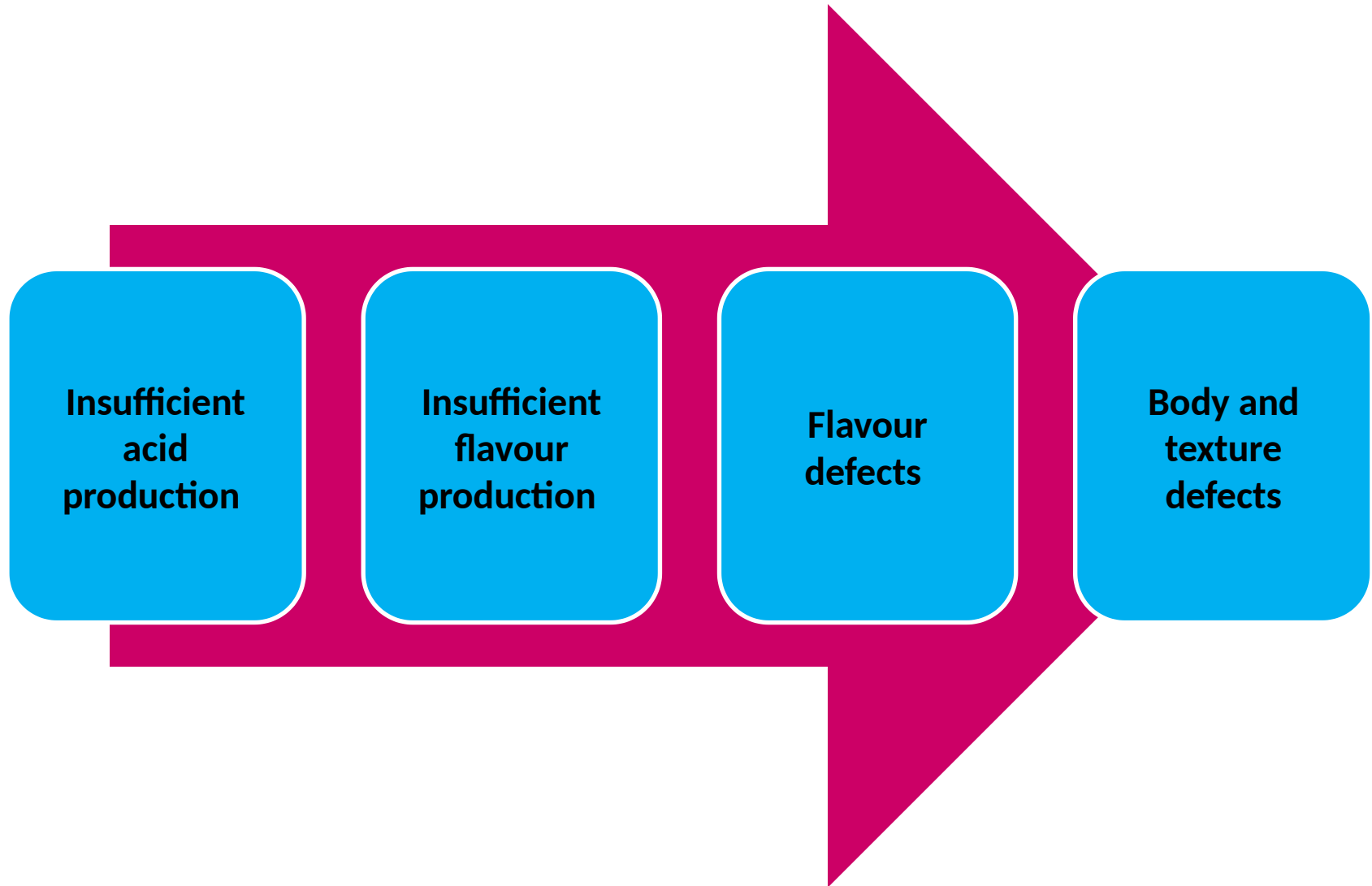




Starter Defects and Failures

Starter Defects:



Insufficient acid production or non-acid curd: One of the most common defects in lactic acid starter cultures. A good starter should produce lactic acid at vigorous and steady rate. The slowness in starters may occur with dramatic suddenness or starter culture may slowly lose its viability. Insufficient acid production could be:

