History, classification and importance of starter cultures in dairy industry
Starter cultures are selected bacterial or fungal strains of food-grade microorganisms of known and stable metabolic activities, either pure or mixed, used to initiate a fermentation process.
Starter cultures are a group of active, carefully selected and desirable microorganisms that are used in the production of cultured dairy products (dahi, yoghurt and cheese). These cultures are needed to produce the desired effect in the finished product through the process of fermentation.

Since natural microflora of milk is inefficient and destroyed by the heat treatments, a starter can produce particular characteristics (appearance, body, texture, flavour) in the milk. They produce lactic acid from lactose. Certain starters are added specifically for their ability to produce flavour compounds (diacetyl). The lower pH of cultured products can be inhibitory to certain spoilage organisms.
<table>
<thead>
<tr>
<th>Function</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Acid production</td>
<td>□ Gel formation</td>
</tr>
<tr>
<td></td>
<td>□ Expulsion (syneresis) of whey for texturing</td>
</tr>
<tr>
<td></td>
<td>□ Preservation of milk</td>
</tr>
<tr>
<td></td>
<td>□ Helps in the development of flavour</td>
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<tr>
<td>Flavour</td>
<td>□ Formation of flavour compounds like diacetyl and acetaldehyde</td>
</tr>
<tr>
<td>Preservation</td>
<td>□ Lowering of pH and redox potential</td>
</tr>
<tr>
<td></td>
<td>□ Production of lactic acid</td>
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<tr>
<td></td>
<td>□ Production of antibiotics</td>
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<td></td>
<td>□ Production of H₂O₂</td>
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<td></td>
<td>□ Production of acetate</td>
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<tr>
<td>Gas formation</td>
<td>□ Eye formation in certain cheeses</td>
</tr>
<tr>
<td></td>
<td>□ Production of open texture Ex. blue veined cheese</td>
</tr>
<tr>
<td>Stabilizer formation</td>
<td>□ Development of body and viscosity</td>
</tr>
<tr>
<td></td>
<td>Ex. Polysaccharide materials</td>
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<tr>
<td>Lactose utilization</td>
<td>□ Reduces the development of gas and off flavours</td>
</tr>
<tr>
<td></td>
<td>□ Suitable for lactose intolerant people</td>
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<tr>
<td>Lowering of redox potential</td>
<td>□ Helps in preservation</td>
</tr>
<tr>
<td></td>
<td>□ Helps in development of flavour</td>
</tr>
<tr>
<td>Proteolysis and lipolysis</td>
<td>□ Helpful in the ripening/maturation of cheeses</td>
</tr>
<tr>
<td>Miscellaneous compounds</td>
<td>□ Production of alcohol in kefir and kumis</td>
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</tbody>
</table>
History of Starter Cultures and Fermented Milks

Fermented foods are of great significance since they provide vast quantities of nutritious foods in a wide diversity of flavors, aromas, and textures enriching the human diet.

Over 3500 traditional, fermented foods exist worldwide. Fermented foods have been with us since early ages and of these fermented milks have long been an important component of nutrition and diet. Originally fermented milks were developed as a means of preserving nutrients.

Our ancestors acquired the knowledge to use milk though they did not know about the existence of microorganisms. When milk was left at room temperature, it was spontaneously fermented and was used as such or after dilution with water. By trial and error, the practice of inoculating milk with a small amount of previously fermented milk was developed. Heating the milk before inoculation increased the quality of the product and specific, traditional fermented milk products were developed.

About 200 years ago, cheese and butter production became industrialized. Traditional products were standardized and starters were grown in manufacturing plants. In the late 19th century companies specialized in starter production were founded where starters were undefined, containing an unknown mixture of different strains of the suitable species. Later, starters were analyzed and defined, composed of specific strains with selected properties selected to give a standard product.
Consumption of fermented milks has generally increased around the globe over a period from 2001-2004. According to another report by Euromonitor, largest fermented dairy market till 2003 was Japan, where the leading brand Yakult is the reference product available for more than 50 years. The next most significant markets are South Korea and Brazil, followed by a number of Western European markets including US probiotic drinking yoghurts (on the basis of portability, snack appeal and health claim).

In the Indian subcontinent, fermented milk products such as dahi, lassi, buttermilk and shrikhand figure prominently in people’s diet.
Significance of Starters in Milk Fermentation.....

• Enrichment of human diet through a wide variety of flavours, aroma and texture of foods.
• Preservation of foods via lactic acid, alcoholic, acetic acid and alkaline fermentations.
• Bio-enrichment of food substantially with proteins, essential amino acids, essential fatty acids and vitamins.
• Detoxification during food fermentation processing.
• Nutritional and physiological benefits such as promotion of growth and digestion, settling effect on the GI tract by deceasing harmful bacteria, improvement of bowel movements, suppression of cancer, suppression of blood cholesterol, suppression of tumors and fulfilling the needs of lactose intolerant people.
• Among various milk products, the fastest market growth is almost six times to that of total dairy sector growth and observed for probiotic based fermented milk products during the last decade. The increasing demand from consumers for dairy products with 'functional' properties is a key factor driving value sales growth in developed markets. This led to the promotion of value-added products (probiotic and other functional yoghurts, reduced-fat, enriched milk products, fermented dairy drinks and organic cheese).

