5 Probiotic foods

1.5.1 Yoghurt with high concentration of viable cells of the probiotic strain *Lactobacillus delbrueckii* subsp. *bulgaricus* NBIMCC 3607

Lactic acid bacteria enriched foods take place a major place in the diet of our contemporaries. 80% of the population used yoghurt as a daily food supplement for direct consumption. A characteristic feature of this product is the addition of starters of pure cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp.*bulgaricus* for conducting lactic acid fermentation. A product with characteristic taste and aroma, physicochemical and biological properties can be obtained from milk as a raw material by applying an appropriate scientific course. These traditional lactic acid bacteria pose good positive impact on the body by forming metabolites, which inhibit the putrefactive and pathogenic flora or of the progress of the use of lactose.[19]

Lactobacilli are chiefly important in the formulation of probiotic foods hence the formulation of functional foods.[20] In the production of yoghurt, cheese and other fermented liquid products, several species of the genus *Lactobacillus* are utilising as starters.[21,22] Properties of the strain itself sometimes allow the decision in favour of selection of probiotic culture. Due to low reproductive capability in the medium or because of their low survival rate in the processes of freezing and freeze-drying, not all probitic strains can be cultured on industrial scale [23] that is why the cultures used in the production of fermented foods must meet certain requirements.

The choice of probiotic strains is based on microbiological criteria for food safety of the last product. This is achieved by applying non-pathogenic strains with clear health effects and suitable hygiene.[24]

Lactobacilli and Bifidobacteria in fermented milk products should have high concentration of viable cells and best survival when passing through the stomach which allows their biological role in the intestine.

Examination of several properties of bacteria such as oxygen sensitivity, storage stability, resistance to the proteases of the digestive system, sensitivity to aldehyde or phenolic compounds formed by the metabolism of amino acids, antioxidant activity, adhesion to the intestinal mucosain involves an *in vitro* testings.[25,26] Strains show the specific properties of lactic acid bacteria in a different degree. The biological action of fermented foods can be enhanced by combining the strains of different qualities which in turn is related to their capability to develop as symbiotic cultures.

Fermented milk products with probiotic characteristics are formulate on the basis of the experience in the field of progress of probiotics. Given that yogurt is the usually popular food after bread a technology that includes the use of a starter culture with the probiotic strain *Lactobacillus delbrueckii* subsp.*bulgaricus* NBIMCC 3607, which has high reproductive capability and meets all the needs for probiotic cultures, has been developed. The technology is piloted for a period of over 1 year in industry.

The data show that the yoghurt formed by using this technology lasts for one month, during which the acidity is maintained within the standard requirements and the concentration of viable cells of *L.bulgaricus* NBIMCC 3607 in 1 gram of the product exceeds 1billion by the end of the prolonged storage. In addition, the ratio of Streptococci to Lactobacilli is within the range of 1:1. By using any of the current technologies, similar results can be obtained. High concentrations of lactobacilli in yogurt augment its healing and preventive characteristics thus the mainly popular product becomes probiotic.

1.5.2 Bio-yoghurt

The application of yogurt during antibiotic remedy and for other medical purposes restricted due to presence of many strains like *Streptococcus thermophilus* and

Lactobacillus delbrueckii subsp. *bulgaricus* which cannot survive in the intestinal tract. Consequently, probiotic bacteria are incorporated in the composition of starter cultures for lactic acid products in addition to the traditional microorganisms *L.bulgaricus* and *Str.thermophilus*, which turns them into products with medicinal properties, recognized as bio-yoghurt (yogurt, dry mixes, ice cream, soft and hard cheeses, products for infant feeding).

The microflora of bio-yoghurt incorporates mainly *L.acidophilus, L.paracasei* ssp.*paracasei, L.paracasei* biovar *shirota, L.rhamnosus, L.reuteri, L.gasseri, Bifidobacterium infantis, Bif.breve, Bif.longum, Bif. bifidum, Bif.adolescentis* and *Bif.lactis.*[27] In addition to these species, some products contain *Bif. animalis*, which grow rapidly than other bifidobacteria, but unlike them it is not isolated from the intestinal tract of humans, although some *in vitro* studies show that some strains of *Bif. animalis* posses the ability to attach to epithelial cells.

