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Nutritional Benefits of Enriching Dairy Foods with Probiotics

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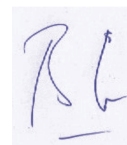
FOREWORD

Probiotics are those beneficial bacteria when consumed in adequate quantities provide a health benefit to the consumer. This definition is further qualified in the Indian Council of Medical Research (ICMR) Guidelines which provides the specific characteristics that are required to confirm a bacterial strain to be a Probiotic. Milk and dairy products are the best vehicles to which Probiotics can be added. Fermented foods also contain beneficial bacteria a majority of which belong to the *Lactobacillus* species. However not all of them have the defined Probiotic properties. This distinction between beneficial bacteria found in fermented foods versus beneficial bacteria that have Probiotic properties is not known to many. Fermented foods also provide a beneficial effect for the consumer as an inherent quality of the food e.g., DAHI but not necessarily due to the Probiotic effect of the fermenting bacteria. This distinction is not known to many.

The authors of this Review have provided a brief historical and regulatory perspective and describe the technical aspects of the various dairy based Probiotic enriched foods, and their nutritional and health advantages. The major strains used by the industry have been categorized and the specific benefits of each are also elucidated. In addition, the Prebiotics that promote the growth of the naturally present bacteria in the gut as well as the consumed Probiotics are also described and listed. There are several products that have a combination of Pre and Probiotics, defined a Symbiotics.

The design of these dairy based Probiotic foods is based on good science and an understanding of the molecular mechanisms as well as the application of these products to benefit gut health as well as overall well-being. Dairy Probiotic foods function as regular foods meant for daily consumption as well as supplements to regular diets.

This Review updates us on these aspects to help understand the intricacies of the science of Probiotics and the range of possibilities for innovative dairy products



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ABSTRACT

Several food products prepared with the help of beneficial microbes can fit into the present day definition of Probiotics which have been consumed by mankind since ancient times. Beneficial microbes are the agents that produce many fermented foods and beverages, which are popular in different parts of the world. Indications about the use of products with healthy microbes are given in *Vedic* literature and are also depicted in ancient Egyptian and European treatises. However, systematic studies on Probiotics started after the publication of a book “Prolongation of life” by Eli Metchnikoff in 1907 (Anukam *et al.*, 2007). During the last century, use of Probiotics as food and as pharma products got substantial rise. The Present Review intends to compile and analyse the literature pertaining to use of Probiotics as food products, especially for nutritional enrichment. The Review will focus on the key issues important to establishing the requirement to re-assure the efficacy and safety after strain development, process standardization, or product formulations, bioavailability of the live cells and biomolecules, effect of processing, etc. Current literatures addressing the mechanisms of action for Probiotic function and the development of Novel Probiotic Foods with particular health claim and meeting the nutritional requirement through fermentation are greatly needed to better understand the product and its application.

Keywords: Probiotics, Prebiotics, Nutritional Enrichment, Fermented Foods, Bioavailability, Efficacy, Regulatory, Safety, Synbiotic, Functional Food.

1. INTRODUCTION

One of the key challenges that is faced in the 21st century is the need to feed an ever-increasing human population with increasingly limited natural resources by providing balanced diet. It is estimated that roughly 1 out of 9 people in the world are undernourished, mainly due to protein-energy malnutrition (Torres-Tijet *et al.*, 2020). The role of a balanced nutrition in the maintenance of health is of interest to the scientific community and numerous studies have proved the efficacy of some foods in the reducing the risk of several diseases including certain metabolic syndromes. Consequently, there has been an increase in the research to find out new natural components and the development of novel foods, enabling innovation in the food area and the creation of new market niches, mainly related to functional products. Primarily, diet not only satisfies satiety but also provides adequate quantities of nutrients to meet metabolic needs of the consumer. Additionally, advancements in nutrition have revealed that diet also plays an important role in the prevention and cure of various diseases by modulating several physiological functions. Currently, consumers are searching for products with high nutritional and functional properties. The avoidance of products derived from animals (i.e. vegetarian and/or vegan) has become an increasing trend in modern lifestyle. In this scenario, the development of products that are nutritionally balanced and/or that have added value stand out for their practical use. In this niche area, Probiotic products are the best fit as they have proven track record of being healthy, natural, clean and their unique possibility to be dispensed through various food matrices. The

efficacy of Probiotic cultures is associated with their viability in the food systems and hence various factors affect their final fate. Therefore, several approaches are undertaken to improve and sustain microbial cell viability, starting from selection of Probiotic organisms to raw materials, processing technology, food matrices, packaging and storage conditions. The development of Synbiotic combinations is another approach to stimulate the growth of Probiotics (Sengupta *et al.*, 2011). The development of sustainable foods with balanced nutrition brings enormous benefits for the planet and to the society.

Probiotics are one of the most consumed food supplements and they constitute relentlessly burgeoning billion dollars industry worldwide. **The word Probiotics comes from the Latin Pro (“for”) and the Greek Bios (“life”). Metchnikoff is considered to be the “Father of Probiotics,”** but there are numerous other Scientists who have individually been credited with coining the word itself viz., Kollath (1953), Lilly and Stillwell (1965), Parker (1974), Fuller (1989), and others. They each had their own definition of the term. The World Health Organization/ Food Agricultural Organization (WHO/FAO) developed a widely accepted definition in 2001 (Binda *et al.*, 2020), which was slightly modified by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014 as **“Probiotics are live microorganisms, that, when administered in adequate amounts, confer a health benefit on the host”** (Hill *et al.*, 2014). However, for Indians, Probiotics and Synbiotic are not new concepts, as we have an evidence of a product called as **Panchamrut**, which is in vogue since *Vedic* times and used in every ritual. This has

concept of using curd, which will has healthy Bacteria and adding Honey, which is a good source of Prebiotics. However, the credit goes to Eli Metchnikoff and Prof. Shirota of Japan for spreading the knowledge of Probiotic science in the present world.

Probiotics have been supplemented in various food products namely Cheese, Ice Cream, Yoghurt, Nutrition Bars, Snacks, Breakfast Cereals, Fruit Juices and Infant Formulas. Moreover, they are also commercialized as pharma products as Lyophilized Capsules, Tablets, etc. Consumption of Probiotics is largely supported by Physicians, specifically Gastroenterologists worldwide (Suez *et al.*, 2019). Over the past few years, Fermented Milks, among the Fermented Foods, have been largely promoted on the media due to their proven Nutritional and Health Benefits. Fermented Milks and Dairy Products provide the best matrices for the Probiotics and hence are subject to multidimensional studies for their technological and functional attributes (Marco *et al.*, 2017). Moreover, consumption of Fermented Milks has been increasing rapidly worldwide because many consumers laud the health benefits associated with Probiotic Fermented Milks (Barros *et al.*, 2020; Songisepp *et al.*, 2004). The main Probiotic preparations currently available in the market belong to a group of Bacteria designated as **Lactic Acid Bacteria (e.g. *Lactobacilli*, *Streptococci* and *Bifidobacteria*)**, which are important normal constituents of the human gastrointestinal microflora and produce Lactic Acid as a major metabolic product. These Probiotics used in foods have previously been primarily added as part of the fermentation process, however increasingly they are added as supplements.

Further there is also an increasing trend in using Probiotics as Nutraceuticals, being available in various formats. However, pure cultures can be used in addition to foods.

The number of species and the abundance of microorganism added in food can vary from a single species to a consortia. Most commercialized Probiotic foods are Dairy-based, which provides an excellent carrier for Probiotic Bacteria, specifically to ***Lactobacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium species*** (Vlasova *et al.*, 2016, Chaudhary and Mudgal, 2020). For Dairy-based Probiotic Foods, they have an inherently supportive matrix; however, for Non-Dairy Probiotic Foods, it is quite complicated. Each food holds a unique food matrix, demanding higher inputs from Food Scientists to design a stable and cost-effective Non-Dairy Probiotic Food with high nutritional and sensory properties. Despite several technical difficulties, many Non-Dairy Probiotic foods have adorned global supermarkets shelves. Some recent Non-Dairy Probiotic Foods such as Fermented Cereals, Meat Products (Sausages, Hams, Lions), Beverages (Alcoholic-Free Beer), and Fruits and Vegetables (Snacks, Juices, Cut Fruits) have drawn much attention in the scientific world and also among consumers (Asaithambi *et al.*, 2021). For successful Probiotic food, the metabolic activity and viability of cultures should be conserved throughout the production process and storage, i.e., from manufacturing to its final survival in the Gastrointestinal Tract. Foods like Fermented Dairy Products contain a significant population of microorganisms, but most of these Bacteria are colonised during transmission through the Gastrointestinal Tract and hence

cannot be called as Probiotic Bacteria. Since many strains of Bacteria added to foods are sensitive to gastrointestinal conditions, novel and high-efficiency protective techniques are developed to improve their viability and survival during transmission through the stomach and intestine. To achieve this goal, encapsulation strategies are known to be efficient in protecting Probiotics by delivering them in nano-carrier systems (Atraki and Azizkhani, 2021). The ability of Probiotic strains to survive passage through the GI tract can be mainly attributed to their acid and bile tolerance. These are intrinsic characteristics of the strain, which can be improved by the protective action of carrier foods and/or by the presence of nutrients such as Metabolizable Sugars. The most common food matrices used as Probiotic vehicles are Dairy Products, which are able to enhance the transit tolerance of Bacteria. Some strains of *Lactobacillus* and *Bifidobacterium* have been shown to tolerate acidic stress when ingested with Milk products (Curto *et al.*, 2011). Most commercially available cultures are preserved as Direct Vat Set (DVS), applied either as Freeze-Dried Powders or as highly concentrated deep-frozen cultures. Among these, DVS cultures are commonly preferred as it is hard to propagate Probiotic cells in an industrial fabrication site. The deep-frozen cultures comprise a microbial load of more than 10^{10} colony forming unit (CFU) /g, while freeze-dried culture carries more than 10^{11} CFU/g. The cell concentration per gram of product differs from the type of organism and the

choice of culture. Therefore, specific information regarding strain properties must be examined in advance for process optimization (Asaithambi *et al.*, 2021). Probiotic products are becoming dear to society because of health benefits and dear to food industry because of premium income and hence the market for the same is increasing. A report by Global Market Insights Inc. indicates that probiotics market will be worth USD 4.15 billion by 2027 and growth is expected to register 9% Compound Annual Growth Rate (CAGR) between 2021 to 2027 (<https://www.globenewswire.com/en/news-release/2021/05/11/2227202/0/en/Probiotics-Market-worth-4-15-billion-by-2027-Says-Global-Market-Insights-Inc.html>). Indian Probiotic market is expected to grow at the rate of 20% per annum, doubling in next 5 years. The Indian market for food ingredient Probiotics is largely import driven. Lot of work done in India on this subject has been compiled and published as status paper by Probiotic Association of India (Grower *et al.*, 2014). Similarly lot of literature is also available on the website of The International Life Sciences Institute (ILSI) (<https://ilsi.org>) and ILSI India (www.ilsi-india.org) in the form of bulletins and other publications.