- a) Spontaneous loss of vitality inherent in starter itself:**
 - Starter strain variation or genetic instability usually associated with plasmids, loss of lac' plasmid
- b) Incompetence in the control of starters**
 - i. allowing the starters becoming contaminated
 - ii. Frequent sub-culturing
 - iii. Use of unsuitable media
 - iv. Use of wrong incubation temperatures

c) Causes of slowness inherent in milk itself

- i. Use of abnormal quality milk i.e. mastitis milk or colostrums milk or late lactation milk or silage milk or winter milk.
- ii. Milk containing antibiotic residues.
- iii. Milk contaminated with bacteriophage
- iv. Milk containing residues of detergents and sanitizers
- v. Seasonal variations in milk composition
- vi. Milk containing natural inhibitory substances like leucocytes, antibiotics etc.,
- vii. Excessive aeration of milk

d) Changes in the method of making fermented milk

Sudden change in the methods adopted for the preparation a particular fermented milk causes slow acid production.

e) Bacteriophage action

Insufficient flavour production: Factors that affect the acid production also affect flavour production. In addition to these the following factors affect the flavour: improper acid production, milk having low citrate ions, milk containing high Gram-negative organisms that reduce diacetyl content by producing diacetyl reductase.

Flavour defects :

- a) **Sharp acid taste:** Due to the over ripening of the culture which may be due to increased inoculum or higher incubation temperature or prolonged incubation period.
- b) **Malty flavour:** Due to the variants of *L. lactis ssp. lactis* var *maltigenes*
- c) **Metallic flavour:** Due to over ripening of the culture/due to the use of poorly maintained containers with metallic contamination or due to the over growth of *Leuconostoc* sp.
- d) **Green flavour:** Due to under development of *Leuconostoc* as result of repeated transfer of culture before adequate development
- e) **Flat flavour:** 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

Body and texture defects:

1. **Weak body:** It is due to insufficient acid production or milk with low total solids or milk subjected to severe heat treatment.
2. **Hard and lumpy:** It is due to over ripening.
3. **Wheying off:** It is due to over ripening or due to low total solids in milk resulting in fracturing, defective starters, use of un-homogenized whole milk, agitation or vibration of culture soon after curd begins to form. Whey may collect on the surface or at the bottom of the culture or beneath the cream layer.
4. **Ropiness:** Due to the contamination of starter with *Alcaligenes viscosus* or due to the ropy strains of starters like *Leuconostocs* or *L. lactis ssp lactis var hollandicus*.
5. **Gassiness:** Production of CO₂ by the coliforms or yeast. CO₂ 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 troublesome because it results in eye formation or bulging of cans.

Problems with milk-antibiotics, abnormal milks etc.: Milk with

abnormalities or other contaminants can create problems for starters:

- Use of abnormal milk i.e. mastitis milk or colostrums milk or late lactation milk.
- Milk containing antibiotic residues.
- Milk contaminated with bacteriophage.
- Milk containing residues of detergents and sanitizers.
- Milk having inhibitory bacteria.
- Seasonal variations in milk composition.
- Milk containing natural inhibitory substances

Problems with production method: Problems encountered during the production of fermented milk products:

- Use of abnormal milk
- Use of poor quality starter cultures
- Use of improperly cleaned utensils and equipment
- Use of milk subjected to improper heat treatment
- Use of unhygienic production practices
- Handling persons with unclean habits and unhealthy persons
- Use of improperly cleaned and sterilized packaging material
- Use of improper storage conditions and temperatures.

Agents	Nature	Mode of action	Control steps
Penicillin, tetracyclin, streptomycin	Pharmacological antibiotics	Bacteriostatic, 0.1 IU/ml usually critical, heat stable	Routine test milk supply, Reject contaminated milk
Chlorine, quaternary ammonium compounds, iodine	Sterilizing chemical	Bactericidal, heat stable	Checking of sanitary practices in farm and and in plant
Bacteriophage	Viruses	Disintegrate bacteria, effectiveness depends on phage type, speed and bacterial growth, heat stable	Change starter, keep whey and dust away from product, heating milk at 85C for 30 mins destroys phage
Natural inhibitors of milk	Bacterial inhibitors	Bacteriostatic, heat labile	Pasteurization of milk at 72C for 15 secs destroys natural inhibitors of milk

Starters Failures

1. Effect of antibiotic residues:

Treatment of mastitis in cows involves application of antibiotics. Milk from treated cows cannot be legally sold, but, occasionally becomes mixed with salable product.