Many researchers believe that the good probiotic effects on the human body is obtained by species and strains isolated from gastrointestinal tract of human. The digestive system of the fetus in the womb is sterile and inhabited within the first 2-3 days after birth. So right after birth the digestive system is colonized by species and strains that form its gastro-intestinal microflora, as a result of natural selection, and these microfloras are better adapted to the conditions available in gastro-intestinal tract. Thus probiotic bacteria enter into the body through the consumption of these types of functional food. Probiotic lactobacilli get attach to special receptors on the epithelial wall and fill the vacant spots in the intestine. In intestine, microbes utilize the nutrients and produce lactic acid and substances have antimicrobial activity.[27] Their prophylactic role consists in changing the conditions, making them inappropriate for the growth of bacteria that cause infections such as *Salmonella* sp.[27] Presence of lactobacilli also leads the enhancement in the level of immunoglobulin Ig A and Ig G,[28] thus protecting the immune system, lower cholesterol levels,[29,30] etc. Bifidobacteria are inhabited on the surface of the colon. In this part of the gastrointestinal tract different types of bifidobacteria use nutrients and produce lactic and acetic acids and bacteriocins with antimicrobial properties. Presence of bifidobacteria stimulates the walls of the colon to excrete the polysaccharide mucin that eases the passage of faeces through the colon, thereby preventing the colonization of cells of *E.coli, Candida* sp. thus protecting the body.

In recent years some yoghurt products have been reformulated to enrich live cells of strains of *L.acidophilus* and species of *Bifidobacterium* (known as AB-cultures) in addition to the conventional yoghurt organisms, *Str.thermophilus* and *L.bulgaricus*. Thus bio-yoghurt is yoghurt that enriched with live probiotic microorganisms, which are posed to exert beneficial health effects.[31] To exert good probiotic effect, the number of viable cells of probiotic bacteria in bio-yoghurt should exceed 1 million[32] (10^8-10^9cfu/g).[33] According to a Japanese standard at least 10^7 viable cells/ml of bifidobacteria must be present in fresh milk. According to the record of National Yoghurt Association (NYA) in the U.S. is apprehensive in the production of bio-yoghurt the concentration of lactic acid bacteria in the finished products must be 10^8 viable cells of lactic acid bacteria / g.

In addition, the culture should not only have rapid growth during fermentation as well as acid tolerance in order to maintain high microbial content during storage. Technologies for obtaining probiotic yogurt from whole milk and lactic acid beverage with bifidobacteria from skimmed cow's milk with the participation of *Streptococcus thermophilus, Lactobacillus bulgaricus* and strains of the genus *Bifidobacterium* have been developed. The microbiological indicators of this probiotic milk are presented in Table 1.

With the addition of *Bifidobacteria* in the starter culture for yoghurt a product with high concentration of active cells (more than 10^8cfu/g) with durability of 30 days is obtained.

Probiotic bacteria in Bread sourdough: Bread is one of the main products in the diet of contemporary people. The quality of bread depends upon several factors like intrinsic parameters of the flour, such as carbohydrate,[34,35] gluten,[36] mineral element,[37] lipid content[38,39] and endogenous enzyme activity,[40] and on the other hand extrinsic parameters referring to the bread making procedure, such as temperature, stages and level of fermentation,[41] water activity,[42,43] redox potential and additives,[44,45,46] and incorporation of nutritional or rheological improvers, such as dairy ingredients,[47] affect the quality of the final product.