The present review is aimed at bringing out the Nutritional and Functional attributes of Probiotic Food Products, so that the stakeholders from Government, Academia and Industry can focus on their benefits and that can be conveyed to the society in an effective way.

2. IMPORTANT PROBIOTIC CULTURES

Probiotic cultures comprise of mainly bacteria and few yeasts and other organisms. Among the bacteria, members of **Lactic Acid Bacteria**, especially **Lactobacilli** and **Bifidobacteria** have been most extensively studied genera as

candidate probiotic organisms because of their association with the intestinal tract of human beings and their generally being regarded as safe (GRAS).

2.1. LACTOBACILLI

Lactobacilli are in general characterized as Gram positive, non-spore forming and non-flagellated rods. The G+C content of their DNA is usually between 32 and 51 mol%. They are either aerotolerant or anaerobic and strictly fermentative. Glucose is fermented to Lactic Acid in the homofermentative case and to equimolar amounts of lactic acid, CO₂ and Ethanol in the heterofermentative counterpart. Of the 56 species of **Lactobacillus genus**, the most commonly suggested for dietary use are **Lactobacillus acidophilus**, **L.casei**, **L.rhamnosus**, **L.bulgaricus**, **L.helveticus**. **Lactobacilli** are commonly used as starter cultures for Fermentation of Milk, Vegetables, Cereals and other foods and most of the species enjoy GRAS status due to their association with foods since long time. **Lactobacilli** provide various health benefits to the host by the production of a variety of Vitamins and Enzymes. Health application of **L. acidophilus**

has been mostly studied as Antimicrobial, Antiviral and towards Eradication of diarrhea. **L. acidophilus** has also been very active against **Rotaviruses**, **Enterobacteriaceae** and **Candida** infection (Minj *et al.*, 2020). Health benefits associated with the **L.casei** group have been reported for a variety of health conditions, ranging from atopic dermatitis to cancer. Potential mechanisms include the production of antimicrobial substances such as **Bacteriocins**, Enhancing the epithelial barrier through attachment, Competition for pathogenic binding sites, or Modulation of the immune system (Hill *et al.*, 2018). **L.rhamnosus** plays a role in Adhesion to mucosal surfaces and normalization of mucosal barrier. **Lactobacillus rhamnosus GG (LGG)** expresses >90 proteins which are involved in Biofilm formation, Phage-related functions, Reshaping the bacterial cell wall, and Immunomodulation (Capurso *et al.*, 2015).

2.2. BIFIDOBACTERIA

Bifidobacteria were first isolated from the feces of breast-fed infants in 1899 by Tissier and since then **Bifidobacteria** have been isolated from a range of different Ecological Niches such as the oral cavity, sewage and the insect gut, the GIT of various mammals and more recently from Kefir (Klijn *et al.*, 2005; Ventura *et al.*, 2007; Laureys *et al.*, 2016). They are rod-shaped, non-gas producing, anaerobic microorganisms with bifid morphology. Generally characterized as gram

positive, non-spore forming, non-motile and catalyse negative anaerobes. The optimum pH for growth is 6-7 with virtually no growth at pH 4.5 – 5.0 or below or at pH 8.0 – 8.5 or above. Ability of **Bifidobacteria** to ferment hexoses by a **Fructose-6-Phosphate Phosphoketolase (F6PPK)** shunt, often termed the “Bifidus Pathway” serve as a biochemical test to differentiate **Bifidobacteria** from morphologically similar genera such as **Lactobacillus**,

Actinomyces*, *Propionibacterium*, *Eubacterium. Presently 30 species are included in this genus, 10 of which are from human sources. All ***Bifidobacteria*** from human origin are able to utilize Galactose, Lactose and usually Fructose as carbon sources. Relevant ***Bifidobacterium sp.*** that act as Probiotics are generally strict anaerobes and are difficult to cultivate in Milk or other food substrates. Using genomic methods it was found that most strains isolated from Probiotic Dairy Products in Germany belonged to ***Bifidobacterium animalis***. Other common species used as Probiotics are ***B. brevis*, *B. infantis*, *B. Longum*, and *B. lactis***. Evidence has emerged to indicate the impact of many

Bifidobacteria on the host's immune system and metabolism, resulting in an association with a range of health benefits such as a Reduced risk of Respiratory Tract Infections and Various Gastrointestinal Disorders and Infections, particularly Antibiotic Associated Diarrhea. One of the Probiotic strains that has been studied for its mechanism of action and clinical benefits is ***Bifidobacterium animalis subsp. Lactis 420 (B420)***. The health benefits that have been shown with B420 consumption include for example Control of body fat mass gain in a human intervention trial. Preclinical data furthermore suggest Enhancement of Mucosal Integrity and Glycemic Control, as well as Improving Host Resistance to Pathogens (Uusitupa *et al.*, 2020).

2.3. SACCHAROMYCES

S. boulardii CNCM I-745 was the first strain that has been studied for use as Probiotic in human medicine, and it is one of the recommended Probiotics for the prevention and treatment of antibiotic-related diarrhea, including ***Clostridium difficile***-associated diarrhea (Czerucka and Rampal, 2019). ***S. boulardii*** and ***S. cerevisiae*** genomes were found to differ in internal regions of lower copy number in three chromosomes: ***S. boulardii*** optimal growth temperature corresponds to the human host temperature (37°C), while ***S. cerevisiae*** grows optimally at 30°C. ***S. boulardii*** is also more resistant to very high temperatures keeping 65% viability after one hour at 52°C, while ***S. cerevisiae*** loses viability down to 45% (Fietto *et*

al., 2004). ***Saccharomyces boulardii*** reduces the Duration of Infectious Diarrhoea and Antibiotic associated Diarrhoea.

A microorganism can only be classified as Probiotic after its potential health claims are proved by robust, rigorous and well-designed studies in animals and humans as well. In order to obtain the claimed benefits from Probiotic Fermented Milks, the Probiotics must be viable and adequately abundant at the time of consumption. Since each strain can have specific benefits, consuming a wide variety of Probiotic-Rich Food can give greater results. Some of the commercially available Probiotic strains are given in **Table 1**.

Table 1. Some Commercial Probiotic Strains

Strain	Source
<i>Lactobacillus acidophilus</i> NCFM	Rhodia, Inc. (Madison, Wisconsin, USA)
<i>Lactobacillus brevis</i> KB290	Kagome Co., Ltd. (Tochigi, Japan)
<i>Lactobacillus acidophilus</i> DDS-1	Nebraska Cultures, Inc. (Lincoln, NE)
<i>Lactobacillus acidophilus</i> SBT-2062 <i>Bifidobacterium longum</i> SBT-2928	Snow Brand Milk Products Co., Ltd. (Tokyo, Japan)

<i>Lactobacillus acidophilus</i> R0011 <i>Lactobacillus rhamnosus</i> R0052	Institut Rosell (Montreal, Canada)
<i>Lactobacillus paracasei</i> CRL 431 <i>Bifidobacterium lactis</i> Bb-12	Chr. Hansen (Horsholm, Denmark)
<i>Lactobacillus casei</i> Shirota <i>Bifidobacterium breve</i> strain Yakult	Yakult (Tokyo, Japan)
<i>Lactobacillus casei</i> DN014001	Danone Le Plessis-Robinson (Paris, France)
<i>Lactobacillus fermentum</i> RC-14 <i>Lactobacillus rhamnosus</i> GR-1	Urex Biotech Inc. (London, Ontario, Canada)
<i>Streptococcus thermophilus</i> MN-ZLW-002	Inner Mongolia Mengniu Dairy Industry Co. Ltd., (Hohhot, China)
<i>Lactobacillus johnsonii</i> La1	Nestlé (Lausanne, Switzerland)
<i>Lactobacillus plantarum</i> 299V <i>Lactobacillus rhamnosus</i> 271	Probi AB (Lund, Sweden)
<i>Lactobacillus reuteri</i> SD2112	BioGaia (Raleigh, North California, USA)
<i>Lactobacillus rhamnosus</i> GG	Valio Dairy (Helsinki, Finland)
<i>Lactobacillus rhamnosus</i> LB21 <i>Lactococcus lactis</i> L1A	Essum AB (Umeå, Sweden)
<i>Lactobacillus salivarius</i> UCC118	University College (Cork, Ireland)
<i>Bifidobacterium longum</i> BB536	Morinaga Milk Industry Co., Ltd. (Zama- City, Japan)
<i>Bifidobacterium lactis</i> HN019 (DR10)	New Zealand Dairy Board
<i>Lactobacillus acidophilus</i> LB	Lacteol Laboratory, (Houdan, France)
<i>Lactobacillus paracasei</i> F19	Arla Dairy (Stockholm, Sweden)
<i>Lactobacillus crispatus</i> CTV05	Gyneologix, Boulder, Colo.
<i>Lactobacillus casei</i> DN 114	Danone, Paris, France
<i>Saccharomyces boulardii</i>	Biocodex Inc. (Seattle, Washington, USA)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> 2038	Meiji Milk Products (Tokyo, Japan)

Source: Prajapati & Behare, 2018

3. MODE OF DELIVERY OF PROBIOTICS

Probiotics can be incorporated in food products and consumed in different ways. The method of incorporation depends upon the Type of Raw Material, Processing Conditions, Type of Cultures and their Biological Activities and Consumer Preferences. Three main ways of incorporation of Probiotics in food are as under:

1. As Sole Starter Culture
2. As Secondary Starter Culture
3. As Ingredient Only

Probiotics can be used as Sole Starter Culture when their metabolic activity can produce the desirable changes required for product formation and does not affect its sensory attributes adversely. For example, if a culture is for making Fermented Milk/Dahi, the culture should be able to produce enough acid to coagulate Milk (form curd) and do not produce any adverse flavour or textural changes that can make the product unacceptable. This has not been very successful option as many proven

Probiotic strains are not good in forming strong curd and usually are flat in taste or even have medical type of flavour.