• Another important global trend is increasing demand for consumer convenience.

• Fermented milks containing various nutrients are being tested as curatives for specific diseases and are approaching medical food effectiveness in conventional food format and will continue to be introduced to the food supply.
Classification /taxonomic groups as per Bergey's Manual

- STARTER CULTURES
  - LACTIC
    - RODS: Lactobacillus
      - Lactococcus
      - Streptococcus
      - Leuconostoc
      - Pediococcus
    - COCCI: Bifidobacteria
      - Brevibacterium linens
      - Acetobacter acetii
      - Propionibacteria
    - BACTERIA: Bacillus
      - Kluuyveromyces
    - YEASTS: Candida kefir
      - Saccharomyces
      - Penicillium camembertii
        - Penicillium roquefortii
        - Aspergillus oryzae
        - Mucor rasmussen
        - Geotricum candidum
Genus *Lactococcus*:

- Consist of *L. lactis subsp. lactis*, *subsp. cremoris* and *subsp. lactis biovar diacetylactis*.
- Share closely related characteristics (a) possession of identical isoprenoid quinones and enzyme, β-phosphogalactase (b) indistinguishable lactic dehydrogenase (c) have identical % of guanine and cytosine.
- *L. lactis* is of significance in dairy fermentations. Cells are cocci, homofermentative, mesophilic in nature that usually occur in chains, although single and paired cells are also found. When grown in milk, more than 95% of their end product is lactic acid.
- Optimum growth temperature is between 25-30°C.
- They are weakly proteolytic and can use milk proteins. Variant, *L. lactis subsp. lactis* var. *diacetylactis* converts citrate to diacetyl, CO2, and other compounds. Some lactococci produce exopolysaccharide.

<table>
<thead>
<tr>
<th><em>Lactococcus lactis subsp lactis</em></th>
<th>Acid producer but non-flavour producer</th>
</tr>
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<tbody>
<tr>
<td><em>Lactococcus lactis subsp cremoris</em></td>
<td>Acid producer but non-flavour producer</td>
</tr>
<tr>
<td><em>Lactococcus lactis subsp lactis biovar diacetylactis</em></td>
<td>Both acid &amp; flavour producer</td>
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</table>
Genus *Streptococcus*:

- Gram-positive organisms that usually form pairs or chains. In 1937, Sherman separated the genus according to physiological and growth characteristics, especially with regards to temperature limitations on growth. Four general groups designated by Sherman are (1) pyogenic (*S. agalactiae*) (2) viridans (*S. thermophilus*) (3) enterococcus (*S. faecalis*) (4) lactic (*S. lactis, S. cremoris, S. diacetylactis*).

- The only species used as starter culture is *Streptococcus thermophilus*. This is a yoghurt culture, which is thermophilic in nature with optimum growth temperature of 38-42 C. All are homofermentative organisms.
Genus *Leuconostoc*:

- *Leuconostoc* spp. used in the dairy industry are heterofermentative organisms capable of producing diacetyl, carbon dioxide, and acetoin from citrate. Do not hydrolyze arginine, require various B vitamins for growth.
- These organisms are normally used along with lactic acid bacteria in multiple or mixed strain cheese starter cultures, which produces flavour compounds.
- They grow poorly in milk; probably because they are adapted to growth on vegetables and roots and therefore lack sufficient proteolytic ability to grow in milk. When used in cultured milk starters, they convert excess acetaldehyde to diacetyl, thus reducing undesirable “green” flavor.
- *L. cremoris, L. citrovorum, L. dextranicum*
Genus *Lactobacillus*:

- *Lactobacillus delbruekii subsp. bulgaricus* is used for the preparation of yoghurt along with *Streptococcus salivarius subsp. thermophilus*. These two organisms exhibit a symbiotic relationship.

- *Lactobacillus acidophilus* is a probiotic culture, used for preparation of acidophilus milk and other probiotic milk products like Bioghurt etc.

- The members of lactobacilli are classified based on fermentation of glucose into 3 groups:
Classification of *lactobacillus* based on glucose fermentation

- **Obligately homofermentative**
  - Ferment hexose sugars to lactic acid by the EMP pathway.

- **Facultatively heterofermentative**
  - Ferment hexose sugars either only to lactic acid or to LA, acetic acid, ethanol. Pentose sugars are fermented to lactic and acetic acid via the phosphoketolase pathway. This group not usually found in starter cultures but is associated with beneficial secondary fermentation during cheese ripening.

- **Obligately heterofermentative**
  - Ferment hexose sugars to LA, acetic acid (or ethanol), and CO2 using the phosphoketolase pathway. These lactobacilli can cause undesirable flavor and gas formation during ripening of cheese.

### Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Products</th>
<th>Growth at 15°C</th>
<th>Growth at 45°C</th>
<th>Lactic acid isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homofermentative</strong></td>
<td></td>
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</tr>
<tr>
<td><em>L. delbrueckii</em></td>
<td>Yogurt, koumiss, kefir, Italian and Swiss cheeses</td>
<td>—</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>subsp. <em>bulgaricus</em></td>
<td></td>
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<td></td>
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<tr>
<td><em>L. acidophilus</em></td>
<td>Hard cheese</td>
<td>—</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>subsp. <em>lactis</em></td>
<td>Acidophilus milk, laban</td>
<td>—</td>
<td>+</td>
<td>DL</td>
</tr>
<tr>
<td><em>L. helveticus</em></td>
<td>Yogurt, Swiss cheese</td>
<td>—</td>
<td>+</td>
<td>DL</td>
</tr>
<tr>
<td><strong>Facultatively heterofermentative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. casei</em> subsp. <em>casei</em></td>
<td>Hard cheese</td>
<td>+</td>
<td>—</td>
<td>L</td>
</tr>
<tr>
<td><strong>Obligately heterofermentative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. kefir</em></td>
<td>Kefir</td>
<td>+</td>
<td>—</td>
<td>DL</td>
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</tbody>
</table>
Genus *Pediococcus*:

- The only strains of this genus to be used in dairy starter cultures is *P. acidilactici* and *P. pentosaceus*.
- It divide alternatively in two perpendicular directions to form tetrads that differentiate them morphologically from other lactic acid bacteria.
- The cells do not form spores or capsules, Gram-positive cocci of uniform size, produce DL-lactate, catalase negative and are non-motile.
- *Pediococcus* spp. have been found in cheese, but represented only a small proportion of the total lactic acid bacteria in the product.
Genus *Bifidobacterium*:

- Produce lactic and acetic acids in the ratio of 2:3. They have the enzyme fructose-6-phosphate phosphoketolase which is lacking in LAB. Found in the gut of infants, intestines of man, various animals and honeybees. These organisms are generally used in preparation of therapeutic fermented milk products in combination with yoghurt, acidophilus milk or yakult starter cultures.

- Members: *B. bifidum*, *B. longum*, *B. infantis*, *B. breve* etc.

- The optimum growth temperature is 37-41°C.

- Anaerobic conditions are essential for optimum growth.

- Milk fermented with bifidobacteria has a distinctive vinegar taste due to the production of acetate plus lactate from the metabolism of carbohydrates.
Genus *Propionibacterium*:

- *Propionibacterium* spp. are non-spore-forming, pleomorphic, Gram-positive rods that produce large amounts of propionic and acetic acid and CO2 from sugars and LA.
- *P. freudenreichii* and *P. shermanii* are used in Swiss cheese. It has the ability to produce large gas holes in the cheese during ripening/maturation period. *P. jensenii*, *P. thoenii* and *P. acidipropionici* are other organisms present in these genera.

Genus *Brevibacterium*:

- *B. linens* is used as starter culture in preparation of bacterial surface ripened cheeses. It imparts distinctive, reddish orange colour to the rind of Brick and Limburger cheese.
Genus *Enterococcus*:

- Gram-positive, catalase-negative cocci, produce L(+) lactic acid homofermentatively from glucose, and also derive energy from degradation of amino acids. They have a phosphoenolpyruvate phosphotransferase (PEP-PTS) system for uptake of lactose and other carbohydrates.
- Used as food safety indicators and have a possible involvement in foodborne illness.
- Enterococci are also used as starter cultures in some southern European cheeses.
- *E. faecalis* and *E. faecium* are probiotics for prevention and treatment of intestinal disorders.
Molds are used for the manufacture of some semi-soft cheese varieties and in some fermented milk products. They enhance the flavour and modify slightly the body and texture of curd.

- **White mold** is used in manufacture of surface mold ripened cheeses (Camembert and Brie cheeses). eg: \( P. \) *camemberti*, \( P. \) *candidum*

- **Blue mold** is used in manufacture of internal mold ripened cheeses (Roquefort, Blue Stilton, Danish blue, Gorgonzola cheeses). eg: \( P. \) *roqueforti*

- **Other molds** like *Mucor rasmusen* – used in Norway for the manufacture of ripened skim milk cheese. *Aspergillus oryzae* – used in Japan for the manufacture of Soya milk cheese. *Geotrichum candidum* is used in the manufacture of Villi (cultured product of Finland) where the mold grows on the surface of the milk to form the white velvety layer.
Mold ripened dairy products
Yeasts are used in the manufacture of Kefir and Kumiss. Kefir grains: Kefir grains consist of a mixture of different microorganisms such as *Candida kefir*, *Kluyveromyces marxianus*, *Saccharomyces kefir*, *Torulopsis kefir*. Kumiss: The important starter microflora of kumiss include *Torulopsis* spp., *Kluyveromyces marxianus var lactis*, *Saccharomyces cerevisiae*.