The effect of these factors can be either direct or indirect, by affecting the microflora, either this is supplied as a commercial starter or in traditional sourdough processes.[48]

Bread is considered to be easily perishable food, microbial spoilage is quite obvious. The growth of molds on bread causes huge economic losses and reduction of the safety of the bread due to the production of mycotoxins. Fungal spoilage of wheat bread is occurring due to the *Penicillium* sp., and causes around 90% of wheat bread spoilage.[49] Other bread spoilage molds known to be belonging to the genera *Aspergillus, Monilia, Mucor, Endomyces, Cladosporium, Fusarium* or Rhizopus.[50] Several alternatives are applied to prevent or minimize microbial spoilage of bread, e.g. modified atmosphere packaging, irradiation, pasteurization of packaged bread and/or addition of propionic acid and its salts.[51,52]

Propionic acid has been known to inhibit moulds and *Bacillus* spores, but not yeasts to a large extent, and has therefore been the traditional chemical of alternative for bread preservation.[53] Legislation implemented under the European Parliament and Council Directive No. 95/2/EC requires that propionic acid should only be added to bread in a concentration not exceeding 3000 ppm.[54] However, recent studies have shown that this concentration of propionic acid is not effective against common bread spoilage organisms.[55] In addition to this, a reduction of preservatives to sub-inhibitory levels might allow the growth of spoilage molds[56] and/or mycotoxin production.[57,58,59]

Recent trends in the bakery industry have involved the requirement for high-quality foods, which are simply processed and do not contain chemical preservatives, thus the interest has been increased toward natural preservation systems.[60]

The use of strains of lactic acid bacteria for preservation of bread is one of the natural means, which are imported in the form of sourdough,[61,62] providing fast and reliable stability of the dominant microflora in the production cycle. Selected strains of homo- and heterofermentative lactic acid bacteria are applied as the components of the starter cultures. The latter utilize substrates with the development of lactic and acetic acid, resulting in acidification of the medium (pH, total titratable acidity (TTK).[34,61,63] Acetate production by heterofermentative metabolism has major importance for the improvement of flavour. The molar ration between 2.0 and 2.7 is considered optimum for lactic to acetic acid in bread (fermentation quotient, FQ).[61] The availability of soluble carbohydrates determines the suitable end-products produced during dough fermentation, which is attacked by the enzymes of the flour and the microbial enzyme systems.[34,64,65,66,67]

It is observed that metabolism of carbohydrates is species specific, even strain specific. It relies on the nature of sugars, the co-presence of yeasts and the condition provide during processing.[67] In addition to weak organic acids, i.e. lactic and acetic acid [68, 69, 70], lactic acid bacteria also produce a wide range of low molecular weight substances,[71] peptides[72] and proteins[73] with antifungal activity.

Sourdough is utilized in the production of classic bread, sour bread, snacks, pizza and sweet baked goods. Sourdough fermentation enhances the performance of the dough, leads improvement in the volume, texture, taste and nutritional value of the final product, slows down the loss of freshness and flavour and protects bread from mold and bacterial spoilage. The proper balance between the metabolism of yeast strains and strains of hetero- and homo-fermentative lactic acid bacteria leads the beneficial effects, which are the predominant microorganisms in natural sourdough. The metabolic activity of lactic acid bacteria leads the production of organic acids and contributes, together with yeasts, to the production of aromatic components.[74,75,76]

The activity of the lactobacilli in the composition of sourdough affects the protein fraction of flour during fermentation. This protein is particularly important for the quality of the bread, as the protein network of the bread determines its rheology, gas retention and thus the volume and texture of the bread. The substrates for the microbial conversion of amino acids in taste precursors and antifungal metabolites[77] are provided by proteolytic reactions.

The levels of some peptides are reduced, which is helpful in the cases of inability to absorb cereal products by some people.[78]

Bread with best quality is obtained by the simultaneous use of homo- and heterofermentative lactic acid bacteria in a certain ratio. Pure cultures of yeasts and lactic acid bacteria, imported in sufficient quantities provide fast and reliable stabilization of the dominant microbiota, normal fermentation process and actively participate in the quality of the finished bread. To observe this effect proper selection of species of lactic acid bacteria and process design, control over the purity and the activity of the cultures are required.