The second option is relatively better, where the product is formed by the Primary Starter Cultures, while added Probiotics have secondary role. They may contribute to fermentation and produce metabolites, but does not affect overall technological and sensory attributes of the product. This type of products are able to deliver healthy and active Probiotic cells to the consumers.

The third option is also good, where the required number of cells of Probiotic organism are added as ingredient at the end of the product manufacture. The cells do not grow in the product and hence do not make any change in the product. The food just serves as a carrier of the cells. Here, the advantage of the metabolites of the Probiotic culture through product is not obtained

3.1. PRODUCTS MANUFACTURED WITH PROBIOTICS AS SOLE CULTURE

Since *Acidophilus* and *Bifidus* were the most popular cultures, the original Probiotic products prepared were employing these cultures alone or in combination. Some products are briefly described below.

3.1.1. *Acidophilus* Products

Acidophilus products are cultured Milk products made by fermentation with *Lb. acidophilus* alone or in combination with some other Bacteria or Yeasts (Kandylis *et al.*, 2016). The remarkable thing is that the culture *Lb. acidophilus* is a natural inhabitant of intestinal tract of humans and animals and is able to establish itself there

(Anjum *et al.*, 2014). This property helps in combating intestinal pathogens and cures several intestinal disorders. Additionally it gives several other health benefits and hence it has become very popular. This culture was one of the oldest candidate as Probiotic and now it is incorporated in several foods and feeds for the improvement of growth and performance of the host. Traditional *Acidophilus* milk is prepared by inoculating *Lb. acidophilus* culture at the rate of 2-5% in autoclaved or severally boiled milk. The milk is incubated at 37°C, till it attains 1 to 1.5% acidity. This Milk gives cooked flavour and unappetizing flat taste. Hence, it did not become popular and

was consumed as medicine only as and when needed. Considering the limitation of traditional Acidophilus Milk, attempts were made to prepare several other products which can supply large numbers of viable *Acidophilus* cells in acceptable forms. These attempts were directed towards supplementation of *Lb. acidophilus* with other flavour producing cultures, concentration and drying of the products or

dispensing cell concentrates into other popular dairy products like Pasteurized Milk or Ice-Cream. **Table 2** shows the list of products prepared using Acidophilus as well as Bifidus cultures. The recommended dose of live culture consumption is 10^8 cells per gram to derive maximum benefits (Shi et al., 2016). Hence, required quantity of the product needs to be consumed to have the recommended dose.

Table 2. Acidophilus-Bifidus Products

Name of the Product	Organisms Involved	Principle of Processing
Acidophilus Sour Milk	<i>Lb. acidophilus</i>	Fermentation
A-38	<i>Lc. lactis</i> + <i>Leuconostoc</i> spp. + <i>Lb. acidophilus</i>	Both Groups are Fermented Separately and Mixed
ACO-Yoghurt	Yoghurt culture + <i>Lb. acidophilus</i>	Both Groups are Fermented Separately and Mixed
Acidophilus Yoghurt	Yoghurt culture + <i>Lb. acidophilus</i>	Fermentation
Acidophilus Bifidus Yoghurt	Yoghurt culture + <i>Lb. acidophilus</i> + <i>B. bifidum</i>	Fermentation
Bioghurt	<i>S. thermophilus</i> + <i>Lb. acidophilus</i>	Fermentation
Bifighurt	<i>S. thermophilus</i> + <i>B. bifidum</i>	Fermentation
Biograde	<i>S. thermophilus</i> + <i>B. bifidum</i> + <i>Lb. acidophilus</i>	Fermentation
Sweet Acidophilus Milk	<i>Lb. acidophilus</i>	Non Fermented added in Chilled Pasteurized Milk
Acidophilus Yeast Milk	<i>Lb. acidophilus</i> + lactose fermenting yeast	Acid & Alcohol Fermentation
Acidophilin	<i>Lb. acidophilus</i> + <i>Lc. lactis</i> + yeast	Mixed Acid & Alcohol Fermentation
Acidophilus Ice-Cream	<i>Lb. acidophilus</i>	Ice-Cream added with Concentrated <i>Lb. acidophilus</i> before Freezing
Acidophilus Paste	<i>Lb. acidophilus</i>	Concentrated after Fermentation by Centrifugation
Dried Acidophilus (Powder, Tablets, Capsules)	<i>Lb. acidophilus</i>	Dried Concentrated Cells in Milk Based Media by Spray Drying, Vacuum Drying or Freeze Drying
Acidophilus Whey	<i>Lb. acidophilus</i>	Fermented Whey

Source: Senan and Prajapati, 2015

The Biological Value of acidophilus Milk is definitely high, because of the pressure on predigested proteins availability of essential Amino Acids of Milk Proteins and Microbial Cell Protein. Functionality depends on the type of Milk used and on the manufacturing process employed. *Lactobacillus acidophilus* have been reported to synthesize Folic Acid, Niacin, Thiamine, Riboflavin, Pyridoxine and Vitamin K which are slowly absorbed by the body. The vitamins of the B-complex are frequently obtained as natural ingredients in foods, so addition of *L. acidophilus* to the diet will more effectively help to meet those requirements. The bio-availabilities to the host of such minerals as Calcium, Zinc, Iron, Manganese, Copper and Phosphorus may also be enhanced upon consumption of Fermented Dairy Products and digestibility of the proteins improved (Tamime and Robinson, 1999).

High heat treatment of Milk during manufacture leads to denaturation of Milk Serum Proteins and release of Peptides that are essential for the growth of *Lb. acidophilus*. Various aroma and flavor compounds may be added to Acidophilus Milk to improve their overall sensory acceptability. Acidophilus Milk has a very distinctive tangy flavor and slightly thick texture. Shiby & Mishra, (2013) investigated the effects of A-38 on Plasma Triacylglycerol (TAG), Glucose and Insulin concentrations in human subjects. Furthermore, incorporation of Green Banana Pulp into Fermented Milk was investigated and found to cause a faster decrease in the pH, Albeit Fiber Content, Protein, Lipid, and Carbohydrates level did not change and there was no ultimate change units Calorie Value (Vogado *et al.*, 2018).

Apart from the Milk based *Acidophilus* products, *L. acidophilus* fermented Pear Juice

has been suggested recently as a new strategy for Anti-Hyperglycemia and Antihypertensive Therapy that reduce the oxidative stress associated with Type 2 Diabetes and its complications (Ankolekar *et al.*, 2012). **A Novel Probiotic Product, Oblea (Wafer-Type Dehydrated Traditional Mexican Dessert) was developed using Sweet Goat Whey fermented with *B. infantis* or *L. acidophilus* and maintained above the minimum concentration required in a probiotic product** (Santiago *et al.*, 2012). In a functional bread combining the microencapsulation and starch based coatings (dispersed or multilayer) showed that the microencapsulated *L. acidophilus* survived after baking and storage time, although reduction was higher in the sandwich treatment (Starch Solution/Sprayed Microcapsules/Starch Solution) (Altamirano-Fortoulet *et al.*, 2012).

3.1.2. Bifidus Products

Bifidiobacteria are normal inhabitants of intestinal tract of new born and infants. They possess special health properties and increase the resistance of the infant to several disorders. The Milk cultured with *Bifidobacteria* is called **Bifidus Milk**. The Bifidus Milk has its origin in Germany and is produced in small quantities in some of the European countries. Human strains of *Bifidobacterium bifidum* or *Bifidobacterium longum* is used as culture. The coagulated product has a pH of 4.3-4.7 and contains 10^8 - 10^9 /ml viable *Bifidobacteria*. Bifidus Milk can be produced as stirred product also. This product is easily digestible than the Milk from which it is made. It has been used as a protective means against imbalances in the gut microflora, in the treatment of Liver Diseases, Chronic Constipation and also as an aid in the therapy of Gastrointestinal Disorders (Kurmann *et al.*, 1992). True *Bifidobacteria* are strict anaerobes and

slow growers in Milk, hence it is difficult to prepare Bifidus Milk. However, the product is made from severely heat treated Milk taking greater aseptic precautions and using high rate of inoculum. Protein enrichment and Fat standardization are common practices of Bifidus Milk Production. Bifidus Milk has a slightly acidic flavor and a characteristic aroma with a Lactic Acid to Acetic Acid ratio of 2:3 (Gürakan *et al.*, 2010). The species regularly employed are *B. bifidum* or *B. longum*. However, use of aerolerant strains of *B. adolescentis* have been

found to give better product. Several Humanized Milks or Baby Foods have also been developed by making use of *Bifidobacteria* or *Lb. acidophilus*. In the Dairy Industry, Probiotic strains of *Bifidobacterium* are used in development of Humanized Milks or Baby Foods and they are also used as a component of the starter culture intended for the production of Fermented Beverages, Cottage Cheeses, Ice Creams, etc. (Hati *et al.*, 2013). Bifidus Milk supports the treatments of Gastrointestinal Disorders and Liver Diseases and is digested easily (Yerlikaya, 2014).

3.2. PRODUCTS MADE WITH PROBIOTICS AS SECONDARY CULTURE

Several popular products are prepared by primary start cultures and added with different types of Probiotic Bacteria as supplementary cultures. Yoghurt is the best example of such products, which is available across the Globe.

