



**Types of Starters, Probiotics and
Special cultures (exopolysaccharide
production)**

Types of Starters.....

Starters are grouped under different categories based on composition of microflora, growth temperature, type of products, flavour production and type of fermentation.

Based on the composition of micro flora/ organisms:

Single starter: Always used as a single organism in the preparation of dahi or cheese. The only problem is there will be sudden failure of starter due to bacteriophage attack which leads to heavy loss to the industry.

Paired compatible strain starters: Two strains of cultures with complementary activities in knowing proportion are used. This will reduce chances of culture failures. In bacteriophage attack, only one type of organism will be affected and the other organism will carry out the fermentation without any problem.

Mixed strain starters: Two or more than two organisms which may have different characteristics (acid production, flavour production, slime production etc).

Multiple strain starters: Mixtures of known compatible, non-phage related, carefully selected strains are used.

Based on the growth temperature:

Mesophilic starter cultures: The optimum growth temperature of these cultures is 30°C and they have a growth temperature range of 22-40°C. The mesophilic starter cultures generally contain the organisms of lactococci. eg., Dahi cultures : *Lactococcus* spp. Cheddar cheese: *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar *diacetylactis*

Thermophilic starter cultures: The optimum growth temperature of these cultures is 40°C and they have a growth temperature range of 32-45°C. eg: *Streptococcus thermophilus*, *L. casei* , *L. plantarum* and *L. mesenteroides* subsp. *cremoris*

Based on the flavour production:

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

- B (L) type: *Leuconostocs* as flavour producer
- D type: *L. lactis* subsp. *lactis* biovar *diacetylactis*
- BD (LD) type: Mixer of both of the above cultures
- N or O type: Absence of flavour producing organism (*L. lactis* subsp. *lactis* and subsp. *cremoris*)

Based on the type of fermentation:

The starters are classified as homo or heterofermenter based on end products resulting from glucose metabolism.

- Homofermentative culture eg. *L. lactis* subsp. *lactis*
- Heterofermentative culture eg. *Leuconostoc dextranicum*

Factors affecting activity of starter cultures

Temperature: Lactic starters (*S. lactis*, *S. cremoris* grow optimally at 27-32 C where *S. thermophilus* grow at 38-42 C).

pH: Both externally and internally controlled pH media required for preparation of bulk starters. Cultivation of lactic starters is taken care to prevent the detrimental effect of low pH.

Strain compatibility: Mixed starters should be maintained to avoid decrease in numbers.

Growth medium: MRS, M-17 (excellent growth medium for streptococci). It contains beta-disodium glycerophosphate as a buffer that neutralizes much of the acid produced by the growing organisms.

Inhibitors: The growth and activity of starter cultures in milk is adversely affected by presence of residual antibiotics, sanitizers as well as the production of antibiotic like substances by certain wild strains. Milk should be monitored for the presence of residual antibiotics before addition of starter cultures.

Bacteriophages: Slow or inhibit acid production by LAB. Use of phage resistant mutants for production of fermented foods.

Degree of aeration: Acid production is faster at the bottom of container or under controlled oxygen tension condition. Aeration, agitation and surface culture usually depress the activity of lactic acid bacteria.

Effect of CO₂: The optimum initial concentration of CO₂ needed for LAB is 0.2-2.3% by volume. Sterilized skim milk may contain 0.3-0.5% of CO₂ which may lead to prolonged lag phase. Incorporation of yeast extract in milk at a concentration of 0.5% can get rid of this problem.

Storage conditions: For the routine maintenance of lactic starters, it is desirable not to store the mature cultures in presence of acid that they have produced during growth. Storage under this conditions can lead to cellular injury and loss of plasmids of cells. Mature culture should be transferred to fresh milk and stored refrigerated or kept in stabs of selective media.

