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Review Article

Starter Cultures Used in the Production of Probiotic Dairy Products and Their Potential Applications: A Review

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Abstract: The preservation of food by fermentation is one of the oldest methods known to mankind. Fermented dairy products are popular due to their differences in taste and their favorable physiological effects. A typical example is lactic acid fermentation, which is widely used for the preparation of several fermented milk products, such as dahi (curd), yoghurt, sour cream, kefir, acidophilus milk and various varieties of cheeses. Starter cultures have a multifunctional role in dairy fermentations. Dairy starter cultures are carefully selected microorganisms, which are deliberately added to milk to initiate and carry out desired fermentation under controlled conditions in the production of fermented milk products. Most of them belong to lactic acid bacteria (*Lactococcus, Lactobacillus, Streptococcus* and *Leuconostocs*). In some cases, few non-lactic starters (bacteria, yeast and mold) are also used along with lactic acid bacteria during manufacturing of specific fermented milk products. Today, fermented dairy products in general are produced locally by using traditional methods. Recently, due to the increased demand for natural nutrients and probiotic products, fermented dairy products have reached a different position and are considered to have an important impact on human health and nutrition.

Keywords: Starter Culture, Lactic Acid Bacteria, Fermentation, Dahi

1. Introduction

Starter culture is a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of lactic acid bacteria (LAB) occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product [1]. LAB widely distributed in nature and found naturally as indigenous micro flora in raw milk are Gram positive, catalase negative microorganisms that play an important role in many food and feed fermentation like dahi. Dahi analogue to yoghurt is a popular fermented milk product of Indo-Pak subcontinent. Its consumption stands next to whole milk especially during summer. Dahi has been reported to contain a mixture of LAB in addition to *Lactobacillus bulgaricus* and *Streptococcus thermophilus*mostly used cultures for yoghurt making [2].

The production of lactic acid, by fermenting lactose is the major role of dairy starters. The acid is responsible for development of characteristic body and texture of the fermented milk products, contributes to the overall flavour of the products, and enhances preservation. Diacetyl, acetaldehyde, acetic acid, also produced by the lactic starter cultures, contribute to flavor and aroma of the final product [1]. Dairy starters are also having some direct or indirect functional health promoting attributes, such as live probiotics, prebiotic exopolysaccharides and oligosaccharides, bioactive peptides and lipids, etc. Most of the cultured dairy products are produced using commercial starter cultures that have been selected for a variety of desirable properties in addition to rapid acid production. These may include flavor production, lack of associated off flavors, bacteriophage tolerance, ability to produce flavor during cheese ripening, salt tolerance, exopolysaccharide production, bacteriocin production, temperature sensitivity, etc. [3].

When examining worldwide, various dairy products which are different in name but similar in content can be found and those products are an important part of human diet [4, 5, 6, 7 and 8]. Fermented dairy products have favourable effects on human health such as, reducing lactose intolerance, prevention of diarrhea and constipation, increase in the effectiveness against *Helicobacter pylori* infection, preservation of oral health, partial prevention of cancer, cholesterol lowering, enhancement of mineral absorbtion. Along with their extensive effects on human health, they have the ability to form low molecular weight components such as conjugated linoleic acid (CLA), gamma aminobutyric acid (GABA) and bacteriocin [9, 10, 11 and 12].

2. Probiotic Dairy Products

Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora. In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, Helicobacter pylori infections, urinary tract infections, hyperlipidemia and assimilation of cholesterol [13].

Probiotic foods enhance health after consumption and contain microorganisms which are viable, specific and effective on main systems of nutritional physiology. Fermented milks have long been used as the main vehicles for probiotic strains. Less frequently, cheeses have been used for incorporation of probiotic microorganisms, but they may offer a number of advantages compared with fermented milks [14, 15]. Cheese has higher pH, more solid consistency, and relatively higher fat content compared with fermented milks such as yoghurt [16]. Several factors must be considered when using probiotic bacteria in fermented products like yoghurt. Primarily, the probiotics must be viable and present in high count at time of consumption to achieve the desired benefits.

3. Microorganisms Used in Fermentation of Dairy Products

3.1. Lactobacillus Acidophilus

L. acidophilus is a gram positive, anaerobic or facultative anaerobic, nonmotile, catalase (-), rod-shaped bacteria. It is a homofermentative bacteria which has an optimum growth temperature of 35-38°C and optimum pH interval of 5, 5-6 [4, 17]. It doesn't produce ammonia from arginine but ferments amygdalin, cellobiose, fructose, glucose, galactose, mannose, trehalose, saccharose, esculin and maltose. It doesn't metabolisemannitol [17, 18]. It was first isolated from children's faeces by Ernst Moro in 1900 and in 1970 it was officially acknowledged as L. acidophilus by Hansen and Mocquat. This term means lactic acid bacteria which can show growth in an acidic enviroment [19, 20].