3.2.1. Yoghurts

A wide range of fermented Milk products are made in different countries, but the classical example is Yoghurt, which is manufactured as either Set-, Stirred- and/or Drinking-Types, with the products being flavoured by adding Fruit Preparations or Fruit Essences Plus Colouring Matter (Tamime *et al.*, 2005). Classical Yogurt is prepared by fermenting heat treated Milk with the help of a Symbiotic culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (Shah, 2003). The manufacturing stages of Probiotic Yoghurt are very similar to 'Classical' Yoghurt, but Natural/Plain Probiotic Yoghurt is slightly sweeter in taste and the fermentation time is slightly longer compared with the 'Classical' product. Increasingly Yogurts have been prepared with Probiotic microorganisms with varying viability over a range of shelf lives. Numerous scientific investigations have been

carried out to increase the viability and functionality of Probiotic strains in Yoghurt preparations. The most common Probiotic cultures added to Yoghurt are selected strains of *Lactobacilli* and *Bifidobacteria* (Yilmaz-Ersan and Kurdal, 2014). However, several factors may affect the survival of *Lactobacillus* and *Bifidobacterium spp.* in Yogurt. These include strains of Probiotic Bacteria, pH, Presence of Hydrogen Peroxide and Dissolved Oxygen, Concentration of Metabolites such as Lactic Acid and Acetic Acids, Buffering capacity of the media as well as the storage temperature. Viability of Probiotics in Yogurt depends on the availability of Nutrients, Growth Promoters and Inhibitors, Concentration of Solutes, Inoculation Level, Incubation Temperature, Fermentation Time and Storage Temperature. Yoghurt has about 0.8% to 1.2% Lactic Acid and Shelf-life of 3-4 weeks at refrigeration temperature. Supplementation with different substances has showed variable effects on the viability of Probiotic bacteria in yoghurt. The Bio-Yogurt supplemented with ascorbic acid improved the viability of *L. acidophilus* (Tamime *et al.*, 2005). Oxygen scavenging effect of ascorbic acid is one of the possible mechanisms that may help to

improve the viability of Probiotic bacteria. Moreover, due to their buffering capacity, the addition of whey protein may enhance the viability of some Probiotic bacteria, especially in yogurts with added fruit pulp. The incorporation of Prebiotics like Fructooligosaccharides and Inulin (Vinderola *et al.*, 2000), nutraceuticals combination containing Isoflavones, Phytosterols and Omega-3-Fatty Acids (Kailasapathy *et al.*, 2008) in yoghurt formulations stimulated the viability and activity of Probiotic bacteria. Also, addition of cysteine at 250 and 500 mg/L to yogurt was associated with higher viability of *L. acidophilus* during manufacture and storage while viability of *Bifidobacteria* was adversely affected by the same levels in different starter cultures. However, in mixed culture products, antagonistic and Symbiotic interactions among Probiotic cultures and between Probiotic and standard starter cultures are very important. The Probiotic cultures must be compatible with each other and with the standard starter cultures, since these microorganisms could produce inhibitory substances and affect viability of Probiotic bacteria (Vinderola *et al.*, 2000; Vinderola *et al.*, 2002).

3.2.2. Probiotic Dahi

Dahi is the traditional fermented Milk widely consumed in the Indian sub-continent. Traditional Dahi is made with *Mesophilic Lactic Acid Bacteria* as the starter cultures, but modern commercial Dahi has many thermophilic cultures with health benefits too. A Probiotic Dahi contains additional culture of Probiotic *Lactobacilli* or *Bifidobacteria*.

Some initial studies on Probiotic Dahi include the preparation of *Acidophilus* Dahi at Sheth MC College of Dairy Science, Anand where the

product had live *Lactobacilli* count of 200 millions/g at the end of the shelf life which makes it more beneficial. This effect had been also validated by human feeding trials of *Acidophilus* Milk. There was average or significant reduction in the Serum Total and LDL Cholesterol. In majority of the cases, a significant change in Lipid Profile Parameters (LPP) was observed after 10 and 20 days of feeding, indicating a gradual effect (Gohel *et al.*, 2016; Sreeja and Prajapati, 2015; Senan and Prajapati, 2015; Sreeja *et al.*, 2017). Similar works have also been conducted in National Dairy Research Institute (NDRI) on Dahi with increased functionality. Two types of Probiotic Dahi namely DI (containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactococcus lactis ssp*) *Lactis Biovar diacetylactis* (DRC-1) and DII (containing *Lactobacillus casei* mixed Dahi culture BD4) were evaluated for Immunomodulatory property in mice in terms of Non-Specific and Specific immune responses. The cultures significantly enhanced the Non-Specific immune response by increasing Phagocytic activity as well as Lysosomal enzymes activity of Peritoneal Macrophages. It also effectively enhanced the protective immune response against *Salmonella enteritidis* (Yadav *et al.*, 2007; Prajapati & Behare, 2018).

Cereal based Dahi developed by Kale *et al.* (2011) was a value added Dahi prepared by incorporating Fruit Pulps, Oat Flour and Whey Proteins Concentrate (WPC) to increase the nutritional and sensory quality. Dahi with 1% Oat Flour and 1% WPC was found to be highly acceptable by sensory evaluation and in terms of Curd tension of Dahi. Rajni *et al.* (2014) prepared a Dahi by incorporating Foxtail Millet Flour (1%) in Double Toned Milk. They further studied the effect of addition of Whey Protein Concentrate (WPC) to it. On sensory scores

basis it was found that Dahi made by the addition of 1% Foxtail Millet Flour and one percent WPC could safely be stored up to 27 days at $4\pm1^{\circ}\text{C}$ temperature. Singh (2017) prepared Fibre, Vitamin C and Iron rich Dahi by incorporating roasted Amaranth Flour and Orange Juice, the contents were increased by using natural ingredients. The product storage stability was found acceptable upto 10 days at refrigeration temperature.

3.2.3. Kefir

Kefir is a foamy, effervescent Milk product resulting from mixed Lactic Acid and Alcoholic Fermentation of Milk by Kefir grains. It is an old and historic product from Caucasian mountains in Russia. Kefir grains are Gelatinous, white or cream coloured, irregular grains of varying size (0.5 – 2.0 cm diameter), added with Wheat grain or Walnut (Kotova *et al.*, 2016). They are made of Polysaccharide called 'Kefiran' and are insoluble in water. Within the folds of granules, Bacteria and Yeasts reside in Symbiotic relationship. It is found that Kefir grains contain at least six functionally different groups of organism's viz., (i) **Mesophilic Homofermentative Lactic Streptococci**, (ii) **Mesophilic Heterofermentative Streptococci**, (iii) **Thermo-philic Lactobacilli**, (iv) **Mesophilic Lactobacilli** (v) **Yeasts**, and (vi) **Acetic Acid Bacteria**. All these organisms grow in association during Kefir manufacture and produce Lactic Acid (0.91 -1%), and Alcohol (0.5 -1.0%) and CO_2 (0.03 – 0.07%) as major end products (Behare *et al.*, 2015).

Kefir is expected to contain several potentially Probiotic organisms and it has been found to show health benefits. It improves digestion, prevents constipation and regularizes Bowl Movement. Being a natural antioxidant it helps to keep skin youthful and glowing. Kefir can enhance the functioning of Brain and aids in reducing stress. Regular use of Kefir helps to

reduce high Cholesterol level. Similarly Kefir is found effective for Heart health, respiratory and immune system. Kefir has been associated with Cholesterol metabolism and Angiotensin-Converting Enzyme (ACE) inhibition, antimicrobial activity, tumor suppression, increased speed of wound healing, and modulation of the immune system including the alleviation of allergy and asthma. These reports have led to increased interest in Kefir as a focus of research and as a potential Probiotic-containing product (Bourrie *et al.*, 2016).

3.2.4. Koumiss

Koumiss is a product similar to Kefir made by Acid and Alcoholic Fermentation of Milk. It is very popular in Russia and Central Asia, and traditionally made from Mare's Milk. However, due to increased demand of Koumiss and short supply of Mare's Milk, now the product is made from Cow's Milk after some modifications. Koumiss culture consists of ***Lb. delbrueckii* subsp. *bulgaricus***, ***Lb. acidophilus*** and ***Kluyveromyces lactis* or *K. marxianus* subsp. *marxianus***. Yeast and ***Lactobacilli*** grow in association in this product and produce 1 to 1.5% Lactic Acid, 1-2% Alcohol and 0.5-0.9% Carbon Dioxide. The Koumiss is graded as weak, medium and strong based on acidity and alcohol contents. In Russia, Koumiss is used for the treatment of Pulmonary Tuberculosis. Zha (1987) mentioned that Koumiss was used to cure Tuberculosis effectively during every summer and autumn at the Ximeng Mongolian Medical Research Institute. It was proved that the causative agent of Tuberculosis (***Mycobacterium tuberculosis***) cannot survive in the Mare Milk because of the Antituberculosis element generated by the microflora of Koumiss. When Koumiss is used in clinical practice to treat Tuberculosis, a 60%–91% rate of recovery was reported, which was confirmed by lab techniques such as X-rays and Tuberculin test, and the disappearance of the symptoms are an indication of effective treatment (Dhewa *et al.*, 2015). Yoghurt or Kefir are Fermented Milk that originated in Middle-Eastern

countries. The sour Milk is stored in porous earthenware or hanged in cloth bags to allow its concentration. In some area it is rolled into balls and sun dried. The product contains mixed microflora consisting of *Lc. Lactis*, *S. thermophilus*, *Lb. bulgaricus*, *Lb. plantarum*, *Lb. brevis*, *Lb. casei* and Yeast.

3.2.5. Probiotic Lassi & Butter Milk

True Butter Milk is the fluid remaining after the Cream is churned into Butter. However, more commonly the cultured Butter Milk is produced by Souring Skim Milk with *Mesophilic Lactococci* and *Leuconostocs* (Oberman and Libudzisz, 1998). They produce mild sour taste with acidity generally around 0.8 to 0.9% and give typical Diacetyl flavour. Generally Mesophilic Mixed Starter Cultures comprising *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *Cremoris*, *Lactococcus lactis* subsp. *Lactis Biovar. Diacetylactis* and *Leuconostoc mesenteroides* subsp. *Cremoris* are used. Certain slimy/ropy Lactic strains are also used

that give better consistency to the product.. Sometimes Milk may be supplemented with 0.1-0.2% Citrate to impart high flavour intensity in Butter Milk (Barukcic *et al.*, 2019). Agitation and Cooling also affect the flavour of the product.

Lassi (a refreshing drinkable, Yoghurt like beverage popular in the Indian Subcontinent) is prepared from Milk with 1.5 to 4.5% Fat from Dahi, followed by vigorous stirring to break the Curd and addition of Sugar syrup plus additional flavour (Behare *et al.*, 2010). It is a white to creamy-white, viscous liquid with a sweetish rich aroma and mild to acidic in taste. Starter culture used in the manufacture of Lassi is similar to those that are used in Dahi.

