Lecture Notes

By

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Starter Culture and Fermented milk Products

Lesson-1

Introduction to Starter Cultures and Fermented Milks

1.1 INTRODUCTION

Starter cultures are those microorganisms that are used in the production of cultured dairy products such as dahi, yogurt and cheese. The organisms selected for this purpose of need to produce the desired effect in the finished product.

Starters are a group of active and desirable microorganims capable of bringing about desirable changes in the milk product through the process of fermentation. These are carefully selected microorganisms that are deliberately added to milk to initiate ("Start') and carry out the desired fermentation in the production of fermented milk products. In other words starters bring about the specific changes in the appearance, body, texture and flavor characteristics of the final products.

The natural micro flora of the milk is inefficient, uncontrollable and unpredictable or is destroyed altogether by the heat treatments given to the milk. A starter culture can produce particular characteristics in a more controlled and predictable fermentation when added to milk.

The most common use of starter cultures is for the production of lactic acid from latose (milk sugar). Which in most cases causes or assists in the coagulation of milk protein by lowering pH of milk. Cultures that produce lactic acid are generally referred to as "lactic acid lacteria" (LAB). Certain starter organisms are added specificaly for their ability to produce flavor compounds such as diacetyl. Starter organisms can also influence texture of cultures and/or aged products though the breakdown of proteins, fats and other milk constituents in addition to the pH effect. The lower pH of cultured products can be inhibitory to certain spoilage organisms, although inhibition is associated with other by – products of growth with some starters.

More recently, probiotic cultures are finding their way into cultures milk products. These organisms posses some claimed health benefit for the consumer. e.g., better digestion, anti cancer compounds and presentation of heart disease etc. Probiotic cultures may be added as adjuncts or they may be directly involved in the fermentation process.

1.2. HISTORY OF STARTER CULTURES AND FERMENTED MILKS

Fermented foods and beverages have long been manufactured without the use of commercial starter cultures. Traditional methods of production include back slopping, or using a small amount of the finished specifically preserved product to inoculate a new batch, the use of microorganisms found naturally on the product, and the use of special containers that allow for the survival of the starter culture microorganisms within cracks and pores. These traditional methods allow for the development of individual varieties of fermented foods and beverages, and they are still practiced today for small- to mid-scale production facilities, as well as in less developed countries and in homemade-type products. Traditional methods, however, are prone to slow or failed fermentations, contamination, and inconsistent quality. In contrast, modern large-scale industrial production of fermented foods and beverages demands consistent product quality and predictable production schedules, as well as stringent quality control to insure food safety. (Durso, and Hutkins, 2003)

The pure culture techniques in microbiology were not developed until Pasteur in the 1860s Similarly Lactic acid bacteria (LAB), were identified by Lister in the 1870s, it is important to note that an industry developed technique to produce pure cultures only a short time later. Storch (Denmark) Weigman (Germany), and Conn (USA) in late 1880s showed that pure cultures could be used to ripen cream, and soon the role of flavor-producing bacteria (i.e., citrate-fermenting diacetyl-producers) was established. By 1878 Christian Hansen began a culture business that continues even today to be a major supplier of starter cultures for the dairy, meat, brewing, baking, and wine industries.

Initially, starter strains were prepared by the manufacturer by growing pure strains in heat-sterilized milk. Calcium carbonate was often added as a buffer in order to maintain a neutral pH. These liquid cultures remained popular until relatively recently, even though they had a relatively short shelf-life due to the loss of cell viability and fermentative activity. Eventually, rather crude dry culture preparations were produced which required several transfers in milk to revive the culture to an active state. Freeze-dried cultures also became available, but the early product also required growth in intermediate or mother cultures. Frozen cultures, now the most common form for dairy cultures, were not introduced until the 1960s. Significant improvements in freezing and freeze-drying technologies have led these types of cultures to dominate the starter culture market.

The modern starter culture industry provides cultures for nearly every type of fermented food and beverage. Most culture houses also produce and sell the media used to propagate

starter cultures, the enzymes used to coagulate milk for cheese production, and other ancillary products. In addition, the starter culture companies maintain a staff of highly trained microbiologists who provide expert technical service and support when issues or problems related to culture performance arise. And although there are many small culture manufacturers throughout the world, specializing in cultures for specific products or applications, the industry is dominated by a small number of large companies.

1.3.**ADVANTAGES OF MILK FERMENTATION/ROLE OF FERMENTATION IN FOOD**

The Feremented food have following advantages as listed below:

1. Enrichment of human diet through a wide variety of flavours, aroma and texture of foods.
2. Preservation of foods via lactic acid, alcoholic, acetic acid and alkaline fermentations
3. Bio-enrichment of food with proteins, essential amino acids, Essential fatty acids and vitamins.
4. Detoxification during food fermentation processing.
5. Nutritional and physiological benefits such as
    a. Promotion of growth and digestion
    b. Settling effect on the GI tract by deceasing harmful bacteria.
    c. Improvement of bowel movements
    d. Suppression of cancer.
    e. Suppression of blood cholesterol
    f. suppression of tumours
    g. Catering to the needs of lactose intolerant people.

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THE END

LESSON – 2

Role, Function and Classification of Starter Culture

2.1 INTRODUCTION

Starter cultures consist of microorganisms that are inoculated directly into food materials in order to bring about desired and predictable changes in the finished product. As a result the food may have enhanced preservation property, improved nutritional value, modified sensory qualities, and increased economic value. Although many fermented foods can be made without a starter culture, the addition of concentrated microorganisms, in the form of a starter culture, provides a basis for insuring that products are manufactured on a consistent schedule, with consistent product qualities.

2.2 DEFINITIONS

Starter cultures are bacterial or fungal strains either pure or mixed, used to initiate a fermentation process. The culture include selected strains of food-grade microorganism of known and stable metabolic activities and that is used to produce fermented foods of desirable appearance, body, texture and flavor. Stater culture means the microoganims that are selected based on their ability to produce lactic acid for curd production and a low pH to prevent spoilage, produce metabolites that give desirable flavours or produce enzymes that ripen the dairy product.

2.3 ROLE/FUNCTIONS OF STARTER CULTURES.

The primary function of lactic starters is the production of lactic acid from lactose. In addition to lactic acid production the starter cultures are also useful in different ways as stated below.

Table 2.1 : Functions of starter cultures (Agrimoon.com)

Function	Result
Acid production	○ Gel formation ○ Expulsion (syneresis) of whey for texturing ○ Preservation of milk ○ Helps in the development of flavor

Flavour	o Formation of flavor compounds like diacetyl and acetaldehyde
Preservation	o Lowering of pH and redox potential o Production of lactic acid o Production of antibiotics o Production of H_2O_2 o Production of acetate
Gas formation	o Eye formation in certain cheeses o Production of open texture Ex. blue veined cheese
Stablizer formation	o Development of body and viscosity o Ex. Polysaccharide materialas
lactose utilization	o Reduces the development of gas and off flavours o Suitable for lactose intolerant people.
Lowering of redox potential	o Helps in preservation o Helps in development of flavor
Proteolysis and lipolysis	o helpful in the ripening/maturation of cheeses
Miscellaneous compounds	o Production of alcohol in kefir and kumis

2.4 CLASSIFICATION / TAXONOMIC GROUPS AS PER BERGEY'S MANUAL

Starter cultures are generally classified based on their ability to utilize the lactose as shown in Fig. 2.1

Classification of starter cultures

RODS	COCCI	BACTERIA	YEASTS	MOLDS
Lactobacillus	*Lactobacillus* *Streptococcus* *Leuconostocs* *Pediococcus*	*Bifidobacteria* *Brevibacterium linens* *Acetobacter acetii*	*Candida kefir* *Kluyveromyces* *Toruloxpora* *Saccharomyces*	*Penicillium camembertti* *Penicillium roquefortii* *Aspergillus*

		Propionibacteria		*oryzae* *Mucor* *rasmusen* *Geotricum* *candidum*

2.4.1. Bacteria

2.4.1.1 Genus Lactococcus : Bergey's Manual or Systematic Bacteriology (1986), combined all the mesophilic lactic acid bacteria (LAB) with Lactococcus lactis to form a single species as they posses.

1. Identical isoprenoid quinines and the enzyme phosphotase
2. Indistinguishable lactic dehydrogenase
3. Identical percentage of guanine and cytosine.
4. High DNA homology

The only properties that distinguish them are plasmid controlled.

ILactococcus lactis subsp lactis	Acid producer but non flavor producer
Lactococcus lactis subsp cremris	Acid producer but non-flavour producer
Lactococcus lactis subsp lactis biovar diacetylactis	Both acid & flavor producer

All the above organisms are mesophilic in nature and their optimum growth temperature is between 25-30^{0}C. all are homofermentative organisms.

2.4.1.2.Genus Streptococcus : The members of the Streptococcus are Gram positive organism that usually form paris or chains. In 1937, Sherman separated the genus according to physiogical and growth characteristics, especially with regards to temperature limitations on growth. Four general groups designated by Sherman are (1) Pyogenic. (2) Viridians (3) Enterococcus and (4) lactic. This categorization has become somewhat obsolete as

relationships between species have been shown to overlap. The only species used as starter culture is Streptococcus salivarius subsp themophilus. This is a yoghurt culture, which is thermophilic in nature with optimum growth temperature of 38.42^0C. All are homofermentative organisms.

2.4.1.3. **Genus Leuconostoc :** All are heterofermentative organisms capable of producing lactic acid, CO2 and aromatic compounds (ethanol and acetic acid) from glucose. These organisms are normally used along with lactic acid bacteria (LAB) in multiple or mixed strain cheese starter cultures, which produces flavor compounds.

Leuconostoc creamoris

Leuconostoc citrovorum

Leuconostoc dextranicum

2.4.1.4 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 Bifighurt, Bioghurt, etc. The members of lactobacillus are classified based on fermentation of glucose into 3 groups as shown in Figure given below:

Obligate Homofermentative Group-I	Facultative Homofermentative Group II	Obligate HeteroFermentative Group-III	
Orla Jensen Group	Thermobacterium	Streptobacterium	Betabacterium
Growth at 15°C	-	+	+
Growth at 45°C	+	-	±
Pentose Fermentation	_	+	+
CO_2 from Glucose	-	-	+
CO_2 from Gluconate	-	+	+

Phosphoketolase	Absent	Inducible by pentose	Present
FDP aldolase	Present	Present	Absent
Example	*Lactobacillus acidophilus*	*Lactobacillus caseii*	*Lactobacillus brevis*

Fig. 2.2 Classification of Lactobacillus based on glucose fermentation.

2.4.1.5 Genus Bifidoacterium : 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.

Eg: Bioghurt, Biograde, Bifighurt, Cultura 'AB, Yakult, Miru-Miru.

Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, etc.

