UNIT II

VIRUSES

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- **Classification of viruses**
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Virus:

The name 'virus' came from a Latin word virus which means venom or poisonous fluid. Although plant diseases like leaf roll of potato and human diseases like yellow fever, small pox etc., were known for long time, the nature of causative agent was known to us quite later.
Adolph Meyer (1886), an agriculture chemist of Holland, observed a diseased tobacco plant showing mottling of leaf and named it mosaic. He was able to demonstrate the infectious nature of the sap of infected plant by grinding, filtering through double filter paper and then applying the sap to the healthy plants.

The infective capacity of the fluid (sap) was lost by heating at 80°C and he concluded that certain microbes are the causative agent of tobacco mosaic. D. Iwanowski (1892), a Russian scientist, was the first to demonstrate the transmission of tobacco mosaic virus disease from infected to healthy plant through sap, even the sap was filtered through Chamberland filter candle, which is sufficient enough to remove bacteria.

General Characters of Virus:

The viruses are non-cellular, self-replicating, obligate, intracellular parasitic agents essentially composed of a protein that covers a central nucleic acid molecule, either RNA or DNA. The amount of protein varies from 60 to 95% and the rest is nucleic acid. A simple virus particle is often called a virion. They grow and multiply only in living cells.

They cause diseases of animals including man, and plants of different groups, except bryophytes and gymnosperms. All known viruses are the pathogen of either plants or animals.

They are the smallest among the infective agents, even much smaller than the smallest bacteria and varying over a wide range from 18-400 nm (Parvo virus– 18-26 nm, Tobacco mosaic virus – 1 7.5 nm x 300 nm, Tulip mosaic virus – 28 nm, Polio virus – 27 nm x 30 nm, Influenza virus – 80 nm x 120 nm, Small pox virus – 400 nm, etc.).

According to electron microscopic observation they are of different forms such as rod shaped, spherical, cubical etc. Within the host cell, the virus can grow, multiply and undergo mutation, but it does not respire. They depend completely on the enzyme system of the host cell for their activity.

Characters of Virus:

In brief the important characters of viruses are:

(a) They are non-cellular, self-replicating agents.

(b) They can grow and multiply intracellularly as an obligate parasite (i.e., grow only in living host) or remain inert outside the host.

(c) Depending on the symmetry, they are of three types: cubical, helical and complex.

(d) The viruses consist of two parts: the centrally placed nucleic acid, covered by protein coat.

(e) The nucleic acid is either DNA or RNA, but both do not remain together.

(f) The nucleic acid may be single or double stranded.

(g) The outer covering i.e., shell or capsid is made up of protein units, called capsomeres; except some animal viruses which are with additional polysaccharides.

(h) They have no machinery of their own for protein synthesis and thereby they use host machinery for the synthesis of protein.

(i) During replication their nucleic acid directs the host cell to make different parts of virus and when these parts assemble together they form a complete infectious particle, the virion.

(j) They are transmitted very easily from one organism to another organism.

Viruses have both living and non-living characters.

Living characters of viruses:

(a) They have the nucleic acid (DNA or RNA) i.e., the genetic material that can replicate.

(b) Mutation is well-established by the availability of mutant forms in some viruses.

(c) They are sensitive to stimulants like radiation, chemical substances etc.

(d) They can multiply in the living cells of the host.

(e) The viruses have antigenic property.

(f) They can attack specific host.

Non-living characters of viruses:

(a) The viruses remain as inert material outside their host.

(b) They are autocatalytic in nature.

(c) They are devoid of cell membrane and cell wall.

(d) The viruses are devoid of cellular organelles like ribosomes, mitochondria etc.

(e) The viruses can be crystallised.

Structure of virus:

1. Being ultramicroscopic, the viruses can be seen only by an electron microscope.

2. Generally, plant viruses are smaller than animal of bacterial viruses.

3. The shape of the virions is highly variable in different groups of viruses. They may be rod shaped, bullet shaped, brick-shaped, oval, irregular and pleomorphic, or even like a piece of coir rope.

4. In 'tailed' or T-bacteriophages the virion is made up of complex head and an attached tail.

5. A virus particle or virion consists of nucleic acid core surrounded by a protein coat or capsid. The capsid with the enclosed nucleic acid is called nucleocapsid. The capsid is made up of many morphological units, called capsomeres. Chemically, the units of capsid are polypeptide molecules, which form an impenetrable shell around the nucleic acid core.

6. The envelope of the viruses is derived from the host-cell membrane, and is lipoproteinaceous in nature. Recent researches have shown that the lipid of the lipoprotein is of host-cell origin, whereas its protein is of viral origin.

7. The tail consists of a hollow core (Figs. 300, 301) surrounded by a contractile sheath.

8. At the terminal end of the tail is present an end- plate which has attached tail fibres.

9. At the head end the tail connects the head through a thin disc or collar.

10. The nucleic acid in the head of the T-even bacteriophages is double stranded DNA.

11. The end-plate is hexagonal. It has a pin at every corner and remains connected to six very long tail fibres. Bacteriophages remain attached to the host cell through these tail fibres.

The virus consists of two parts:

(i) Nucleic acid (centrally placed), and (ii) Protein coat, sometimes with additional envelope.

(i) Nucleic acid:

Viruses contain only one type of nucleic acid i.e., either DNA or RNA. The DNA containing viruses are called Deoxyviruses, whereas viruses having RNA are called Riboviruses. They vary in the structure of their nucleic acid. Most of the plant viruses have RNA either single (TMV) or double stranded (Rice ragged stunt viruses), except a few have DNA either single (Gemini viruses) or double stranded (Dahlia mosaic virus).

Animal viruses have mostly double stranded DNA or either single (Polio virus) or double (Reo virus) stranded RNA and bacteriophages contain mostly double stranded DNA, but they also have single stranded RNA (f_2, R_{17}, fr) or single stranded DNA (f_1, fd, M_{13}). Each virion contains only one molecule of nucleic acid, called genome, consisting of nucleotide pairs whose number ranges from 1000-250,000 pairs. The amount of nucleic acid of a virion usually depends on its size. The number of genes per virion ranges from 4-8 for small viruses and 100-200 for the large viruses.

(ii) Protein coat:

The protein coat surrounding the genome is called capsid and the capsid together with the enclosed nucleic acid is called nucleocapsid. The capsid is made up of a large number of protein subunits, called capsomeres (Fig. 2.39A). Many mammalian viruses have envelope made up of a bilayered lipoprotein, mainly of host cell origin that surrounds the nucleocapsid (Fig. 2.39B).

Symmetry:

The capsid is symmetrically arranged around the central nucleic acid.

Based on symmetry of capsid, the viruses are grouped into three categories:

(a) Cubical (icosahedral),

(b) Helical, and

(c) Complex.

(a) Cubical (icosahedral) capsids:

They have a polygon with 12 corners (vertices), 20 sides (facets) and 30 edges. Each side is an equilateral triangle. They are of two types — Pentons (pentagonal capsomeres at the corners) and Hexons (hexagonal capsomeres at the corners), e.g., herpes and toga viruses are enveloped and papova and adenoviruses are naked.

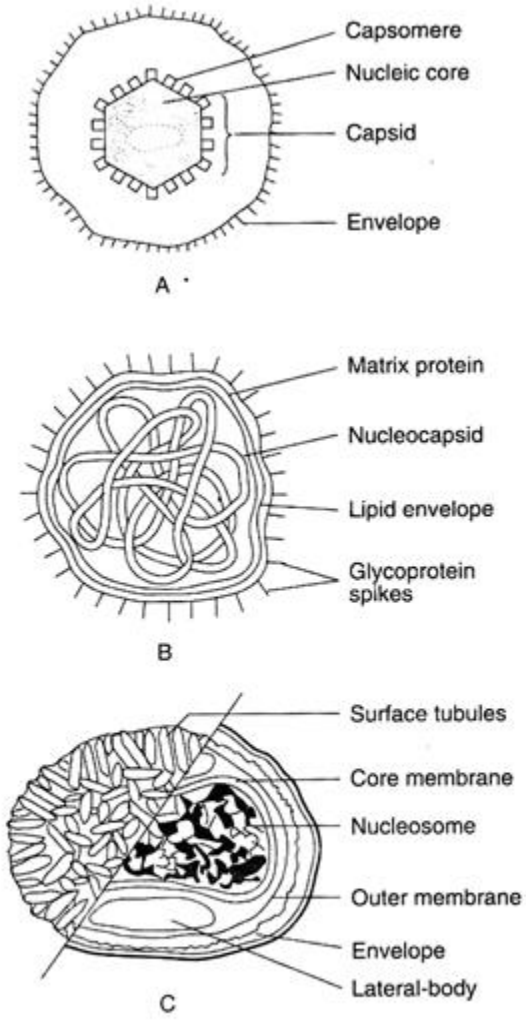


Fig. 2.38 : Diagram of virus particles. A. Enveloped virus with icosahedral symmetry, B. Virus with helical symmetry, C. Complex capsid

(b) Helical capsids:

Both nucleic acid and capsomeres are coiled together and form a spiral or helical tube. All the helical types are RNA viruses and most of them are enveloped, e.g., Tobacco mosaic virus (TMV), Influenza virus, etc.

(c) Complex capsids:

Viruses which do not conform to either of the above two types due to complexity of their structure are called complex capsids, e.g., pox virus and bacteriophages like T_2, T_4, and T_6.