The strains *Lactobacillus casei* C, *Lactobacillus brevis* I, *Lactobacillus plantarum* NBIMCC 2415 and *Lactobacillus fermentum* J are isolated from naturally fermented sourdough, which defines their ability to grow in the mixture of flour and water, reaching high levels of viable cells and accumulating acid.

1.5.4 Soy probiotic foods

Soy foods are essential in the diet of the people in the Far East. These categories of food are good source of protein, and also supplying the body with all the essential amino acids for building and maintaining the tissues.[79] Soy probiotic foods are a good source of flavones and isoflavones that exhibit antioxidant activity and can reduce the damage caused by free radicals.[80] Soybeans have stachyose and raffinose, oligosaccharides which are known as bifidogenic factors. Traditional soy foods supplied the vitamins from groups B and D, mineral elements calcium, magnesium, iron, etc. by traditional soy foods. Anti-cancer agents - protease inhibitors, saponins, phytosterols, phenolic acids, phytic acid and isoflavones, most of which are important flavones and isoflavones, which are polyphenolic compounds and have connection to the group of plant estrogens, phytoestrogens, are also found in soy foods. The term phytoestrogens refers to substances which have the effect of female hormones, but are not steroids. Soy foods are also known to play an important role in preventing chronic diseases such as menopausal disorders, cancer, osteoporosis, atherosclerosis.

Dried, ripened, whole soybeans are generally used to obtain soy milk.

Lactose is absent in soya milk hence good for the peoples have lactose intolerance. It is good replacement of cow's milk for all people who suffer from allergies, lactase deficiency and milk protein intolerance. It can be used to carry out lactic acid fermentation with suitable strains of lactic acid bacteria (*Lactobacillus acidophilus, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus casei, Leuconostoc mesenteroides, Lactococcus lactis* ssp. *lactis, Bifidobacterium longum, Bifidobacterium bifidum*) to obtain various fermented soy foods. It is a suitable environment for the formulation of new probiotic supplements. Having in mind the fact that it contains oligosaccharides, the obtained concentrates are synbiotics.

Soy milk yoghurt has been studied extensively.[81,82,83] Fermented soy milk products provide economic and nutritional benefits, because they can be prepared at higher protein levels at comparable or lower cost than regular fermented milk products.[84] There is favourable amino acid balance, meets of the essential amino acid, requirements, except for methionine has been observed in soy proteins.[85] Research[86,87,88] show that soy milk products have lots of advantages in the nutrition of children and adults, suffering from allergies, diabetes, cancerous, heart and renal diseases. Soy milk products and soy milk yoghurt can successfully replace fermented milk products from cow's milk.[87,88] By selection of strains of lactobacilli (*Lactobacillus acidophilus* A) and bifidobacteria (*Bifidobacterium bifidum* L1) alone and in a combination with streptococci (*Streptococcus thermophilus* T3) soy probiotic milk and beverages, characterized by high concentration of active cells of lactobacilli and bifidobacteria (10^{11} - 10^{14}cfu/g) and moderate titratable acidity, which allows 20 days of storage under refrigerated conditions, are obtained. The antioxidant activity of fermented soy foods observed to be significantly higher as compare to unfermented soy foods.

Wang et al., 2006[78] observed the influence of spray-drying and freeze-drying on fermented soy milk with *L.acidophilus* and *Str.thermophilus* and bifidobacteria - *Bif. longum* and *Bif. infantis*. The authors described the increased antioxidant activity in fermented soy milk which was observed to be species specific. Freeze-drying of soy milk leads to lower reduction of the antioxidant activity. This opens up new revenue to use soy milk for obtaining probiotic supplements and probiotic enriched soy milks and beverages.

Soy cheese can be produced by coagulation of soy milk, result of the action of lactic acid bacteria. Soy cheese is the result of fermentation with starter cultures for soy cheese and the probiotic strain *L.rhamnosus*. Other non-fermented soy foods can be enriched with Probiotic lactobacilli and bifidobacteria to produce soy mayonnaise, soy delicacies, etc.in concentration 10^{6}-10^{7}cfu/g, which provides greater durability of soy foods.