The resulting low-level antibiotic contamination may be sufficient to inhibit starter culture microorganisms. As antibiotic levels in milk increase, acid production decreases.

Lactic acid bacteria are very sensitive to antibiotics (penicillin, cloxacillin, streptomycin and tetracycline) commonly used for mastitis treatment. Milk testing negative for antibiotics, using *Bacillus stearothermophilus* as an indicator, can still have sufficient antibiotic to cause starter culture inhibition.

Sensitivity of starter cultures to antibiotics is highly strain and species dependent. *S. thermophilus* is more susceptible to penicillin and cloxacillin (β -lactam antibiotics) than lactococci (sensitive to streptomycin and tetracycline).

Swiss (Emmental) cheese made with antibiotic-contaminated milk (0.005 IU/mL) exhibited abnormal eye formation, presumably from inhibition of propionibacteria.

2. Effect of sanitizers:

1. Occasionally, chemical sanitizers may contaminate milk, usually as a result of human error.
2. Chlorine- and iodine-based sanitizers lose their activity in milk and therefore, not likely to cause starter culture inhibition.
3. Quaternary ammonium compounds present more potential problems, because they maintain activity in milk, and lactic acid bacteria are sensitive to low concentrations.
4. *Lactococcus* strains were inhibited by 20 µg/mL and some were inhibited by only 10 µg/mL quaternary ammonium compound. Thermophilic starter cultures are inhibited at 0.5–2.0 µg/mL quaternary ammonium compound.
5. Peracetic acid and acid anionic sanitizers can also maintain some activity in milk.
6. Relatively high concentrations of hydrogen peroxide or quaternary ammonium compound are required to give positive results in antibiotic screening tests. The amount of chemical sanitizer that might enter milk through lack of rinsing should not be sufficient to cause culture inhibition. However, problems can be encountered when sanitizer solution is not drained from tanks or trucks.

3. Effect of bacteriophages:

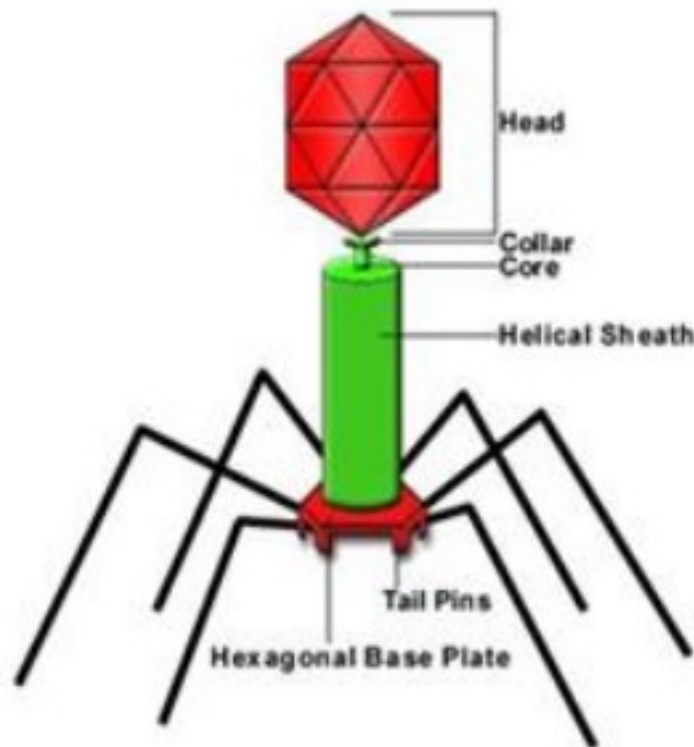
- 1. Bacteriophages are the viruses that infect bacteria. They are the quintessential parasites of the living kingdoms.**
- 2. Bacteriophages are widely distributed in nature and most abundant in intestinal contents of animals.**
- 3. First invented by Twort in 1915.**
- 4. All viruses contain nucleic acid, either DNA or RNA 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 or reproduce.**
- 5. 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.**
- 6. Viruses cannot generate or store energy in the form of ATP, but have to derive their energy from the host cell. They also parasitize the cell for basic building materials, such as amino acids, nucleotides, and lipids (fats).**