The optimum growth temperature is 37^0C-10^0C. Anaerobic conditions are essential for optimum growth. Milk fermtneted with bifidobacteria has a distinctive vinegar taste due to the production of acetate plus lactate from the metabolism of carbohydrates.

2.4.1.6. Genus Propionifacterium : Propionibacterium, freuderveichii and Propionibactrium 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. thoeii and P. acidipropionici are other organisms present in these genera.

2.4.1.7. Genes Brevebacterium : *Brevebacterium lines* is used a as starter culture in preparation of bacterial surface ripened cheese. It imparts distinctive, reddish orange color to the rind of (or formation of smear on) Brick and Limburger cheese or Camembert cheese.

2.4.2. Molds

Moulds are used for the manufacture of some semi soft cheese varieties and in some fermented milk products. Moulds enhance the flavor and modify slightly the body and texture of curd.

2.4.2.1. **White mold** is used in manufacture of surface mould ripened chees like camembert and Brie cheese.

Eg; *Penicillium camemberti, Penicillium* caseicolum, Penicillium condition

2.4.2.2. Blue mold is used in manufacture of internal mould ripened cheeses like Roquerfort, Blue Stilton, Danish blue, Gorgonzol and mycella cheeses.

Eg; Penicillium, roquefortii

2.4.2.3 Other molds

Mucor rasmusen – used in Norway for the manufacture of ripened skim milk cheese.

Aspeigillus oryzae _ used in Japan for the manufacture of Soya milk cheese.

Geotricum candidum – used in the manufacture of villi a cultured product of Finland. The mould grows on the surface of the milk to form the white velvety layer.

2.4.3 Yeasts : Yeasts are used in the manufacture of Kefir and Kumiss

2.4.3.1. Kefir grains : kefir grains consist of a mixture of different microorganisms such as Candida kefir, Kluyeromyces marxianus, Saccharomces kefir, Torulopsis kefir.

2.4.3.2 Kumiss : the important starter microflora of kumiss include Torulopsis spp.

Khuyeromyces marxianus var lactis, Saccharomyces cervisiae.

2.5 TYPES OF STARTERS

Starters are grouped under different categories based on compositon of microflora, growth temperature, type of products, flavor production and type of termentation into the following categories.

2.5.1. Based on the Composition of Microflora/Organisms

a. **Single :** Used of only single organism in the preparation of dahi or cheese. Disadvantage of using single coulture is that there may be sudden failure of starter due to bacteriophage attack which leads to heavy loss to the industry.

b.**Paired Compatible Strain :** Two strains of cultures having complementary activities in know proportion are used. This will reduce chances of culture failures. 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 :** More than two organism which may have different characteristics like, acid production, flavor production, slime production etc. in unknown proportion are used.

d. **Multiple/Mixed Strain :** More than two strains in known proportion are used. The quality and behavior of these strains is predictable.

2.5.2. Based on the Growth temperature. Based on the growth temperature organisms can be divided into mesophilic and themophilic.

Mesophilic Starter Cultures : The optimum growth temperature of these cultures is 300C and they have a growth temperature range of 22-40^{0}C. The mesophilic starter cultures generally contain the organisms of Lactococci.

Ex. Dahi Cultures : Lactococcus spp.

Chedar cheese : Lactococcus lactis subsp lactis, Lactococcus lactis subsp cremoris Lactococcus latis subsp lactis blovar diacetylatis, leuconostoe mesenteroides subsp cremoris.

Themophillic Starter Cultures : The optimum temperature of these cultures is 40^{0}C and they have a growth temperature range of 32.-45^{0}C.

Ex. *Streptococcus themophilus*

Lactobacillus delbrueckii subsp bulgaricus

Lb. delbrueckii subsp lactis

Lb. casei

Lb. helveticus

Lb. Plantarum

2.5.3. Product for which used

Yoghurt : Streptococcus thermophilus

Lactobacillus delbrueckii subsp bulgarious

Swiss cheese : Streptococcus salivarius subsp thermophilus

lactobacillus delbrueckii subsp bulgaricus

lactobacillus helveticus

lactobacillus casei

2.54. 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 : Leuconostocs as flavor producer (old name is Betacoccus)

D. type L. lactis subsp lactis biovar diacetylacis

BD (LD) type : Mixer of both of the above cultures

N or O type : Absence of flavor producing organism

2.5.5. Based on the type of fermentation : The starters are classified as homo or hetero fermenter based on end products resulting from glucose metabolism.

Homo fermentative cultures : eg. *Lactococcus lactis subsp lactis*

Hetero fermentative cultures eg. *Leuconostoc dextranicum*

THE END

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Lesson 3.

Purpose of Propagation, Traditional Methods-Advantage and Limitations.

3.1 INTRODUCTION

Starter propagation: It is the most important operation of the quality control unit of dairy plant as the quality of starter is having a direct bearing on the quality of finished product. The main aim of propagation is to maintain pure cultures and activate cultures without any loss of viability. The culture organisms are preserved in small qualities known as stock cultures. Fermentation process of any cultured dairy product relies on the **'purity'** and **activity** of the starter culture.

An active starter must have the following characteristics

1. Must be active
2. Must contain maximum number of viable organism
3. Must be free from contaminants.

To obtain the above qualities of cultures

1. The inoculums (inoculation) is carried out under aseptic condtions.
2. Growth is initiated in a sterile medium

3.2 TRADITION METHODS

The traditional method is also known as simple microbiological technique. In this method the starter are propagated in the laboratory itself taking the stock cultures which are in in-active state or dormant state. Commercial manufactures provide starter cultures in lyophilized (freeze-dried). Frozen or spray-dried forms. These cultures cannot be added directly in the milk to initiate fermentation process because they are in an in-active state. They need to be brought in to active form and also scale upto prepare bulk quantities.

The dairy product manufactures need to inoculate the culture into milk or other suitable substrate. Number of steps are to be followed during propagation of starter culture for ready to use. They can be seen from the following.

1. Stock culture : can be obtained from
    a. WDCM- The World Directory of collection of cultures of microorganisms.

Situated at the National Institute of Genetics, Shizuoka, Japan

http://wdem.nig.ac.jp

b. NCDC- National Collection of dairy Culture-NDRI, Karnal

c. C. ATCC – American Type Culture Collection

P.O. Box 1549, Manassa, VA 20108, USA

Web: http//www.atcc.org.

d. ECCO- European Culture Collections Organization.
e. NCFB – National Collection of Food Bacteria-UK
f. NCIM – National Collectioin of Industrial Microoganisms – Pune
g. NIZO- Netherlands Dairy Research Institute

2. Educational Colleges

3. Cultural stock Organizations

4. Commercial manufactures

a. Chr. Hansen laboratoriam-Harsholm, Denmark, Specialized in DVS (Direct vat Set) cultures and ready set cultures. In India- ESDEE chemocrats, 46 white Hall, 143 A, Kranti Marg, Mumbai-400 036.

b. Wisby laboratoriam – Tonder, Denmark-Specialized in DIP (Direct- in product) cultures for direct inoculation of milk In India- Food & Pharma specialities D-22. Nizamuddin East, New Delhi.

c. Gist-broaces- Delft, The Nethelands – In Australia & USA

II. Mother culture _ (First inoculatin) : All cultures will originate from this preparation.

I. Intermediate culture (feeder) – is used in preparation of larger volumes of prepared starter.
II. Bulk starter culture – this stage is used in dairy product production.

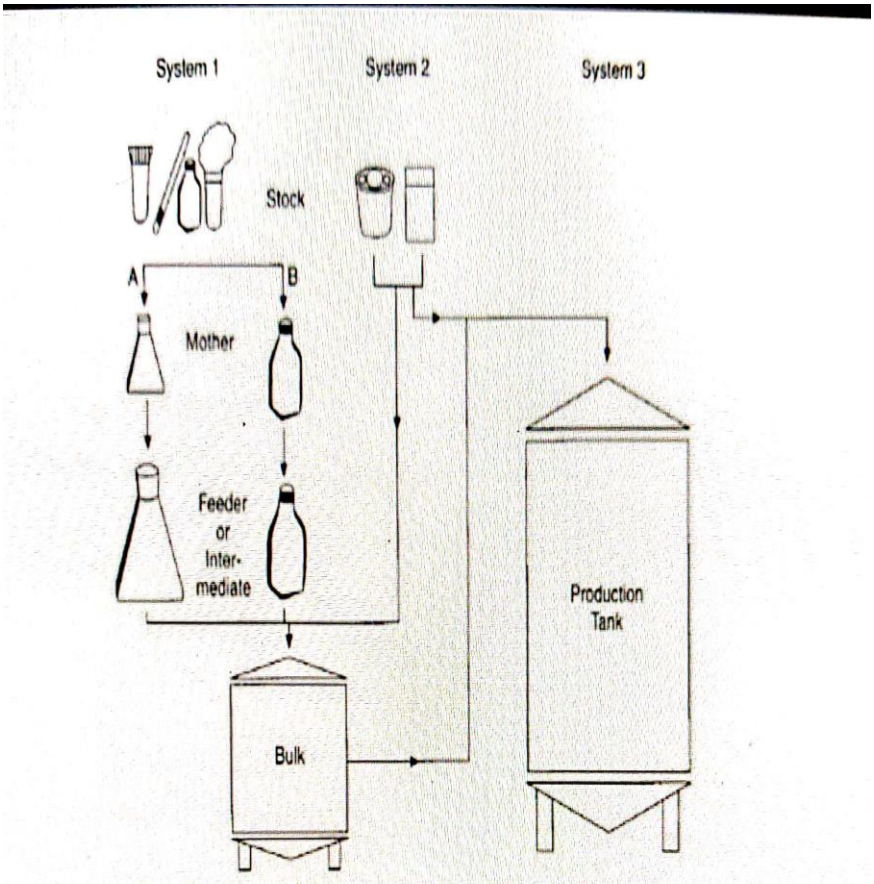

Figure 7.9. Starter culture preparations. *Note*: In System 1, stock culture may be liquid, freeze-dried, or frozen at −196°C for the production of bulk starter and cheese or fermented milks, respectively. In Systems 2 and 3, stock culture may be concentrated freeze-dried or frozen at −60°C to −196°C for the production of bulk starter and cheese or fermented milks, respectively. [After Tamime and Robinson (1999). Reproduced by courtesy of Woodhead Publishing, Cambridge, England.]

Source: Prajapati,V. Sreeja and S.Reddy (Agreemoon.com)

3.2.1 Scale up system of propagation :

Stock and mother cultures are propagated in laboratory and feeder and bulk cultures in the starter room of the dairy plant.

Different stages of starter propagation are :

1. Selection of milk
2. Treatment of milk
3. Inoculation

4. Incubation

5. Cooling of starters

3.2.2. Selection of milk : Milk is a good medium for the propagation of starters because it gives better and milder flavor, better viscosity. The milk meant for the starter propagation should be of high quality.

