Fermented Milk and Dairy Products

4.1 Introduction

Starter cultures are used in the manufacturing of different types of fermented milk and dairy products including yogurt, dahi, cultured buttermilk, sour cream, quarg, kefir, koumiss, and cheese. The use of these cultures for the preparation of products has been practiced since time immemorial. Traditionally, the common method was to use the previous-day's product (i.e., milk, dahi, whey, buttermilk, etc.) as an inoculum to produce the fresh batches of fermented product. Such methods were not reliable and often resulted in off-flavor, inconsistent products, and product failure due to undesirable fermentation. These drawbacks were mainly due to the lack of scientific knowledge of starter culture technology. The ideal of pure culture for making good-quality fermented milk became more visible in the mid-nineteenth century, where starters were widely studied and their metabolisms were well established. This leads to manufacturers handling large volumes of milk started selection of appropriate starters to obtain uniform quality product. This made the selection of appropriate starters even for manufacturers handling large volumes of milk to obtain uniform quality of product. Thereafter, companies started producing pure cultures for commercial application.

Starter cultures may be defined as the carefully selected group of microorganisms that are deliberately added to milk and milk products to bring desirable fermentative changes. These have multifunctional role in dairy fermentation; the primary one is to produce lactic acid, hence, popularly called as lactic acid bacteria (LAB). Besides production of lactic acid, certain cultures perform secondary functions such as the production of acetic, propionic, and folic acids, CO_2, H_2O_2, ethanol, bacteriocins, exopolysaccharides (EPS), and so on (Cintas et al. 2001; Padalino et al. 2012; Yang et al. 2012). This chapter reviews the characteristics of different starters, their types, scaling up, problems associated with starters, and their application.

4.1.1 Functions of Starter Cultures

Different functions of starter cultures are to

- Produce lactic acid and other metabolites (i.e., alcohol, CO_2, propionic acid, acetic acid, etc.).
- Produce aromatic compounds like diacetyl, acetaldehyde, and acetoin.
- Control the growth of pathogens and spoilage causing microorganisms.
- Produce certain vitamins (i.e., folic acid, vitamin B_{12}, niacin, etc.).
- Bring proteolytic and lipolytic activities.
- Improve body and texture of certain products by producing EPS.
- Assist in overall acceptability of the final product.

4.2 Characteristics of Dairy Starter Cultures

Starter cultures form a large group of microorganisms that include bacteria, yeasts, and molds (Table 4.1) for particular fermented milk products. Although starter cultures are genetically diverse, the common characteristics of these groups include Gram-positive, non-spore

Table 4.1 Microorganisms Used as Starter Cultures

MICROORGANISM(S)	MAJOR CHARACTERISTICS
A. BACTERIA	
Lc. lactis subsp. *lactis*, *Lc. lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis* biovar. *diacetylactis*	Cocci shaped, production of lactic acid, exopolysaccharides, riboflavin, bacteriocins, diacetyl, etc.
Leuconostoc mesenteroides subsp. *dextranicum*, *Leu. mesenteroides* subsp. *mesenteroides*, *Leu. cremoris*	Cocci shaped, production of lactic acid, exopolysaccharides (homopolysaccharides, dextran), diacetyl, CO_2, etc.
Streptococcus thermophilus	Cocci shaped, production of lactic acid, exopolysaccharides (homo- or heteropolysaccharides), bacteriocins, folate, etc.
Lactobacillus delbrueckii subsp. *bulgaricus*, *Lb. reuteri*, *Lb. casei*, *Lb. fermentum*, *Lb. plantarum*, *Lb. helveticus*, *Lb. acidophilus*, *Lb. paracasei*, *Lb. rhamnosus*	Rod shaped, production of lactic acid, exopolysaccharides (homo- or heteropolysaccharides), riboflavin, bacteriocins, etc. Few species are used as probiotic.

(*Continued*)