Table 2.12 : Salient features of some common viruses

Group	Nucleic acid configuration	Symmetry	Size (nm)	Representative viruses	Diseases
A. DNA virus					
Animal virus					
1. Adeno virus	DS	C	70–90	Adeno virus (7 groups, and many serotypes)	Sore throat, Conjunctivitis
2. Pox virus	DS	B	200–400	Variola Cow pox	Small pox Cow pox
3. Hepanda virus	DS	C	42	Hepatitis B	Serum hepatitis
4. Parvo virus	SS	C	18–26	Polyoma virus	Papilloma of dog, rabbits etc.
Plant virus					
1. Dahlia mosaic virus	DS	C	50	DMV	Dahlia mosaic disease
2. Cauliflower mosaic virus	DS	C	50	CMV	Cauliflower mosaic disease
3. Gemini virus	SS	C	20	Gemini virus	Bunchy top of banana, Maize streak
B. RNA virus					
Animal virus					
1. Reo virus	DS	C	60–80	Rota virus Orbi virus	Infantile diarrhoea Veterinary disease
2. Picorna virus	SS	C	20–30	Polio virus Coxsackie virus	Poliomyelitis Meningitis
3. Toga virus	SS	C	50–70	Alpha virus (mosquito-borne)	Encephalitis
4. Flavi virus	SS	C	50–70	Flavi virus (mosquito-borne)	Yellow fever, Dengue fever
5. Rhabdo virus	SS	H	75	Rabies virus	Rabies
6. Bunia virus	SS	H	90–100	Buniya-virus, Phlebo-virus, Nairo-virus, Unko-virus	Fever, headache, C. N. S. involvement
Plant virus					
1. Oryza virus	DS	C	65–70	Rice ragged stunt virus	Rice ragged stunt virus disease
2. Tobacco mosaic virus	SS	H	17 × 300	TMV	Tobacco mosaic virus disease
3. Tulip yellow mosaic	SS	C	28–30	TYMV	Tulip yellow mosaic disease

SS = single stranded, DS = double stranded, C = cubical, B = brick-shaped, H = helical

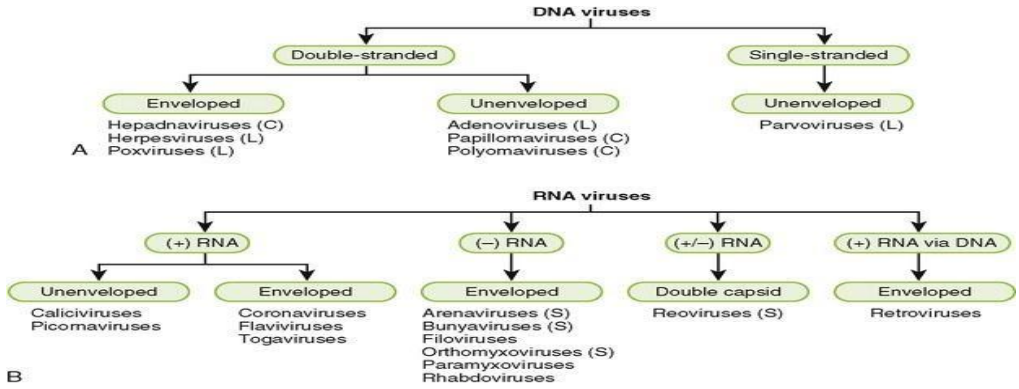
Classification of virus

A] Classification on the basis of nucleic acid

B] Classification on the basis of structure or symmetry

C] Classification on the basis of replication properties and site of replication

D] Classification on the basis of host range
E] Classification on the basis of mode of transmission
Classification of virus on the basis of nucleic acid



1. DNA virus:

- viral genome is DNA

i) Double stranded DNA virus: eg. Adenovirus, Herpesvirus
ii) Single stranded DNA virus: eg. Parvovirus, φ174 virus

2. RNA virus:

- genome is RNA

i) Double stranded RNA virus: eg. Reo virus
ii) Single stranded RNA virus: these are further classified into two groups

- Positive sense RNA (+RNA): Polio virus, Hepatitis A
- Negative sense RNA (-RNA): Rabies virus, Influenza virus

Some examples of DNA and RNA viruses:

dsDNA viruses	ssDNA viruses	dsRNA viruses	ssRNA (+) viruses	ssRNA (-) viruses	RNA and DNA (RT) viruses
Poxviridae	*Circoviridae*	*Reoviridae*	*Picornaviridae*	*Bornaviridae*	*Retroviridae* (RNA)
Asfaviridae	*Anellovirus*	*Birnaviridae*	*Caliciviridae*	*Rhabdoviridae*	*Hepadnaviridae*
Iridoviridae	*Parvoviridae*		*Hepevirus*	*Filoviridae*	(DNA)
Herpesviridae			*Astroviridae*	*Paramyxoviridae*	
Adenoviridae			*Nodaviridae*	*Orthomyxoviridae*	
Polyomaviridae			*Coronaviridae*	*Bunyaviridae*	
Papillomaviridae			*Arteriviridae*	*Arenaviridae*	
			Flaviviridae	*Deltavirus*	
			Togaviridae		

Classification of virus on the basis of structure

1. Cubical virus:

- they are also known as icosahedral symmetry virus
- Eg. Reo virus, Picorna virus

2. Spiral virus:

- they are also known as helical symmetry virus
- Eg. Paramyxovirus, orthomyxovirus

3. Radial symmetry virus:

- eg.Bacteriophage

4. Complex virus:

- eg. Pox virus
-

Classification of virus on the basis of replication properties and site of replication

1. Replication and assembly in cytoplasm of host:

- Eg. All RNA virus replicate and assemble in cytoplasm of host cell except Influenza virus

2. Replication in nucleus and assembly in cytoplasm of host:

- Eg. Influenza virus, Pox virus

3. Replication and assembly in nucleus of host:

- All DNA viruses replicate and assemble in nucleus of host cell except Pox virus.

4. Virus replication through ds DNA intermediate:

- Eg. All DNA virus, Retro virus and some tumor causing RNA virus replicates through ds DNA as intermediates.

5. Virus replication through ss RNA intermediate:

- Eg. All RNA virus except Reo virus and tumor causing RNA viruses.

Classification of virus on the basis of host range:

1. Bacteriophage:

- Phage are virus infecting bacteria. Eg, λ phage, T2, T4, φ174, MV-11

2. Plant virus:

- Those virus that infects plants. Eg. Tobacco mossac virus (TMV), cauliflower mosaic virus (CMV)

3. Animal virus:

- Those virus that infects animals. Eg. Polio virus, Retro virus, Herpes virus, Adeno virus

4. Insect virus:

- Virus that infects insects. Eg. Baculovirus, Sacbrood virus, Entomopox virus, Granulosis virus

Classification of virus on the basis of mode of transmission:

1. Virus transmitted through respiratory route:

- Eg, Swine flu, Rhino virus

2. Virus transmitted through faeco-oral route:

- Eg. Hepatitis A virus, Polio virus, Rota virus

3. Virus transmitted through sexual contacts:

- Eg. Retro virus

4. Virus transmitted through blood transfusion:

- Eg. Hepatitis B virus, HIV

5. Zoonotic virus:

- virus transmitted through biting of infected animals;
- Eg. Rabies virus, Alpha virus, Flavi virus

Chemical composition of virus
Nucleic acid

- Any particular virus contains only a single kind of nucleic acid. However, this may be DNA or RNA; indeed, the RNA viruses provide the only instance in nature in which RNA is the exclusive repository of genetic information. All viral genomes are haploid, i.e., they contain only one copy of each gene, except for retrovirus genomes, which are diploid. Viral DNA or RNA can be double-stranded (ds) or single-stranded (ss).
- DNA The genome of all DNA viruses consists of a single molecule, which is double-stranded except in the case of the parvoviruses, and may be linear or circular.
- First, the DNA of most of the larger viruses—like that of cells—contains what appears to be redundant information, in the form of (1) repeat (reiterated) sequences and (2) introns, i.e., regions which are spliced out and discarded from the RNA transcript.

- Viral DNAs contain several kinds of non coding sequences, in addition to introns and various types of terminal repeat sequences, described above. Consensus sequences, which tend to be conserved through evolution because they serve vital functions, include those of RNA splice sites, polyadenylation sites, RNA polymerase recognition sites and promoters, initiation codons for translation, and termination codons.
- Single-stranded viral nucleic acid, which is generally RNA, can also be defined according to its sense (also known as polarity).

Protein

- Some virus-coded proteins are structural, i.e., they are part of the virion; some are nonstructural and are concerned with various aspects of the replication cycle. A major role of structural proteins is to provide the viral nucleic acid with a protective coat.
- The virions of all viruses of vertebrates contain several different proteins, the number ranging from 3 in the case of the simplest viruses to over 100 in the case of the complex poxviruses. In isometric viruses, the structural proteins form an icosahedral capsid which sometimes encloses a polypeptide core that is intimately associated with the nucleic acid. Some virions, e.g., those of reoviruses, appear to have two concentric capsids.

Lipid

- Lipid constitutes about 30–35% of the dry weight of enveloped viruses, the viral envelope being derived from cellular lipids. As a consequence, the composition of lipids of particular viruses differs according to the composition of the membrane lipids of the cells in which they have replicated. About 50–60% of the envelope lipid is phospholipid, and most of the remainder is cholesterol.
- The poxviruses, ranaviruses, and African swine fever virus contain cellular lipid in their envelopes, and other lipids in the inner part of the virion. Lipid occurs in the outer membrane of poxviruses, and has a different composition from that of host cell lipids. In ranaviruses and African swine fever virus the additional viral lipid occurs within the icosahedral capsid.

Carbohydrate

- Apart from that associated with viral nucleic acid, carbohydrate occurs as a component of viral glycoproteins, which usually occur as peplomers, with their hydrophobic ends buried in the lipid bilayer of the envelope, while their glycosylated hydrophilic ends project into the medium. Poxviruses also contain internal glycoproteins, in the membrane of the core, and one of the outer capsid proteins of rotaviruses is glycosylated.

Replication of Virus by Lytic Cycle:

This type of cycle is seen in T-even phages (T_2, T_4 etc.) which attack Escherichia coli.

The lytic cycle consists of five steps

(a) Adsorption,
(b) Infection
(c) Synthesis of phage components in host cell,
(d) Formation of new phage particle, and
(e) Liberation of phages from the host cell.

(a) Adsorption:

The interaction between the phage specific organelle — the tail and the receptor site of the host cell is called the adsorption. The adsorption is facilitated by the negatively charged carboxyl groups on the host surface and the positively charged amino-group of protein present at the tip of the phage tail.
In T-even phages, the tip of the tail fibre first attaches to the cell surface. The tail fibre then bends and allows the tail pins to attach on the host surface that makes an irreversible attachment.