L. acidophilus has an antimicrobial effect due to the formation of organic acids (lactic acid, acetic acid, etc.), H_2O_2 and antibiotic substances (Lactocidin, Acidophilin, Acidolin, Lactocin B). As a result of *L. acidophilus*'s traits, intestinal infections and disesases can be brought under control and negative effects of antibiotic treatment can be eliminated. *L. acidophilus* is resistant to bile acid and has a strong antibiotic effect on fecal *E. coli* strains and other intestinal pathogens [21, 22].

Due to originating from intestinal microbiota, *L. acidophilus* is usually isolated from infant's faeces. Studies has shown that ingested L. acidophilus, either through a product or pure culture, can be absorbed in intestines, can adapt and survive in gastric juice for two days and longer in bile secretion and faeces [19, 23].

3.2. Bifidobacteriums sp.

In 8th Bergey's Manual, B*ifidobacterium*ssp. was defined as an independent genus by taxonomists and named *Bifidobacterium*and was included in Actinomycetaceae family. Some of the defined 24 species in 9th Bergey's Manual are *B. bifidum*, *B. breve, B. infantis, B. thermophilum, B. adolescentis, B. longum, B. pseudolongum, B. coryneforme, B. indicum*and *B. dentim* [24].

The use of *Bifidobacterium* species in fermented and cultured milk and growing knowledge of their taxonomy and ecology resulted in an increase in their popularity in the late 1970s. They grew in popularity considering low acid formation during their shelf life and higher consumption of L(+) lactic acid in comparison with D(-) lactic acid. Among the many probiotic traits that have been attributed to bifidobacteria are a) the induction of immunoglobulin production, b) improvement of food nutritional value by assimilation of substrates not metabolized by the host, c) anti-carcinogenic activity and d) folic acid synthesis [25]. Within various probiotic bacteria, *Bifidobacteriumlactis*has been studied intensively and its beneficial roles for host health has been described. *B. lactis*is preferred for industrial production because of the oxygen and acid tolerance compared with other bifidobacteria species [26, 27 and 28].

3.3. Lactobacillus Casei

L. casei is in Streptobacteria subgenus and has a diameter smaller than 1.5 µm, has tendency to form chains and does not have flagella, it is rod shaped, nonmotile and homofermentative. By metabolising pentose, it occasionally forms L(+) lactic acid and acetic acid. *L. casei*shows rapid growth in media containing %4 gluconate and forms CO_2. It has an optimum growth temperature of 28-32°C and can grow under 15°C and in some conditions can even show growth at 6-7°C. *L. casei* can utilise sorbitol and sorbate but shows low fermentation rates with maltose and saccharose. It requires riboflavine, folic acid, Capantothenate and niacin for growth. It doesn't form gas and shows strong proteolytic effect after lysis [23, 29 and 30].

L. casei is a lactic acid bacteria which can be used in foods worldwide. *L. casei* is being used as culture or in mixtures to improve sensorial properties and in traditional dairy products such as kefir ve Laban Zeer, cheese such as provolone and parmesan, recent products such as yakult, actimel, gefilus and vifit.

3.4. Lactobacillus Rhamnosus

Due to its probiotic traits, *Lactobacillus rhamnosus* GG or *Lactobacillus* GG is the most common microorganism used in dairy products marketed for infant's and children's consumption. *Lactobacillus rhamnosus* GG, was isolated from human faeces in 1983 and was patented in 1985. *Lactobacillus rhamnosus* GG is one of the most studied strains and is one of the most common bacteria that is used in probiotic preparations and foods. It has the suffix "GG" because it was discovered in Tufts University by Sherwood Gorbach and Barry Goldin. *Lactobacillus rhamnosus* GG was first used in the studies at 1990 and was found beneficial to children's health. Due to its favourable impact on children's health it is widely used with products for infants and children. Some of the main traits of *Lactobacillus rhamnosus* GG are being indigenous to human intestinal flora, resistance to low pH values and adherence to gastrointestinal track [31].