Table 4.1 (*Continued*) Microorganisms Used as Starter Cultures

MICROORGANISM(S)	MAJOR CHARACTERISTICS
P. acidilactici	Cocci shaped, forms tetrads, production of lactic acid, bacteriocins, etc.
Bifidobacterium adolescentis, *B. brevis*, *B. bifidum*, *B. infantis*, *B. lactis*, *B. longum*	Anaerobic heterofermentative, non-spore forming rods, production of two molecules of lactate and three molecules of acetate. Species are also used as probiotic.
Brevibacterium linens, *B. casei*	Rods, pleomorphic, obligate aerobes, impart reddish-orange color in cheeses.
Propionibacterium freudenreichii subsp. *freudenreichii*, *P. freudenreichii* subsp. *shermanii*	Rods, pleomorphic, production of propionic acid, acetic acid, and CO_2.
Enterococcus faecium, E. faecalis, *E. durans*	Cocci shaped, production of lactic acid, bacteriocins.
B. YEASTS	
Candida kefir	Short ovoid to long ovoid, budding yeast-like cells or blastoconidia, production of ethanol and CO_2.
Kluyveromyces marxianus	Formation of pseudomycelium, fermentation of lactose and inulin, produces aroma compounds such as fruit esters, carboxylic acids, ketones, furans, alcohols, monoterpene alcohols, and isoamyl acetate.
Saccharomyces cerevisiae	Forms blastoconidia (cell buds), produces ascospores, production of alcohol and CO_2.
Torulospora delbrueckii	Forms buds, produces ethanol and CO_2.
C. MOLDS	
Penicillium roquefortii, *P. camemberti*	Production of asexual spores in phialides with a distinctive brush-shaped configuration, production of mycotoxins like roquefortine and PR toxin.
Geotrichum candidum	Produce chains of hyaline, smooth, one-celled, subglobose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae. Septate hyphae that disarticulate into arthroconidia and do not form budding yeast cells. Contributes to an aroma.
Aspergillus oryzae	Filamentous fungus, highly aerobic and are found in almost all oxygen-rich environments.
Mucor rasmusen	Spores or sporangiospores can be simple or branched and form apical, globular sporangia that are supported and elevated by a column-shaped columella.

forming, non-pigmented, and unable to produce catalase and cytochrome, growing anaerobically but are aero-tolerant and obligatorily ferment sugar with lactic acid as the major end product. The nutritional requirement of these cultures varies from species to species. Most of the cultures are nutritionally fastidious, often requiring specific amino acids, vitamin B, and other growth factors, while unable to use complex carbohydrates.

4.2.1 Bacteria

Lactococci have been widely used for manufacturing a variety of fermented milk products. So far, five species are recognized but only *Lactococcus lactis* is used as starter culture that has practical significance in dairy fermentations. There are two subspecies, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris,* and one variant, *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*, which are commonly used as single or in mixed cultures. Lactococci are homofermentative and mesophilic, and when grown in milk, more than 95% of their end product is lactic acid, L(+) isomer. However, being weakly proteolytic, they can use milk proteins and grow at 10°C but not at 45°C. *Lc. lactis* subsp. *lactis* is more heat and salt tolerant than other subspecies. It ferments maltose, grows at 40°C, and in pH 9.5, produces ammonia from arginine, whereas *Lc. lactis* subsp. *cremoris* did not show these characteristics. *Lc. lactis* biovar. *diacetylactis* shows a close relationship with *Lc. lactis* subsp. *lactis* but differs by exhibiting citrate positive ability and does not produce as much lactic acid in milk as the latter. Nisin and diplococin are produced by *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris,* respectively, while bacteriocins produced by *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* are not named. Some lactococci can produce EPS and improve textural properties of cultured dairy products (Cerning 1990; Behare et al. 2009a).

Leuconostoc ssp. occur in pairs and chains of cocci and are often ellipsoidal. It is difficult to differentiate *Leuconostoc* from lactococci. Both are catalase negative and form chains of coccal to oval shaped cells. However, a useful method by which one can fairly distinguish these two is by growing them in litmus milk. Lactococci reduce litmus before coagulation, whereas leuconostocs do not. Fundamentally, leuconostocs are heterofermentative and produce D-lactate, and with the exception of *Leuconostoc lactis*, these show no change in litmus milk

(Garvie 1960). These also do not hydrolyze arginine and require various B vitamins for growth. *Leuconostoc* ssp. grows at 10°C but not at 40°C, and can ferment lactose, galactose, fructose, and ribose. The end product produced includes diacetyl, carbon dioxide, and acetoin from citrate. The species that are widely used as dairy starters include *Leu. mesenteroides* subsp. *cremoris* (previously referred as *Leu. cremoris* or *Leu. citrovorum*), *Leu. mesenteroides* subsp. *mesenteroides, Leu. mesenteroides* subsp. *dextranicum*, and *Leu. lactis*. They are primarily used as flavor producers in butter, cheeses, and flavored milks. These cultures are often used in combination with other fast growing lactic cultures.