(b) Infection:

After adsorption, the phage particle secretes an enzyme which hydrolyses the murin complex of the host cell wall and forms a pore. The sheath of the tail then contracts and pushes the central tubular part, i.e., core of the tail, into the host wall, like an injection needle. The nucleic acid of the phage then passes through the core and enters the host bacterium.
The empty protein shell of the phage is called ghost, which may remain attached even after release of nucleic acid. Once the bacterial cell receives the nucleic acid of a phage, it becomes resistant to the other phages.

(c) Synthesis of Phage Components in Host Cell:

Once the phage nucleic acid takes the entry inside the bacterial cell, it suppresses the synthesis of bacterial protein and directs to synthesize the proteins of the phage particle.
The DNA of phage replicates following the semi-conservative process. Majority of the DNA acts as a template for its own synthesis and the rest is used as template for the synthesis of viral specific m-RNA by utilizing the RNA-polymerase of the host.

The newly formed m-RNA directs the host cell to synthesize the proteins which are used to build up the protein coat of the phage particle. Almost at the end of replication of phage nucleic acid, a protein, the phage lysozyme, is synthesized.

(d) Formation of New Phage Particle:

The new phage particles are formed by the assemblage of nucleic acid and protein. This process is called maturation, which is controlled by viral genome. In this process, initially the condensation of nucleic acid molecule takes place.
The protein sub-units then aggregate around the nucleic acid molecule and form the head of the phage. By this time the tail formation starts. Initially the core tube is attached with the basal plate and then sheath becomes assembled around the core tube. In this stage, the tail becomes attached to the base of the head taking a collar in between. At last, the tail fibers are attached to the basal plate.

(e) Liberation of Phages from the Host Cell:

In a cycle of phage development, about 200 phages are formed which take about 30-90 minutes. In the host cell, the phage DNA secretes lysozyme (an enzyme) which causes the lysis of host cell wall. As a result of lysis the phage particles are liberated.

Transmission of virus
1. Mechanical Transmission:

In nature plant viruses are mechanically transmitted from diseased to healthy plants by rubbing leaves together, injecting plant extract, by action of animals, etc. Viral particles remain adhered to plant surfaces, epidermis or hairs.

During rubbing the cells are broken and viral particles are liberated in the damaged cells. Transmission through this mechanism occur in such plants which are closely planted.

Similarly viral particles attached on surface of animal body are transmitted when they rub their body first on infected plants and then on healthy plants. Viral particles enter through the injuries made by animals. Similarly birds also transmit viruses by this method, for example TMV.

2. Vegetative and Graft Transmission:

Generally viral particles are present almost in all parts of systematically infected plants. Virus will certainly be transmitted to the progeny, if any part of such mother plants in used for vegetative propagation through rhizomes, bulbs, corns, tubers, cuttings, etc. Therefore, one must not use the infected vegetative part of vegetatively propagating plants such as dahlia, chrysanthemums, carnations, potatoes, etc.

Grafting technique (placing the cut end of one plant onto immediate contact of tissues of other plants to establish a union product in one plant) has been well practiced in India since time immemorial. There is wide variety of grafting techniques such as stem grafting or wedge grafting, tuber grafting (in potatoes), root grafting, etc. Grafting is widely used commercially for propagation of plants.

3. Pollen Transmission:

When pollens consisting for viruses fall on stigma of female plants, they germinate and eventually facilitate the virus to infect the ovules of plants. Such viruses are called pollen-borne viruses. Example of pollen-transmitted viruses are: barely stripe virus, tobacco ring spot virus, bean common mosaic virus, fruit ring spot virus. Dhatura mosaic virus is transmitted through pollen to seeds in 79% offspring.

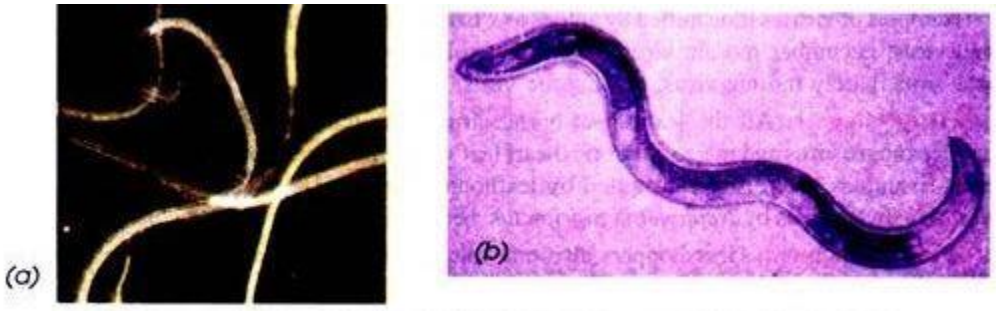
4. Seed Transmission:

Seed transmission of viruses is very rare but many viruses are known to be seed transmitted. However, a very low level (0.1%) of seed transmission had epidemiologically been found out.

Some example of seed transmitted viruses are bean mosaic virus, tomato ring spot virus, tobacco ring spot virus, cowpea mosaic virus, cucumber mosaic virus, mung and urd bean mosaic viruses, etc. Seed transmitted viruses are present in embryo, endosperm or seed coat. After seed germination, virus- infected seedlings are produced.

5. Nematode Transmission:

There are some plant parasitic nematodes that feed roots of plants. Such nematodes also act as vector for some viral pathogens. Vectors are the organism that assist in transmission of viruses. Examples of some plant parasitic nematodes are: Longidorus, Paratrichodorus, Trichodorus and Xiphinema. These nematodes transmit viruses for about 10 months but transmission does not involve viral replication inside the vector.



Xiphinema (a) and Trichodorus (b) are virus transmitting nematodes.

In soil two types of virus transmission can be observed:

(a) Some of the viruses remain active in debris of roots are leaves. Healthy plants that come in contact of debris are infected, e.g. TMV,

(b) Virus is transmitted to healthy plants by nematodes that inhabit the soil e.g. potato rattle virus.

6. Fungal Transmission:

There are many soil-inhabiting fungi which transmit several viruses, for example Olpidium brassicae, Polymyxa graminis, Synchytrium endobioticum and Spongospora subterranean. These fungi are obligate endoparasite of higher plants.

Their zoospores infect the roots of new hosts, introduced viruses and produce virus specific symptoms. These fungi acquire virus from virus- infected plants which persists in soil for several months or years. O. brassicae transmit tobacco necrosis virus, P. graminis transmit wheat mosaic virus and S. subterranean transmit potato mop top virus.

7. Insect Vector Transmission:

Vectors are highly mobile and play an important t role in natural ecology of viruses. In many cases relationship between virus and vector is an intimate biological association, besides mere mechanical transfer.

Most of the viruses are transmitted by vectors. They have slender, needle-like mouthparts to which they pierce the cells and suck cell sap of host plants. Virus is transmitted to healthy plants when the viruliferious insects feed the plant tissue.

Viruses are divided into three groups, on the basis of length of the period and relationship with insect:

(a) Non-persistent

(b) Semi-persistent

(c) persistent

8. Dodder Transmission:

Dodders are the trailer or climber parasitic plants which grow forming bridge between two plants. Cuscuta reflexa is the most famous dodder plant that lacks leaves. They belong to the family Convolvulaceae.

Dodders wind around the host and penetrate its haustoria into host tissue sending up to vascular tissue. Haustoria acquire virus from the infected plants that are eventually transmit to the new hosts. Dodder transmitted viruses are sugar beet curly top virus, tomato bushy stunt virus, tobacco rattle virus, etc.

Economic Importance of Virus

- In preparing antidotes/vaccine: Pox, mumps, polio, jaundice etc diseases can be controlled by penetrating using or dead virus in the human body as vaccines.
- In controlling harmful animals and insects: Some animals and insects which are harmful to humans can be controlled by some special virus.
- Control of disease: T2 bacteriophage virus saves humans from dysentery by spoiling some harmful bacteria, like, e-coli. In Virotherapy, viruses use as vectors to treat several diseases, as they can explicitly target cells and DNA. It intimates hopeful use in the treatment of cancer and in gene therapy.
- In the laboratory: Virus is used in the lab, as the simplest living model. In the research of genetics, the virus is used mostly. It is an important subject in genetic engineering.
- In the evidence of evolution: Virus plays a vital role to acquire knowledge about the trend of evolution and the process of formation of living organisms because the virus contains both living and non-Living characteristics.

- In nanotechnology, viruses can be considered as organic nanoparticles. Because of their size, shape, and structures have been used as a template for organizing materials on the nanoscale.
- In Seawater: A spoon of seawater contains about a million viruses, making them the most plenteous natural substance in aquatic ecosystems. They are useful in the disposal of saltwater and freshwater ecosystems. Viruses increase the number of Photosynthesis in Oceans and are effective for reducing the amount of carbon dioxide in the atmosphere by approximate 3 gigatonnes of carbon per year.
- Viruses are much smaller than bacteria. A virus particle is called virion. The virions vary widely in size. The smallest virus measures about 10 mm in diameter (e.g., foot-and-mouth disease virus). The largest virus, (e.g., poxvirus) measures about 250 nm, i.e., as large as the smallest bacteria or mycoplasma.

PATHOGENIC VIRUS

Tobacco mosaic virus

1. Introduction to Tobacco Mosaic Virus:

This is the best known of all virus diseases. The tobacco mosaic virus affects all dicotyledonous plants of which most important are tobacco and tomato. But it does not affect any monocotyledonous plants.

Although Adolph Mayer in 1886 first pointed out the mosaic pattern on leaves of affected tobacco plants, it was not until 1898 the first scientific proof of the existence of a virus was given by Beijerinck.

He was able to demonstrate that a diseased tobacco plant juice was able to induce mosaic disease in healthy tobacco plants.

Whereas, C. A. Knight showed that the tobacco mosaic virus is made up of sixteen amino acids.

The tobacco mosaic virus affects photosynthetic tissue of the host leading to distortion, blistering and necrosis. It also causes dwarfing of affected plants. It is one of the most damaging viruses of plants, causes enormous loss of tobacco crop by reducing yield and quality.

2. Symptoms of Tobacco Mosaic Virus:

The symptom is systemic mosaic type. The primary symptom on young leaves is faint circular chlorotic lesions appear with gradual vein clearing.