Lot of work has been done on Probiotic Lassi or *Chaach* or Butter Milk in India. Some of the products developed at the Faculty of Dairy Science, Anand Agricultural University Anand are given in **Table 3** (Gohel *et al.*, 2016; Sreeja and Prajapati, 2015; Senan and Prajapati, 2015).

Table 3. Probiotic and Synbiotic Products Developed at Anand

Product	Ingredients	Remarks
Synbiotic Dahi	Milk, Inulin, Sugar	Set Coagulated Product with 10^8 Viable Cells of Probiotic <i>Lactobacilli</i> per gram.
Synbiotic Raita	Milk, Inulin, Fructooligosachharide, Tomato, Cucumber, Onion, Banana, Sapota, Sugar	Stirred Yoghurt Type Products Fermented by Probiotic <i>Lactobacilli</i> and Garnished with Fruits and Vegetables.
Synbiotic Lassi	Milk, Oat, FOS, Carrot, Mango, Sugar, Honey	Thick Liquid with Probiotics and Shelf Life of 3 weeks at 5°C.
Whey Drink	Whey, Sugar, Pineapple	Beverage with Fruit Pieces and 10^8 cells/ml of Probiotic <i>Lactobacilli</i> .
Herbal Probiotic Lassi	Milk, Safed Musli, Sugar, Honey	Milk Fermented by Probiotic <i>Lactobacilli</i> and Supplemented with Herbs.
Protein Rich Lassi	Milk, Spirulina, Sugar	Fermented Milk Enriched in Protein by Spirulina.

Acidophilus Banana Powder	Acidophilus Milk, Banana, Sugar, Elachi	Dried Product with 10 million/g Viable Cells of <i>Lb. acidophilus</i>
Acidophilus Wheat Malt Powder	Acidophilus Milk, Wheat Malt, Sugar, Cocoa Powder	Dried Product with 10 million/g Viable Cells of <i>Lb. acidophilus</i>
Milk-Rice Probiotic Food	Milk, Rice, Freeze Dried Probiotic Culture	Milk and Rice were Fermented and Spray Dried and Blended with Freeze Dried Probiotic <i>Lactobacillus</i> Cells.
Probiotic Carbonated Beverage	Milk, Sugar	Milk Fermented by Probiotic <i>Lactobacilli</i> and Supplemented with Artificial Carbonation.
Carbonated Probiotic Beverage Employing Yeast	Paneer Whey, Sugar	Whey Fermented with <i>L.helveticus</i> and <i>K.marxianus</i> @ 2 % v/v).
Probiotic Ragi Ice Cream	Probiotic Culture, Finger Millet, Milk Solids	Ice-Cream Supplemented with Finger Millets (Ragi) and added with Indigenous Probiotic Culture.
Probiotic Oat Based Lassi	Probiotic Culture, Oat, Whey Protein Concentrate	An Oat based Fermented Beverage using Oat Bran, Cow Milk and Probiotic Bacteria.
Probiotic Smoothie	Probiotic Culture, SMP, WPI, Oat, Sugar, Mango Pulp	Smoothie was Prepared using Dry Dairy Ingredients.

Patel *et al.* (2015) reported a product where Standardized Milk was coagulated using *S. thermophilus*, *L. bulgaricus* and the Probiotic organism *L. paracasei* as free cell or in the immobilized form. Aloe-Vera Supplemented Probiotic Lassi (APL) showed stupendous role in prevention of *Shigella* Infection in terms of Reduced Pathogen Load, Elevated s-IgA Level, increased RBC Count, improved Haemoglobin Level and enhanced WBC Count (Hussain *et al.*, 2017). The Bioactive Peptide found in Lassi showed partially or completely homology to that the Milk Protein Bioactive Peptides having ACE-Inhibitory, Immunomodulatory, Antioxidant, Opioid and Cytomodulatory Activities (Padghan *et al.*, 2017). Several commercial Probiotic Drinks (Drinkable Fermented Milks including Cultured ButterMilk, Yoghurt Drink, Dairy Drink) found in the European markets and markets in other Countries. In the Indian scenario, the markets have started exhibiting many Probiotic Drinks and are widely accepted among Indians.

3.2.6. Probiotic Cheese

Cheese is one of the most versatile food products available today, appealing to many palates and suitable for all age groups. Its versatility offers opportunities for many marketing strategies, as a Probiotic Food carrier. However, the development of Probiotic Cheeses implies obligatory knowledge of all their processing steps, as well as on their level of influence positive or negative on the survival of these microorganisms throughout their shelf life. Cheeses have a number of advantages as Probiotic carrier over Yogurt and Fermented Milks because they have Higher pH and Buffering Capacity (Karimi *et al.*, 2011), Highly Nutritious, has High Energy, contains more Solid Consistency, has Relatively Higher Fat Content, and Longer Shelf Life. Several studies have demonstrated a high survival rate of Probiotics in Cheese at the end of shelf life and high viable cells. Probiotics in Cheese were found to survive the passage through the simulated Human Gastrointestinal Tract and significantly

increase the numbers of Probiotic cells in the gut. However, comparing the serving size of Yogurt to that of Cheese, Cheese needs to have higher density of Probiotic cells and higher viability to provide the same health benefits. Cheese was introduced to Probiotic Industry in 2006 when a company decided to test the growth and survival of Probiotic strains in Cheese (Song *et al.*, 2012). At that time, only few Probiotic Cheese Products were available in the market. The test showed that less than 10% of the Bacteria were lost in the Cheese Whey. Based on the process, commercial Probiotic Cheese was first developed by the Mills DA, in Oslo, Norway. Over 200 commercial Probiotic Cheeses in various forms, such as Fresh, Semi-Hard, Hard Cheese in the marketplaces. Semi-Hard and Hard Cheese are available, compared to Yogurt as a carrier for Probiotics. It has relatively low Recommended Daily Intake and need relatively high inoculation level of Probiotics (about 4 to 5 times). Fresh Cheese like Cottage Cheese has high Recommended Daily Intake, limited shelf life with refrigerated storage temperature. It may, thus, serve as a food with a high potential to be applied as a carrier for Probiotics (Songisepp *et al.*, 2004). Milk fermented with with *L. acidipiscis*, *L. pentosus* and mixed i.e combination of these two cultures have shown high ACE-Inhibitory Activity. The *L. plantarum* and mixed fermentations have shown higher Iron-Binding Activity, whereas the *L. acidipiscis* and mixed fermentations had the highest Calcium-Binding Activity. This demonstrates that Probiotic microorganisms isolated from Double Cream Cheese have great potential to be used in the production of Functional Foods (Hern *et al.*, 2021). Mozzarella Cheese is a prominent member of Pasta Filata Cheeses, originating from Italy. Pasta Filata Cheeses are famous for their exceptional Stretchability, Shredability, and Meltability which are due to unique Texturing and Plasticizing treatments of Curd in hot water. Songisepp *et al.*, (2004) developed an original Probiotic Cheese based

on the Estonian Open-Texture, Smear-Ripened, Semisoft Cheese "Pikantne." Cheese was produced by two methods using Cheese Starter Cultures (Probat 505) in combination with 0.04% of Probiotic *Lactobacillus fermentum* strain ME-3 (10^9 CFU/mL) with high Antimicrobial Activity and Antioxidative properties. Gut *Lactobacilli* can affect the metabolic functions of healthy humans. The Probiotic Cheese prepared with *Lactobacillus plantarum* TENSIA (Deutsche Sammlung Für Mikroorganismen, DSM 21380) could reduce some symptoms of Metabolic Syndrome in Russian Adults with Obesity and Hypertension. Microencapsulation may be used to protect Probiotic organisms and improve viability and encapsulated *B. bifidum*, *B. infantis* and *Bifidobacterium longum* have been used in the manufacture of Crescenza Cheese (Kailasapathy, 2002). Another method of introduction, particularly into Semi-Hard and Hard Cheeses, is through the addition of a dried culture during salting of Curd, as is done with some accelerated ripening enzyme preparations. This method minimises the losses of Bacterial cells to Whey and eliminates the effects of competition with *Lactic Acid Bacteria* during Milk ripening.

Table 4. Probiotic Products Available in the Market have Following Strains

Probiotic Strains
<i>L. casei shirota</i>
<i>L. johnsonii</i> , <i>L. helveticus</i>
<i>L. casei defensis</i>
<i>L. casei</i>
<i>L. acidophilus</i> , <i>L. casei</i>
<i>L. casei</i>
<i>L. casei</i>
<i>B. animalis</i> DN173010
<i>B. animalis subsp. lactis</i>
<i>B. animalis subsp. lactis</i> , <i>L. acidophilus</i>
<i>B. animalis subsp. lactis</i>

Source: Sreeja and Prajapati, 2015

3.3. INCORPORATION OF PROBIOTICS AS SUPPLEMENTS

Probiotics, are one of the most consumed Food Supplements and they constitute relentlessly burgeoning billion dollars Industry worldwide. Probiotics have been supplemented or added as one of the ingredients in various food products namely Cheese, Ice Cream, Yoghurt, Nutrition Bars, Snacks, Breakfast Cereals, Infant Formulas, and Juices. Moreover, they are also commercialized as Lyophilized Cells packed in different formats. Consumption of Probiotics is largely supported by physicians, specifically Gastroenterologists worldwide (Sakandar *et al.*, 2021).

3.3.1. Fermented Dairy Products

Microencapsulation is one of the important tools to improve the viability of Probiotic cells in different products. For example, Microencapsulated Probiotic Cells added to Yogurt fermented by Yogurt cultures may circumvent possible negative sensory attributes due to a Probiotic strain (Nayanzi, *et al.*, 2021). Qi (2019) found 60% increased survival rate of Probiotics under simulated Gastrointestinal conditions compared to free cells (25%) and concluded that the encapsulation by using Biopolymers offers an effective way to protect Probiotics in adverse processing and In Vitro conditions.

The potential of Lactose-free Greek-style Yoghurt as a new matrix for incorporation of Spray-Dried Microcapsules containing the Probiotic *Bifidobacterium lactis* BB-12 has been evaluated. All formulations showed encapsulation yield above 96% and good Probiotic viability (>8 log CFU/g) throughout 30 days of storage (4°C). Microcapsules produced with Gum Arabic and Inulin showed the best physical properties (Lowest Moisture, Solubility

and Hygroscopicity Values, and Highest Bulk and Tapped Densities), it did not affect the viability of the starter cultures and thus were selected to be added to the Yoghurt. After 30 days of storage, Probiotic viability was above 6.5 log CFU/g, indicating that Lactose-free Greek-style Yoghurt may be an appropriate matrix for *B. lactis* BB-12 (Pinto, *et al.*, 2019).