6. Phages attack Lactic acid bacteria & lyse 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.
7. Bacteriophagic infections are caused by either lytic or temperate phages. Infection with lytic (virulent) phages results in release of infectious viral particles (virions) into the environment, whereas temperate phages incorporate their DNA into the host chromosome and do not immediately produce new virions.
8. In lytic cycle, phages attach themselves to living cells and as they divide phages also multiply. The infective material is injected (nucleic acid) through hollow tail of the phage particle into the bacterial cell. The rate of phage proliferation is greater than that of bacterium. Following, a point is reached at which lysis begins. Lysis is preceded by swelling of the bacteria to 6-16 times their normal size. Irreversible adsorption of phage to the cell triggers a no. of steps which lead to the transport of viral nucleic acids into the cytoplasm and nucleic acid gets metabolically active transforming cellular function to one of synthesizing viral components. As the phage assembly completed, lysis of the host cell occur.

Ultra structures : Typical bacteriophage has a structure like tadpole.

Head – enclosing nucleic acids in protein sheath.

Tail – a hollow tube of proteins & bearing tail plate & tail fibers.



Head

Tail

Nucleic acid

Capsid

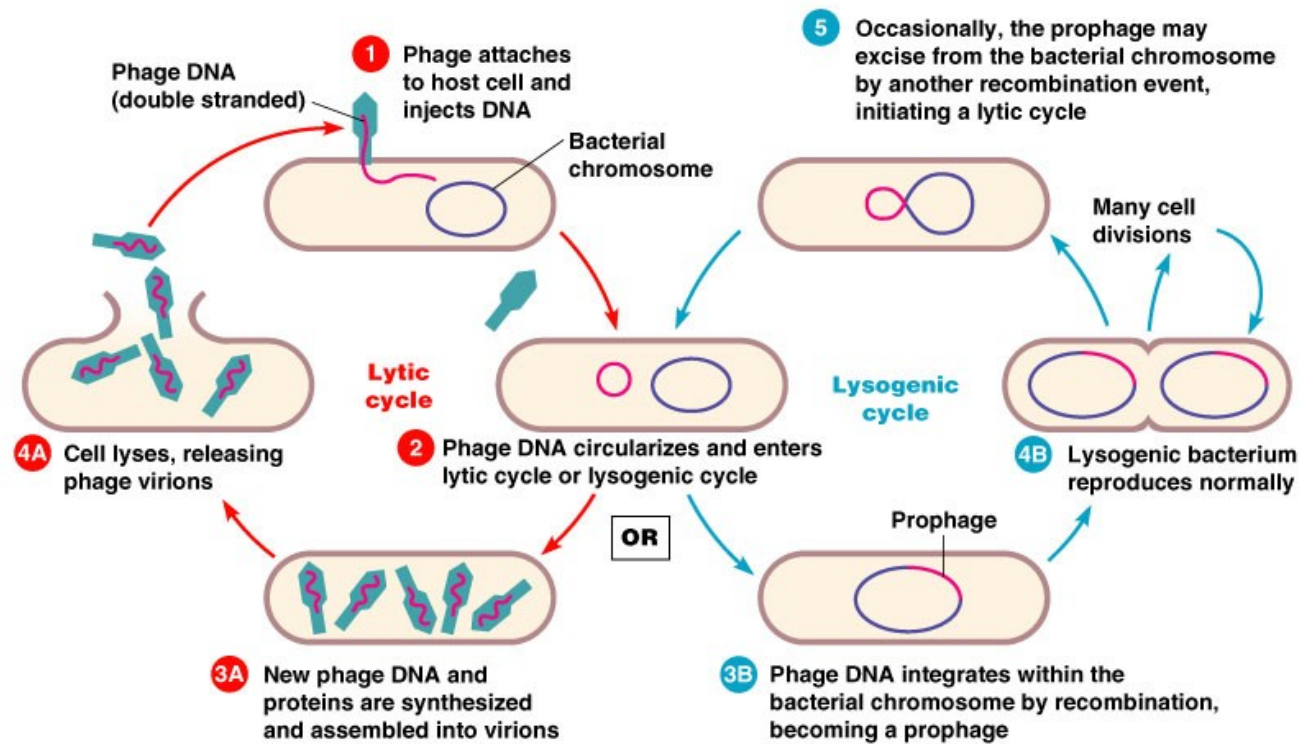
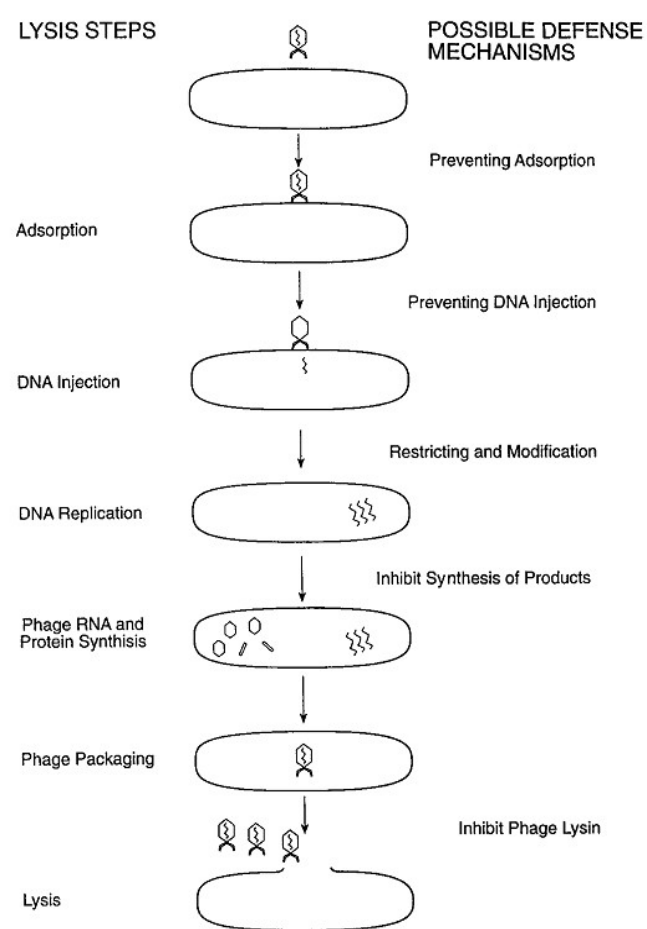
Collar