For example, one strain of tobacco mosaic virus may cause yellow mottling on the leaves, a second may cause necrosis only, whilst a third induces a gross malformation. Another variable factor is the variety of plant affected. In flowers, petals show mosaic symptoms. Severe strains cause streaking of stem. The disease is seldom fatal to the host.

3. Causal Organism of Tobacco Mosaic Virus:

The typical tobacco mosaic virus is Tobacco mosaic virus 1, Marmor tabaci Holmes.

The virus remains active in extracted host plant juice even up to 25 years. It is a very resistant virus, can stand desiccation for 25 years or more. It occurs in very high concentration in plant and its dilution end point is 10^{-6}. The thermal inactivation point of the virus is 90°C.

The virus particles are rod-shaped measuring 280µ in length by 15µ, in width. The ribonucleic acid thread intertwines more or less centrally between the protein subunits.

The cells of tobacco plants infected with tobacco mosaic virus are characterized by the presence of certain cell inclusions. They are: (i) two types of intracellular inclusions, and (ii) intra-nuclear inclusion. The intracellular inclusions are: (a) X- bodies and (b) striate material of crystalline plates.

The X-bodies are amorphous, protoplasmic more or less vacuolate inclusions. Whereas striate material of crystalline plates gives protein reaction. These crystals resemble the purified virus-protein crystals. The intra-nuclear fibrous and crystalline inclusions are produced by a yellow-mottling strain of tobacco mosaic virus.

4. Disease Cycle of Tobacco Mosaic Virus:

The virus perennates in infected tobacco plant debris, tobacco refuse from warehouses, cigarettes, cigars, pipe and chewing tobacco and in perennating hosts which form the source of primary inoculum.

This is one of the most infectious of the plant viruses. The virus is disseminated from plant to plant by mechanical transmission, by handling tobacco plants during transplanting; through other field operations; and contact by man and cultivation implements. The virus enters in the host tissue, it multiplies very rapidly producing disease symptoms.

5. Control of Tobacco Mosaic Virus:

Following are some of the suggested control measures:

(i) Seed beds should be located at a great distance from the tobacco warehouses.

(ii) Seed beds should be free from any tobacco refuse.

(iii) Seed bed soil should be sterilized by steam.

(iv) Care should be taken to avoid contamination through hands and cultivation implements.

(v) Since pipe tobacco, cigarettes and chewing tobacco are all sources of primary inoculum, smoking or chewing of any kind of tobacco should be avoided.

(vi) Susceptible hosts, weed or otherwise in which virus may harbour, should be destroyed.

(vii) Previous year's plant debris should be destroyed by burning.

(viii) Diseased plants should be removed and burnt to stop further spread of the disease.

(ix) Growing resistant varieties produces good results.

General Account of Mycoplasmas:

Mycoplasmas are the smallest among the known aerobic prokaryotes (Fig. 2.50). They were first discovered by Pasteur in 1843, during his work on the possible causal agent of pleuropneumonia of cattle. Thus they were called pleuro- pneumonia-like organism (PPLO).

Pasteur was unable to isolate them in pure culture. Later, Nocard and Roux (1898), the French microbiologists, were successful in growing them in pure culture-medium containing serum and confirmed . by inoculation and subsequent expression of disease in healthy cattle.

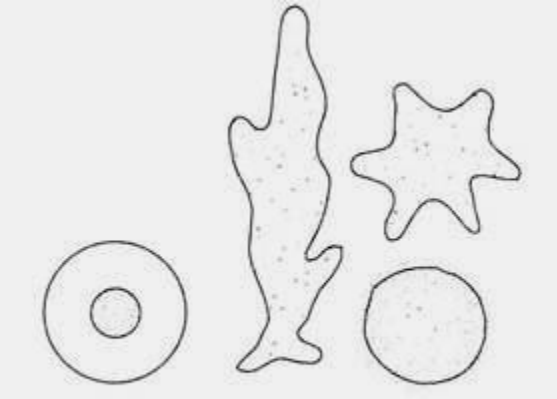


Fig. 2.50 : Mycoplasmas : A. Single colony, B. Cells of various shapes

Mycoplasmas are commonly found in soil, hot spring, sewage water and also in plants and animals including man. Borrel (1910) named these organisms Asterococcus mycoides. Later, in 1929, Nowak placed them under the genus Mycoplasma.

Classification of Mycoplasmas:

Based on nutritional requirement, mycoplasmas are divided into the following three genera:

1. Mycoplasma:

They require cholesterol for their growth. They parasitise on animals including man by causing damage to the mucous membranes and different joints of the body.

2. Acholeplasma:

They do not require cholesterol for their growth. They are available in sewage water and soil as saprophytes and in vertebrates and also in plants as parasites.

3. Thermoplasma:

They also do not require cholesterol for their growth. They are aerobic microorganisms showing good growth in acidic pH between 0.96-3.0, with an optimum temperature of 59°C.

Structure of Mycoplasmas:

The cell is devoid of cell wall which makes them readily deformable showing irregular and variable shapes. They may be ring-like, granular, coccoid, pear-shaped, filamentous, etc. (Fig. 2.50). The filaments are of two types: unbranched or branched. The cells are very small and measure 0.3-0.9 μm in diameter.

The cells are covered by cytoplasmic (lipoprotein) membrane (Fig. 2.51). Cell membrane covers the cytoplasm which contains nucleoplasm like structure and ribosomes. The genetic material is composed of DNA and RNA. It is about less than 50%, the amount present in other prokaryotic organisms. The amount of RNA (8%) is more than DNA (4%).

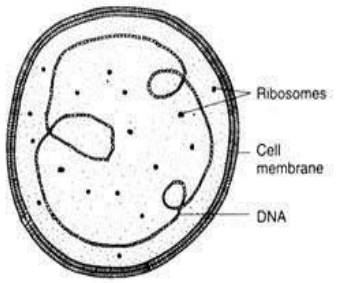


Fig. 2.51 : Mycoplasma : Structure of a cell (Electron micrograph)

They are usually non-motile, but some forms show gliding movements. They reproduce by vegetative means i.e., by binary fission and budding.

They are sensitive to antibiotics like chloramphenicol, streptomycin, erythromycin etc., but are insensitive to penicillin, ampicillin etc., due to the absence of cell wall.

Diseases Caused by Mycoplasma:

Mycoplasmas cause different serious diseases in plants and animals including man.

Some of these are:

(a) Plant Diseases:

(i) Little leaf disease of brinjal,

(ii) Bunchy top of papaya,

(iii) Big bud of tomato,

(iv) Witches broom of legumes,

(v) Yellow dwarf of tobacco,

(vi) Strip disease of sugarcane,

(viii) Cotton viruses-

(b) Human Diseases:

(i) Primary atypical pneumonia (PAP) by Mycoplasma pneumoniae,

(ii) Mycoplasma hominis causes pleuropneumonia, prostatitis, inflammations of genitals etc.

(iii) Mycoplasma fermentants causes infertility in man.

(c) Animal Diseases:

(i) Mycoplasma a galactia causes a galactia of goat and sheep,

(ii) Mycoplasma mycoides causes pleuropneumonia of cattle,

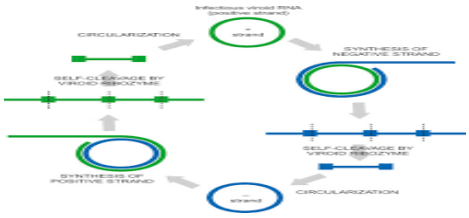
(iii) M. bovigenitalium causes inflammation of genitals of different animals.

Viroids:

Viroids are much smaller than viruses and are also considerably simpler, for they consist of no more than a single strand of RNA.

The RNA is not enclosed in any structure, and except during infection is not associated with any other chemical substances.

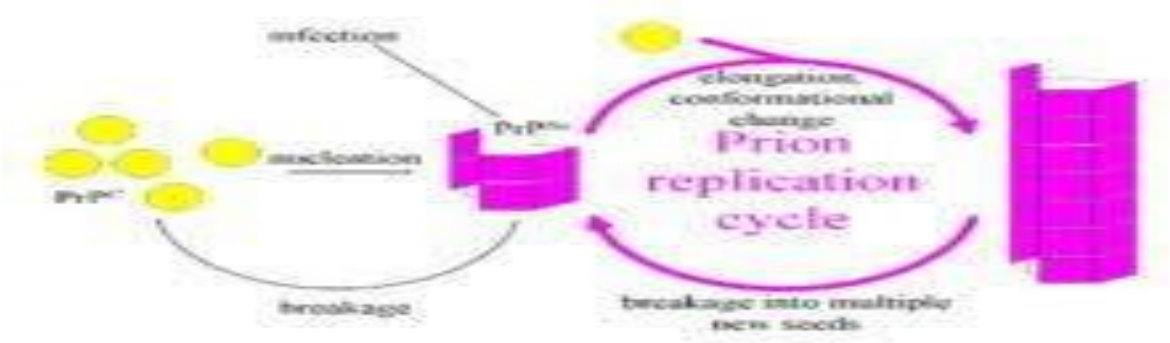
The typical viroid is an RNA molecule about 50 nm in length. Though at the present time viroids are only suspected of being the agents of certain diseases in animal cells, they are known to be the cause of a number of plant diseases including spindle tuber disease in potatoes.



Prions:

Generally, infectious agents are either viroids, viruses, prokaryotic cells (e.g., bacteria), or eukaryotic cells (e.g., certain protists). What these infectious agents have in common is that their identity is defined by the nucleic acid that each carries. Although there is still considerable debate on the matter, it now appears that there may be an exception to this rule.

Scrapie (a disease of goats and sheep) and a disease of the nervous system in humans called Creutzfeldt-Jakob disease) appear to be caused by agents consisting only of protein; the agents of these diseases are called prions. The protein comprising a prion has a molecular weight between 50,000 and 100,000, corresponding to a particle size that is 100 times smaller than the smallest viruses



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INDUSTRIAL APPLICATION OF MICROORGANISMS

Acetic acid

Acetic acid systematically named **ethanoic acid** is a colourless liquid organic compound with the chemical formula CH_3COOH (also written as CH_3CO_2H, $C_2H_4O_2$, or $HC_2H_3O_2$). When undiluted, it is sometimes called *glacial acetic acid*. Vinegar is no less than 4% acetic acid by volume, making acetic acid the main component of vinegar apart from water. Acetic acid has a distinctive sour taste and pungent smell. In addition to household vinegar, it is mainly produced as a precursor to polyvinyl acetate and cellulose acetate. It is classified as a weak acid since it only partially dissociates in solution, but concentrated acetic acid is corrosive and can attack the skin.