Encapsulated Probiotic Bacteria in Alginate Microcapsules to increase their survival during manufacturing of “Mozzarella” Cheese has been reported. (McMahon *et al.*, 2012). The freeze-dried culture *Streptococcus thermophilus* was used, as primary starter, in the Cheese-making trials. Five Lyophilized commercial Probiotic Bacteria (*L. casei* DG, CASDG; *L. paracasei* F19, PARF19; *L. paracasei* B21060, PARB21060; *L. rhamnosus* GG, RHAMGG; *L. rhamnosus* IMC 501 plus *L. paracasei* ssp. *Paracasei* IMC 502, SYNBIO) were screened in the study and used as adjunct cultures. Both in “Mozzarella” and “Scamorza” no significant change in load was observed during refrigeration or ripening time both in control and Probiotic samples.

A Probiotic Fermented Milk popular in Japan contains Probiotic culture, *Lb. casei* strain *Shirota* discovered by Dr. Minoru Shirota, a Japanese Scientist in 1930, which has scientifically proven several health benefits. It was first launched in Japan in 1935, and now it is available in several countries including India. It contains more than 6.5 billion *Lactobacillus casei* strain *Shirota* in 65 ml bottle (concentration of 10⁸ CFU/ml). It has more than 80 years of research to support its safety and efficacy and has been proven to improve Bowel movement, aid in digestion, maintain balance of Gut Microflora, reduce toxins in body and helps in building immune system. There are many suggested mechanisms of the Probiotic action of *LcS* in the intestine, but

aside from Immune Modulation, the production of Lactic Acid (resulting in a reduction of local pH) and the competitive adhesion or displacement of pathogenic bacteria have been quoted most often in the literature (Schwandt *et al.*, 2005)

3.3.2. Ice-Cream and Frozen Desserts

Ice-Cream and Frozen Desserts have potential as carriers of Probiotic organisms, but freeze stress must be considered with respect to viability during manufacture and extended storage. Various combinations of *Lactobacilli* and *Bifidobacteria* have been used in Synbiotics. Addition may be direct (i.e. blending of Ice-Cream mix and Probiotic cells immediately prior to freezing), or it may involve Fermentation of the Milk for proliferation of Probiotic cells prior to blending with the Ice Cream mix (Tamime *et al.*, 2005). In either case, the protection of cells against freeze damage is of importance. Encapsulation and freeze-drying, and co-encapsulation of different micro-organisms have been evaluated with the result that free cells and freshly encapsulated cells without freeze-drying demonstrated the best survival rates. Properly selected strains, such as *Lactobacillus johnsonii* La1, survive the relatively high Sugar content of Ice-Cream as well as the sub-lethal injuries caused by freezing. Because Ice-Cream is a non-fermented product, the impact of the Probiotic micro-organisms on flavour should be a consideration

3.3.3. Probiotic Chocolates

Probiotic applications into Chocolate could offer a good alternative to common Dairy Products and allow broadening the Health Claims of Chocolate based Food Products. Indeed, recent market research on Functional Food has shown that, in relation to Chocolate, Digestive Health is one of the most important drivers of consumer acceptance (Callebaut, 2007;

<https://www.barry-callebaut.com/en-IN/group/media/news-stories/barry-callebaut-launches-first-probiotic-chocolate-industrial-scale>). The development of Probiotic containing Chocolate involves a good understanding of the selected Probiotic strains, the Chocolate manufacturing process and the different critical points of the process for Probiotic survival, as well as the application of specific protective technology. One of the major challenges to incorporating Probiotics into Liquid Chocolate and Chocolate related products on an industrial scale is that these products need to be maintained at a temperature that does not kill the Probiotics. This requires a narrow temperature range lower than that normally used in Chocolate manufacturing, making it difficult to achieve effective mixing and proper Probiotic dispersion. To take care of these difficulties a patented process has been reported where two selected Probiotic strains were protected with a Specific Micro-Encapsulation Technology (Patent US 2003/0109025 A1, 2003).

Mandal *et al.* (2013) reported study on incorporation of Micro-Encapsulated *Lactobacillus casei* NCDC 298 and Inulin in Milk Chocolate and the Efficacy of the Milk Chocolate in delivering the live *Lactobacilli* in modulating the intestinal microenvironment of mice. They observed that after 30 days of storage at room temperature, the *Lactobacilli* counts were decreased by approximately 3 and 2 log cycles from an initial level of ~8 log CFU/ g in Milk Chocolate with free and Encapsulated *Lactobacilli*, respectively; however, at refrigeration temperature the viability of free as well as Encapsulated *Lactobacilli* was unchanged in Chocolate till 60 days. Supplementation of Milk Chocolate with Inulin (5%) and free or Encapsulated *Lactobacilli* (~8 log CFU/ g) increased the Faecal *Lactobacilli*, Decreased Coliforms and b-Glucuronidase Activity. Ghadia *et al.* (2015) prepared a Probiotic Chocolate using Probiotic culture

***Lactobacillus helveticus* MTCC 5463.** The culture was added in the form of either concentrated or freeze dried state. It was concluded that the Probiotic Chocolate with ***L. helveticus* MTCC 5463** could be prepared by incorporation of freeze dried culture at the rate 3% (w/w) of the ingredient mix just before tempering. The Chocolate had acceptable Organoleptic quality till 30 day of storage but the viability of Bacteria (2.42×10^8) remained good only up to 15 days of storage at $10 \pm 2^\circ\text{C}$.

3.3.4. Infant Foods

Probiotics and Prebiotics have been used in development of Infant Foods since long time. The basic concept for the product development is to make a product similar to Human Milk that will nurture the baby. Initially attempts were to modify the Cow's Milk composition to bring it closer to Human Milk. Then Probiotic addition was done with strain of ***Bifidobacteria*** as the organisms is supposed to play key defensive role in baby's gut by modulating the gut flora. Some of the earlier products that came from Europe were prepared as dried formula product containing Prebiotic Lactulose and viable ***B. bifidum***. It is produced from Modified Milk and developed in Germany in 1964. One of the product developed in USSR in 1982 is a liquid formula containing viable ***Bifidobacteria***. A Milk formula was used for making Russian product. The heat treated, Homogenized Milk formula is fermented with 5% starter culture containing 0.5% Corn extract at 37°C for 8-10h until coagulation and followed by cooling. The final product has about 0.6% Titratable Acidity and 10^7 - 10^9 /g viable ***Bifidobacteria*** (Sreeja and Prajapati, 2015).

A dried formula developed in Czechoslovakia in 1984 was made by fermenting heat treated Cream (12% fat) with 2-5% mixed culture consisting of ***B. bifidum***, ***L. acidophilus*** and ***P.***

acidilactici in 1:0.1:1 ratio at 30°C till desired acidity followed by cooling (https://sist.sathyabama.ac.in/sist_coursematerial/uploads/SMB1603.pdf). To this, heat treated Vegetable Oil, Lactose, Whey Proteins and Vitamins are added and the mixture is homogenized and spray dried. The final reconstituted product has a Titratable Acidity of 0.25% and contains 10^8 - 10^9 /ml viable culture (Kurmann *et al.*, 1992). Yet another commercialized Milk Powder (i.e.) containing ***B. animalis subsp. Lactis* BB-12** was manufactured for older infants (Playne, 2002, Chouraqui *et al.*, 2004).

B. animalis subsp. lactis* BB12:** BB12 has been commercially available for over 25 years and is one of the most thoroughly studied Probiotic strains available. It has been administered to infants either alone or in combination with a number of other Probiotic strains and has proven to be well tolerated and has a number of beneficial effects (Garrigues, *et al.*, 2010). Cesarean-delivered infants consuming formula supplemented with BB12 have higher levels of ***Anti-Poliovirus Specific and ***Anti-Rotavirus*** Specific IgA following Immunization in comparison with a control group indicating the Immunomodulating properties of the Probiotic (Holscher, *et al.*, 2012).

Table 5: Some Examples of Incorporation of Probiotics in Infant Foods

Name	Deposit Code
<i>L. acidophilus</i> NCFM	ATCC SD5221
<i>B. animalis subsp. lactis</i> BB12	DSM 15954
<i>L. rhamnosus</i> GG	ATCC 53103
<i>L. reuteri</i>	DSM 17938

Source: Mugambi *et al.*, 2012

4. USE OF PREBIOTICS AS A MEANS OF NUTRITIONAL ENRICHMENT

In 2008, the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined “**Dietary Prebiotics**” as “**a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health**” (Davani-Davari *et al.*, 2019).

An integration of synergistically acting Probiotics and Prebiotics thus is defined in terms of “Synbiotic.” The mode of action of Synbiotics is directly linked with the type of live culture being used for the purpose. The Synbiotic products offer health benefits in two ways: (1) By Improving the Viability of the Probiotic Cells and ensuring that they Reach the Colon in Satisfactory Populations to give a Beneficial Health Effect and (2) With Prebiotics, Probiotic Cells can Survive and Increase in Numbers. A true Probiotic cannot survive well and is vulnerable to variations in pH, oxygen, and temperature in the human digestive system

without presence of specific Prebiotic. In other words, the Prebiotics are basically supportive and preservative agents for the live cultures (Shafi *et al.*, 2014).

Synbiotics are mostly prepared by adding Prebiotic ingredients/Dietary Fiber or value-added ingredients along with Probiotics (**Table 6**). More than 350 products have been approved under Food for Specific Health Use as of April, 1, 2011, with a Claim to control GI functions (Helps to Maintain Good GI Condition and to Improve Bowel Movement), which include **Oligosaccharides, Lactobacillus, Bifidobacteria** and **Dietary Fibers/Nondigestible Oligosaccharides**. Among all, preparation with **Cassava Starch or Inulin**, Milk and three different **LAB; Lactulose, Inulin** with ***L. acidophilus* LA-5** and ***Bifidobacterium bifidum* BB-12**; **inulin, FOS** and **Honey with *L. acidophilus***; **Sugar with Inulin** and **Probiotics; Raftiline, Raftilose** with **Honey, Milk**, and **LAB** are worth mentioning (Ghosh, 2012).