Streptococcus thermophilus used as a starter is fairly close with *Str. salivarius,* a common inhabitant of the mouth. Earlier, *S. thermophilus* was combined with *Str. salivarius,* but analysis of DNA hybridization data indicated these two as different and *S. thermophilus* got separate species status (Axelsson 1993). *S. thermophilus* can be easily distinguished from lactococci and *Leuconostocs* by sugar fermentation profile and growth temperature. It can grow at 45°C (but no growth at 10°C), while lactococci and leuconostocs cannot grow during these conditions. Additionally, *S. thermophilus* strains differ in their ability to utilize galactose. Use of non-galactose fermenting strains will result in high levels of this reducing sugar in products. Since galactose and other reducing sugars react with amino acids in the Maillard reaction, it is usual to only select galactose-utilizing strains to reduce the probability of undesirable color changes in heated products. Most strains of *S. thermophilus* hydrolyze aesculin, lactose, and saccharose. It is one of the fastidious starter cultures that coagulate milk in very short time. Several dairy products subjected to high temperatures (>40°C) during fermentation are acidified by the combined use of *S. thermophilus* and *Lactobacillus* ssp. Streptococci are generally isolated from milk and milk products. But *Enterococcus* species, which also resembles *Str. thermophilus* by growing at 45°C and having similar morphological features, often created confusion for isolation of *S. thermophilus* from milk and milk products. However, most enterococci can grow at 10°C in 6.5% NaCl and at pH 9.6 and contain the lancified group D-antigen while *S. thermophilus* does not show these characteristics.

Lactobacillus consists of a genetically and physiologically diverse group of rod shaped LAB. These are generally found in milk, cheeses,

butter, and traditional fermented milk products (Bettache et al. 2012). Orla-Jensen (1931) classified lactobacilli into three groups: *thermobacterium*, *streptobacterium*, and *betabacterium*. *Lactobacillus* was further divided into three main groups, I, II, and III resembling the classification of Orla-Jensen (1931), but designating them as subgeneric taxa (Kandler and Weiss 1986). Lactobacilli are the most acid tolerant of the starter cultures, liking to initiate growth at acidic pH (5.5–6.2) and lowering the pH of milk to below 4.0. Some species are homofermentative, while others are heterofermentative. While some species produce mainly L-lactate from glucose, others produce D-lactate. Since certain strains exhibit significant racemase activity and a racemase is an isomerase, D/L lactic acid is also produced (Table 4.2). In pure

Table 4.2 Selected Characteristics of *Lactobacillus* ssp.

	LACTIC ACID	GROWTH AT		CARBOHYDRATE UTILIZATION							
LACTOBACILLI	ISOMER	15°C	45°C	ESC	AMY	ARA	CEL	GLU	GAL	MAN	XYL
GROUP I (THERMOBACTERIUM)—OBLIGATE HOMOFERMENTATIVE											
Lb. acidophilus, *Lb. gasserie*	DL	−	+	+	+	−	+	+	+	−	−
Lb. delbrueckii subsp. *bulgaricus*	D(−)	−	+	−	−	−	−	+	−	−	−
Lb. helveticus	DL	−	+	−	−	−	−	+	+	−	−
Lb. johnsonii	DL	+	+	ND	+	ND	+	+	+	−	−
Lb. kefiranofaciens	D(L)	−	−	ND	−	ND	−	+	+	−	−
GROUP II (STREPTOBACTERIUM)—FACULTATIVE HETEROFERMENTATIVE											
Lb. paracasei subsp. *paracasei*	L/DL	+	d	+	+	−	+	+	+	+	−
Lb. rhamnosus	L(+)	+	+	+	+	d	+	+	+	+	−
Lb. plantarum	DL	+	−	+	d	+	+	+	+	+	−
GROUP III (BETABACTERIUM)—OBLIGATE HETEROFERMENTATIVE											
Lb. brevis	DL	+	−	d	−	+	−	+	d	−	−
Lb. fermentum, *Lb. reuteri*	DL	−	+	−	−	d	d	+	+	−	−
Lb. viridescens	DL	+	−	−	−	−	−	+	−	−	−

ESC, aesculin; AMY, amygdalin; ARA, arabinose; CEL, cellobiose; GLU, glucose; GAL, galactose; MAN, mannitol; XYL, xylose; d, 11%–89% strains showed positive reaction; ND, not detected.

cultures, many lactobacilli are slow growers, and due to this reason, these are generally combined with other fast growing cultures. Few lactobacilli are used as probiotics for treatment of various disorders (Delzenne et al. 2011).