Thus, addition of these microbes leads the increased durability of soy foods as well as their biological effect on the body since they deliver beneficial microflora as well. By this way preparation of healthy foods can be achieved without the application of chemical preservatives. The role of the chemical preservatives is conducted by the imported probiotic cultures.

1.6 Probiotic bacteria in the fermentation of fruit, vegetables, fruit and vegetable juices

Almost all fruits and vegetables inhabited by many types of lactic acid bacteria and can undergo natural fermentation. The fermentation process may vary as a function of the inhabited microflora of the raw material, the temperature and the storage condition. Currently fermented cabbage, olives, cucumbers, carrots, lettuce, peas, corn, tomatoes, onions, pickles, radishes, brussels sprouts, etc. are being produced mainly by natural fermentation. They allow fermentation with starter cultures as well. Lactic acid bacteria including the probiotic strains are incorporated as components of the starter cultures for fermented fruits and vegetables. they have the ability to grow in the fruit matrix and the cell vitality depends on the strain, the type of the substrate, the acidity of the product,[99] their resistance to high concentrations of salt in the medium, their capability to cultivate at temperatures around 18ºC, to reproduce quickly and to accumulate acids, which leads the acidification of the environment and inhibit the growth of extraneous microflora. During its growth in vegetable juice *Leuconostoc* synthesize dextranase which helps in the growth of other lactobacilli and bifidobacteria.

Different strains have different sensitivity towards the pH of the juice, to the acidification as a result of the fermentation, to the metabolic products, to the environmental conditions such as temperature, etc.[89,90] It has been observed that the optimum temperature for the growth of probiotic strains is 35-40ºC and pH varies between 4.0 and 3.6. Agar, alginate and chitosan are used to protect the cells from the effects of the environmental factors.[90,91,92] A probiotic banana product fermented with *Lactobacillus acidophilus*, included in alginate gel structures, is obtained. The bacteria included in alginate gel and carrageenan matrices for the protection of cells from the damages caused by freezing and freeze-drying.[93] Encapsulation is also commonly employed in the production of probiotics as well.[94]

To make the nutrient media suitable for the growth of probiotics, many processed fruits and vegetables are added to enhance the nutrients, mineral elements, vitamins and antioxidants in nutrient media.[95] Tomato juice is a suitable medium for the growth of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*,[95] which for 48 hours of growth at 30ºC reach concentration of 10^{8}cfu/ml. This

probiotic beverage can be stored at 4 degree temperature and maintains the amount of viable cells for 4 weeks. According to Rakin et al., 2007[96] addition of yeast autolysate to the vegetable juices before lactic acid fermentation leads stimulation of the growth of *Lactobacillus plantarum* and *Lactobacillus delbrueckii*. *Lactobacillus acidophilus* and *Lactobacillus plantarum* can grow in red beet juice, reaching up to10^9 cfu/ml viable cells and reducing the pH from 6.3 to 4.5.

of course during the growth of probiotic bacteria in fruit and vegetable juices it is possible to obtain a product with specific flavor and aroma. In such cases the addition of fruit juices, which remove the off flavor, is needed. All this suggests that probiotic bacteria represent a potential for obtaining fruit and vegetable functional foods because of their ability to grow in them and their resistance to acidic environments.

Table 1: Probiotic strains used in the production of fermented milk.[97,98,99]

Genus	Species
Lactobacillus	*L. acidophilus; L. delbrueckii subsp. bulgaricus; L. casei; L. crispatus; L. johnsonii; L. lactis; L. paracasei; L. fermentum; L. plantarum;L. rhamnosus; L. reuteri; L. salivarius.*
Bifidobacterium	*B. adolescentis; B. bifidum; B. breve; B. essensis; B. infantis; B. lactis; B. Longum*
Enterococcus	*E. faecalis; E. Faecium*
Pediococcus	*P. acidilactici*
Propionibacterium	*P. freudenreichii*
Saccharomyces	*S. boulardii*
Streptococcus	*S. thermophilus*

Table 2: Some criteria applied in the selection of probiotic strains for fermented foods.