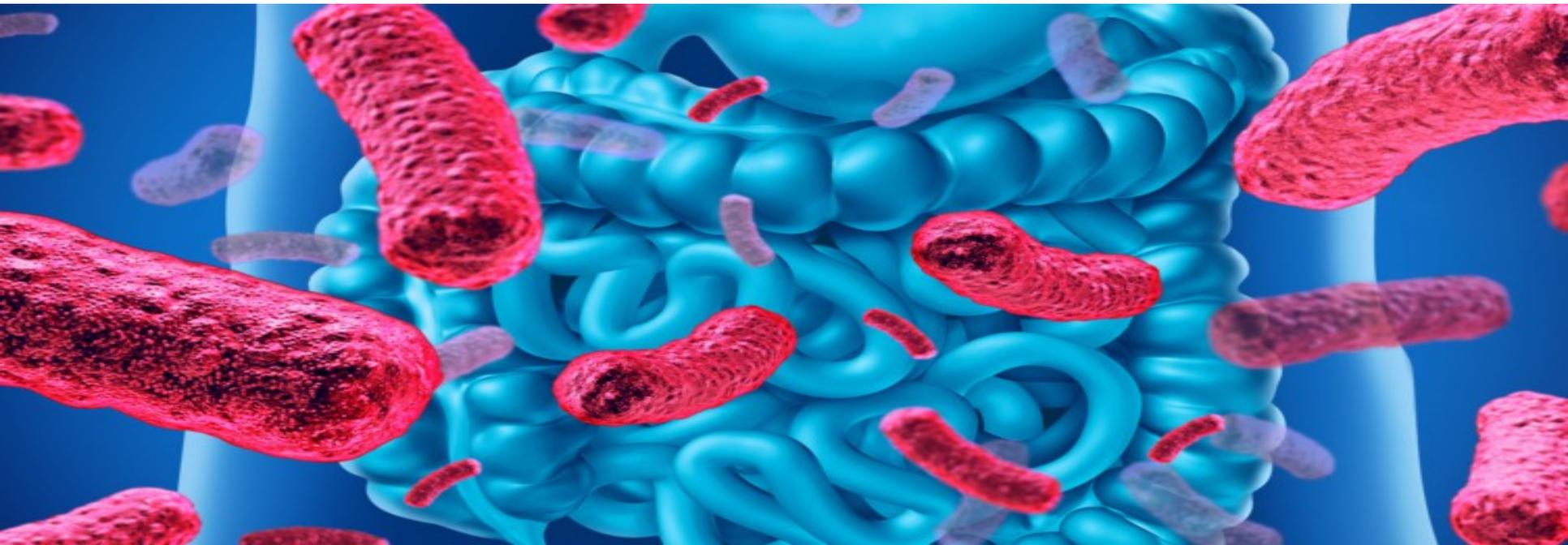
Exopolysaccharide Production (EPS)

- **EPSs are produced by a variety of bacteria and are present as capsular polysaccharides (CPS and LPS) bound to the cell surface, or are released into the growth medium.**
- **These polymers consist of a single type of sugar (homopolysaccharides) or regular, repeating units consisting of different sugars (heteropolysaccharides).**
- **Homopolysaccharides are composed of one type of monosaccharide, which is either D-glucose or D-fructose; these fructans and glucans have a common feature in being synthesised by extracellular transglycosylases (glycansucrases) using sucrose as the glycosyl donor. The heteropolysaccharides, common among the yoghurt starter cultures, consist of a backbone of repeated subunits of three to eight monosaccharides. D-galactose, D-glucose and/or L-rhamnose are almost always present, but ratios vary.**

- Major role in the production of yoghurt, cheese, fermented cream and milk-based desserts where they contribute to texture, mouth-feel, taste perception and stability of the final products.
- Capsular EPSs form capsules around the cell wall and are not secreted into the medium.
- Unattached EPSs are secreted outside the cell wall of the producer and are responsible for the “ropy” phenotype observed in fermented milks.
- The genetic machinery responsible for EPSs production in lactococci is generally encoded on large operons containing more than 10 genes.
- EPSs are considered food-grade or fermented milks containing these EPSs are active as prebiotics, cholesterol lowering and immunomodulants.
- EPS-producing strains of *S. thermophilus* and *L. delbreuckii* subsp. *bulgaricus* enhance the texture and viscosity of yoghurt and reduce syneresis.
- Genetic machinery encoding EPS production in *S. thermophilus* may possess dozen or more unique EPSs gene clusters which range in size from 15-20 kb and are chromosomally encoded.