- Milk of first grade should be used.
- Milk of abnormal quality i.e. mastitis milk or colostrums milk or late lactation milk should not be used
- Milk should be free from antibiotic residues
- Milk should be free from bacteriophage
- Milk should not contain residues of detergents and sanitizers
- Milks should have a low bacterial load
- Milk should have high SNF content.
- Milk should have clean flavor and odour

Milk of NFDM (SMP) is used for the propagation of starters as it avoids daily variations and also Flavor and odor defects are easy to detect if skim milk is used for the starter propagation. Skim milk with a total solids content of 10-12% is desirable for the propagation of starters.

3.2.3.Treatment of Milk : Milk is usually subjected to heat treatment before using for the inoculation of starters.

Treatment of milk will serve the following Purpose :

- Inactivation of harmful organisms and bacteriophage in milk.
- Inactivation of natural antibacterial properties of milk such as immunoglobulin, LP system, lactoferrin and lysozyme.
- Reduction of the oxidation reduction potential of milk by removing dissolved oxygen
- Denaturation of proteins to make available which in small concentration act as stimulants for the growth of starters.
- Improvement of the viscosity of the finished culture due to denaturation of whey proteins by improving their water retention properties to minimize the wheying off.

Heating milk at 90^0C for 1 hour or boiling/steaming for 30 min is usually desirable. Autoclaving of Milk at 121^0C for 15 min may be followed but this may result in the formation of curd with soft and sloppy body and texture also it may give rise to scorched flavor of curd So Tyndalization of reconstituted skim milk is the bestpractice to be followed.

Tyndalization

a. On First day the reconstituted skim milk is autoclaved at 110^0C under 10 lbs pressure for 10 minutes. The milk samples are to be kept at room temperature to check the germination of spores which might be escaped the heat treatment and cause curdling of milk if they are present in large number.
b. On the second day the samples are again examined for any curdling. The curdled samples are discarded and the other samples are re steamed for 30 minutes.
c. After steaming the milk samples are kept at room temperature.
d. On the third day the samples are again examined for any curdling. The curdled samples are discarded and the other samples are again steamed for 30 minutes.
e. The samples so sterilized are either kept at room temperature or in refrigerator till they are used.

3.2.4.Inoculation: All the microbiological safety and sterilization methods should be used to avoid any least contamination. Inoculation should be carried out in separate room having Laminar flow system. Freeze dried ampoule is sterilized with alcohol and liquid culture tubes are shown to flames. The amounts of inoculums depend on the activity of starter, temperature of incubation and time of incubation. Normally 0.5% to 2.0% is used subject to variations depending upon the situation. Possibilities of external contamination are minimized as far as possible by working quickly.

3.2.5 Incubation: Incubation, temperature depends on the amount of inoculums and on the type of starter culture. For the Mesophillic organisms the temperature is 20-25^0C for 12-14 hours and for the Thermophilic organisms it is 40-45^0C for 3-4 hours. Change in temperature may affect the composition of starters in mixed population.

3.2.6 Cooling: After incubation the culture is cooled to stop further development. Refrigeration appears to give appropriate cooling effect.

Drawbacks of traditional method of propagation is

1. It is Time consuming

2. It Requires skilled operators and Personals

3. It May lead to contamination by bacteriophages.

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Lesson-4

Mechanically and Chemically Protected Systems for Starter Propagation

4.1 INTRODUCTION

Traditional method is most commonly used method for propagation of Starter cultures in spite of that it is a cumbersome process involving several steps. As we have learned in the above chapter that this method requires always a technical person is to propagate the active and pure culture without any contamination. Several methods have been practiced to avoid starter contamination and variation since long days.

The detailed are giving below.

4.2. Production system for Bulk starter cultures:

Production systems used for bulk starter cultures aimed to produce a pure active culture free from contamination especially from bacteriophages.

It uses

1. Simple Microbiological technique.
2. Employment of Mechanically protected equipment.
3. Propagation in Phage Resistant medium/Phage Inhibitory Medium (PRM/PIM)

4.3. MECHANICALLY AND CHEMICALY PROTECTED SYSTEMS

4.3.1. Mechanically protected Equipment:

While employing mechanically protected equipment two things should be kept in mind. i.e.,

a) Growth medium should be heated and cooled at incubation temperature in a completely closed vat.

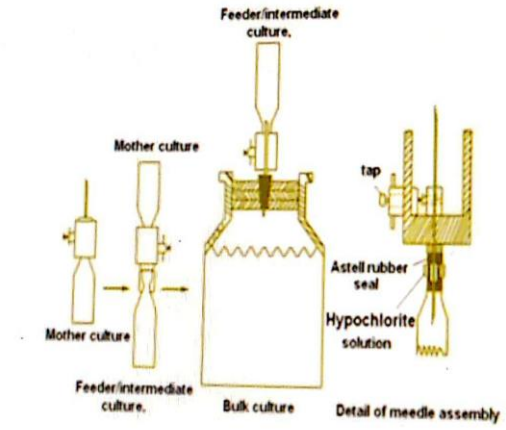
b) Inoculation of the starter should be done only in a complete safe and sterilize place where there is a complete control of entry of contaminated external air.

4.3.2. Lewis System :

1. The system involves the use of a two way hypodermic needle system to carry out the transfer of stock cultures to mother culture, mother to feeder and then feeder to bulk starter.

2. All inoculations take place through a barrier of chlorinated water. Re-usable and collapsible polythene bottles are used at each stage.

3. The bottles are fitted with Astell rubber seals and a screw cap.



l: Schematic illustration of the Lewis system for starter culture transfer

Source: Prajapati,V. Sreeja and S.Reddy (Agreemoon.com)

i. **Alfa-Laval Sytem :-** The system is similar to the Lewis System but the tank is of different design. Sterilized air is used instead of squeezing. In this system Two types of needles are used.

- Short needle : For sending sterilized air in.
- Long needle : It is used to force the culture to the next container

4.3.2. **Propagation in Phage Resistant Medium/Phage Inhibitory Medium (PRM/PIM).**

- The PRM/PIM medium is used mostly for mesophilic starter cultures. The medium is used the ingredients like milk-solids, sugar, stimulatory compound (yeast extract,

pancreatic extract), phosphate citrate buffer, chelating agents (ammonium or sodium phosphate).

- The chelating agents bind with Ca++ and Mg++ as these ions are required by the bacteriophage during the proliferation and replication.
- The bacteriophage also requires these ions for the phage adsorption onto the bacterial cell.
- So the Bacteriophage unable to bind with the bacterial cell due to absence of ca++ and mg++ ion.

4.4. STARTER SYSTEMS

4.4.1. Bulk Set : In this system the supplier provides the user with a small amount of culture which is then fermented to the user through one or two stages to produce a volume of bulk starter. Typically fermentation takes place at 22-26^{0}C for 14-16 hr for Mesosphilic and at 43^{0}C for 4.5 to 5 hr for Themophilic starter. A significant portion of this time is taken up by the lag phase as cells repair themselves from their storage mode. This starter then may be used immediately by inoculating into production milk (1% for cheddar, 3% for yoghurt).

4.4.2. Direct Vat Inoculation : This is made available in more concentrated form in large volumes such that the product can be inoculated directly into the production milk. In both cases inoculation into pasteurized medium ensures an active starter dominating the fermentation.

4.5 MAIN OPERATING METHODS

The main starter operating systems for controlling the bacteriophages are

a) Rotational
b) Non-rotation
c) Dutch P/L system

4.5.1. **Rotational :** The concept is that the regular change of a culture would prevent the build-up of a bacteiophage and thus avoid starter failure because phages are generally but not exclusively virulent against one host type. Traditionally this is the method of choice.

Different culture is used for each fill of the vat and the same culture could not be reintroduced for 5 days or for one production week. The effectiveness of the system can be improved by regular monitoring of phage to ensure any attack by them and to made starter effective and active.

4.5.2 Non-Rotational : Originally developed in New Zealand. This is based on the identification of single strain phage resistant bacteria. These individual strains are introduced into the plant normally as blend of three, where they are used continuously. Regular testing is done to monitor the development of phage against any of the strains. When the phage levels are likely to affect acid production, the strain is removed and replaced by a suitable alternative. This systems required maintenance of high hygiene and production discipline. Such systems reported to do outstanding in terms of elimination of bacteriophage problem. But it required trained and a strict good plant hygienic system.

4.5.3. Dutch P/L system : This system work on the principal of development of phage resistant starter mixture. Mixed cultures are transferred in an environment and deliberately exposed with bacteriophage. The resulting mix of cultures may include new phage resistant mutants and this will help to operate satisfactorily in the production environment. But the method required high skilled microbiologists/Scientist and staff on site to mange the system.

4.6. EFFECTIVE STARTER SYSTEM MUST SATISFY FOLLOWING CRITERIA:

- Clear cut objectives in the plant.
- Physical as well as Human constraints must be dealt with.
- All involved staff should understands the system and the requirement of Starter.
- All staff should understand the bacteriophage, its seriousness and the knowledge to protect them.
- Proper movements of air, water,
- Potential check on people for as a channel for phage attack.
- Following HACCP.
- Strict hygiene and production disciplines associated with starter production and usage.
- Regular phage monitoring by daily testing of culture.
- Proper Results against the objectives defined.

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Lesson-5

Metabolism in Starter Cultures (Carbohydrates, Citrate and Vitamin)

5.1. INTRODUCTION

Lactic Acid Bacteria (LAB) produce lactic acid by fermenting lactose present in milk . Fermentation of lactose is carried out by different pathways by different types of starter cultures. Microbial cells derive their energy requirements via Fermentations, Tri-carboxylic acid cycle, Cytochrome system for terminal electron transport, and an anapleortic pathway etc

5.2. CARBOHYDRATES METABOLISM:

Milk contain Lactose which is is a disaccharide. Lactose constitutes about 40% of milk solids. There are two strategies to hydrolyze lactose by Lactic acid bacteria.

1. β-D Glactosidase (Lactase or β-gal).
2. β-D Phosphogalactosidase (β-p-gal)

Lactic acid bacteria possess both β- gal and β-p-gal enzymes. Most Lactobacilli have gal enzyme except *L. casi* which exhibits only β-p-gal activity. In natural system *Lactococci* posses both activity but in starter cultures only p-gal activity is seen.

Transportation of Lactose is into cell membrane of the lactic acid bacteria occur via two systems i.e. through Permeases and through Phospho-enol-pyruvate dependent phospho-transferase system (PEPPTS). Lactose is broken down to glucose and galactose by catalytic action of galactosidase on β,1-4 galactosidic bond in lactose. Glucose is the common substrate used by both aerobic and anaerobic organisms. Aerobic have complete mechanisms to complete the catabolism of glucose and end up with the formation of CO_2 and H_2O. Anaerobic pathway leads to incomplete breakdown of glucose releasing small amounts of energy. TCA cycle is aerobic and in this glucose or acetate is oxidized to CO_2 and H_2O. Electrons are removed from each steps of substrates metabolism , as they get oxidized and then through a series of oxidation reduction process in an Electron Transport chain molecular oxygen to form finally water.