Acetic acid is the second simplest carboxylic acid (after formic acid). It consists of a methyl group attached to a carboxyl group. It is an important chemical reagent and industrial chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is controlled by the food additive code E260 as an acidity regulator and as a condiment. In biochemistry, the acetyl group, derived from acetic acid, is fundamental to all forms of life. When bound to coenzyme A, it is central to the metabolism of carbohydrates and fats.

The global demand for acetic acid is about 6.5 million metric tons per year (Mt/a), of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from methanol.[] Vinegar is mostly dilute acetic acid, often produced by fermentation and subsequent oxidation of ethanol.

Production

Purification and concentration plant for acetic acid

Acetic acid is produced industrially both synthetically and by bacterial fermentation. About 75% of acetic acid made for use in the chemical industry is made by the carbonylation of methanol, explained below. The biological route accounts for only about 10% of world production, but it remains important for the production of vinegar because many food purity laws require vinegar used in foods to be of biological origin. Other processes are methyl formate isomerization, conversion of syngas to acetic acid, and gas phase oxidation of ethylene and ethanol.[22] Acetic acid is often a side product of different reactions, i.e. during heterogeneous catalytic acrylic acid synthesis[23][24][25] or fermentative lactic acid production. As of 2003–2005, total worldwide production of virgin acetic acid was estimated at 5 Mt/a (million tones per year), approximately half of which was produced in the United States. European production was approximately 1 Mt/a and declining, while Japanese production was 0.7 Mt/a. Another 1.5 Mt were recycled each year, bringing the total world market to 6.5 Mt/a. Since then the global production has increased to 10.7 Mt/a (in 2010), and further; however, a slowing in this increase in production is predicted.[30] The two biggest producers of virgin acetic acid are Celanese and BP Chemicals. Other major producers include Millennium Chemicals, Sterling Chemicals, Samsung, Eastman, and Svensk Etanolkemi.

Oxidative fermentation

For most of human history, acetic acid bacteria of the genus *Acetobacter* have made acetic acid, in the form of vinegar. Given sufficient oxygen, these bacteria can produce vinegar from a variety of alcoholic foodstuffs. Commonly used feeds include apple cider, wine, and fermented grain, malt, rice, or potato mashes. The overall chemical reaction facilitated by these bacteria is:

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

A dilute alcohol solution inoculated with *Acetobacter* and kept in a warm, airy place will become vinegar over the course of a few months. Industrial vinegar-making methods accelerate this process by improving the supply of oxygen to the bacteria.

The first batches of vinegar produced by fermentation probably followed errors in the winemaking process. If must is fermented at too high a temperature, acetobacter will overwhelm the yeast naturally occurring on the grapes. As the demand for vinegar for culinary, medical, and sanitary purposes increased, vintners quickly learned to use other organic materials to produce vinegar in the hot summer months before the grapes were ripe and ready for processing into wine. This method was slow, however, and not always successful, as the vintners did not understand the process.

One of the first modern commercial processes was the "fast method" or "German method", first practised in Germany in 1823. In this process, fermentation takes place in a tower packed with wood shavings or charcoal. The alcohol-containing feed is trickled into the top of the tower, and fresh air supplied from the bottom by either natural or forced convection. The improved air supply in this process cut the time to prepare vinegar from months to weeks.[1]Nowadays, most vinegar is made in submerged tank culture, first described in 1949 by Otto Hromatka and Heinrich Ebner. In this method, alcohol is fermented to vinegar in a continuously stirred tank, and oxygen is supplied by bubbling air through the solution. Using modern applications of this method, vinegar of 15% acetic acid can be prepared in only 24 hours in batch process, even 20% in 60-hour fed-batch process.

Anaerobic fermentation

Species of anaerobic bacteria, including members of the genus *Clostridium* or *Acetobacterium* can convert sugars to acetic acid directly without creating ethanol as an intermediate. The overall chemical reaction conducted by these bacteria may be represented as:

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$$

These acetogenic bacteria produce acetic acid from one-carbon compounds, including methanol, carbon monoxide, or a mixture of carbon dioxide and hydrogen:

$$2\ CO_2 + 4\ H_2 \rightarrow CH_3COOH + 2\ H_2O$$

This ability of *Clostridium* to metabolize sugars directly, or to produce acetic acid from less costly inputs, suggests that these bacteria could produce acetic acid more efficiently than ethanol- oxidizers like *Acetobacter*. However, *Clostridium* bacteria are less acid-tolerant than *Acetobacter*. Even the most acid-tolerant *Clostridium* strains can produce vinegar in concentrations of only a few per cent, compared to *Acetobacter* strains that can produce vinegar in concentrations up to 20%. At present, it remains more cost-effective to produce vinegar using *Acetobacter*, rather than using *Clostridium* and concentrating it. As a result, although acetogenic bacteria have been known since 1940, their industrial use is confined to a few niche applications.

Uses

Acetic acid is a chemical reagent for the production of chemical compounds. The largest single use of acetic acid is in the production of vinyl acetate monomer, closely followed by acetic anhydride and ester production. The volume of acetic acid used in vinegar is comparatively small.

Vinyl acetate monomer

The primary use of acetic acid is the production of vinyl acetate monomer (VAM). In 2008, this application was estimated to consume a third of the world's production of acetic acid The reaction consists of ethylene and acetic acid with oxygen over a palladium catalyst, conducted in the gas phase.[45]

$$2\ H_3C{-}COOH + 2\ C_2H_4 + O_2 \rightarrow 2\ H_3C{-}CO{-}O{-}CH{=}CH_2 + 2\ H_2O$$

Vinyl acetate can be polymerised to polyvinyl acetate or other polymers, which are components in paints and adhesives.[45]

Ester production

The major esters of acetic acid are commonly used as solvents for inks, paints and coatings. The esters include ethyl acetate, *n*-butyl acetate, isobutyl acetate, and propyl acetate. They are typically produced by catalyzed reaction from acetic acid and the corresponding alcohol:

Most acetate esters, however, are produced from acetaldehyde using the Tishchenko reaction. In addition, ether acetates are used as solvents for nitrocellulose, acrylic lacquers, varnish removers, and wood stains. First, glycol monoethers are produced from ethylene oxide or propylene oxide with alcohol, which are then esterified with acetic acid. The three major products are ethylene glycol monoethyl ether acetate (EEA), ethylene glycol monobutyl ether acetate (EBA), and propylene glycol monomethyl ether acetate (PMA, more commonly known as PGMEA in semiconductor manufacturing processes, where it is used as a resist solvent). This application consumes about 15% to 20% of worldwide acetic acid. Ether acetates, for example EEA, have been shown to be harmful to human reproduction.

Acetic anhydride

The product of the condensation of two molecules of acetic acid is acetic anhydride. The worldwide production of acetic anhydride is a major application, and uses approximately 25% to 30% of the global production of acetic acid. The main process involves dehydration of acetic acid to give ketene at 700–750 °C. Ketene is thereafter reacted with acetic acid to obtain the anhydride

Acetic anhydride is an acetylation agent. As such, its major application is for cellulose acetate, a synthetic textile also used for photographic film. Acetic anhydride is also a reagent for the production of heroin and other compounds.

Medical use

Acetic acid (medical use)

Acetic acid injection into a tumor has been used to treat cancer since the 1800s. Acetic acid is used as part of cervical cancer screening in many areas in the developing world. The acid is applied to the cervix and if an area of white appears after about a minute the test is positive Acetic acid is an effective antiseptic when used as a 1% solution, with broad spectrum of activity against streptococci, staphylococci, pseudomonas, enterococci and others. It may be used to treat skin infections caused by pseudomonas strains resistant to typical antibiotics. While diluted acetic acid is used in iontophoresis, no high quality evidence supports this treatment for rotator cuff disease

As a treatment for otitis externa, it is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system.

Foods

Vinegar

Acetic acid has 349 kcal per 100 g. Vinegar is typically no less than 4% acetic acid by mass. Legal limits on acetic acid content vary by jurisdiction. Vinegar is used directly as a condiment, and in the pickling of vegetables and other foods. Table vinegar tends to be more diluted (4% to 8% acetic acid), while commercial food pickling employs solutions that are more concentrated. The proportion of acetic acid used worldwide as vinegar is not as large as commercial uses, but is by far the oldest and best-known application.

Citric acid

Citric acid is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industries. It is produced mainly by submerged fermentation using ***Aspergillus niger*** **or** ***Candida*** **sp**. from different sources of carbohydrates, such as molasses and starch based media. However, other fermentation techniques, e.g. solid state fermentation and surface fermentation, and alternative sources of carbon such as agro-industrial residues have been intensively studied showing great perspective to its production.

INTRODUCTION

Citric acid ($C_6H_8O_7$, 2 - hydroxy - 1,2,3 - propane tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid in the field of food (60%) and pharmaceuticals (10%). It has got several other applications in various other fields. Currently, the global production of citric acid is estimated to be around 736000 tones/year (Química e Derivados, 1997), and the entire production is carried out by fermentation. In Brazil, almost the entire demand of citric acid is met through imports. There is constant increase (3.5-4%) each year in its consumption, showing the need of finding new alternatives for its manufacture.