Probiotics

- Fermented milks and dairy products containing beneficial or “probiotic” cultures, such as lactobacilli and bifidobacteria, are currently among the best-known examples of functional foods.
- Defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”.
- Propionibacteria, *Leuconostoc*, pediococci, and enterococci, have also received attention as candidate probiotic cultures.



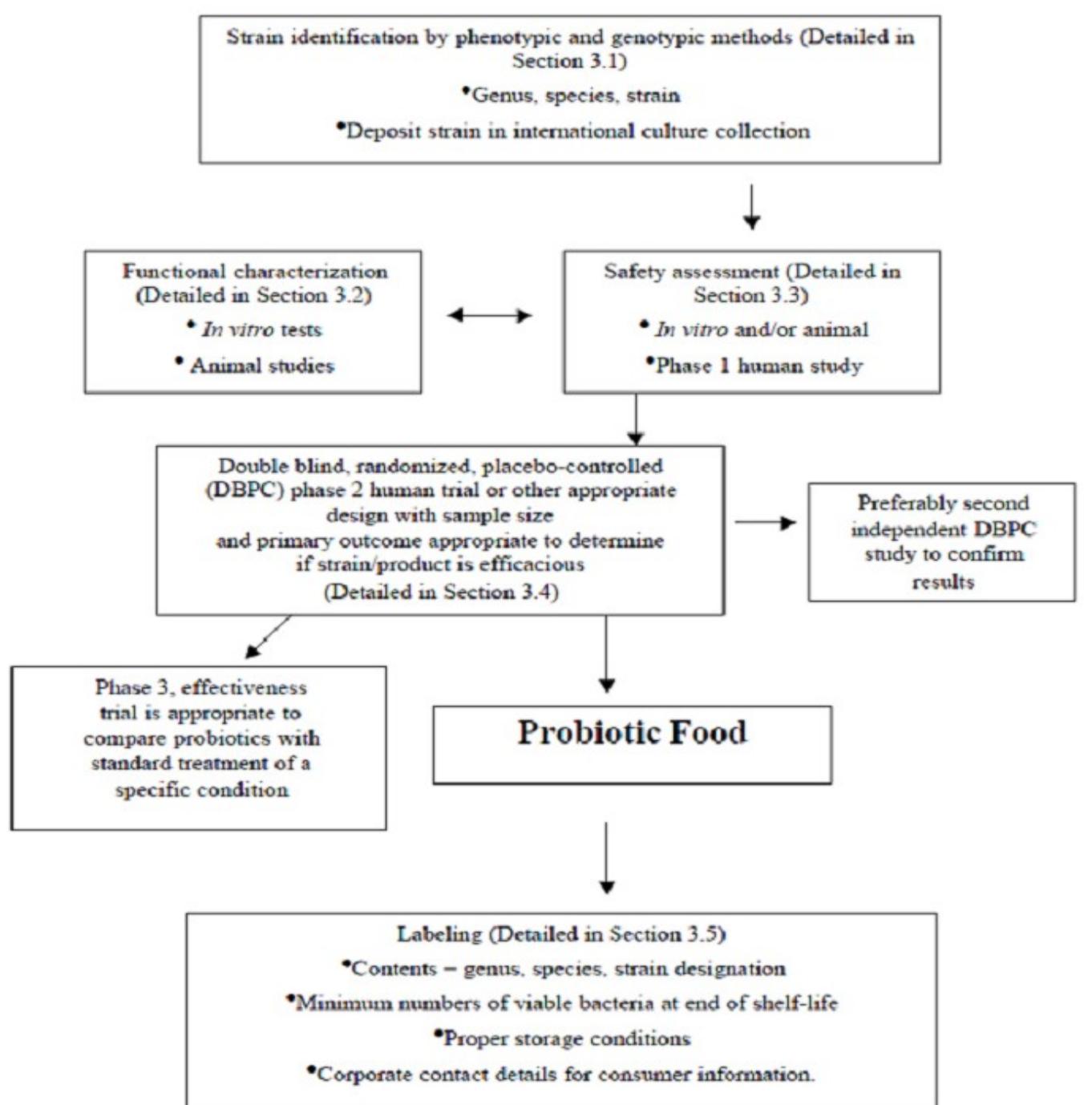
<i>Lactobacillus</i> species	<i>Bifidobacterium</i> species	Other lactic acid bacteria	Nonlactic acid bacteria
<i>L. acidophilus</i>	<i>B. adolescentis</i>	<i>Enterococcus faecalis</i> ²	<i>Bacillus cereus</i> var. <i>toyoi</i> ^{2,3}
<i>L. amylovorus</i>	<i>B. animalis</i>	<i>Enterococcus faecium</i>	<i>Escherichia coli</i> strain <i>nissle</i>
<i>L. casei</i>	<i>B. bifidum</i>	<i>Lactococcus lactis</i> ⁴	<i>Propionibacterium freudenreichii</i> ^{2,3}
<i>L. crispatus</i>	<i>B. breve</i>	<i>Leuconostoc mesenteroides</i>	<i>Saccharomyces cerevisiae</i> ³
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> ⁴	<i>B. infantis</i>	<i>Pediococcus acidilactici</i> ⁴	<i>Saccharomyces boulardii</i> ³
<i>L. gallinarum</i> ²	<i>B. lactis</i> ⁵	<i>Sporolactobacillus inulinus</i> ²	
<i>L. gasseri</i>	<i>B. longum</i>	<i>Streptococcus thermophilus</i> ⁴	
<i>L. johnsonii</i>			
<i>L. paracasei</i>			
<i>L. plantarum</i>			
<i>L. reuteri</i>			
<i>L. rhamnosus</i>			