Pediococci divide to form tetrads that differentiate these morphologically from other LAB. Only *Pediococcus pentosaceus* and *P. acidilactici* are used as dairy starters but these are less important than other LAB. However, *P. acidilactici* is used with therapeutic properties along with other starter cultures *P. acidilactici, Lb. acidophilus,* and *Bifidobacterium bifidum* in the ratio of 1.0:0.1:1.0 (Tamime and Marshall 1997). The important characteristics of *P. acidilactici* and *P. pentosaceus* are shown in Table 4.3.

Only six (*B. adolescentis*, *B. breve*, *B. bifidum*, *B. infantis*, *B. lactis*, and *B. longum*) out of 30 *Bifidobacterium* species are used in the dairy industry. These produce lactic acid and acetic acid in the ratio of 2:3. These are catalase negative, Gram-positive, irregularly shaped (pleomorphic) rods, many of which form branched cells. These are anaerobic in nature and can grow poorly in milk possibly because of the lack of small peptidases. The optimum temperature for growth is 37°C–41°C, while no growth occurs below 20°C and above 46°C. Growth at 45°C seems to discriminate between animal and human strains. The optimum pH is between 6.5 and 7.0 and no growth is recorded at pH lower than 4.5. These can utilize lactose, galactose, fructose, maltose, and sucrose. Bifidobacteria differ with other LAB by possessing the enzyme fructose-6-phosphate phosphoketolase, the key enzyme of the bifid-shunt. Some of the members of these groups

Table 4.3 Selected Characteristics of Pediococci

CHARACTERISTICS	*P. PENTOSACEUS*	*P. ACIDILACTICI*
Lactic acid isomer	D	DL
Growth at 10°C	–	–
45°C	–	+
Growth in 10% NaCl	V	–
Acid from		
Arabinose	+	V
Xylose	V	+
Maltose	+	–
Trehalose	+	V

V, Variable; +, positive reaction; –, negative reaction.

are also used as probiotics as these find a suitable environment in the human host and provide beneficial health effects by improving intestinal disorders like diarrhea, constipation, and irritable bowel syndrome (Grandy et al. 2010; Guglielmetti et al. 2011). Bifidobacteria are the first organisms to establish in new borne babies.

Brevibacterium linens and *Brevibacterium casei* are used in cheeses to impart a distinctive reddish-orange color to the rind or cause the fermentation of smear on brick and Limburger cheese (Olson 1969; Reps 1993). The bacteria are Gram positive, pleomorphic rods, and obligate aerobes, and optimum growth temperature is 20°C–25°C (*B. linens*) or 30°C–37°C (*B. casei*). These do not use lactose or citrate but can grow on the lactate produced during cheese fermentation. These organisms are salt tolerant and non-motile and produce no endospore.

Propionibacterium ssp. are non-spore forming, pleomorphic, Gram-positive rods that produce large amounts of propionic and acetic acids, and carbon dioxide from sugars and lactic acid. These are anaerobic to aerotolerant mesophiles and are closely related to coryneforms in the *Actinomycetaceae* group. The most useful species for dairy is *P. freudenreichii* (subsp. *freudenreichii* and *shermanii*) widely used in Swiss cheeses (i.e., Emmental and Gruyere) predominantly for producing large gas holes in cheese during maturation. Propionibacteria grow on lactic acid produced during cheese fermentation. Lactate is oxidized to pyruvate, which is then converted to acetate and carbon dioxide or propionate. The other species in dairy products are *P. jensenii*, *P. thoenii*, and *P. acidipropionici*.

Enterococci are generally found in milk and milk products like other LAB. Based on 16SRNA sequencing within *Enterococcus*, three species, that is, *E. faecium*, *E. faecalis*, and *E. durans*, are revealed. These are Gram positive, catalase negative cocci, and produce L(+) lactic acid from glucose. These are normal inhabitants of the human intestinal tract and, hence, are indicators of fecal contamination, and some species are pathogenic. Enterococci are not widely used as starter cultures due to the role of some species in causing food borne illness. However, in some of the Southern European countries, these are used as starter culture in some cheese varieties and fermented milk products (Tamime and Marshall 1997). In addition, selected *Enterococcus* ssp. are commercially available as probiotics for the prevention and control of intestinal disorders.

