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Review Article

Selection and Application of Starter Culture for Milk and Milk Products: A Review

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Abstract

Starter cultures are fundamental to the fermented dairy industry due to their essential role in driving the fermentation process and enhancing the overall quality of dairy products. These cultures-comprising selected strains of bacteria, yeasts, and moulds-are intentionally introduced into milk and other dairy substrates to initiate controlled fermentation. Their primary function involves the production of lactic acid, which not only preserves the product by lowering pH but also contributes to desirable changes in texture, flavour, and aroma. Furthermore, starter cultures significantly improve the consistency and shelf-life of fermented dairy products such as yogurt, cheese, and kefir. Beyond their technological importance, many starter cultures possess probiotic properties, offering a range of health benefits. These include supporting gut health, aiding digestion, and strengthening the immune system, thereby increasing the nutritional value of the final product. As demand grows for functional foods with natural health-promoting properties, the role of starter cultures in developing probiotic-rich dairy items has become increasingly important. This review explores the microbiological composition, functional roles, and health implications of starter cultures in fermented dairy products, highlighting their indispensable contribution to food quality, safety, and human wellness.

Keywords: *Starter Cultures; Exo-Polysaccharides; Probiotic; Nutritional Value; Dairy Products*

Introduction

Starter cultures are beneficial bacterial or fungal strains, either pure or mixed, used to initiate fermentation in food production. These active microorganisms promote desirable characteristics in products like cheese, dahi, fermented sausages, fish, alcoholic beverages, and soy-based foods. They play a key role in creating flavors, textures, and preserving the products [1].

Starter cultures can consist of one or several strains of microorganisms, including lactic acid bacteria, various other bacteria, and yeasts [2]. They can aid in: Improving flavor, supporting preservation, ensuring high product quality, preventing fermentation issues, accelerating the fermentation process, generating lactic acid through the fermentation of sugars, and eliminating harmful microorganisms [3].

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Role/functions of starter cultures: The main role of lactic starters is to convert lactose into lactic acid. Besides producing lactic acid, the starter cultures offer several other benefits, as outlined below (Bintsis T, 2018).

Function	Result
Acid production	Gel formation Expulsion (syneresis) of whey for texturing Preservation of milk Helps in the development of flavour
Flavour	Formation of flavour compounds like diacetyl and acetaldehyde
Preservation	Lowering of pH and redox potential Produces lactic acid for fermentation Production of antibiotics Production of H_2O_2 Production of acetate
Gas formation	Eye formation in certain cheeses Open texture formation (e.g., blue cheese)
Stabilizer formation for texture control	Enhances body and viscosity (e.g., polysaccharides)
Lactose utilization	Limits gas, off-flavours; suitable for lactose intolerant
Lowering of redox potential	Helps in preservation
Proteolysis and lipolysis	Helpful in the ripening/maturation of cheeses
Miscellaneous compounds	Production of alcohol in kefir and kumis

Table: *Functions of starter cultures.*

Source: www.agriMoon.com [4].

Types of starters

Starters are grouped under different categories based on composition of micro flora, growth temperature, type of products, flavor production and type of fermentation into the following categories [4].

Based on the composition of micro flora/organisms

a. Single: Used of only single organism in the preparation of dahi or cheese. Disadvantage of using single culture is that Bacteriophage attacks can cause sudden starter failure, leading to significant industry loss.

b. Paired compatible strain: Using complementary culture strains in balanced proportions reduces failure risk. In case of bacteriophage attack, only one type of organism will be affected and the other organism will carry out the fermentation without any problem.

c. Mixed strain: Multiple organisms with varied traits (acid, flavor, slime) used in unknown proportions.

d. Multiple/mixed strain: More than two strains in known proportion are used. The quality and behaviour of these strains is predictable.

Based on the growth temperature: Based on the growth temperature organisms can be divided into mesophilic and thermophilic.

a. Mesophilic starter cultures: Optimum growth temperature: 30°C and they have a growth temperature range of 22 - 40°C The mesophilic starter cultures generally contain the organisms of *Lactococci*. E.g. Dahi cultures: *Lactococcus* spp. Cheddar cheese: *Lactococcus lactis sub sp lactis, Lactococcus lactis sub sp cremoris, Lactococcus latis sub sp lactis blovardiacetylatis, Leuconostoc mesenteroides sub sp cremoris*.

b. Thermophilic starter cultures: The optimum temperature of these cultures is 40°C and they have a growth temperature range of 32- 45°C E.g. *Streptococcus thermophilus, Lactobacillus delbrueckii sub sp bulgaricus, Lb. Delbrueckii sub sp lactis, Lb. casei, Lb. helveticus, Lb. plantarum*.