5.2.1 Homo-fermentative pathway by Lactic Acid Bacteria:

In homo-fermentative pathway the lactose is transported across the cell membrane which involves the Phospho-enol-pyruvate dependent phospho-transferase system. The lactose is phosphorylated to lactose – P (glycosyl β galactoside – 6 Phosphate) during its translocation (Figure- 5.1)

In this system the lactose- P is hydrolyzed by β – phospho-galactosidase (-β– D) phosphogalzctoside – galactohydrolyse) to D-glucose and galactose-6 phosphate. Glucose is metabolized to pyruvate via Embden Meyerhof Pathway (EMP). In the metabolism of galactose it is first converted to glyceraldehydes-3 phosphate via D-tagatose-6 phosphate pathway. So A overall two pathways are involved.

a. **Metabolism of Glucose by EMP pathway**

b. **Metabolism of Galactose by tagatose 6-phophate pathway**

Lactose in organisms like themophilus and L.b. bulgariucus is transported into the cell by the enzyme Permease and or PEP: PTS (Phosphoenol phosphorylase: Phosphotransferase) sytem. These organisms posess β -D galactosidase (β -gal enzyme which hydrolyses lactose into β -D galactose and glucose. Glucose is coverted into lactic acid via EM (Embden Meyerhof) pathway. Galacotose is excreted from the cell or may be utilized by other bacteria producing other than lactic acid. But some of the homofermentative organism under some special conditions such as absence or limiting glucose or lactose or particular pH under take heterofermetations.

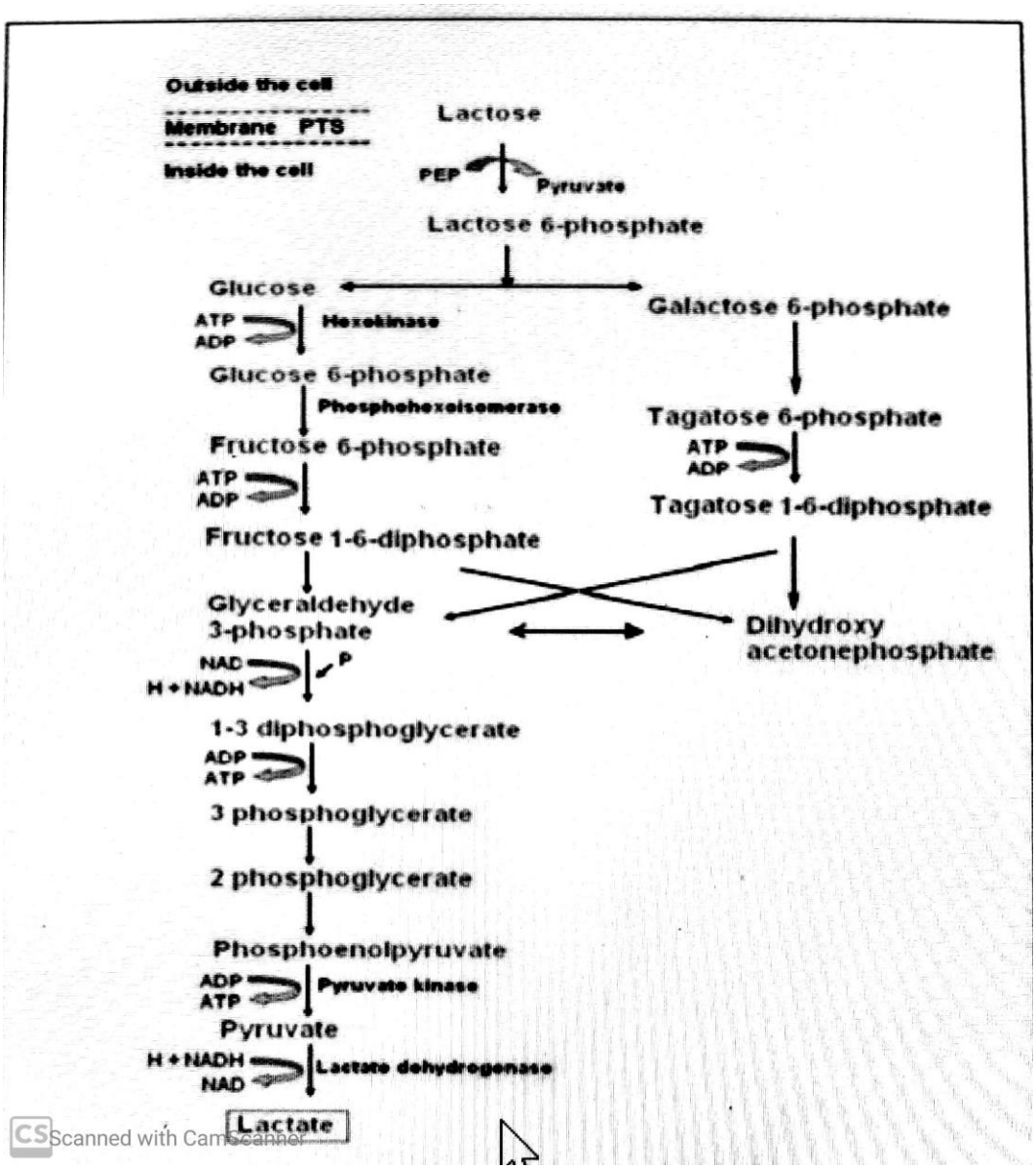

Fig: Homofermentation pathway

Source: Prajapati,V. Sreeja and S.Reddy (Agreemoon.com)

5.2.2. Hetero fermentation : Heterofermentative Lactic acid bacteria produces large quantities of ethanol, CO_2 in addition to lactic acid when grown on lactose or glucose. The microorganisms gone through three distinctive chemical reactions to obtain end products as CO2 lactic acid and ethanol during heterofermentation as depicted in Figure 5.2.

5.2.3. CO_2 is produced by the oxidative decarboxylation of 6-phosphogluconate

Ethanol is produced by reduction of acetaldehyde.

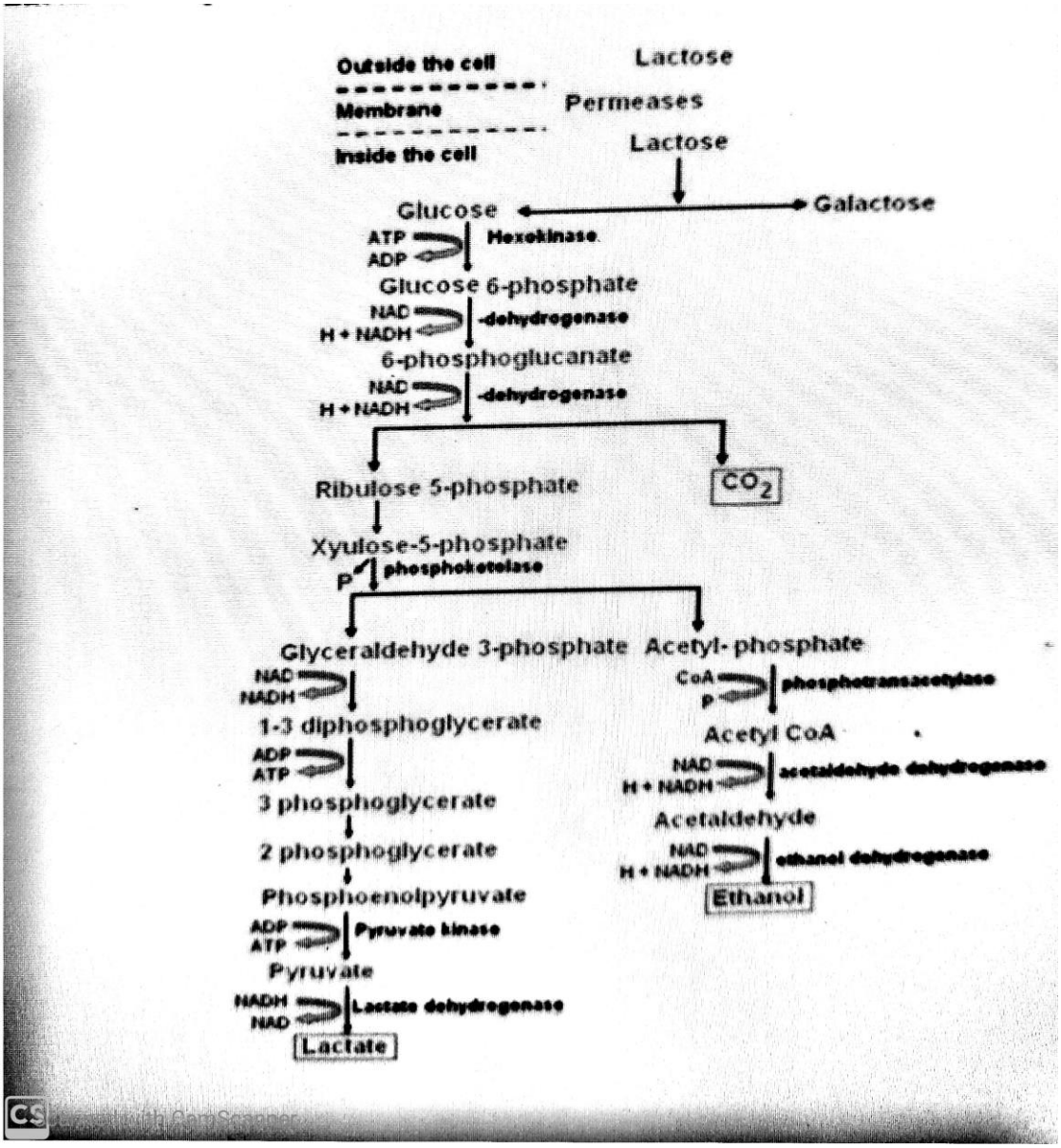

Fig: Heterofermentation pathway

Source: Prajapati,V. Sreeja and S.Reddy (Agreemoon.com)

5.2. CITRATE METABOLISM: PRODUCTION OF FLAVOUR COMPOUNDS

Milk contain about 1.4-2.0 g citrate / kg of milk. Cellular uptake of citrate is done by enzyme citrate permease (Plasmid encoded enzyme) in *Lactococcus lactis ssp lactis biovar diacetylactis.* Citrate lyase is a inducible enzyme produced only in the presence of citrate. There are two pathways for the formation of diacetyl, as shown in Figure 5.3.

Chemical-By oxidative decarboxylation of a acetolactate which is excreted into milk by bacterial cells.

Enzymatic-Diacetyl is formed in cells by the reaction of acetyl Co-A and activated acetaldehyde.

Diacetyl is an intermediate product. So concentration varies during manufacturing of cultured dairy products and it may disappear subsequently. There is relationship between the concentration of diacetyl and acidity of the product.