3.5. Enterococcus Faecium/Enterococcus Faecalis

Enterococci are singular, double or short chained gram positive cocci. *Streptococcus faecalis*was defined by Andrewea and Horder in 1906 and *Streptococcus faecium*was defined by Orla-Jensen in 1919. In 1984 Schleifer and Kilpper-Balz suggested that *S. faecalis*and *S. faecium*should be distinguished from *Streptococcus* genus and considered in *Enterococcus* genus. Later on, bacteria that are studied in this genus were divided into various species such as; *E. faecalis, E. faecium, E. durans, E. avium, E. casseliflavus, E. malodoratus, E. hirae, E. gallinarum, E. mundtii, E. raffinosus, E. pseudoavium, E. flavescens, E. dispar, E. sulfureus, E. saccharolyticus, E. columbaeve E. cecorum* [32, 33, 34, 35 and 36].

These bacteria can be found with high amounts in dairy products and other foods and although having extensive biotechnological properties such as; capability to produce bacteriocin, having probiotic traits and usage in dairy industry, there isn't a consensus on to consider them foodborne pathogens. However recent studies have shown that *E. faecalis* and some lactic acid bacteria species can cause clinical infections, especially infective endocarditis. *E. faecalis* can be found not only in human and animal faeces but also on plants and this largely reduces it's usage as a sanitation indicator [33, 37, 38 and 39].

Among *Enterococcus* genus, *Enterococcus faecium* and *Enterococcus faecalis* are stated to have probiotic traits. *Enterococcus faecium*'s usage on diarrhea treatment is considered to be an alternative for antibiotic use. The probiotic effect of *Enterococcus faecium*on humans arise out of reducing the absorbtion of cholesterol from digestive system [40].

3.6. Lactobacillus Gasseri

Lactobacillus gasseri is a rod shaped, non spore forming lactic acid bacteria. This bacterium is a prolific autochthonous microorganism that colonizes the GIT, oral cavity, and vagina in humans. *Lactobacillus gasseri*is classified as a group B acidophilus complex microorganism, and can be differentiated from group A members by the way of genetic determination and the apparent absence of major surface-layer (S-layer) proteins. The niche-related phenotypes involved incolonization of the human mucosa, including the oral cavity, GIT, and vagina are exhibited by LAB such as *L. gasseri* and may contribute to or potentiate probiotic activity [41]. *L. gasseri*shows to be beneficiary to gastrointestinal system and is stated to have the capability to reduce fecal mutagenic enzyms due to its probiotic acitivity. It has the ability to adhere to intestines and has a role in bacteriocin formation and macrophage stimulation. In the view of its probiotic traits, it can be used in the production of fermented diary products and in commercial preparations [42].

3.7. Streptococcus Thermophilus

S. thermophiles is a gram positive, circular or elliptical bacteria which has a diameter of 0,7 - 0,9 micron. Morphology of bacteria depends on the genus, enviroment and growth temperatures. For instance, they have short chains at 45°C, are diplococcus at 30°C but have long chains in cultures that have high acidity. Bacteria in question show symbiotic relationship with *L. bulgaricus* at yoghurt production. At first *S. thermophiles* shows activity in the milk fermented to produce yoghurt, and slightly increases the acidity of the media and consumes oxygen. *L. bulgaricus* starts to grow rapidly in this medium and forms valine which is a necessity for *S. thermophilus*'s growth [43, 44]. Besides yoghurt production, they are present in various starter cultures that are used to produce fermented dairy products and cheese [44].

3.8. Propionibacterium Species

In 1906, Freudenreich and Orla-Jensen isolated bacteria from Emmantel cheese and named them *Bacterium acidi-propionici* and *Bacillus acidi-propionici*. These organisms are defined based on their capability to form propionic acid. The class Propionibacterium was first introduced by Orla-Jensen in 1909. Classical PAB, also known as "dairy-propionibacteria" are usually present in raw milk, butter and are known for their significant role in maturation process of Swiss type cheeses. Because of their presence in natural flora and starter culture, PAB have a signicant role in nonconventional cheese production. Further, by forming propionic acid and acetic acid

PAB contributes to the sensorial properties of these products. In milk and other dairy products, *Propionibacterium ferudenreichii*, *Propionibacterium jensenii* and *Propionibacterium acidipropionici* are present prior to the other species of this genus [45].