Table 6: Examples of Prebiotics and Synbiotics Used in Human Nutrition

Prebiotics	Probiotic Organism
FOS	<i>Lactobacillus</i> Genus Bacteria
GOS	<i>Lactobacillus, Streptococcus</i>
Inulin	and <i>Bifidobacterium</i> Genus Bacteria + FOS
XOS	<i>Lactobacillus, Bifidobacterium, Enterococcus</i> Genus Bacteria + FOS
Lactitol	<i>Lactobacillus</i> and <i>Bifidobacterium</i> Genus Bacteria +
Lactosucrose	Oligofructose <i>Lactobacillus</i> and <i>Bifidobacterium</i>
Lactulose	Genus Bacteria + Inulin
Soy Oligosaccharides	
TOS	

Source: Sáez-Lara *et al.*, 2016

Some of the Health Benefits of Synbiotics are as Under (Mugambi *et al.*, 2012).

- Increased Expression or Change in the Composition of Short-Chain Fatty Acids to Colonocytes during Fermentation of Prebiotics Carbohydrates.
- Increased Fecal Weight and a Mild Reduction in Luminal Colon pH.
- A more Acidic pH and Modulation of the Intestinal Flora, especially Growth Stimulation of Carbohydrate-Fermenting Bacteria.
- Decreased Concentration of Putrefactive, Toxic, Mutagenic, or Genotoxic Substances and Bacterial Metabolites, as well as of Secondary.
- The *bifidobacteria* and *lactobacilli* (Increased by Oligosaccharides) Exhibit Low β -Glucuronidase and Nitroreductase Activity.
- Decreased Nitrogenous End Products and Reductive Enzymes.
- Production of Butyric Acid Reinforcing the Regeneration of the Intestinal Epithelium (i.e., Through its Pro-Apoptotic Potency).
- Increased Expression of the Binding Proteins or Active Carriers Associated with Mineral Absorption.
- Enhanced Immunity and Modulation of Mucin Production.

5. FUNCTIONAL EFFECTS OF PREBIOTICS ON HUMAN HEALTH

In recent years, poor eating habits have had a pronounced impact on people's health and quality of life. The excessive consumption of Fats, especially Saturated Fats, Surplus Sugar and Salt, and low consumption of Dietary Fibers, have led to an increasing incidence of Chronic Degenerative Diseases, such as Cardiovascular Problems, Cancer, Diabetes, and Obesity (Choque-Delgado *et al.*, 2011). Prebiotics are the functional compounds of foods, which play an important role in the prevention and treatment of Gastrointestinal Diseases. Prebiotics consumption could result in some advantages to the host, due to their selective metabolism in the intestinal tract (Gibson *et al.*, 2010). The metabolites produced by healthy microbiota interact with the immune system in the prevention or reduction of Intestinal Inflammatory Disease (Damaskos and Kolios, 2008; Mohamadzadeha *et al.*, 2011).

The food industry is adding value to products by supplementing them with Prebiotics. The health-promoting effects of Prebiotics include benefits to host nutrition, the growth inhibition of pathogens, and the promotion of beneficial microbiota (Choque-Delgado *et al.*, 2011) (**Table 7**). The latter causes fermentation of Nondigestible Fibers, Energy Saving, Synthesis

of B and K Vitamins, Metabolism of Plant Compounds, and Pharmaceuticals, Production of SCFA and Polyamines, Improvement in Gastrointestinal Motility and Function, Cholesterol Reduction, and Stimulation of the Local Immune System (Penders *et al.*, 2016).

The regular intake of Prebiotic Fructans, such as the **FOS** and **Polysaccharides** (e.g., **Inulin**), sustain health, and overall well-being by (i) Improving Blood Parameters, (ii) Enhancing Resistance against Intestinal as well as Extra-Intestinal Pathogens, (iii) Modulating Immune Responses, and, Finally, (iv) Decreasing Allergies (Choque-Delgado *et al.*, 2011; Peshev and Van den Ende, 2014).

Fructo Oligo Saccharides (FOS) and **Inulin** are thought to exert their beneficial activities mainly in the proximal part of the Colon, but there is a great interest in finding different Prebiotics or mixtures that exert their biological activity in the Distal Colon, where many Chronic Diseases originate. **Branched Fructans** (e.g., **Cereal Graminans**) and **Arabinogalactans** may be beneficial in this respect (Allsopp *et al.*, 2013; Terpend *et al.*, 2013; Yang *et al.*, 2013). Usually, the effects of these compounds are attributed to indirect mechanisms via their positive influence on intestinal microbiota.

Table 7: Use of Synbiotics in Product Development

Products	Probiotic	Prebiotic	Use of Prebiotic and Their Effect in Synbiotic Products	References
Synbiotic Semi-Hard Cheese	<i>Lb. paracasei</i> INIA P272	Fructo-Oligosaccharides (FOS)	Increasing the Probiotic Strain Viability in the Host. Organic Acid Detection and Volatile Fraction Analysis of the Cheese Revealed a Stimulation of <i>Lb. paracasei</i> Metabolism when FOS was Present.	Langa <i>et al.</i> , 2019
Synbiotic Yogurt	<i>Lb. rhamnosus</i> and <i>Lb. reuteri</i>	Inulin, Lactulose, and Oligofructose	Inulin and Lactulose showed the Lowest Syneresis. Inulin - <i>Lactobacillus rhamnosus</i> and <i>Lactobacillus reuteri</i> , showed the Best Viability and Provides a Microbial Balance in the Gut. Inulin and Lactulose - showed the Most Desirable Sourness.	Shaghaghi <i>et al.</i> , 2013

Synbiotic Ice Cream	<i>Saccharomyces boulardii</i> C NCM I-745	Inulin	<p>Addition of Inulin Increased the Viability of <i>S. boulardii</i> C NCM I-745 with Viable Count of 6.16 log CFU/g in the Synbiotic Ice Cream after 120 d of Storage.</p> <p>Improved Firmness and Melting Property and Stability.</p> <p>About 27 Volatile Compounds Identified in Different Ice Cream Formulations and their Concentration was the Highest (3983 µg/L) in Synbiotic Ice Cream as Compared to Control (616.4 µg/L).</p>	Sarwar <i>et al.</i> , 2021
Synbiotic Lassi Synbiotic Yoghurt	<i>Str. thermophilus</i> MTCC 5460	Honey (at 5% level) Honey Contains 4-5 % Fructo-Oligosaccharides which may Serve as Prebiotic Agents	Slightly Higher <i>Lactobacilli</i> and <i>Streptococci</i> Counts and a Significantly (P<0.05) Higher Sensory Scores.	Sharma <i>et al.</i> , 2016
Synbiotic Yoghurt	<i>L. rhamnosus</i>	Fibre Source (0.5 % oat flour)	<p>Good Storage Stability.</p> <p>The Viability of Probiotics was Good in Synbiotic Yoghurt Made with both the Probiotic (<i>L. rhamnosus</i>) and Prebiotic Fibre Source (0.5 % oat flour) up to 21 Days of Storage.</p> <p>The pH, Acidity, Syneresis, Protein, Fat and Crude Fibre Content of the Synbiotic Yoghurt was High among all the Other Yoghurt Samples.</p>	Ranjitham and Poornakala, 2020
Synbiotic Mousse	<i>Lb. acidophilus</i> La-5	Inulin and Fructo Oligosaccharides	Significant Reductions of Total Cholesterol and HDL-Cholesterol, as well as of Immunoglobulins (A and M), and Interleukin-1β in both Groups.	D. Xavier-Santos <i>et al.</i> , 2018
Synbiotic Low-Fat Yogurt	<i>Lb. casei</i>	Pectinase Hydrolyzed Fraction of Tragacanth Gum (PHFTG)	Low Molecular Weight Tragacanth Gum (TG) was Easily Fermented as Indicated by Concomitant Increases in <i>L. casei</i> Population. TG can act as Substitutes for Conventional Stabilizers or Fat Replacer in the Food Industry that can also Enhance the Health Benefits of Products.	Ghaderi-Ghahfarokhi <i>et al.</i> , 2020

6. GENETIC IMPROVEMENT OF PROBIOTICS FOR ENHANCING THE HEALTH ATTRIBUTES

Lactic Acid Bacteria and *Bifidobacteria* are Generally Recognized as Safe (GRAS) according to Food and Drug Administration (FDA), US. Application of genetic engineering technology to improve characterized strains or develop new strains, is a global area of active research. Advances in gene technology allow us to modify strains by introducing new genes or altering their metabolic functions. Over the last 20 years, numerous ways to introduce heterologous genes into *Lactic Acid Bacteria strains* have been demonstrated (Rondanelli *et al.*, 2015; Papadimitriou *et al.*, 2015).

By means of recombination and later chromosomal integration and Food-Grade Vectors and the development of CRISPR-Cas9 genome editing in combination with ssDNA recombineering, Kojic *et al.* (2011) could express the aggregation factors of *L. lactis* in the Probiotic Bacteria with interest, to enhance its adhesion to the intestinal epithelium. Interleukins or Human Cytokines in the Probiotic strain could be expressed in order to increase immune system stimulation; vaccines using Probiotic Bacteria could be produced (Shi *et al.*, 2014); expression of enzymes such as bile salt hydrolase from *Lactobacillus fermentum* in Probiotic Bacteria which would also help to reduce cholesterol (Kumar *et al.*, 2013); and gastro intestinal problems in patients with Celiac Disease could be reduced by expressing a Prolyl Endopeptidase in Probiotic bacteria (Alvarez-Sieiro *et al.*, 2014). Only a few nucleotides have been modified by CRISPR system in *Lactic Bacteria* (Oh & van Pijkeren, 2014). However, the development of this technique would allow editing much longer gene sequences, allowing

us to introduce new heterologous genes in *Lactic Acid Bacteria* and *Bifidobacteria* (Stefanovic *et al.*, 2017). Organisms modified by CRISPR system are not considered as GMOs but are considered safe and suitable for application in food manufacture and human health.