4.2.2 Yeasts

The presence of yeasts in dairy products is unacceptable and considered as contaminants (IDF 1998). These are quite common in the environment and are often isolated from sugar-rich materials. These are Gram positive, short to long ovoid, form blastoconidia and produce ethanol and CO_2 during fermentation of lactose. Yeasts are chemo-organotrophs, as these use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from glucose and fructose, or disaccharides (i.e., sucrose and maltose). Few species can metabolize pentose sugars like ribose alcohols and organic acids. Yeast species either require oxygen for aerobic respiration or are anaerobic, but also have aerobic methods of energy production (i.e., facultative anaerobes). Unlike bacteria, there are no known yeasts that grow only anaerobically. These grow best in a neutral or slightly acidic environment. Although yeasts are commonly used in bread and wine production, few species are used in the manufacture of kefir and koumiss, where these carry out yeasty-lactic fermentation. The important species of yeasts, which are used in fermented milk products, include *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Torulaspora delbrueckii*, *Kluyveromyces marxianus*, and *Candida kefyr.*

4.2.3 Molds

Molds are used in the cheese industry for making some semisoft cheese varieties. The major role of molds is to enhance the flavor and aroma and modify the body and texture of the curd slightly. *Penicillium camemberti* and *P. roquefortii* are used in mold ripened cheeses. *P. camemberti*, also called white mold, grows on the surface of the cheese (Camembert, Brie, and similar varieties), while *P. roquefortii*, called blue mold, grows in the interior of the blue-veined cheeses (Roquefort, Stilton, Gorgonzola). Both species are lipolytic and proteolytic and produce methyl ketones and free fatty acids that impart distinctive flavor and aroma to the cheeses. Other molds have limited application but are used in some parts of the world including *Mucor rasmusen* in Norway for ripened skim milk cheese (Kosikowski and Mistry 1997) and *Geotrichum candidum* in viili, a fermented milk product of Finland. *G. candidum* is cosmopolitan in distribution (i.e., air, water, plants, and milk and

milk products). Lipases and proteases of *G. candidum* release fatty acids and peptides that can be metabolized by subsequent microbial populations and contribute to the development of distinctive flavors (Litthauer et al. 1996; Holmquist 1998).

4.3 Types of Starter Cultures

Basically, starters can be grouped as lactic or non-lactic starters. The starter cultures used in the manufacture of cheese can be classified into different groups.

4.3.1 Composition of Starter Flora

4.3.1.1 Single Strain Starter It consists of only one type of microorganism and a lactic acid producer is commonly used to achieve the desirable changes. However, use of such culture is always at risk if a culture fails due to inherent or external factors, for example, *Lc. lactis* subsp. *lactis* or *Lc. lactis* subsp. *cremoris* or *S. thermophilus.*

4.3.1.2 Mixed Strain Starters These consist of two or more strains in an unknown proportion. The advantage of using a mixed strain starter is if one strain fails due to any reason the other performs. It also gives a wider tolerance to other factors like temperature and pH changes. However, mixed starters are difficult to maintain as in repeated transfer one strain may become dominant over another. Consequently, it is advisable to use such cultures in a correct proportion or distinctly grow those and mix just before the inoculation of milk, for example, *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, and *Leuconostoc* ssp.

4.3.1.3 Paired Compatible Starters Two single strains are used in a desired ratio to have a better performance. Compatible strains are selected by monitoring their growth in a liquid medium. However, none of the strains should produce an inhibitory substance that may inhibit the growth of another, for example, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*.

4.3.1.4 Multiple Strain Starters These are a mixture of known compatible, non-phage related, carefully selected strains that give consistent

product. Starters can be used for extended periods as the numbers of strains are known, for example, *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*.

4.3.2 Growth Temperature of Starter Culture

4.3.2.1 Mesophilic Starters These have an optimum growth temperature between 20°C and 30°C and comprise mainly of *Lactococcus* and *Leuconostoc* species. Apart from the production of lactic acid, certain cultures also produce diacetyl. These are widely used in the making of dahi, culture buttermilk, butter, lassi, and so on.

4.3.2.2 Thermophilic Starters These cultures exhibit a higher optimum growth temperature that lies between 37°C and 45°C and are useful in the manufacture of products that require acidification at higher temperature (>40°C). These are used in the making of yogurt, dahi, acidophilus milk, and high scalded cheeses. The examples of thermophilc starters are *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, and *Lb. fermentum*. *S. thermophilus* produces lactic acid at a faster rate and are therefore generally combined with other thermophilic starters.

4.3.3 Production of End Product by the Starter Culture

4.3.3.1 Lactic Starters Starter cultures that produce lactic acid as the principal end product from lactose. *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, and *S. thermophilus* are the typical examples of lactic cultures.