Industrial field	Criteria	Product	References
Suppliers of probiotic culture	Cheap cultivation	Cultures for all groups of products	Charteris et al., 1998[100]
	Easy concentration for obtaining high cellular density	Cultures for all groups of products	Charteris et al., 1998[100]
Production of probiotic foods	Possibility for industrial production	Products, produced in high quantities (cheese)	Gomes and Malcata, 1999[101]
	Compatibility with other lactic acid bacteria	All fermented products	Samona and Robinson, 1994 [102]
	Stability during storage at acidic conditions	Acidophilous milk, yoghurt, cheese	Micanel et al. 1997;[103] Gobbeti et al, 1998[104]
	Stability during storage in non-fermented milk	Sweetened acidophilous milk	Brashears and Gilliland, 1995 [105]
	Resistance to bacteriophages	All fermented products	Richardson, 1996[106]
	Survival in the conditions during the maturation and freezing of the ice cream	Ice-cream	Christiansen et al., 1996[107]
	Tolerance to preservatives	Non-sterilized products	Charteris et al., 1998[100]
	Stability during storage at temperatures under -20°C	Ice-cream, frozen products	Christiansen et al., 1996[107]
	Tolerance towards oxygen during growth	All fermented products	Gomes and Malcata, 1999[101]
	Low activity at temperatures under 15°C	Cultures for all groups of products	Gomes and Malcata, 1999[101]
	Utilization of pentanal and n-Hexanal	Soya products	Scalabrini et al., 1998;[108] Murti et al., 1993[109]
	Stachyose	Soya products	Scalabrini et al.,1998[108]

Table 3: Physicochemical and microbiological indicators of yogurt produced using the new technology.

Day	Titrable	Concentration of viable cells (cfu/cm^3)		Proportion	Extraneous
		Str. thermophilus	*L. bulgaricus*	*L.bulgaricus*	
1	104	$5.00x10^{11}$	$5.00x10^{11}$	1:1	Not found
15	106	$6.5x10^{11}$	$6.45x10^{11}$	1:1	Not found
30	108	$6.00x10^{11}$	$6.00x10^{11}$	1:1	Not found

Table 4: Physicochemical and microbiological indicators of the probiotic yogurt with bifidobacteria during storage at 4 ± 2°C.

Day	Titrable acidity	Concentration of Viable cells,cfu/cm^3			Proportions *Str.thermophilus*: *L.bulgaricus*	Extraneous microflora
		Str.thermophilus	*L.bulgaricus*	*Bifidobacteria*		
1	114	$7x10^{10}$	$3,5x10^{10}$	$7x10^{9}$	1:2	Not found
15	120	$7,7x10^{10}$	$3,75x10^{10}$	$2,9x10^{9}$	1:2	Not found
30	126	$6x10^{9}$	$3x10^{9}$	$5x10^{8}$	1:2	Not found

CONCLUSION

Health awareness enhancing the interest in probiotic products at a rapid pace due to globalization in urban lower middle class and rural masses. Beneficial microorganisms (lactobacilli and bifidobacteria) interact with other members of the intestinal microflora. The ability of the selected strains of lactobacilli and bifidobacteria to inhibit the growth of most representatives of *Enterobacteriaceae* which cause toxemia and toxicoinfections and some molds is a criterion that the microbial strains in the composition of probiotics and probiotic foods must meet.

Consumers should understand that anything in excess is not always good though probiotic has shown a lot of potential in aiding number of illness and conditions. In the past few decades, lot of research has been carried out in this direction but no concrete conclusion could be drawn till date and have to still wait more as research is going on in this direction. Till then, consumers should understand the concept, "probiotic rather than medicine". Besides this, in the existing situation, excellent growth opportunities are stored for both domestic as well as for foreign companies to venture their capital in the probiotic industry and to make a mark for the betterment of the society.

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DECLARATION

This manuscript is free from ethical issues ad conflict of interest.

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