Table 1. Applications of citric acid

Industry	Applications
Beverages	Provides tartness and complements fruits and berries flavors. Increases the effectiveness of antimicrobial preservatives. Used in pH adjustment to provide uniform acidity.
Jellies, Jams and Preserves	Provides tartness. pH adjustment.
Candy	Provides tartness. Minimizes sucrose inversion. Produces dark color in hard candies. Acts as acidulant.
Frozen fruit	Lowers pH to inactivate oxidative enzymes. Protects ascorbic acid by inactivating trace metals
Dairy products	As emulsifier in ice creams and processed cheese; acidifying agent in many cheese products and as an antioxidant.
Fats and oils	Synergist for other antioxidants, as sequestrant.
Pharmaceuticals	As effervescent in powders and tablets in combination with bicarbonates. Provides rapid dissolution of active ingredients. Acidulant in mild astringent formulation. Anticoagulant.
Cosmetics and toiletries	pH adjustment, antioxidant as a metallic–ion chelator, buffering agent.
Industrial applications	Sequestrant of metal ions, neutralizant, buffer agent
Metal cleaning	Removes metal oxides from surface of ferrous and nonferrous metals, for preperational and operational cleaning of iron and copper oxides
Others	In electroplating, copper plating, metal cleaning, leather tanning, printing inks, bottle washing compounds, floor cement, textiles, photographic reagents, concrete, plaster, refractories and moulds, adhesives, paper, polymers, tobacco, waste treatment, etc.

CITRIC ACID PRODUCTION

A large number of micro-organisms including bacteria, fungi and yeasts have been employed to produce citric acid. Most of them, however, are not able to produce commercially acceptable yields. This fact could be explained by the fact that citric acid is a metabolite of energy metabolism and its accumulation rises in appreciable amounts only under conditions of drastic imbalances.

Strains selection and improvement

The two principal methods of selecting populations, namely, "the single-spore technique" and the "passage method" have been used for selecting citric acid producing micro-organisms The single-spore technique has the disadvantage that mineral acid or organic acids (gluconic acid, oxalic acid) simulate the presence of citric acid. Rohr et al. (1979) improved this method by incorporating a specific stain for citric acid (para-di-methylamino benzaldehyde), instead of using the indicator.

PRODUCTION TECHNIQUES AND RAW MATERIALS

Although citric acid is mostly produced from starch or sucrose based media using liquid fermentation, a variety of raw materials such as molasses, several starchy materials and hydrocarbons have also been employed. Rohr et al. (1983) classified raw materials used for citric acid production in to two groups: (i) with a low ash content from which the cations could be removed by standard procedures (e.g. cane or beet sugar, dextrose syrups and crystallized dextrose); (ii) raw materials with a high ash content and high amounts of other non sugar substances (e.g. cane and beet molasses, crude unfiltered starch hydro-lysates).

Several attempts have been made to produce citric acid using molasses, which is preferred due its low cost and high sugar content (40-55%). The composition of molasses depends on various factors, e.g. the kind of beet and cane, methods of cultivation of crops and fertilizers and pesticides applied during cultivation, conditions of storage and handling (e.g. transport, temperature variations), production procedures, etc. Both, cane and beet molasses are suitable for citric acid production. However, beet molasses is preferred due to its lower content of trace metals. Generally, cane molasses contains calcium, magnesium, manganese, iron and zinc, which have a retarding effect on the synthesis of citric acid. Consequently, some pre-treatment is required for the removal/reduction of trace metals. Despite that, cane molasses posses difficulties in achieving good fermentation yields.

Liquid fermentation

Submerged fermentation: The submerged fermentation (SmF) process is the commonly employed technique for citric acid production. It is estimated that about 80% of world production is obtained by SmF. Several advantages such as higher yields and productivity and lower labour costs are the main reasons for this. Two types of fermenters, conventional stirred fermenters and tower fermenters are employed, although the latter is preferred due to the advantages it offers on price, size and operation (Rohr et al., 1983). Preferentially, fermenters are made of high-grade steel and require provision of aeration system, which can maintain a high dissolved oxygen level. Fermenters for citric acid production do not have to be built as pressure vessels since sterilization is performed by simply steaming without applying pressure. Cooling can be done by an external water film over the entire outside wall of the fermenter.

In SmF, different kinds of media are employed such as sugar and starch based media (Table 3). Molasses and other raw materials demand pre-treatment, addition of nutrients and sterilization. Inoculation is performed either by adding a suspension of spores, or of pre-cultivated mycelia. When spores are used, a surfactant is added in order to disperse them in the medium. For pre-cultivated mycelia, an inoculum size of 10% of fresh medium is generally required. Normally, submerged fermentation is concluded in 5 to 10 days depending on the process conditions. It can be carried out in batch, continuous or fed batch systems, although the batch mode more frequently used.

Table 3. Raw materials employed in submerged fermentation for citric acid production

Raw material	Strain	Citric acid	Yield, %	References
Brewery wastes	*A. niger* ATTC 9142	19 g/L	78.5	Roukas & Kotzekidou, 1986
Beet molasses	*A.niger* ATTC 9142	109 g/L	-	Ogawa & Fazeli, 1976
	Yarrow lipolytica A101	54 g/L	68.7 [a]	Kautola et al., 1992
Cane molasses	*A. niger* T 55	-	65	Kundu et al, 1984
Wood Hemicellulose	*A. niger* IMI- 41874	27 g/L	45 [a]	Maddox et al., 1985
	S. lipolytica IFO 1658	9 g/L	41	Maddox et al., 1985
Date syrup	*A. niger* ATTC 9142	-	50	Roukas & Kotzekidou, 1997
Corn starch	*A. niger* IM-155	-	62	Nguyen et al., 1992
Starch hydrolysate	*Y. lipolytica* DS-1	-	-	Shah et al., 1993
	Y. lipolytica A-101	-	75	Wojtatowicz et al., 1993
Rapeseed oil	*Y. lipolytica* A-101	-	57	Wojtatowicz et al., 1993
Soybean oil	*Y. lipolytica* A-101	-	63	Wojtatowicz et al., 1993
Coconut oil	*C.lipolytica* N-5704	-	99.6 [b]	Ikeno et al., 1975
Palm oil	*C.lipolytica* N-5704	-	155 [b]	Ikeno et al., 1975
Olive oil	*C.lipolytica* N-5704	-	119 [b]	Ikeno et al., 1975
Soybean oil	*C.lipolytica* N-5704	-	115 [b]	Ikeno et al., 1975
Glycerol	*C.lipolytica* N-5704	-	58.8 [b]	Ikeno et al., 1975
n-Paraffin	*C.lipolytica* N-5704	-	161 [b]	Ikeno et al., 1975

[a] *based on sugar consumed;* [b] *based on oils and fatty acids*

FACTORS AFFECTING CITRIC ACID PRODUCTION

Medium and its components

Carbon source: Citric acid accumulation is strongly affected by the nature of the carbon source. The presence of easily metabolized carbohydrates has been found essential for good production of citric acid. Sucrose was the most favourable carbon source followed by glucose, fructose and galactose. Galactose contributed to a very low growth of fungi and did not favour citric acid accumulation.Other sources of carbon such as sorbose, ethanol, cellulose, manitol, lactic, malic and a -acetoglutaric acid, allow a limited growth and low production. Starch, pentoses (xyloses and arabinoses), sorbitol and pyruvic acid slow down growth, though the production is minimal

Initial sugar concentration was critical for citric acid production and other organic acids produced by *A. niger*. *A.niger* strains needed an initial sugar concentration of 10-14% as optimal; no citric acid was produced at sugar concentration of less than 2.5%. Immobilized cells of *A.niger* needed lower concentrations of sucrose than free cells culture, in order to obtain high yields (200 g of citric acid/L for free cells culture, and 120 g/L for immobilized cells). Maddox et al. (1985) reported the influence of different sources of carbon on citric acid production by *A. niger* and *Saccharomycopsis lipolytica*. Glucose, maltose, galactose, xylose and arabinose were tested. Fermentation was carried out in 8 and 4 days, respectively, at 30°C and 180 rpm.

Several raw materials can be employed successfully for citric acid production. There are some critical factors (costs, need of pretreatment), which should be considered for substrate determination. One another aspect is the presence of trace elements, which can act as inhibitors or stimulants. Consequently, sometimes it is necessary to conduce a pre-treatment, e.g.; precipitation of trace metals of molasses by potassium ferrocyanide.

Nitrogen source: Citric acid production is directly influenced by the nitrogen source. Physiologically, ammonium salts are preferred, e.g. urea, ammonium sulfate, ammonium chlorure, peptone, malt extract, etc. Nitrogen consumption leads to pH decrease, which is very important point in citric acid fermentation .However, it is necessary to maintain pH values in the first day of fermentation prior to a certain quantity biomass production. Urea has a tampon effect, which assures pH control. The concentration of nitrogen source required for citric acid fermentation is 0.1 to 0.4 N /liter. A high nitrogen concentration increases fungal growth and the consumption of sugars, but decreases the amount of citric acid produced.

Phosphorous source: Presence of phosphate in the medium has a great effect on the yield of citric acid. Potassium dihydrogen phosphate has been reported to be the most suitable phosphorous source. Phosphorous at concentration of 0.5 to 5.0 g/L was required by the fungus in a chemically defined medium for maximum production of citric acid. Phosphate is known to be essential for the growth and metabolism of *A. niger*. Low levels of phosphate favour citric acid production, however, the presence of excess of phosphate was shown to lead to the formation of certain sugar acids, a decrease in the fixation of CO_2, and the stimulation of growth.

Trace elements: Trace element nutrition is probably the main factor influencing the yield of citric acid. A number of divalent metals such as zinc, manganese, iron, copper and magnesium have been found to affect citric acid production by *A. niger*.

Lower alcohols: Addition of lower alcohols enhances citric acid production from commercial glucose and other crude carbohydrate. Appropriate alcohols are methanol, ethanol, iso-propanol or methyl acetate. The optimal amount of methanol/ethanol depends upon the strain and the composition of the medium, generally optimum range being 1-3%.

Miscellaneous: Some compounds which are inhibitors of metabolism such as calcium fluoride, sodium fluoride and potassium fluoride have been found to accelerate the citric acid production, while, potassium ferrocyanide has been found to decrease the yield. There are many compounds, which act in many ways to favour citric acid accumulation. Some of them are capable to impair the action of metal ions and other toxic compounds influence growth during the initial phase. Some of these are: 4-Methyl-umbelliferone, 3-hydroxi-2-naphtoic, benzoic acid, 2-naphtoic acid, iron cyanide, quaternary ammonium compounds, amine oximes, starch, EDTA, vermiculite, etc.