4.3.3.2 Non-Lactic Starters Non-lactic starters produce other end products like acetic acid, carbon dioxide, ethanol, and propionic acid. Such cultures can also produce lactic acid as one of the end products. The examples of non-lactic starters include *B. bifidum*, *P. freudenreichii* subsp. *freudenreichii*, and some lactobacilli.

4.3.4 Physical Forms of Starter Culture

4.3.4.1 Liquid Starter This form of culture is more popular and handled in a dairy plant on routine basis. Cultures are available in fluid

form and normally preserved in small volumes for a few days but their quantities can be scaled up as per the requirement. These cultures have a limited shelf life and require periodic transfer to maintain them active. Commonly, starter cultures are made in sterile reconstituted skim milk or litmus chalk milk.

4.3.4.2 Frozen Starter Culture These are made in a frozen state by deep-freezing (–20°C to –40°C) or freezing in liquid nitrogen (–196°C). Cultures have more shelf life than liquid state and can be used for few months. However, at low temperature mechanical process affects the performance of cultures that can be sorted out by adding cryoprotective agents (like sucrose, gelatin, and glycerin).

4.3.4.3 Frozen Concentrated Starter Culture Cells are first concentrated to have a high number of cell population approximately 10^{10}–10^{13} CFU/g followed by rapid freezing that is done in liquid nitrogen. The protection to cells is given by appropriate cryoprotective agents. These are preserved for longer duration and used for large quantities of milk. These cultures are often used as direct-vat-set (DVS) cultures.

4.3.4.4 Dried Starter Cultures Starter cultures are dried to increase their shelf-life for longer times and even more than a year. The dried form of the culture is achieved by vacuum-drying, spray-drying, and freeze-drying. In all types of drying, viability of the culture is most important. Drying should not affect the characteristics of the cultures. Spray-drying and vacuum-drying give much lower survivability of the cultures than the freeze-drying, hence freeze-drying becomes the method of choice. In freeze-drying, the culture of interest is grown overnight in an appropriate medium, and the cell pellet is obtained by centrifugation. The cell pellet is resuspended in a minimal quantity of milk containing a cryoprotective agent. The cell mass is then subjected to freeze-drying (–40°C). The entire process takes 8–10 h.

4.3.4.5 Dried Concentrated Cultures These are the cultures usually dried by lyophilization after concentration of cells by techniques like high speed centrifugations or diffusion culture. These cultures contain active cells in a range of 10^{11}–10^{14}CFU/g and can be used as DVS cultures.

Table 4.4 Starter Cultures Named as Per the Intended Use

STARTERS/PRODUCTS	CULTURES EMPLOYED
Yogurt culture	*S. thermophilus* + *Lb. delbrueckii* subsp. *bulgaricus*
Dahi culture	Lactococci, *Leuconostoc* ssp., *Lb. delbrueckii* subsp. *bulgaricus, Lb. acidophilus, S. thermophilus*
Cheddar cheese culture	Lactococci
Cottage cheese culture	*Leu. mesenteroides* subsp. *cremoris*, *Lb. casei*
Swiss cheese culture	Thermophilic lactobacilli, *S. thermophilus, P. freudenreichii* subsp. *shermanii*
Acidophilus culture	*Lb. acidophilus*
Bifidus culture	*Bifidobacterium bifidum*
Yakult culture	*Lb. casei*
Koumiss culture	*Lb. delbrueckii* subsp. *bulgaricus, Lb. acidophilus, Kluyveromyces fragilis, K. marxianus*
Kefir culture	Kefir grains (contain lactobacilli, yeasts, lactococci and acetic acid bacteria)
Brick cheese culture	Lactococci, *Brevibacterium linens*
Roquefort cheese culture	Lactococci, *Penicillium roquefortii*
Camembert cheese culture	*Penicillium camemberti*
Cultured buttermilk culture	Lactococci, *Leuconostoc* ssp.
Bulgarian milk culture	*Lb. delbrueckii* subsp. *bulgaricus*
Leben/Labneh culture	*Lc. lactis* subsp. *lactis, S. thermophilus, Lb. delbrueckii* subsp. *bulgaricus,* lactose fermenting yeasts
Probiotic cultures	*Lb. acidophilus, B. bifidum, B. longum, B. infantis*

4.3.5 Product for Which Starters Used

Starter cultures can also be categorized on the basis of intended use (Table 4.4). The cultures are named after the product for which they are meant, for example, yogurt culture, dahi culture, kefir culture, and cheese culture.

4.4 Propagation of Starters

Propagation is the process of multiplication of pure or mixed starter cultures. This is essentially required to prepare large quantities of product commercially. A typical flow diagram for propagation of starters in dairy is given in Figure 4.1.