Product for which used

- Yoghurt: *Streptococcus thermophilus, Lactobacillus delbrueckii subsp bulgaricus.*
- Swiss cheese: *Streptococcus salivarius sub sp thermophilus, Lactobacillus delbrueckii sub sp bulgaricus, Lactobacillus helveticus, Lactobacillus casei.*

Based on the flavor production

The starters are grouped into B, D, BD and N type based on their ability of flavor production:

- B (L) type: *Leuconostoc* as flavor producer (old name is *Betacoccus*).
- D. type *L. Lactis sub sp lactis biovar diacetylactis.*
- BD (LD) type: Mix of both cultures for optimal growth at 30°C.
- N or O type: Lack of flavor-producing organism.

Based on the type of fermentation: Starters are classified as homo or heterofermenters based on glucose metabolism products:

- Homo fermentative cultures: e.g. *Lactococcus lactis sub sp lactis*.
- Hetero fermentative cultures: e.g. *Leuconostoc dextranicum*.

Selection criteria of starter culture

The selection of starter cultures is based on technological relevant traits but most especially in some requirements like Safety: The culture should be recognized as safe: Stability: The culture should remain viable and stable during storage. Scalability: The culture should be capable of being produced on a large scale. Technological traits: The culture should have technological relevant traits, such as absence of amino acid decarboxylase activity. Lack of toxicogenic activity or antimicrobial resistance. Stability under processing conditions, salt tolerance, bacteriocin production [5].

Metabolism and activities: The culture's metabolism and activities should be studied, as their effects and properties may vary between laboratory conditions and food products.

Optimal temperature: The culture should be classified as either mesophilic (temperate-loving) or thermophilic (heat-loving) based on its optimal temperature for growth and acid production. Starter cultures are microorganisms, bacteria, molds, yeast, that are added to food to improve its quality improve the taste aroma preservation of food [5].

Application of starter culture milk and milk products

- **Metabolites produced by LAB:** LAB exhibits a straightforward homo- or hetero fermentative metabolic process. These bacteria depend on lactose as their primary carbohydrate source. Dairy LAB comprises species from the genera *Lactobacillus, Lactococcus, Leuconostoc*, and *Streptococcus*. The fermentation process carried out by LAB predominantly produces lactic acid, which is essential for preserving food items. The metabolic activity of LAB positively influences the texture and taste of fermented products [6].

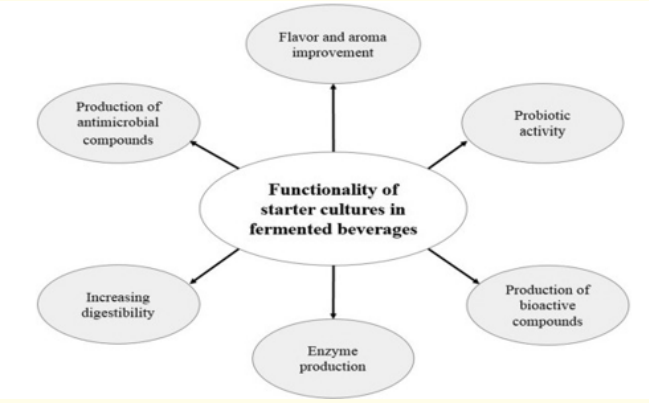

Figure 1

- **Production of aroma and flavour:** LAB are crucial for aroma and flavor in fermented foods. They acidify products, creating a tangy taste, and contribute via proteolytic and lipolytic activities. LAB also produce volatile compounds that define the characteristic flavours of various fermented products like sourdough, kefir, koumiss, butter, and yogurt produce compounds like lactate, ethanol, diacetyl, and acetaldehyde. Acetaldehyde is a key flavor compound in yogurt, produced by starter cultures in fermented milks. In mesophilic cultures, it originates from threonine, while in thermophilic cultures, lactic acid bacteria produce acetaldehyde from sugar, nucleic acids, lipids, and aromatic compounds in milk [6].

Figure 2

- **EPS production and textural improvement:** Polysaccharide-based bio thickeners, derived from plants (e.g. starch, pectin, guar gum) and seaweeds (e.g. carrageenan, alginate), are commonly used in the food industry. They help with emulsification, stabilization, suspension of particulates, and control of crystallization. These thickeners enhance viscosity, firmness, and texture, reduce syneresis, and improve the mouthfeel of low-fat products [6].

- **Bacteriocins production:** Bacteriocins are antimicrobial peptides or proteins produced by bacteria, including many lactic acid bacteria [7]. These bacteriocins have broad inhibitory spectra and can be used in food preservation, reducing reliance on chemical preservatives and intense heat treatments, while preserving the organoleptic and nutritional quality of foods [6].

Figure 3

- **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 [6].
- **Production of nutraceuticals:** Nutraceuticals are food components that promote health through specific physiological actions. Bacterial-origin nutraceuticals, particularly from LAB, are added to foods like fermented dairy products. Strain selection and process optimization can enhance their content, with health benefits attributed to their low-calorie, fiber-like nature, and bifidogenic effects [6].
- **Reduction of toxic or anti nutritive factors:** The fermentative action of specific LAB strains can remove toxic or antinutritive factors, such as lactose and galactose, from fermented milks, helping prevent lactose intolerance and the accumulation of galactose [6].
- **Production of vitamins metabolism:** Certain starter cultures can synthesize vitamins, increasing their content in fermented milk, while others may utilize vitamins, reducing their levels. This effect varies by strain. For example, yogurt bacteria produce folic acid, niacin, and vitamin B6, while propionic bacteria produce vitamin B12 [6].