Microorganisms reported as probiotics

***Lactobacillus* and *Bifidobacterium* are central to both probiotic and prebiotic (non-digestible food ingredients that stimulate the growth and/or activity of certain colonic bacteria) approaches to dietary modulation of the intestinal microflora. This is due to (a) the association of these bacterial groups with human health, (b) their presence in fermented foods (c) possess generally regarded as safe (GRAS) status.**

1. Survival in the environmental conditions in the intended host
 2. Proliferation and/or colonization under host environmental conditions
 3. Survival in association with the host immune system and noninflammatory
 4. Immunostimulatory for the mucosal immune system
 5. Production of antimicrobial substances
 6. Antagonism against cariogenic and pathogenic bacteria
 7. Safety tested; nonpathogenic, nontoxic, nonallergic, nonmutagenic, or anticarcinogenic, even in immunocompromised hosts
 8. Genetically stable, no plasmid transfer
 9. Technologically suitable for process applications
 10. Desirable metabolic activity and antibiotic resistance/sensitivity
 11. Desirable health effects
 12. Potential for delivery of recombinant proteins and peptides
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Guidelines for the Evaluation of Probiotics for Food Use



Health Effects of Probiotics

Well-substantiated effects

- Alleviation of lactose maldigestion
- Prevention/treatment of infections
- Reduction of serum cholesterol
- Chemopreventative effects
- Modulation of the immune system

Potential effects

- Treatment/prevention of inflammatory bowel disease
 - Alleviation of constipation
 - Improvement of dermatitis
 - Liver disease therapy
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