Process parameters

pH: The pH of a culture may change in response to microbial metabolic activities. The most obvious reason is the secretion of organic acids such as citric, acetic or lactic acids, which will cause the pH to decrease. Changes in pH kinetics depend highly also on the micro-organism. With *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., pH can drop very quickly until less than 3.0. For other groups of fungi such as *Trichoderma*, *Sporotrichum*, *Pleurotus* sp., pH is more stable (between 4 and 5). Besides, the nature of the substrate also influences pH kinetics

Generally, a pH below 2.0 is required for optimum production of citric acid. A low initial pH has the advantage of checking contamination and inhibiting oxalic acid formation. A pH of 2.2 was reported to be optimum for the growth of the mould as well as for the production of citric acid whereas, a higher pH i.e. 5.4 and 6.0-6.5 has been found optimum for citric acid production in molasses medium

Aeration: Aeration has been shown to have a determinant effect on citric acid fermentation (Rohr et al., 1983; Dawson et al., 1986). Increased aeration rates led to enhanced yields and reduced fermentation time (Grewal and Kalra, 1995).

The influence of dissolved oxygen concentration on citric acid formation has been examined. It is important to maintain the oxygen concentration above 25% saturation and interruptions in oxygen supply may be quite harmful (Kubicek et al., 1980). The high demand of oxygen is fulfilled by constructing appropriate aeration devices, which is also dependent on the viscosity of the fermentation broth. This is an additional reason why small compact pellets are the preferred mycelial forms of *A. niger* during fermentation (Kubicek and Rohr, 1986). When the organism turns into filamentous developments, e.g. due to metal contamination, the dissolved oxygen tension rapidly falls to less than 50% of its previous value, even if the dry weight has not increased by more than 5%. Aeration is performed during the whole fermentation with the same intensity through the medium at a rate of 0.5 to 1.5 vvm. However, because of economic reasons, it's usually preferred to start with a low aeration rate (0.1 to 0.4 vvm). High aeration rates lead to high amounts of foam, especially during the growth phase. Therefore, the addition of antifoaming agents and the construction of mechanical "defoamers" are required to tackle this proble

Product recovery

The recovery of citric acid from liquid fermentation is generally accomplished by three basic procedures, precipitation, extraction, and adsorption and absorption (mainly using ion exchange resins). Citric acid extracted by this method has been recommended suitable for use in food and drugs. Precipitation is the classical method and it is performed by the addition of calcium oxide hydrate (milk of lime) to form the slightly soluble tri-calcium citrate tetrahydrate. The precipitated tri-calcium citrate is removed by filtration and washed several times with water. It is then treated with sulphuric acid forming calcium sulphate, which is filtered off. Mother liquor containing citric acid is treated with active carbon and passed through cation and anion exchangers. Several anion-exchange resins are commercially available. Finally, the liquor is concentrated in vacuum crystallizers at 20-25°C, forming citric acid monohydrate. Crystalization at temperatures higher to this is used to prepare anhydrous citric acid.

Applications of citric acid

Citric acid is mainly used in food industry because of its pleasant acid taste an high solubility in water. It is worldwide accepted as "GRAS" (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives. The pharmaceutical and cosmetic industries retain 10% of its utilization and the remainder is used for various other purposes. Table 1 presents main applications of citric acid.

WINE

Fermentation

The process of alcoholic fermentation requires careful control for the production of high quality wines. Requirements include suppression of the growth of undesirable microorganisms, presence of adequate numbers of desirable yeasts, proper nutrition for yeast growth, temperature control for prevention of excessive heat, prevention of oxidation, and proper management of the cap of skins floating in red musts.

Grape skins are normally covered with bacteria, molds, and yeast. The wild yeasts such as *Pichia*, *Kloeckera*, and *Torulopsis* are often more numerous than the wine yeast *Saccharomyces.* Although species of *Saccharomyces* are generally considered more desirable for efficient alcoholic fermentation, it is possible that other yeast genera may contribute to flavour, especially in the early stages of fermentation. *Saccharomyces* is preferred because of its efficiency in converting sugar to alcohol and because it is less sensitive to the inhibiting effect of alcohol. Under favourable conditions, strains of *Saccharomyces cerevisiae* have produced up to 18 percent (by volume) of alcohol, although 15 to 16 percent is the usual limit.

Use of the yeast *Schizosaccharomyces pombe* has been proposed for the early stages of alcoholic fermentation. Because it metabolizes malic acid, this yeast would be useful in excessively acid musts, but commercial applications have not yielded consistently favourable results. The addition of lactic-acid bacteria to musts, using strains metabolizing malic acid, is now common.

The number of undesirable microorganisms is greatest in partially rotted or injured grapes. Such damage may occur in harvesting or during transportation, particularly in warm climates. Suppression of undesirable microorganism growth is required, and the most common method used is the addition of sulfur dioxide to the freshly crushed grapes at the rate of about 100 to 150 milligrams per litre. Sulfur dioxide is more toxic to undesirable microorganisms than to desirable microorganisms. When it is used in musts, an inoculum of the desired yeast strain, usually called a pure yeast culture, is added. Musts are rarely pasteurized, although this process may be applied when they contain undesirable amounts of oxidizing enzymes from moldy grapes.

Enologists, technicians in the science of wine making, do not agree on the most desirable yeast species and strain, but strains of *S. cerevisiae* are generally used. The chosen strain is allowed to multiply as much as possible in sterilized grape juice and is then transferred to larger containers of sterilized grape juice, where it continues to grow until the desired volume is reached. Suitable pressed yeasts of desirable strains are added directly, avoiding the troublesome practice of building up and maintaining a pure yeast culture. About 1 to 3 percent of a pure yeast culture, or sufficient pressed yeast to provide a population of 1,000,000 cells per millilitre, is used.

Temperature control during alcoholic fermentation is necessary to (1) facilitate yeast growth, (2) extract flavours and colours from the skins, (3) permit accumulation of desirable by-products, and (4) prevent undue rise in temperature, killing the yeast cells.

Optimum temperature for growth of common wine yeasts is about 25 °C (77 °F), and in many viticultural areas of the cooler temperate zone, grapes are crushed at about this temperature. Fermentation is seldom started at so high a temperature, however, because it is then difficult to prevent the temperature from exceeding 30 °C during fermentation.

Extraction of flavours and colours is not a problem in white musts; the crushed grape mass is usually separated from the skins before fermentation. Fermentation of white musts at relatively cool temperature (about 10 to 15 °C [50 to 60 °F]) apparently results in greater formation and retention of desirable by-products. An undesirable feature of such relatively low-temperature fermentations is the longer period required for completion (six to 10 weeks compared to one to four weeks at higher temperatures) and the tendency for the fermentation to stop while residual sugar remains. (This is not always considered undesirable—i.e., in German wine production.) In practice white table wines are usually fermented at about 20 °C.

In red wine musts, the optimum colour extraction consistent with yeast growth occurs at about 22 to 28 °C (72 to 82 °F). Alcoholic fermentation produces heat, however, and careful temperature control is required to prevent the temperature from reaching a point (about 30 °C) where yeast growth is seriously restricted. At still higher

temperatures, growth will stop completely. Modern temperature control is accomplished by use of heat exchangers. Older methods include placing the fermenters in a cold room; using cold pipes in the fermenter; pumping the must through double-walled pipes, with cold water in the surrounding pipe; pumping the must through a sump containing cooling coils; and pumping the coolant through jackets surrounding the tank.

Contact with air must be restricted to prevent oxidation during fermentation. In very large containers, the volume of carbon dioxide given off is sufficient to prevent entry of air. In small fermenters, fermentation traps are inserted, preventing entry of air but permitting exit of carbon dioxide. These traps are particularly desirable during the final stage of fermentation, when carbon dioxide evolution is slow. Following fermentation, small amounts of sulfur dioxide are added to help prevent oxidation. Ascorbic acid (50 to 100 milligrams per litre) is sometimes employed to decrease the oxidation and thus the amount of sulfur dioxide required as an antioxidant, but is not generally recommended.

The cap of skins and pulp floating on top of the juice in red-wine fermentation inhibits flavour and colour extraction, may rise to an undesirably high temperature, and may acetify if allowed to become dry. Such problems are avoided by submerging the floating cap at least twice daily during fermentation. This operation, comparatively easy with small fermenters, becomes difficult with large, tall fermenters of up to 100,000-gallon (380,000-litre) capacity. In large units the fermenting must is drawn off near the bottom and pumped back over the cap. The use of small fermentation vessels permits a greater percentage of heat loss to the surrounding atmosphere, simplifying temperature control.

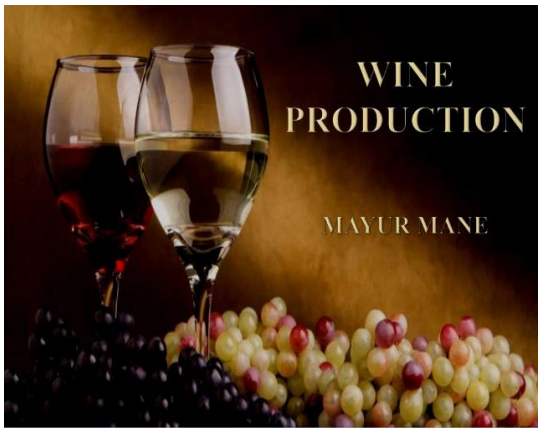
Post fermentation treatment

With appropriate must composition, yeast strain, temperature, and other factors, alcoholic fermentation ceases when the amount of fermentable sugar available becomes very low (about 0.1 percent). Fermentation will not reach this stage when (1) musts of very high sugar content are fermented, (2) alcohol-intolerant strains of yeast are used, (3) fermentations are carried on at too low or high temperatures, and (4) fermentation under pressure is practiced. Fermentation of normal musts is usually completed in 10 to 30 days. In most cases, the major portion of the yeast cells will soon be found in the sediment, or lees. Separation of the supernatant wine from the lees is called racking. The containers are kept full from this time on by “topping,” a process performed frequently, as the temperature of the wine, and hence its volume, decreases. During the early stages, topping is necessary every week or two. Later, monthly or bimonthly fillings are adequate.

Normally the first racking should be performed within one to two weeks after completion of fermentation, particularly in warm climatic regions, as the yeasts in the thick deposit of lees may autolyze (digest themselves), forming off-odours.