Antimicrobial compounds and interactions produced by starter cultures:

The primary compounds responsible for such effects are [8]:

1. Organic acids: Lactic acid, acetic acid, and propionic acid are organic acids produced by starter cultures the reduce pH and inhibit the growth of microorganisms.
2. Hydrogen ion concentration (pH): Some lactobacilli produce hydrogen peroxide, which oxidizes enzymes and increases membrane permeability, causing antimicrobial activity.
3. Low oxidation reduction potential.
4. H_2O_2 and CO_2.
5. Aroma compounds: Starter cultures produce diacetyl and acetaldehyde, which improve the flavour and aroma of dairy products.
6. Fatty acids: *Lactobacillus* and *Lactococci* with lipolytic activity may produce significant fatty acids under certain conditions.
7. Bacteriocins: Bacteriocins from starter cultures enhance dairy product safety.

Properties of ideal starters

1. A good starter should have the ability to produce lactic acid at vigorous and steady rate.
2. A starter must be pure, free of contaminants.

3. It should grow rapidly in suitable organic substances [9].
4. It can be easily cultivable in large quantities.
5. It should maintain physiological constancy.
6. It should produce necessary enzymes abundantly to drive desired chemical changes.
7. It should transform easily with simple environmental adjustments [10].

Activity and purity tests of starter cultures

1. **Organoleptic tests:** Organoleptic tests require high degree of training and experience. These tests assess appearance, flavor, and consistency [11]:

   a. **Body:** The body should be firm like custard, with a texture that holds shape during gentle tapping and breaks smoothly with stronger tapping.

   b. **Texture:** It should be smooth, viscous, and free of lumps and whey pockets after agitation.

   c. **Taste:** The taste should be clean, acidic, and free from bitterness or saltiness.

   d. **Flavour:** Should have a typical aroma, free from off-flavors like malty, bitter, rancid, yeasty, or putrid.

2. **Chemical tests:** Chemical parameters for assessing starter activity include:

   a. Titratable acidity

   b. Volatile fatty acids

   c. Tests for diacetyl and acetyl methyl carbinol

   d. Tests for acetaldehyde starter cultures and fermented milk products.

3. **Microbiological methods:** Microbiological methods to assess starter activity and purity include: Plate count, direct microscopic count (rod/cocci ratio), selective plating, and tests for contaminants (coliforms, yeasts, molds). Starters must be free from foreign microbes, as contaminants may outcompete starter bacteria.

4. **Purity tests:** The purity of the starters is evaluated by using microscopic or chemical tests.

   a. **Microscopic examination:** This is performed by grams staining or Newman‘s stain. Gram -ve bacteria, spore formers, yeasts and molds and staphylococci etc should be absent. The lactic bacteria should appear as Gram +vecocci or thin rods.

   b. **Catalase test:** Adding 3% hydrogen peroxide; effervescence indicates contamination, as true starters (LAB) are catalase-negative.

5. **Activity rating:** The starters are evaluated for their activity using different methods:

   a. **Titratable acidity test:** The cultures lose their activity due to acid injury if they are allowed to over ripen. A developed acidity in the range of 0.7 to 0.85% LA is optimal [4].

   b. **Horral Elliker’s test:** Used for mesophilic cultures: Inoculate sterilized milk with 0.3 ml culture, incubate at 37.7°C. Incubate inoculated tubes at 37.7°C for 3 1/2 hours. At end of incubation period, the entire contents of each tube are titrated with N/10 sodium hydroxide and phenolphthalein indicator. A titration value of 0.4% LA or higher indicates that the culture should be well suited for cheese making. A titration value of 0.30% to 0.35% LA indicates a slow culture. Starter developing acidity of less than 0.30% LA invariably graded as inactive as it produces little or no acid during cheese manufacture.

c. **Whitehead-Cox activity test:** Whitehead and Cox test starter suitability by ripening 10 ml culture in 1,000 ml pasteurized milk at 30°C for 1 hour. Add rennet, cut and cook curd (38 - 39°C), drain whey, and measure acidity to assess final starter activity.

d. **Creatine test:** Creatine test indicates diacetyl levels in mesophilic cultures by forming a pink complex.

e. **Dye reduction test:** Excellent cultures reduce resazurin/methylene blue in 35 minutes; good ones in 50 - 60 minutes.

Conclusion

- Milk contains many health promoting constituents including immunoglobulin, 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, flavored milk drinks, cheeses, yoghurt etc.
- LAB ferment lactose into acids, lowering pH and preventing spoilage in milk.
- LAB produce secondary metabolites affecting flavor, aroma, texture, and antimicrobial activity.
- These bacteria produce proteases and peptidases to help digest milk proteins.
- *Bifidobacteria* and *Lactobacilli* are widely used in probiotic dairy products.

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