To achieve the highest concentration of diacetyl in fermented milk the following criteria should be fulfilled

1. pH should be <4.6 for controlling diacetyle.
2. Complete fermenatation of citric acid.

In Dairy products diacetyl concentration is required in small quantity to achieve acceptable flavor. Active diacetyl reductase reduces flavor by converting diacetyl to acetoin. The potential source of the diacetyl reductase is the contaminating bacteria especially Gram negative psychrotrophs, coliforms and yeasts.

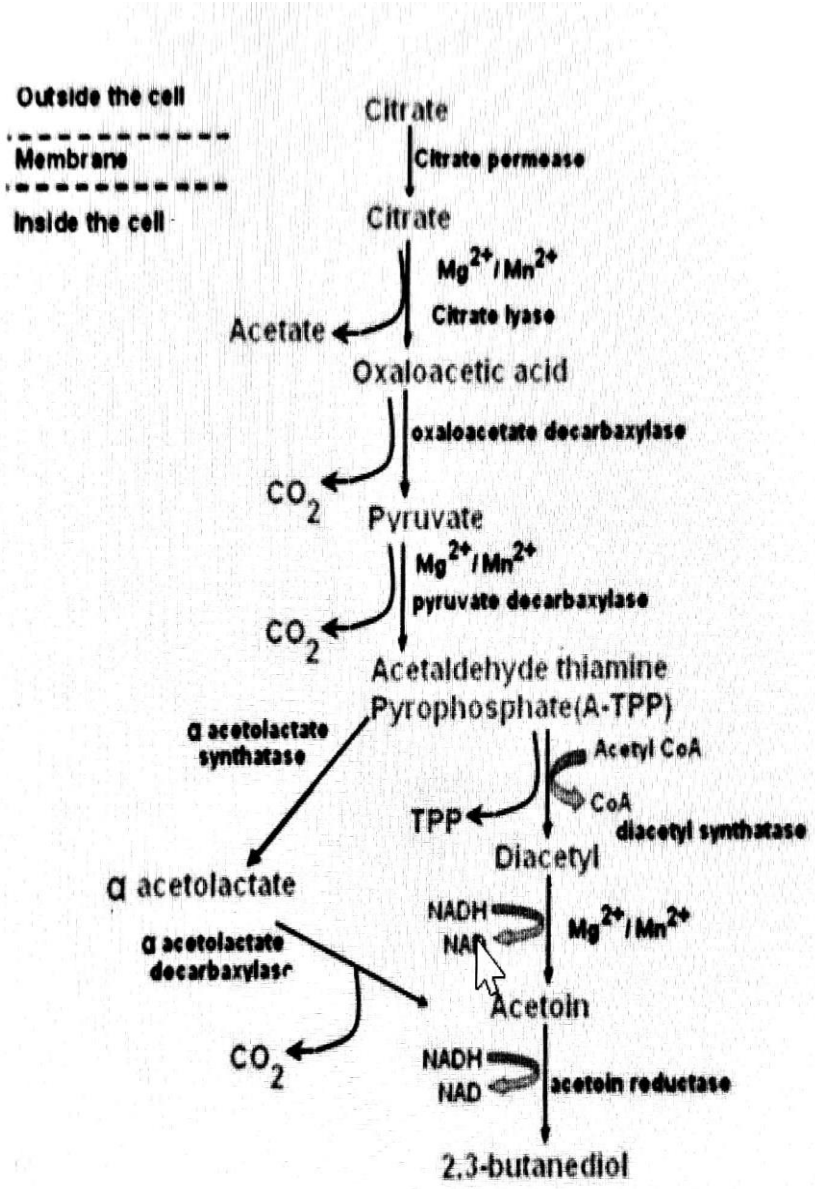

Fig: Citrate Metabolism

Source: Prajapati,V. Sreeja and S.Reddy (Agreemoon.com)

5.4 PROTEIN METABOLIM

The hydrolysis Protein yields amino acids. The range of products released by proteolysis is dependent on two main factors.

1. The components of the milk protein fraction.

2. Types of Proteolytic enzymes that the starter organism may possess.

Proteolytic enymes : These enzymes as the name suggests, are specific in their action and their main function is to catalyse the hydrolytic cleavage of the peptide bonds which from the backbone for the protein molecule.

Enzymes acting on peptide bonds are known as peptide hydrolases. A large number of such enzymes have been identified till date. In the previous date the name of enzyme were given by the type of substrate but now a day name is given on the action on peptide bond. ie. the petidases and the proteinases.

The Peptidases are also known as Exopeptidases which specifically catalyse the hydrolysis of terminal amino acid or a – carboxy) groups of the peptide bonds.

The Proteinases: are also known as proteolytic enzymes, endopeptidases or peptidyl-peptide hydrolases, which catalyse the hydrolysis of the internal peptide linkages of protein.

Yoghurt starter cultures are weak in proteolysis fermentation. *S. thermopiles and L. bulgaricus* may produce proteolysis enzymes during the fermentation which causes a significant degree of proteolysis and this activity may be important for the following reasons.

1. The enzymatic hydrolysis of milk proteins results in the liberation of peptides of varying sizes and free amino acids.
2. The possible changes due to proteolysis activity can affect the physical structure of yoghurts.
3. The liberation of amino acids into the milk is essential to the growth of *S. thermopiles.*
4. Although amino acids and peptides may not contribute directly towards the flavor of yoghurt, they act as precursors for a series of reactions which produce flavor compounds.

5.5. Vitamin Metabolism:

INTRODUCTION

Milk and yoghurts contains both fat and water-soluble vitamins and various antimicrobial compounds synthesized by the starters. The content of these vitamins changes during the growth of starter cultures in fermented milk products preparation. The change in vitamin content could be either an increase or decrease in the finished product as explained below.

5.5.1. INCREASE IN VITAMIN CONTENT

Niacin and folic acid are vitamins which increase during the actual manufacture of yoghurt because they are actively synthesized by the starter cultures. The increases in folic acid and niacin in yoghurt is amounting as 3.946 and 22ug/100g respectively and the losses in storage may exceed these gains in due course of time. Although there is a decerese in vitamin B12 during yoghurt production, but some species of Lactobacillus and strains of yoghurt starter culture synthesize vitamin B_{12}. *S. Thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* synthesise niacin and folic to a lesser extent than vitamin B6 during the production of yoghurt.

5.5.2.DECREASE IN VITAMIN CONTENT

Heating of milk even at moderate temperature or an excess of dissolved oxygen may reduce significantly the vitamin content. The most susceptible ones are vitamin C, B6, B12 and folic acid.

- Excessive heat treatments of the milk. e.g. boiling for 5 minutes, cause even greater losses of the above vitamins; for example, vitamin B12 is reduced to 1.78 ug/liter.
- The yoghurt starter bacteria utilize some of the vitamins present in milk during the fermentation period to meet their growth requirement. This factor contributes, to some extent, to a reduction of the nutritional properties of the product. However, the quantities consumed are dependent on the rate of inoculation, the strain of yoghurt starter and the conditions of fermentation.
- Some vitamins decrease during the storage of yoghurt at 4^0C. During the storage of yoghurt at 5^0C for 16 days, loss of folic acid and vitamin B12 is 28.6 and 59.9% respectively.

- A decrease in the biotin, niacin and pantothenic acid contents are noticed due to the combined effect of microbial catabolism during the incubation period, and chemical decomposition of these vitamins during cold storage.

5.5.3.BIOSYNTHESIS OF FOLIC ACID (FOLACIN)

The "folic acid group" (or "folates") is a generic name given to around ten different compounds which share a basic structural unit connected to "conjugates" of different numbers of glutamic acid residues. Many organisms require folacin as a growth factor. It functions as a coenzyme in many different biochemical reactions, i.e. as an activator and carrier of carbon units during oxidation and it participates in the metabolism of purines, pyrimidiness and some amino acids.

5.5.4.BIOSYNTHESIS OF NIACIN

The niacin activity was exhibited by nicotinic acid and nicotinamide. the former compound constitutes part of the structure of the two important coenzymes, ie.. NAD and nicotinamide adenine dinucleotide phosphate (NADP). These two coenzymes are composed of adenylic acid and nicotinamide ribotide linked through their phosphate groups. As NAD and / or NADP are essential for many oxidative/reductive biochemical reactions, the niacin synthesized by *S.thermophilus* and *Lactobacillus delbrueckii* subsp bulgaricus may originate from nicotinamide fraction arising during formation of NAD and / or NADP. The biosynthesis of these nucleotides involves, basically, the following steps : firstly, the synthesis of a sugar moiety and secondly, the synthesis of the pyrimidine or purine base. alternatively, after this formation of NAD and / or NADP, the nicotinamide fraction could be released as a result of the degradation of these nucleotides.

Nicotinic acid is derived by a few bacteria from the metabolism or breakdown of tryptophan, a pathway which is dependent on the availability of certain vitamins, e.g, thiamine, riboflavin and vitamin B_6 to activate the required enzymes. As *S. thermophilus and lactobacillus delbrueckii subsp bulgaricus* utilizes these vitamins and tryptophan does not accumulate during yoghurst production, it is possible that these organism use the vitamins for the synthesis of niacin.

5.5.5.Biosynthesis of Vitamins B_6 : The activity of vitamins B6 is exhibited equally by the following compounds : pyridoxine, pyridoxal and pyridoxamine. The basis structure of these compounds is similar which consists of a pyridine ring, but they differ in the respect of the radical components as follows :

No information is available on the biosynthesis of the pyridine ring in microorganisms, plants or animals : however, the different forms of vitamin B_6 are inter convertible by microorganisms. In view of the limited knowledge of the synthesis of vitamins B_6 are their in general, it is difficult to suggest any possible metabolic pathway by which S. thermophilus and lactobacillus delbrueckii subsp bulgaricus might synthesis this vitamin.

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Lesson – 6

Antimicrobial Compounds Produced By Starters and Interactions among Starter Cultures

6.1. INTRODUCTION

A number of antimicrobial compounds produce from starter culture during the preparation of fermented milk products. These antimicrobial compounds are very effective against many Gram positive bacteria and exhibit therapeutic properties. The starter cultures grow in association with other bacterial cultures and such association will vary depending upon the characteristics of the organisms grown in a particulars environment.

6.2. ANTIMICROBIAL COMPOUNDS

Fermented milks are well known for their therapeutic properties. This is primarily due to their ability to fight against intestinal pathogens. The effect is exerted by LAB through production of several antimicrobial compounds and / or creation of unfavorable conditions for other bacteria. The primary compounds responsible for such effects are.