Martin *et al.* (2011) described a chromosomally integrated expression system in *Lactobacillus paracasei* based on the aggregation-promoting factor gene (*apf*) of *Lactobacillus crispatus* (Marcotte *et al.*, 2004). *Lb. paracasei* produced antibodies directed against the rotavirus. Alvarez-Sieiro *et al.* (2014) engineered a food-grade strain of *Lb. casei* to deliver *Myxococcus Xanthus* Prolyl Endopeptidases into the gut environment. Steidler *et al.* (2003) also showed that *L. lactis* strains expressing and secreting murine Interleukin-10 (IL-10) could be used to treat inflammation in mouse colitis models. *L. lactis* strains secreting human IL-10 was approved by the Dutch authorities for use in a small clinical trial as an experimental therapy for use in humans with Inflammatory Bowel Diseases (van Pijkeren *et al.*, 2012). Genetic Manipulation of the Pathway for Diacetyl Metabolism in *Lactococcus lactis*. Benson *et al.*, (1996) reported that the level of Diacetyl (0.53 mM) achieved by a combination of *aldB* (Alpha- Acetolactate Decarboxylase) deletion and *ilvBN* (Alpha-Acetolactate Synthase form Part of the Operon Involved in the Biosynthesis of Isoleucine, Leucine, and Valine). Increased expression compares favorably with the level produced by *L. lactis ssp. Lactis Biovar Diacetylactis* (0.087 to 0.17 mM) from Citrate fermentation. When cultivated aerobically at an initial pH of 6.8, overexpression of the *als* gene in

L. lactis NZ2700 resulted in the conversion of more than 60% of the Pyruvate into Acetoin, while no Butanediol was formed. Moreover, at an initial pH of 6.0, similar amounts of Acetoin were obtained, but in addition, approximately 20% of the Pyruvate was converted into Butanediol. These metabolic engineering studies indicate that more than 80% of the Lactose can be converted via the activity of the overproduced α -Acetolactate Synthase in *L. lactis* (Boels *et al.*, 2001).

Polyols, such as Mannitol and Sorbitol, are Low-Calorie Sugars that can replace Sucrose, Lactose, Glucose or Fructose in food products as they display equivalent sweetness and taste. Heterofermentative LAB such as *Leuconostoc mesenteroides* are known for their ability to produce Mannitol in the fermentation of Fructose. These Bacteria convert part of the Fructose for energy generation via the usual Heterofermentative pathway, while the rest of the Fructose is reduced directly to Mannitol. Overproduction of the Mannitolphosphate Dehydrogenase (MPDH) in LDH-Deficient *L. Lactis* strains or a decrease in Phosphofructokinase (PFK) activity. Higher production of Mannitol by *L. lactis* would also be expected if excretion of this Polyol, by introducing the Mannitol transporter as found in *Leuconostoc Mesenteroides* (Kleerebezem *et al.*, 2000). Overexpression of the gene coding for SDH Sorbitol Dehydrogenase, in combination with disruption of the MPDH and the LDH, considerable Sorbitol production has been observed in *L. plantarum* strains. Also in this case, even higher production of Sorbitol is expected when the Dephosphorylation and the transport (export) of Sorbitol is enhanced (Kleerebezem *et al.*, 2000). Kleerebezem & Hugenholtz, (2003) attempted the engineering of *Lactococcus Lactis* to produce the Amino Acid i.e. L-Alanine by rerouting the carbon flux toward Alanine by expressing the *Bacillus Sphaericus*

Alanine Dehydrogenase, L-AlaDH, (Pyruvate + NADH + NH_4^+ \rightarrow L-Alanine + NAD^+ + H_2O). Expression of L-AlaDH in an L-LDH-deficient strain permitted production of Alanine as the sole end product (Homoalanine Fermentation). The Dairy starter *Bacterium Lactococcus Lactis* is able to synthesize Folate and accumulate large amounts of Folate. Five genes involved in Folate biosynthesis were identified in a Folate gene cluster in *L. lactis* MG1363: *folA*, *folB*, *folKE*, *folP*, and *folC*. The gene *folKE* encodes the Biprotein 2-Amino-4-Hydroxy-6-Hydroxy Methylidihydro Pteridine Pyrophosphokinase and GTP Cyclohydrolase. The overexpression of *folKE* in *L. lactis* was found to increase the extracellular Folate production almost 10-fold, while the total Folate production increased almost 3-fold (Burgess *et al.*, 2004). It has been shown that metabolic engineering can be used to increase Folate levels in *L. lactis*, *L. gasseri* and *L. reuteri*. Several of Folate biosynthetic genes have been overexpressed individually or in combination in *L. lactis* strain NZ9000 using the NICE system. Overproduction of the first enzyme in Folate biosynthesis, GTP Cyclohydrolase, led to a 3-fold increased production of Folate. Overexpression of *folKE* that encodes the biprotein 2-Amino-4-Hydroxy-6-Hydroxymethylidihydropteridine Pyrophosphokinase and GTP Cyclohydrolase I in *L. lactis* ssp. *cremoris* MG1363, was found to increase the extracellular Folate production almost 10-fold and the total production 3-fold (Burgess *et al.*, 2004). The gene coding for this enzyme, *ribA*, has been brought to overexpression in *L. lactis* using the NICE-system resulted in a 3-fold overproduction of Riboflavin. Increased Riboflavin-production by selecting for strains or variants that are resistant to analogues of Purine, or their biosynthetic precursors, such as 8-Azaguanine and Decyainine. Substantial Riboflavin overproduction was reported when all four biosynthetic genes (*ribG*, *ribH*, *ribB* and *ribA*) were over expressed simultaneously in *L. lactis* (Papagianni, 2012) (Table 8).

Table 8. Overview of Relevant Metabolic Engineering Approaches Resulting in the Design of New Strains with Improved Properties

Biomolecules	Producers	Specific Genes	Functional Benefits
Alanine	<i>Lactococcus lactis</i>	<i>AlaD, ldh, alr</i>	Low Calorie Sweetener, Improve the Flavor of Fermented Food and Save the Cost of Sugar.
Mannitol and 2,3-Butanediol	<i>Lactococcus lactis</i>	<i>Ldh, ldhB, ldhX</i>	Mannitol is Used as a Sweet-Tasting Bodying and Texturing Agent. It Reduces the Crystallization Tendency of Sugars and is Used as such to Increase the Shelf Life of foodstuffs. 2,3-Butanediol Used for Production of Moistening and Softening Agents.
Sorbitol	<i>Lactobacillus casei</i>	<i>GutF, ldhL</i>	Used as a Low-Calorie Sweetener, is An Excellent Humectant and Texturizing Agent.
Xylitol	<i>Lactococcus lactis</i>	<i>XylI, xylT</i>	Used as Sweetener in Food and Pharmaceutical Products, the Metabolism of Xylitol in Humans is Independent of Insulin, which Makes it a Suitable Sugar Substitute for Use by Diabetics.
Folate	<i>Lactococcus lactis</i>	<i>FolKE</i>	A Cofactor in Normal Cellular Functions and Also in Growth and Development.
EPS Production	<i>Lactococcus lactis</i>	<i>GalU</i>	Used to Improve the Texture of Fermented Dairy Product, Also have a Potential Application as Food Additives.
Histidine Decarboxylation Pathway	<i>Lactococcus lactis</i>	<i>HdcA, hdcP, hdcB</i>	Role in Gastric Acid Secretion, Neurotransmission and Immune Response.
Lactic Acid	<i>Lactobacillus helveticus</i>	<i>ldhD, ldhL</i>	Used as an Acidulant and a Preservative, as well as a Substrate for the Production of Biodegradable Plastics and Some Other Organic Acids.

Ethanol Production	<i>Lactococcus lactis</i>	<i>Pdc, ldh, ldhB</i>	Ethanol is Used as a Natural Product to Extract and Concentrate Flavours and Aromas, and can help evenly Distribute Food Coloring.
Butanol	<i>Lactobacillus brevis</i>	<i>Crt, bcd, etfA, etfB</i>	-
Acetaldehyde	<i>Streptococcus thermophilus</i>	<i>GlyA, glyB</i>	Flavour Components of Dairy Products.
Diacetyl	<i>Lactococcus lactis</i>	<i>Nox, aldB</i>	Gives Aroma in Dairy Product Especially Butter, Cheese.
Diacetyl and Acetoin	<i>Lactobacillus casei</i>	<i>Ilvbn, ldh, pdc</i>	Important Flavor Compounds Responsible for the Buttery Aroma of Some Food Products and are Used as Additives in the Food Industry.

Source: Peters et al., 2019

7. CONCLUSIONS

Probiotics are becoming increasingly popular in the society. Any solution that keeps the society happy and healthy and reduces the disease burden is most welcome by the society and the Governments. Consumption of foods with healthy microbes is not new to the world, but preparing novel products with clinically tested Probiotics is more specific and reliable to give desired health attribute. Probiotics can be consumed as live cells in form of pills, but their consumption through food system brings many more benefits. Incorporation of Probiotics in different food matrices is therefore important. Probiotic foods will deliver the inherent nutrients present in the carrier foods apart from metabolites produced by culture. They also help in improving the biological and functional value of the food.

Different types of microorganisms have been used as Probiotics, but the strains belonging to the Genera *Lactobacilli* and *Bifidobacteria* are most popular. These Bacteria have been involved in natural fermentation of several food materials too. Systematic incorporation of Probiotic Bacteria in foods can be done in three ways viz., As Sole Culture, As Secondary Culture or As Dietary Supplement or Ingredient. More than 60% Probiotic have been Dairy

based. Milk has been an excellent raw material for Probiotics to grow and it is known for its Nutritional Value, being nearly a complete food. Further, Milk Proteins give very good Buffering Activity apart from being nutritionally superior. They have been responsible for releasing several Bioactive Peptides too in the medium. Other raw materials like Cereals, Pulses, Fruits, Vegetables, Tubers, Fish and Meat have also been used to prepare Probiotic foods. However, the concept of preparing blends foods is better, as the mixing can nutritionally enrich the product and removes the deficiencies of some raw materials. Probiotic foods have been proved to provide excellent quality proteins rich in essential Amino Acids, Vitamins, easily absorbable Minerals, Vital Fatty Acids and many more Micronutrients for the body. Application of Probiotics in prevention and supplementary treatment of several ailments is well known and several clinical trials to prove their worth are going on. FAO/WHO has given clear Guidelines about Probiotics and different Countries have Regulatory Framework for Ensuring Safety, Efficacy and Labelling Requirements for such Foods. ILSI, ISAPP and several other organizations have published extensive literature on this subject. This Review will further enhance this pool and our understanding about Probiotics and Nutrition.

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