For the manufacture of fermented milk products, starters are grown in heat-treated milk or milk-based media. Starters are added to the milk medium at a predetermined rate so as to allow conventional stages of manufacture. Strict asepsis is required to maintain the purity of

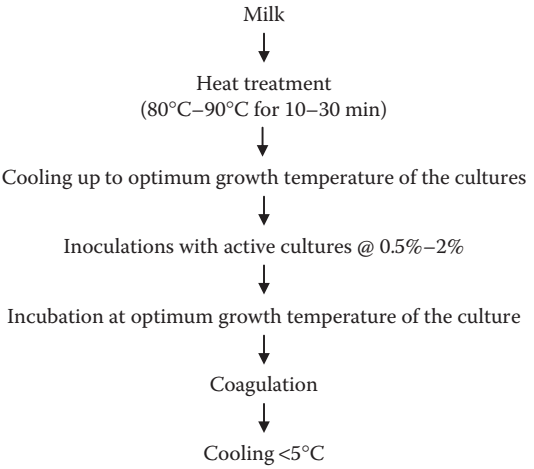

Figure 4.1 Methods of propagation of culture.

starters during propagation. Similarly, the medium (milk) should be free from antibiotic residues or any other substances harmful to the starter. Control of temperatures during incubation and cooling are also important factors affecting the activity of the culture. The conventional method of starter preparation includes a stepwise increase in volume of starters from stock culture (0.4 ml) (liquid, dried, or frozen) to mother culture (40 ml), mother culture to working culture (4 liters), and working culture to bulk culture (200 liters).

Preparation of mother culture is a very important step in the production of bulk starter for commercial application. The methods of bulk starter production are illustrated in Figure 4.2. The traditional method is time consuming, laborious, requires skilled personnel, and is more prone to contamination. The other method makes use of concentrated cultures sufficient to inoculate a large quantity of milk for bulk starter production or directly for product manufacture.

Several new techniques have been developed in the recent past to prevent contamination during starter propagation and bulk starter production, especially from bacteriophage. These include the Lewis system, the Jones system, and the Alfa-Laval system, which employ mechanical and chemical control measures in the design of equipment and bulk starter tank to prevent the entry of contaminants during propagation. Starter propagation in specially designed media, devoid of calcium

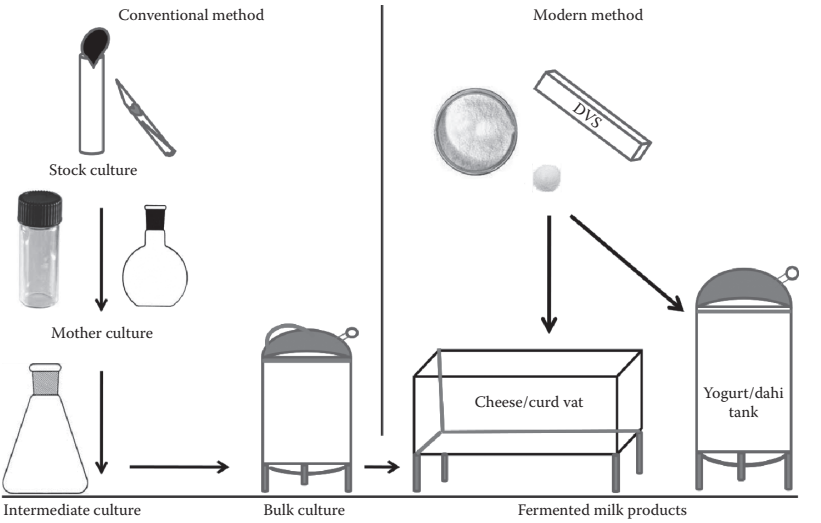

Figure 4.2 Production of starter cultures.

ions, also helps in controlling phage infection. Such media are called phage-inhibitory media or phage-resistant media.

Many professional laboratories now supply DVS or DVI (direct-vat-inoculation) cultures, which are highly concentrated cultures, having cell population of about 10^{10} to 10^{11} cells per gram which are used directly for setting the vat or for the preparation of bulk culture. These cultures omit the need of culture propagation at the factory.

4.5 Problems Associated with Production of Starter Culture

Starter cultures may show a number of defects with respect to growth and performance. Among these, the defects called weak acid, no coagulation, flat flavor, and thin body are due to slowness or sluggishness of the starter. This gives inadequate performance during product manufacture leading to a delay in manufacture, poor quality product, and economic loss. The slowness in starters comes due to a number of factors (Table 4.5) that can adversely affect the growth, leading to reduced rate of acid production or sometimes complete cessation of acid production.