Sheath

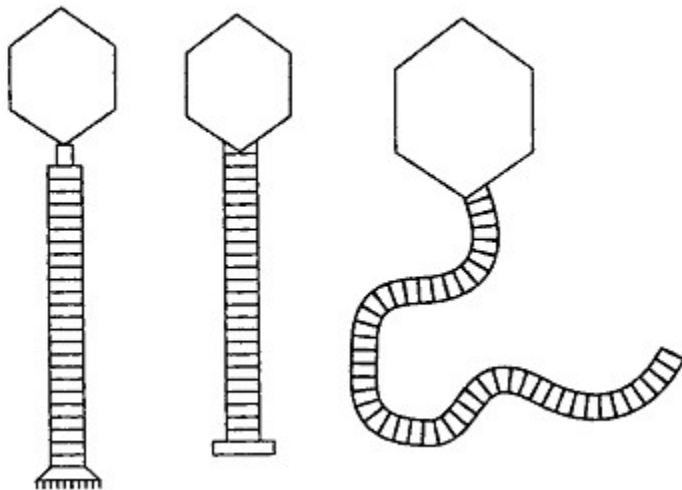
Tail fiber

Spikes

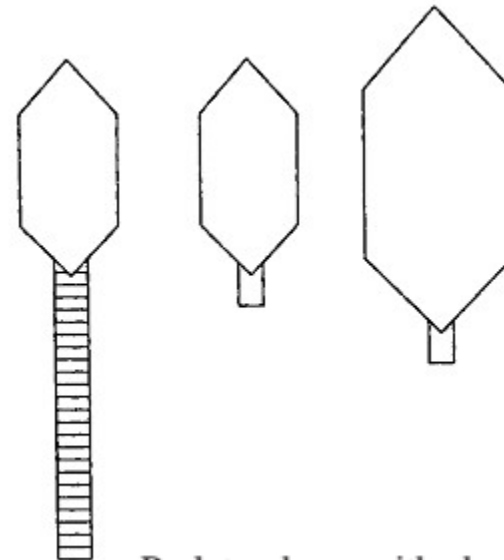
Base plate



8. Phages of LAB are having either prolate or isometric heads with contractile tails in some cases and a variety of terminal fibres or plates in addition to collars and other structures along their length.
9. Phages of *L. lactis* ssp *lactis* and *L. lactis* ssp *cremoris* have isometric heads of 45-65 nm diameter with non-contractile tails 100-250 nm long or, less frequently, prolate heads (55-65 nm X 40-48 nm) with shorter non-contractile tails (80-110 nm long).
10. Phages of *S. thermophilus* contains isometric heads of 50-70 nm diameter with non-contractile tails 200-300 nm long.
11. The bacteriophages of *L. helveticus* show isometric heads (49-56 nm diameter) and contractile tails (150-230 nm long) with sheaths. *L. bulgaricus* phages have isometric heads (44-55 nm diameter), non-contractile tails 120-215 nm in length with cross-bars and collars and base plates sometimes present.



Isometric phage with long tails



Prolate phage with short and long tails

Classification of Bacteriophages: On the basis of presence of single or double strands of genetic material, the bacteriophages are categorized as under:

1. The ssDNA phages
2. The dsDNA phages
3. The ssRNA phages
4. The dsRNA phages

Sources of Phage: **Whey** is the most important source of phage.

Whey can form foci of infection and contaminate air, utensils etc. Hence, separation of whey is probably the biggest single factor in spreading phages; **air** being the most important factor for the spread of phagic infections. Phages could be inadvertently brought into cheese factories by **milk in cans** previously used to take whey to farms. Phage contamination of starter by **wind borne droplets** is also possible not only whey from factory but also from the effluent which may be flowing near the factory. The main growth niches for bacteriophages in a cheese plant are raw milk, pools of water, stagnant floor drains, equipment, and soiled walls.

Chemical protected systems: Calcium ions are essential for the proliferation of phage and specifically for its penetration into streptococcal cells. If starters are grown in a calcium deficient medium, not only starter is protected but phage also dies. Hence, calcium deficient medium may be quite useful for the maintenance of starter cultures to avoid phage contamination as the phage will not grow in such medium but starters will grow. The following medium is shown:

Lactose	0.5%
K₂HPO₄	0.5%
KH₂PO₄	0.3%
Peptone	0.25%
Sodium citrate	0.2%

Measures to control bacteriophage attack:

- 1) Use aseptic techniques for propagation and production of starter cultures
- 2) Ensure effective sterilization of equipment and utensils
- 3) Ensure proper heat treatment of the milk
- 4) Restrict movement of plant personnel in starter handling room, and locate starter room far away from production area
- 5) Check filtration of air into the starter room and production area
- 6) Fog the atmosphere in the starter room with hypochlorite solution/
use of laminar flow cabinets for small scale culture transfers
- 7) Grow starter cultures in phage inhibitory/resistant medium
(PIM/PRM): Growth of phages during production of bulk starter can be controlled by using phage-inhibitory media. These media rely on the ability of phosphate and citrate salts to bind ionic calcium, thus inhibiting phagic absorption. The chelating agents can slow growth of the starter culture. Phage-control media often contain deionized whey, protein hydrolysates, ammonium and sodium phosphate, citrate salts, and yeast extract.

- 8) Use phage resistant strains:** LAB vary widely in their susceptibility to bacteriophagic infection, so the use of resistant strains is an important aspect of phage control. Exopolysaccharide-producing strains are more resistant to phage. Phages that infect and lyse strains producing exopolysaccharide possess a polysaccharide depolymerase enzyme specific for this particular exopolysaccharide.
- 9) Produce the bulk starter culture using a direct-to-vat system**
- 10) Culture rotation:** Culture rotations control bacteriophagic infection by limiting the length of time that a specific strain or mixture of strains is used. Cultures following each other in the series are susceptible to different phage types and are therefore unaffected by phages that may have infected the previous culture.
- 11) Genetically modified resistance strains:** Since phage-resistance plasmids are transferrable by conjugation, application of genetic engineering technology can introduce industrially significant phage resistance starter strains.