1. Organic acids : Lactic acid, acetic acid, prop ionic acid etc.
2. Hydrogen ion concentration (pH)
3. Low oxidation reduction potential
4. H_2O_2 and CO_2
5. Aroma compounds diacetyl, acetaldehyde
6. Fatty acids
7. Bacteriocins

6.2.1.1.Organic acids : The main metabolites of LAB fermentation is lactic acid. It is un dissociated at low pH therefore highly toxic to many microorganisms. Acetic acid and propionic acid have more antimicrobial activity than lactic acid due to its higher pKa values. The antimicrobial effect of organic acid lies in the reduction of pH as well as the undissociated forms of molecules. The low external pH causes acidification of cell

cytoplasm, while the undissociated form of molecule may cause alternation in the cell membrane permeability, collapsing the electrochemical proton gradient that results in disruption of substrate transfer system.

6.2.1.2 H_2O_2 and CO_2 : H_2O_2 is mainly produced by some lactobacilli in the presence flavoprotein oxidase or NADH Peroxidase form glycerol, under aerobic conditions. The antimicrobial activity of H_2O_2 lies in the oxidation of –SH groups causing denaturation of a number of enzymes. Also the peroxidation of membrane lipids increases membrane permeability. H_2O_2 also acts as a precursor for the production of bactericidal free radicals such as superoxide O_2^-, and hydroxides –OH radicles that can damage DNA.

Lactobacillus and Lactococcus species can produce H_2O_2 while CO_2 is mainly produced by heterofermentative LAB. The precise mechanism of its action is still unknown, but it is believed that it may play a role in creating an anerobic environment which inhibits enzymatic decarboxylation reactions and the accumulation of CO_2 in the membrance lipid bilayer may cause disruption in permeability.

6.2.1.3.**Aroma compounds**

The aroma compound like diacetyl produced by LAB inhibits the growth of Gram negative bacteria. Diacetyl react with the arginine (Arg) binding protein, thus affecting Arg utilization that finally interfere with protein synthesis. Similarly Acetaldehyde can also be toxic to many bacteria as it is converted to H_2O_2 in the presence of xanthine oxidase or glucose oxidase activities that may damage DNA and other metabolic activity of some of the bacteria.

6.2.1.4.Fatty acids: Some Lactobacillus and Lactococci sp possess lipolytic activity under certain special condition that may produce significant amount of fatty acids. These unsaturated fatty acids are active against many Gram +ve bacteria.

6.2.1.5 Bacteriocins

These are low molecular weight proteinaceous compounds produced by some group of bacteria which are inhibitory to other closely related bacterial species.

As shown in Table given below many LAB produces bacteriocins & other antibiotic like proteineous substances. These substances are found to be active against many spoilage and pathogenic microorganisms. Their nature and structure is not well known, except for nisin.

Researchers claim that these are proteineous in nature, and is encoded by plasmid DNA. which are stable at low pH, high temperature and many other adverse conditions.

Table . Bacteriocins produced by lactic acid bacteria

S.N.	**Species**	**Compound**
1.	Lactococcus lactis subsp lactis	Nisin
2	Lactococcus lactis subsp cremoris.	Diplococcin
3	Lb. acidophilus	Acidolin
4.	Lb. helveticus.	Lactacin 27

Nisin : It has a narrow spectrum anti-bacterial activity and does not inhibit Gram –ve bacteria, yeast, or fungi. It inhibits closely relative organisms like *Lactococcus lactis subsp cremoris.* It is effective against spore formers. Its synthesis occurs as pronisin in the cell and it further converted to Nisin in the outer layer of the cell.

6.3. **TYPES OF INTERACTION AMONG STARTER CULTURES**

The interactions among various type of microorganism in a particular starter culture may be Neutralism, Antagonism (negative interaction) and Symbiosis (Positive interaction).

1. Neutralism : Type of association in which two organisms living in a close proximity but not affected by each other. This may be possible due to their different growth requirement hence do not affect each other as there will be no competition between these two organisms for nutrients.

2. Antagonism : When an organism adversely affects the environment of another organism it is said to be antagonistic interaction. Antibiosis is antagonistic association between two organisms in which one is adversely affected by production of antibiotic or inhibitory substances produced by other organism for eg Lactic acid bacteria produce lactic acid that is inhibitory to other spoilage organisms.

3. Symbiosis: Symbiosis is defined as the relationship between two (or more) organisms that live in a close association. In the majority of symbiosis one or both partners gain something

positive from the association. Although the pair of symbionts may be able to live separately, but they always do better in the long run by living together.

3. Mutualism – It is a form of positive interaction in which each partner get benefits from the association but the manner in which benefited varies between them. There may be exchange of nutrition between two species i.e is known as Syntrophism. Association results in desired end products which is not possible by the organism separately.

4. Commensalisms: It refers to a relationship between microorganisms in which only one partner get benefit from the association but the other organism in turn is get nothing. Host organism by its growth affects the physical or physiological environment in such a way that favors the growth of dependent partner.eg. Facultative aerobes organism grows and produces anaerobic conditions that favour the growth of other anerobic organism. Similarly growth of yeasts in sugar solutions reduces the concentration of sugar thus permitting growth of bacteria.

5. Synergism: The ability of two or more organisms to bring about an effect greater than the sum of their individual effects. The changes are usually chemical in nature that cannot be accomplished alone. The best example is the association of two microorganism in the yoghurt starter culture. The two lactic acid bacteria grown in association in milk and their growth is considered symbiotic because the rate of acid development is greater when mixed yoghurt cultures of *S. thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* bacteria were used as compared to the single strains.Thus the initial acid production is mainly due to activity of *S. thermophilus* and later on by *Lactobacillus delbrueckii subsp bulgaricus.*

Lactobacillus delbrueckii subsp bulgaricus stimulates *S. thermophilus* by releasing several amino acids like histidine, leucine, lysine, cystine, valine etc. The most important amino acid for the growth of *S.thermophilus* is Valine. *S. thermophilus* produces formic acid, pyruvic acid and carbon dioxide which promote the growth of *Lactobacillus delbrueckii subsp bulgaricus.*

LESSON-7.

Activity & Purity test and Standards for Starter cultures

7.1. INTRODUCTION: For the production of a fermented milk product, the starter cultures, should be chosen based on certain properties. A starter culture should be assessed regularly at its each steps for better production. They should be imposed a quality control tests that include organoleptic, chemical and microbiological tests.

7.2. PROPERTIES OF IDEAL STARTERS

1. A good starter should have the ability to produce lactic acid at vigorous and steady rate.

2. A starter should be pure without any contaminants

3. It should be able to grow rapidly in suitable organic substances

4. It can be easily cultivable in large quantities

5. It should be able to maintain physiological constancy

6. It should be able to produce necessary enzymes readily and profusely in order to bring about the desired chemical changes

7. It should have the ability to carry out transformation under comparatively simple and workable modifications of environmental conditions

7.3. ACTIVITY AND PURITY TESTS OF STARTER CULTURES: Activity and purity of starter cultures can be assessed by organoleptic, chemical and microbial tests such as:

1. Organoleptic tests

2. Chemical tests

3. Microbial tests

4. Purity tests

5. Activity rating tests

7.3.1 Organoleptic tests: This type of tests require high degree of training and experience. These tests measures the following Qualities i.e appearance, flavour and consistency

8.3.1.1 Body: The body should be firm like custard. The texture of curd could be able to retain its shape during gentle tapping of culture and on pronounced tapping it should break clearly and smoothly.

7.3.1.2 Texture: It should not have lumps and whey pockets. It should be smooth and viscous after thorough agitation.

7.3.1.3 Taste: The taste should be clean and acidic, without any bitterness and saltiness.

7.3.1.4 Flavour: Should have typical aroma and should be free from any off flavor and aroma like maltiness, bitterness, rancidity, unclean, yeasty, fruity and putrid etc.

7.3.2 Chemical tests: Various chemical parameters used for assessing the activity of starters include

Titratable acidity

Volatile fatty acid

Tests for diacetyl and acetyl methyl carbinol

Tests for acetaldehyde

7.3.3 Microbiological methods: The microbiological methods used for determining the activity and purity of starter microorganism are

• Plate count test

• • Direct microscopic count test: The ratio between rods and cocci can be obtained

• Selective plating techniques

• Tests for the contaminants like coliforms, yeasts and molds (both should be absent in 1ml of culture). Starter must be free from foreign bacteria, yeasts and molds.

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

7.3.4.1 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 +ve cocci or thin rods.

7.3.4.2. Catalase test-

Tube method: 1.Add 1.o ml of hydrogen peroxide (3%) to about 5 ml of starter culture in a test tube,The apperence of gas bubbles indicates a positive test the absence of bubbles indicates negative test.As lactic acid bacteria are catalase negative a positive reaction indicates gross contamination

2: Slide Mthods: An alternative procedure for doing catalse test is tio perform by slide methods.Transfer two to three drops of actively growing culture on a clean glass slide and add equal drops of 3% hydrogen peroxide on the starter culture. Presence of effervescence indicates possible contamination of the given culture. Lactic acid bacteria are negative for catalase and thus catalase positive test indicates contamination of starters

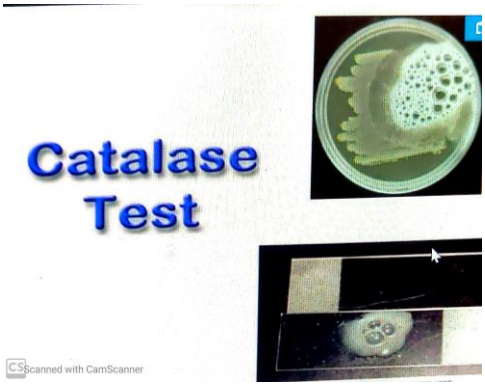

Fig: Catalsae test in Lab

7.3.5. Activity rating: An activity or vitality test help helps to determine the rate of cid development by a starter culture prior to its use in the processing Vat for example A stimulated cheese making process in the laboratory

For determining the activity of starter culture following tests should be performed in the laboratory

7.3.5.1. Titratable acidity test: The activity of Starter culture is directly related to its ability to increase titratable acidity in milk during specific intervals of time. However for better

reliability and reproducibility, the exact condition chosen for the test should be well defined. The active fast acid producer, the Titratable acidity should not be less than 0.8% lactic acid at 30℃ within 16h. 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.

7.3.5.2. Horral Elliker's test: It is used mainly for Mesophilic type of cultures. The test is based on using a higher inoculum i.e 3%in autoclaved reconstituted non fat dry milk and determines the T.A after 3.5 h at 37℃. The test can be understood in the given flow diagramme:

Steps :

Prepare and sterilize the reconstituted milk.

⇩

Take 25 ml of heat treated (90℃, 10 min) reconstituted milk (NFDM) and add of starter culture @ 3% ml into skim milk tube with a sterilized pipettes

⇩

Place tubes of milk in a water bath at a temperature of 30-42°C .

⇩

Incubate at 37.7°C for 3 1/2 hours.

⇩

At the end of incubation period each tube 10 g curd is taken in a porcelain dish and titrated with N/10 sodium hydroxide with phenolphthalein indicator.