3.9. Saccharomyces Cerevisiaeboulardii

Saccharomyces boulardii was discovered by French researcher Boulard in 1923 and is Gram positive yeast which is a member of Saccharomycetaceae family. It is elliptical or spherical shaped and has a size of 4-8 µm. It forms ascospore and grows in standard yeast mediums with and optimal growth temperature of 37°C and has the capability to assimilate and ferment carbohydrates and can prevent microbial pathogen growth. *Saccharomyces boulardii* shows no pathogenic properties and was isolated from a tropical fruit (lychee fruit) which grows in Southeast Asia and was used for the treatment of diarrhea. In 1962, lyophilised commercial preparation of *S. boulardii* was introduced and notably in France, used as a cure for diarrhea ever since. Diverse arguments are ongoing about it's being a subspecies of *Saccharomyces cerevisiae* [46, 47 and 48]. Lyophilised *S. boulardii* is being used clinically in Europe, Asia, Africa and South America. Preclinical and experimental studies have shown that *Saccharomycessboulardii* has anti inflammatory, antimicrobial, enzymatic, metabolic and antitoxic activities [49 and 50].

4. Application and Importance of Dairy Starter Culture

4.1. Metabolites Produced by LAB

LAB has relatively simple homo- or hetero fermentative metabolism. These bacteria rely on lactose as their main carbohydrate source. Dairy LAB includes members of the genera *Lacobacillus, Lactococcus, Leuconostoc* and *Sterptococcus*. LAB fermentation yields primarily lactic acid, which plays a vital function in safeguarding food products. LAB metabolism beneficially affects the texture and flavour of fermented foods. The viscosity and texture of fermented dairy products can be greatly enhanced by the production of polysaccharides by LAB, while compounds such as diacetyl, ethanol, acetaldehyde, etc play vital roles in flavor development. In addition, many LAB produce compounds of human nutritional value as regular end products in their metabolisms, including some B-vitamins. Many strains of LAB and bidifobacteria produce other metabolites that promote human health.

4.2. Production of Aroma and Flavour

LAB contributes to the aroma and flavor of fermented products. They acidify the food, resulting in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities, and produce aromatic compounds. Wild strain starter cultures and NSLAB play an important role in flavor formation because they have a high biosynthetic capacity and produce aromatic compounds. Homo fermentative LAB converts the available energy source (sugar) almost completely into lactic acid via pyruvate to produce energy and to equilibrate the redox balance. However, pyruvate can lead to the generation of many other metabolites such as acetate, ethanol, diacetyl, and acetaldehyde. In this way, LAB produce volatile substances that contribute to the typical flavour of certain fermented products, such as sourdough (determined by the lactate/acetate ratio), kefir and koumiss (ethanol), butter and buttermilk (diacetyl), and yoghurt (acetaldehyde).

Acetaldehyde is one of the important flavour compounds produced by starter cultures in fermented milks and is a major flavour compound in yoghurt. In mesophilic cultures, the precursor of acetaldehyde is threonine while in thermophilic cultures, the precursor is sugar. Apart from this, the acetaldehyde may also be produced by lactic acid bacteria from nucleic acids, lipids and aromatic compounds in milks.

4.3. EPS Production and Textural Improvement

Long-chain, high-molecular-mass polymers that dissolve or disperse in water to give a thickening or gelling properties are indispensable tools in food products formulation. Such polymers are also used for secondary effects that include emulsification, stabilization, and suspension of particulates, control of crystallization, and inhibition of syneresis, encapsulation, and film formation in foods. Most of the biothickeners in current use by the food industry are polysaccharides from plants (e.g. starch, pectin, locust bean gum, guar gum) or seaweeds (i.e. carrageenan, alginate). The animal proteinaceous hydrocolloids gelatin and casein are also used. The functional properties of these polymers in foods are determined by quite subtle structural characteristics.

To give a desired texture and mouth feel to yoghurt, skim-milk powder or whey is frequently added to the milk. Although the consumer does not consider this as unnatural, it represents an extra cost for the producer. In some countries, however, gelatine or plant (e.g., starch, pectin, guar gum, and alginate) and microbial polysaccharides (e.g., xanthan and gellan) are added. Polysaccharides increase the viscosity and firmness, improve the texture, reduce susceptibility to syneresis, and contribute to the mouthfeel of lowfat products.

4.4. Bacteriocins Production

Bacteriocins, ribosomally-synthesized peptides or proteins with antimicrobial activity, are produced by different groups of bacteria. Many lactic acid bacteria produce bacteriocins with broad spectra of inhibitions that offer potential applications in food preservation [1]. The use of these bacteriocins in food industry can reduce the dependency on chemical preservation as well as the intensity of heat treatments, resulting in naturally preserved foods rich in organoleptic and nutritional value. The live bacteriocin-producing LAB incorporation into a food gives it its own built in bio-preservation, thereby returning to a more natural method of shelf-life extension and improving the safety of food.