By far the most common reason for slowness is found to be antibiotic residues in milk. When animals are given antibiotics for certain diseases, they are likely to be secreted into the milk. The starter

Table 4.5 Problems Associated with Starter Cultures and Their Remedies

PROBLEMS	REMEDIES
PROBLEM WITH STARTER ITSELF	
Spontaneous loss of vitality	Replace the culture
Strain variation	Check the balance
Physiological state of cells	Inoculate at the correct stage
INCOMPETENCE IN THE CONTROL OF STARTER	
Starter contaminated	Prevent contamination
Too infrequent sub-culturing	Sub-culture regularly at right stage
Use of unsuitable media (milk)	Use high quality reconstituted skim milk for propagation
Culturing at wrong temperature	Ensure proper temperature
PROBLEM WITH MILK	
Abnormal milk, for example, mastitic, colostrum, late lactation	Never allow mixing with normal milk
Antibiotic residues in milk	Never mix milk from antibiotic-treated animal for 3 days
Milk affected by feeds, seasonal factors, aeration, and so on.	Never use such milk or use after treatment
Inhibitory substances in milk, for example, preservative, detergent and sanitizer residues	Avoid such milk
PROBLEMS ASSOCIATED WITH THE PRODUCTION METHODS	
Changes in ripening time and temperature	Ensure proper adjustment of time and temperature
Cooking and clotting temperature in cheese	Use resistant cultures, don't change temperatures
Bacteriophage action	Observe strict asepsis, use starter rotation system, phage inhibitory media or phage resistant media, environmental control, use of modified cultures.

organisms are inhibited depending upon the concentration of antibiotic and the type of strain. *S. thermophilus* is most sensitive to penicillin residues in milk. The other reason contributing to slowness is bacteriophage infection.

4.6 Application of Starter Cultures

Starter cultures are used in manufacturing of variety of fermented milk products. The properties that are required by the lactic cultures for industrial use may differ from product to product (Panesar 2011). Apart from the production of useful metabolites in the product, certain

Table 4.6 Role of Exopolysaccharides Producing Starter Cultures in Fermented Milk Products

EPS PRODUCING STARTER CULTURES	PRODUCTS	EFFECT(S)	REFERENCE
Lc. lactis subsp. *lactis*	Cream cheese	Better firmness, consistency and melting properties	Nauth and Hayashi (2004)
Lb. kefir	Kefir	Improved texture	Micheli et al. (1999)
Lc. lactis subsp. *cremoris* JFR1	Cheddar cheese	Increased moisture retention, improved sensory, textural and melting properties	Awad et al. (2005)
Lc. lactis subsp. *lactis* B6	Dahi	Improved rheological and sensory properties, less syneresis	Behare et al. (2009b)
S. thermophilus (EPS^-) and *Lb. delbrueckii* subsp. *bulgaricus* (EPS^+)	Stirred yogurt	Increased viscosity	Marshall and Rawson (1999)
S. thermophilus (EPS^+) and *Lb. delbrueckii* subsp. *bulgaricus* (EPS^-)	Yogurt	Ropiness, low serum separation, higher viscosity	Folkenberg et al. (2005)
S. thermophilus (EPS^+) and *Lb. delbrueckii* subsp. *bulgaricus* MR-1R (EPS^+)	Mozzarella cheese	Retained moisture, better melting properties	Perry et al. (1997)
S. thermophilus IG16	Lassi	Improved consistency and sensory attributes, higher viscosity	Behare et al. (2010)

cultures are known to produce EPS, which improves textural, rheological, and sensory properties of fermented milk products (Table 4.6). Some starters can also produce bacteriocins that have potential significance as biopreservatives for food application. Bacteriocins are the proteins that are inhibitory to self or closely related species. However, some bacteriocins of starter cultures have broad spectrum activities, especially against spoilage causing and pathogens (Table 4.7). The application of starters in some of the fermented milk products is as explained below.

Yogurt is made by the combination of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* that shows symbiotic association during their growth. *Lb. delbrueckii* subsp. *bulgaricus* produces certain amino acids from casein that stimulates the growth of *S. thermophilus,* whereas *S. thermophilus* stimulates the growth of *Lb. delbrueckii* subsp. *bulgaricus* by removing oxygen, lowering pH, and producing formic acid. One of the major problems of yogurt making is excessive sourness produced