Fig : Flow diagramme of Horral Elliker's test:

Titratable acidity is calculated and expressed as percent lactic acid as follows:

Acidity (% LA)= 9xVolume of NaOH consumed(ml)x Normality of NaOH /Weight of curd sample taken for titration.

Results and Interference:

1. A titration value of 0.4 %LA or higher indicates that the culture should be well suited for cheese making.

2. A titration value of 0.30% to 0.35 % LA indicates a slow culture.

3. Starter developing acidity of less than 0.30 % LA invariably graded as inactive as it produces little or no acid during cheese manufacture.

7.3.5.2. Resazurin Reduction time test: Preparation of dye solution: Autoclave 200ml of distilled water in light resistant flask. Transfer one tablet of Resazurin tablet with clean dry forcep to the flask of hot water. Allow the tablet to dissolve and let cool down. With the pipette, transfer 1 ml of Resazurin solution and label the tube. Now transfer 10 ml of starter culture to the tube. Incubate it at 37℃ till complete reduction of dye to leuco compound and Record the reduction time.

7.3.5.3.Voges Proskauer test /Creatine Test: Creatine test is a biochemical test generally carried out to check aroma and flavor producing mesophilic cultures (Dahi, Lassi, Buttermilk, cultured cream, cottage cheese etc.) and it based on the ability of such culture to produce acetyl methyl carbinol and diacetyl, which form pink colored complex with creatine. This test is also used to detect gas producing organisms in a non gas forming culture.

Procedure: Inoculate active culture @ 1% in 2ml of citrate broth. Now add I ml of 0.5% creatine solution and 1 ml of 5% α-naphthol freshly dissolved in 2.5 N NaOH. Place the tube in dark for 10 minutes and observe the development of pink colour. The appearance of pink color is indicative of flavor production.

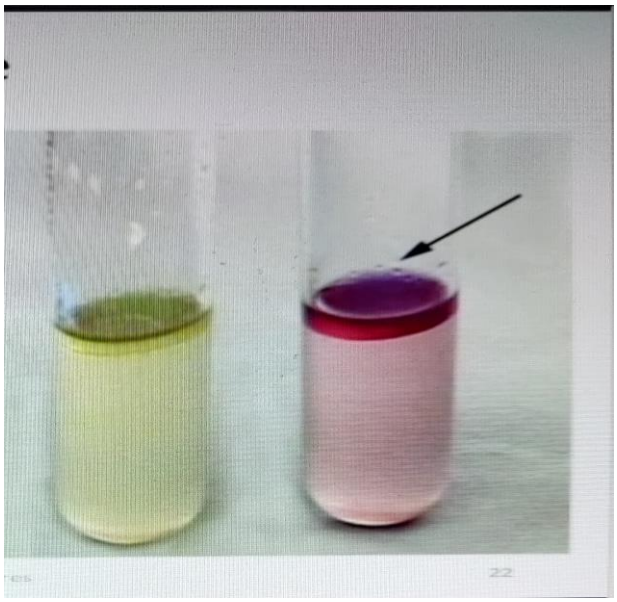

Fig: Vogus-Proskauer Test

7.3.5.3. Whitehead-Cox Activity Test: Whitehead and Cox have recommended an activity test simulating some basic conditions of starter development in cheddar cheese. This method has been extensively used for determining suitability of cultures for this purpose:

Steps:

Take 1,000 ml pasteurized milk and adjust to a temperature of 30°C.

⇩

Add 10 ml of culture, to be tested.

⇩

Ripen for one hour or the usual ripening time.

⇩

Add 2 ml of rennet.

⇩

Cut the curd when set. The curd is cut with a spatula or knife.

⇩

Heat the curd to the usual at temperature 38-39°C, or higher. whatever is commonly used.

⇩

Hold the curd at the final cooking temperature for about 1/2 hour, or a little longer.

⇩

Drain the whey and test its acidity.

⇩

About 25 minutes later, test the whey which has drained from the curd and dump the remaining whey from the container.

⇩

This is assumed to be equivalent to packing.

⇩

Now test the acidity of whey which has drained from the curd, and this is the final activity test value.

THE END

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Lesson 8

Defects in starter culture /Causes of Starter failure

8.1. STARTER DEFECTS: In the previous chapter the things have been discussed for measuring the activity and purity of a good starter culture.The major problems associated with the starter cultures are:

1. Insufficient acid production
2. Insufficient flavor production.
3. Defects in Flavor.
4. Body and texture defects.

8.1.1. **Insufficient acid production or non-acid curd: This is the most common defects in lactic acid starter culture.** A good starter should produce lactic acid at vigorous and steady rate. The reasons for the insufficient acid production could be

a) Spontaneous loss of vitality inherent in starter itself: There may a spontaneous loss of activity of starter culture. The reason may its strain variation or genetic instability that are usually associated with plasmids, or loss of lac plasmid

b) : Non control over the production process: During production process if there is any mistake that allow any mishandling may leads to loss of vitality. The mismanagement may allow contaminants to spoil the starter. Also frequent sub-culturing may also lead to loss of vitality of the starter. Use of unsuitable media and use of wrong incubation temperatures etc are the another reason to allow other contaminants to grow.

C. **Causes of slowness inherent in milk itself:** Milk contain many antimicrobial agents like LP system, Lysozyme, Lactoferin etc that may sometime slow the activity of Starter culture itself

D. **Use of Milk of abnormal quality** i.e. mastitis milk or colostrums milk or late lactation milk or silage milk or winter milk may slow the growth of starter culture.

Another reason for the Slowness of starter Cultures are:

I. Milk containing antibiotic residues

II. Milk contaminated with bacteriophage

III. Milk containing residues of detergents and sanitizers

IV. Milk having inhibitory bacteria

V. Seasonal variations in milk composition

VI. Milk containing natural inhibitory substances like leucocytes, antibiotics etc

VII. Excessive aeration of milk

E. **Changes in the method of production of fermented milk:** Sudden change in the method of production of milk may sometime slow the activity of starter culture.

8.1.2. Insufficient flavor production: Those factors that cause slow acid production also affect flavor production. In addition to these the following factors also affect the flavor

- Improper acid production
- Milk having low citrate ions
- Milk containing high Gram negative organisms that reduce diacetyl content by Producing diacetyl reductase etc.

8.1.3. **Defects in Flavor:** a) **Sharp acid taste** : Sometime taking high inocula may turn over ripening of the culture. Also keeping the Starter at higher incubation temperature or prolonged incubation period may increase the acidity of the product. b**) Malty flavor**: This is due to the variants of *Lactococcus lactis ssp. lactis var maltigenes*

c) **Metallic flavor**: This is due to over ripening of the culture or due to the use of poorly maintained containers with metallic contamination or due to the over growth of *Leuconostoc sp.*

d) **Green flavor:** This is due to under development of *Leuconostocs* as result of repeated transfer of culture before adequate development

e) **Flat flavor:** Due to small amounts of citrates in milk or due to under development of aroma culture

f) **Bitterness:** due to the activity of proteolytic contaminants that breakdown protein and release sulphur compound which cause bitterness in milk

8.1.4. Body and texture defects : a) Weak body : It is due to insufficient acid production or milk with low total solids or milk subjected to severe heat treatment

b) **Hard and lumpy:** It is due to over ripening

c) **Wheying off:** It is due to over ripening or due to low total solids in milk that result in fracturing, and defect in starters. Use of un-homogenized whole milk, agitation or vibration of culture soon after curd begins to form may create wheying off of curd.Whey may collect on the surface or at the bottom of the culture or beneath the cream layer

d) **Ropiness :** Ropiness occurs due to contamination of starter with *Alcaligenes viscolactis* or due to the ropy strains of starters like *Leuconostocs* or *L. lactis ssp lactis var hollandicus* .

e) **Gassiness:** It is due to production of CO_2 by the coli forms or yeast.$CO_2$is also produced by certain strains of *Leuconostocs* and in case of cottage cheese these may contribute to the floating curd defect. In cheddar cheese gas production may be undesirable because it results in eye formation or bulging of cans.

8.1.5. Problems with production method: During production process, Use of abnormal milk of Use of poor quality starter cultures may results in slowness of activity of starter culture. Anothercauses may be as listed below:

1. Use of improperly cleaned utensils and equipment.
2. Use of milk subjected to improper heat treatment.
3. Use of unhygienic production practices.
4. Handling persons with unclean habits and unhealthy persons.
5. Use of improperly cleaned and sterilized packaging material.
6. Use of improper storage conditions and temperatures.

THE END

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Chapter-9

Bacteriophage: Structures, classification, detection and control

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria.). Phage‘ mean to eat and bacteriophage means eating of bacterial cell. Bacteriophages are widely distributed in nature & most abundant in intestinal contents of animals. Bacteriophages were first invented by Twort in 1915. All viruses contain nucleic acid, either DNA or RNA (but not both), and a protein coat, which encases the nucleic acid. Some viruses are also enclosed by an envelope of fat and protein molecules. Without a host cell, viruses cannot carry out their life-sustaining functions to reproduce. They cannot synthesize proteins, because they lack ribosomes and must use the ribosomes of their host cells to translate viral messenger RNA into viral proteins. Viruses cannot generate or store energy in the form of adenosine triphosphate (ATP), but have to derive their energy, and all other metabolic functions, from the host cell. They also parasitize the cell for basic building materials, such as amino acids, nucleotides, and lipids (fats).

9.2. BACTERIOPHAGES

They are significant from processing point of view. Phages attack Lactic acid bacteria & lyses them by multiplying inside the host cell leading to the release of many phages which can re infect the fresh cells in the culture. This results in the failure of starters to act & bring about the changes during preparation of fermented products.

9.3. Control of Phage: Following measures can prevent starter culture by attck of the phages:

- Bacteriophages are highly host specific, a rotation of starter cultures help to control the problem to some extent.
- Ca ion deficient medium for maintaining starter cultures prevent phage attack as the ion helps in phage adsorption to host cell.
- Genetic manipulations to construct phage resistant strains.

9.4. COMPOSITION AND STRUCTURE OF BACTERIOPHAGE

Composition

Although different bacteriophages may contain different materials they all contain nucleic acid and protein. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acids of phages often contain unusual or modified bases. These modified bases protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. The size of the nucleic acid varies depending upon the phage. The simplest phages only have enough nucleic acid to code for 3-5 average size gene products while the more complex phages may code for over 100 gene products. The number of different kinds of protein and the amount of each kind of protein in the phage particle will vary depending upon the phage. The simplest phage have many copies of only one or two different proteins while more complex phages may have many different kinds. The proteins function in infection and to protect the nucleic acid from nucleases in the environment.

Structure

Bacteriophage come in many different sizes and shapes. The basic structural features of bacteriophages are illustrated in Figure given below which depicts the phage called T4.

Size

T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.

Head or Capsid

All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.

Tail

Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is

surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances other structures are involved in binding of the phage particle to the bacterium.