4.5. Lactose-Negative Starters

In yoghurt production, lactose is converted by a yoghurt culture into lactic acid until a final pH of 4.2–4.5 is achieved. Upon storage, the pH can decrease below 4.0. This undesirable post acidification, ascribed to *Lb. delbrueckii subsp. bulgaricus,* leads to an acid and bitter taste. Lactose negative mutants of *Lb. delbrueckii subsp. bulgaricus* enable production of mild yoghurts since such cells can, given their proto-co-operation; only grow in the presence of actively lactose fermenting *S. thermophilus*.

4.6. Reduction of Toxic or Anti Nutritive Factors

The fermentative action of specific LAB stains may lead to the removal of toxic or anti nutritive factors, such as lactose and galactose from fermented milks to prevent lactose intolerance and accumulation of galactose). Other examples are the removal of raffinose, stachyose, and verbascose from soy to prevent flatulence and intestinal cramps, proteinase inhibitors from legumes and cereals to prevent maldigestion, phytic acid and tannins from cereals and legumes to increase mineral bioavailability, and natural toxins such as cyanogenic glucosides from cassava as well as biogenic amines from traditional fermented foods.

4.7. Production of Nutraceuticals

Nutraceuticals are food components that, through specific physiological action, contribute to the health of the consumer. Several nutraceuticals from bacterial origin have been added to food. Through strain selection and process optimisation, the activity of LAB can be modified to increase the content of nutraceuticals in fermented foods such as fermented dairy products. As an example, fermented milks can be produced with LAB starter strains that produce high amounts of low-calorie polyols so as to reduce the sugar content. Also, the use of oligosaccharide-producing LAB that produce sugar polymers with a controlled structure and chain length (and hence molecular mass) may yield fermented products with health applications. Health effects of such oligosaccharides are ascribed to their low-calorie character, their fibre like nature, and their bifidogenic effect. Certain LAB, such as the yoghurt bacteria *Lb. delbrueckii subsp. bulgaricus and S. thermophilus*, are able to produce vitamins such as folate [1]. A controlled use of these bacteria may lead to dairy products with increased folate content. The proteolytic system of LAB can contribute to the liberation of health enhancing bioactive peptides from milk. The latter may improve absorption in the intestinal tract, stimulate the immune system, exert antihypertensive or antithrombotic effects, display antimicrobial activity, or function as carriers for minerals, especially calcium.

4.8. Vitamins Metabolism

Milk contains several water or fat-soluble vitamins. When starter cultures are growing in milk, some vitamins may be utilized by them, leading to their decrease. On the other side some vitamins may be synthesized also, leading to increased content in fermented milk. This increase or decrease depends greatly on the strain of starter. However, generally it is reported that yoghurt bacteria synthesize folic acid, niacin and vitamin B6. Propioni bacteria are known to produce vitamin B12.

4.9. Production of Bacteriocins

Lactic acid bacteria are exerting antagonistic effect against several other organisms, due to production of several antimicrobial substances. These include lactic acid, acetic acid, other organic acids, hydrogen peroxide, diacetyl, reduced pH and EH and a number of bacteriocins.

Bacteriocins are the proteins produced by the bacteria that are inhibitory to closely related species. However, some of the bacteriocins of lactic acid bacteria have shown wide spectrum activities. The exact mechanism for synthesis and other characteristics of many bacteriocins are still not clear. However, the nisin is the only one, which is fully characterised and used as food preservative, other bacteriocins produced by lactic acid bacteria are Acidophilin, Lactocidin, Brevicin, Helveticin, etc.

5. Conclusion

Milk contains many health promoting constituents including immunoglobulins, bioactive fatty acids and peptides amongst others. The healthy image of milk has resulted in dramatic growth in the diversification of dairy products in recent years and in huge increase in the varieties of products such as dairy desserts, flavoured milk drinks, cheeses, yoghurt etc. Apart from the milk components, the health attributes are associated with fermented and probiotic milks and dairy products. Milk has been preserved by fermentation through the action of lactic acid bacteria (LAB), which convert lactose to lactic acid and other organic acids, thereby lowering the pH and subsequently inhibiting the growth of pathogenic and spoilage bacteria. Moreover, these LAB produce a range of secondary metabolites, that can influence the products' flavour, aroma and texture as well as antimicrobial peptides. These bacteria also possess a diverse complement of proteases and peptidases that aid in digestion of milk proteins. In addition, many bifidobacteria and lactobacilli are increasingly exploited in probiotic dairy products such as cheeses, yoghurt, milk drinks etc.

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