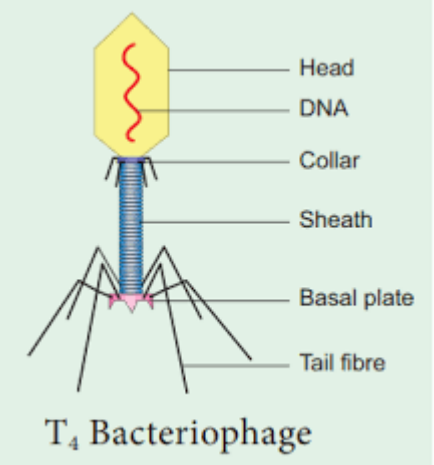

Fig: Structure of Bacteriophage

9.5. Virus classification is the process of naming viruses and placing them into a taxonomic system. Similar to the classification systems used for cellular organisms, virus classification is the subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which is to say they are non-living particles with some chemical characteristics similar to those of life, or non-cellular life. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Viruses are mainly classified by phenotypic characteristics, such as morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause. The formal taxonomic classification of viruses is the responsibility of the International Committee on Taxonomy of Viruses (ICTV) system, although the Baltimore classification system can be used to place viruses into one of seven groups based on their manner of mRNA synthesis. Specific naming conventions and further classification guidelines are set out by the ICTV.

9.5.1.The Baltimore Classification of viruses is based on the method of viral mRNA synthesis

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope. Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+ strand or "sense") DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+ strand or sense) RNA (e.g. Coronaviruses, Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)**:**

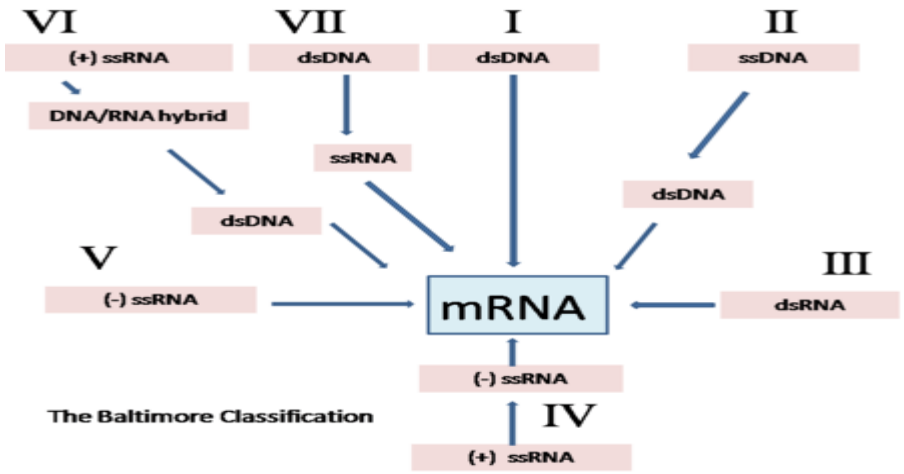

Fig: Baltimore Classification of Virus (Source Wikipedia)

ICTV Classification:

Order	Family	Morphology	Nucleic acid
Caudovirales	*Myoviridae*	Non-enveloped contractile tail	Linear dsDNA
	Siphoviridae	Non-enveloped, long non-contractile tail	Linear dsDNA
	Podoviridae	Non-enveloped, short non- contractile tail	Linear dsDNA
Unassigned	*Tectiviridae*	Non-enveloped, isometric	Linear dsDNA
	Corticoviridae	Non-enveloped, isometric	Circular dsDNA
	Lipothrixviridae	Enveloped, rod-shaped	Linear dsDNA
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA
	Rudiviridae	Non-enveloped, rod-shaped	Linear dsDNA
	Fuselloviridae	Non-enveloped, lemon-shaped	Circular dsDNA
	Inoviridae	Non-enveloped, filamentous	Circular ssDNA
	Microviridae	Non-enveloped, isometric	Circular ssDNA
	Leviviridae	Non-enveloped, isometric	Linear ssRNA
	Cystoviridae	Enveloped, spherical	Segmented dsRNA

Fig: ICTV Classification of Virus

Source:Prajapatian ,Shreja ans Sankarareddy(Agrimoon.com)

A method for detection,identification and/or quantification of bacteriophage of bacterial host specificity for bacterial genus, species or serotype, based upon the occurrence of release of cell contents, particularly nucleotides e.g. ATP, on lysis of bacterial cell walls on incubation with bacterial host cells. When new phage particles are released at the end of the phage replication cycle nucleotide levels are measured and compared with controls. The method provides for the detection of specific phages which is faster and more sensitive than known techniques. The method is only limited by the availability of host bacteria/target phage pairings. Viruses are ubiquitous in natural environments where they can exist as natural inhabitants or as contaminants from the disposal of human and animal wastes. A modified hybridization assay is available for detection of bacteriophage, which employs D Nase protection and slotb lot methods to measure quantitatively the concentration of soluble and bacteriophage encapsulated DNA in fluid samples. The potential use of this assay for estimating virus viability was tested with a model system consisting of inactivating bacteriophage lambda particles. These experiments show that the new hybridization assay provides upper-limit estimates of bacteriophage viability when inactivation results in the release of DNA.

Life cycle of Bacteriophage: The life cycle of phage can be under stand by the picture given below:

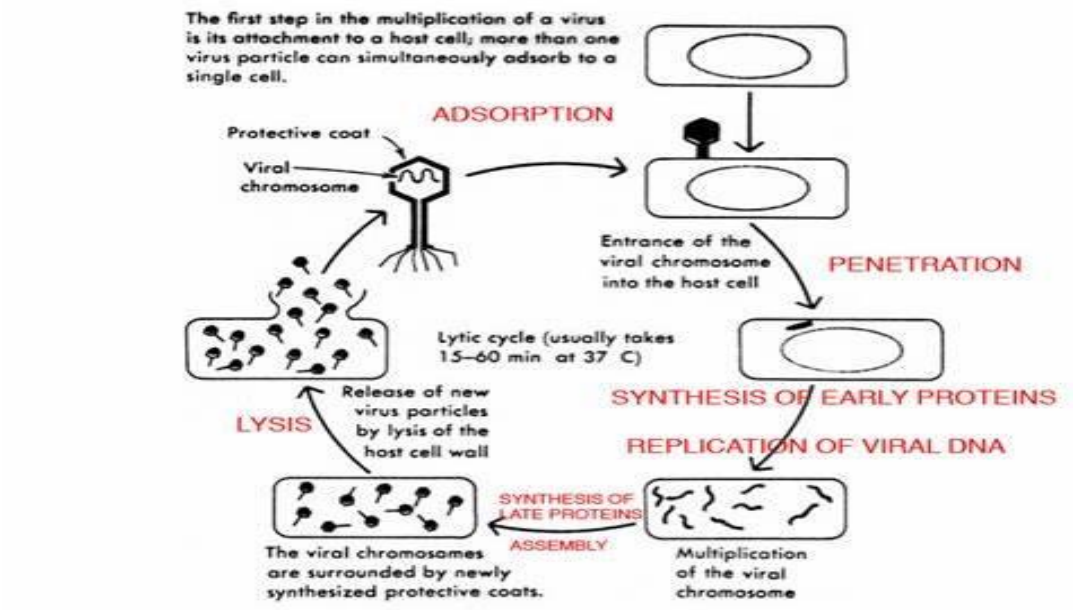

Fig: Lytic Cycle of Bacteriophage

The life cycle completed in five steps as: Adsorption of phage on the bacterial surface then penetration of DNA inside the host cell leaving protein capsid outside then Control of phage DNA over the Bacterial DNA then Synthesis of Phage DNA and Protein afterward their assembly and release of phage by lysing the bacterial cell and release of bacteriophage ready to infect another cell of bacteria..

9.6. Measures to Control Phage Attack

1) Strict Aseptic techniques for propagation and production of starter cultures

2) Effective sterilization of equipment and utensils

3) Proper heat treatment of the milk

4) Restricted movement of plant personnel in starter handling room,

5). Locate starter room far away from production area

6) Check filtration of air into the starter room and production area

6) Fog the atmosphere in the starter room with hypochlorite solution, or the use of laminar flow cabinets for small scale culture transfers

7) Grow starter cultures in phage inhibitory/resistant medium (PIM/PRM)

8) Use a daily rotation of phage un related strains (or phage resistant strains)

9) Produce the bulk starter culture or even the retail product using a direct-to-vat system

THE END

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Chapter-10

Starter Distillates

Starter Distillate

- Starter distillate is made from fermentation of Skim Milk or non fat dry milk by several bacteria.
- According to FDA, starter distillate or butter starter distillate is a steam distillate of the culture of any or all of the following species of bacteria grown on a medium consisting of skim milk usually fortified with 0.1% citric acid : L*actococcus lactis ssp. lactis, L. lactu ssp cemoris and L. lactis SSP biovar diacetylactis, leucodostoc citrovorun and L. dextranacium.*

  The ingredients contains more the 98% water, and reminder is a mixture of butter like flavoring organic compound. Besides diacetyl, startar distillate contains minor amounts of acetylaldehyde, ethylformate, ethylacelate, acetone, ethyl alcohol. 2-butanone, acetic acid and accretion.

  These all natural butter flavor systems posses a smooth, rich, fresh butter character making them ideal for application in humorous food formulations.
- Dairy products
- Baked goods
- Confections
- salads dressings
- Snack foods
- Marinades.
- They are excellent natural replacement for synthetic diacetyl.
- Starter distillate is considered as GRAS (generally recognized as safe) by FDA and regulated under code of food regulation 27 part 184.1848.

Production And Application :

Starter distillates are derived from selective lactic culture fermentation of milk and further purified by steam distillation.

1. **Bacterial Distillates :-**

Natural butter aroma distillate can be prepared by growing the mixed cultures consisting of *Lactococcus lacti ssp. lactis*, *Lactococcus lactis ssp cremoris,* and lactococcus in the culture medium containing skim milk or whey as substrate. The cultures will produce substantial amount of acetolactie acid and is acidified to a pH of about 3.5. Water vapour distillation is carried out effectively in the presence of oxygen to convert the x-acetolactie acid to diacetyl and to distill off approximately 5-15% of the fact bacerial culture medium. The resultant distillate has a diacetyl content ranging from 1369-2666 mg/kg.

2. Synthtic Distillates :-

The synthetic distillates contains edible fats which replaces souring by starter cultures.

Advantages :-

- Results in yield of sweet buttermilk.
- Greeter freedom when optimizing the temperature treatment of cream before churning.
- Yields more consistent product quality in particular as regards odour and flavor.

Disadvantage :-

Addition of pure synthetically prepared diacetyl gives a harsh undesirable taste of diacetyl.

A balanced butter flavor can be obtained by adding other important aroma substances, such as acetic acid, dimethyl sulphide, acetylaldehydes and certain lactones.