Early racking is not required for wines of high total acidity—i.e., those produced in cool climatic regions or from high-acid varieties. Such wines may remain in contact with at least a portion of the lees for as long as two to four months, permitting some yeast autolysis in order to release amino acids and other possible growth factors favouring growth of lactic-acid bacteria. These bacteria then induce the second, or malolactic, fermentation.

Wine



Wine Production

1. Viticulture

Factors which inflence grape's flavor:

- •climate of the vineyard's region
- •drainage around the vines
- •humidity of the region
- •sun exposure.
- •soil quality

2. Harvesting

- •Grappes are picked up by hand or mechanically
- •Descision of harvest informed by level of sugar and acid
- •weather forecasts

3. Stemming/Crushing

Stemming is the separation of the stems and grapes (which are sends to the press)

Crushing: A horizontal press squeezes the broken grapes, separating the fresh juice (must) from the skins (marc)
After crushing starts the fermentation process.

4. Fermentation

- •sugar and acids that naturally react with wild yeasts
- •Vineyard adding their own yeasts
- •fermentation can take from 10 to 30 days to convert natural sugar to alcohol.

5. Draining

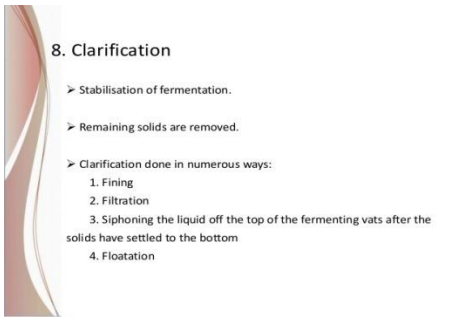
Liquid wine is drained from the vat without being pressed and go into barrels (**free-run wine**). The remaining pulp retains about 20% of the wine.

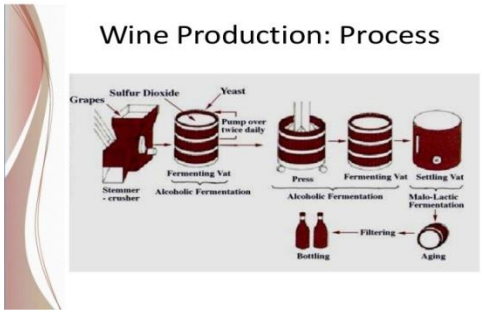
6. Pressing

The remaing pulp, after draining, is pressed to squeeze out the **press wine**.

7. Mixing

The free-run wine and press wine, always from the same source, are mixed together in appropriate ratios to obtain the desired balance.





Milk Products

Cheese

Cheese Definitions

Cheese comes in many varieties. The variety determines the ingredients, processing, and characteristics of the cheese. The composition of many cheeses is defined by Standards of Identity in the U.S. Code of Federal Regulations (CFR).

Cheese can be made using pasteurized or raw milk. Cheese made from raw milk imparts different flavors and texture characteristics to the finished cheese. For some cheese varieties, raw milk is given a mild heat treatment (below

pasteurization) prior to cheese making to destroy some of the spoilage organisms and provide better conditions for the cheese cultures. Cheese made from raw milk must be aged for at least 60 days, as defined in the CFR, section 7 CFR 58.439, to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk. For some varieties cheese must be aged longer than 60 days.

Cheese can be broadly categorized as acid or rennet cheese, and natural or process cheeses. Acid cheeses are made by adding acid to the milk to cause the proteins to coagulate. Fresh cheeses, such as cream cheese or queso fresco, are made by direct acidification. Most types of cheese, such as cheddar or Swiss, use rennet (an enzyme) in addition to the starter cultures to coagulate the milk. The term "natural cheese" is an industry term referring to cheese that is made directly from milk. Process cheese is made using natural cheese plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life.

Ingredients

The main ingredient in cheese is milk. Cheese is made using cow, goat, sheep, water buffalo or a blend of these milks. The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid source such as acetic acid (the acid in vinegar) or gluconodelta-lactone (a mild food acid) is used. For rennet cheeses, calf rennet or, more commonly, a rennet produced through microbial bioprocessing is used. Calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk.

Flavorings may be added depending on the cheese. Some common ingredients include herbs, spices, hot and sweet peppers, horseradish, and port wine.

Bacterial Cultures

Cultures for cheese making are called lactic acid bacteria (LAB) because their primary source of energy is the lactose in milk and their primary metabolic product is lactic acid. There is a wide variety of bacterial cultures available that provide distinct flavor and textural characteristics to cheeses. Starter cultures are used early in the cheese making process to assist with coagulation by lowering the pH prior to rennet addition. The metabolism of the starter cultures contribute desirable flavor compounds, and help prevent the growth of spoilage organisms and pathogens. Typical starter bacteria include *Lactococcus lactis* subsp. *lactis* or *cremoris*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbruckii* subsp. *bulgaricus*, and *Lactobacillus helveticus*.

Adjunct cultures are used to provide or enhance the characteristic flavors and textures of cheese. Common adjunct cultures added during manufacture include *Lactobacillus casei* and *Lactobacillus plantarum* for flavor in Cheddar cheese, or the use of *Propionibacterium freudenreichii* for eye formation in Swiss. Adjunct cultures can also be used as a smear for washing the outside of the formed cheese, such as the use of *Brevibacterium linens* of gruyere, brick and limburger cheeses.

Yeasts and molds are used in some cheeses to provide the characteristic colors and flavors of some cheese varieties. Torula yeast is used in the smear for the ripening of brick and limburger cheese. Examples of molds include *Penicillium camemberti* in camembert and brie, and *Penicillium roqueforti* in blue cheeses.

General Manufacturing Procedure

The temperatures, times, and target pH for different steps, the sequence of processing steps, the use of salting or brining, block formation, and aging vary considerably between cheese types. The following flow chart provides a

very general outline of cheese making steps. The general processing steps for Cheddar cheese are used for illustration.

General Cheese Processing Steps

- Standardize Milk
- Pasteurize/Heat Treat Milk
- Cool Milk
- Inoculate with Starter & Non-Starter Bacteria and Ripen
- Add Rennet and Form Curd
- Cut Curd and Heat
- Drain Whey
- Texture Curd
- Dry Salt or Brine
- Form Cheese into Blocks
- Store and Age
- Package

The times, temperatures, and target pH values used for cheddar cheese will depend on individual formulations and the intended end use of the cheese. These conditions can be adjusted to optimize the properties of Cheddar cheese for shredding, melting, or for cheese that is meant to be aged for several years.

1. Standardize Milk

Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield

2. Pasteurize/Heat Treat Milk

Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Some varieties of milk are made from raw milk so they are not pasteurized or heat-treated. Raw milk cheeses must be aged for at least 60 days to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk.

3. Cool Milk

Milk is cooled after pasteurization or heat treatment to 90°F (32°C) to bring it to the temperature needed for the starter bacteria to grow. If raw milk is used the milk must be heated to 90°F (32°C).

4. Inoculate with Starter & Non-Starter Bacteria and Ripen

The starter cultures and any non-starter adjunct bacteria are added to the milk and held at 90°F (32°C) for 30 minutes to ripen. The ripening step allows the bacteria to grow and begin fermentation, which lowers the pH and develops the flavor of the cheese.

5. Add Rennet and Form Curd

The rennet is the enzyme that acts on the milk proteins to form the curd. After the rennet is added, the curd is not disturbed for approximately 30 minutes so a firm coagulum forms.

6. Cut Curd and Heat

The curd is allowed to ferment until it reaches pH 6.4. The curd is then cut with cheese knives into small pieces and heated to 100°F (38°C). The heating step helps to separate the whey from the curd.

7. Drain whey

The whey is drained from the vat and the curd forms a mat.

8. Texture curd

The curd mats are cut into sections and piled on top of each other and flipped periodically. This step is called **chattering**. Chattering helps to expel more whey, allows the fermentation to continue until a pH of 5.1 to 5.5 is reached, and allows the mats to "knit" together and form a tighter matted structure. The curd mats are then milled (cut) into smaller pieces.

9. Dry Salt or Brine

For cheddar cheese, the smaller, milled curd pieces are put back in the vat and salted by sprinkling dry salt on the curd and mixing in the salt. In some cheese varieties, such as mozzarella, the curd is formed into loaves and then the loaves are placed in a brine (salt water solution).

10. Form Cheese into Blocks

The salted curd pieces are placed in cheese hoops and pressed into blocks to form the cheese.

11. Store and Age

The cheese is stored in coolers until the desired age is reached. Depending on the variety, cheese can be aged from several months to several years.

12. Package - Cheese may be cut and packaged into blocks or it may be waxed.

YOHURT

Yohurt also spelled yoghurt, yogourt, or yoghourt is a food produced by bacterial fermentation of milk. The bacteria used to make yogurt are known as *yogurt cultures*. Fermentation of sugars in the milk by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tart flavor. Cow's milk is commonly available worldwide and, as such, is the milk most commonly used to make yogurt. Milk from water buffalo, goats, ewes, mares, camels, yaks and plant milks are also used to produce yogurt. The milk used may be homogenized or not. It may be pasteurized or raw. Each type of milk produces substantially different results.

Yoghurt is produced using a culture of Lactobaccillus delbrueckii subsp. Bulgaricus and streptococcus bacteria. In addition, other lactobacilli and bifidobacteria are sometimes added during or after culturing yogurt. Some countries require yogurt to contain a certain amount of colony-forming units (CFU) of bacteria; in China, for example, the requirement for the number of lactobacillus bacteria is at least 1 million CFU per milliliter.

To produce yogurt, milk is first heated, usually to about 85 °C (185 °F), to denature the milk proteins so that they do not form curds. After heating, the milk is allowed to cool to about 45 °C (113 °F). The bacterial culture is mixed in, and that temperature of 45 °C is maintained for 4 to 12 hours to allow fermentation to occur.

Production

Yogurt is made by heating milk to a temperature that denaturates its proteins (scalding), essential for making yogurt,[50] cooling it to a temperature that will not kill the live microorganisms that turn the milk into yogurt, inoculating certain bacteria (starter culture), usually *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, into the milk, and finally keeping it warm for several hours. The milk may be held at 85 °C (185 °F) for a few minutes, or boiled (giving a somewhat different result). It must be cooled to 50 °C (122 °F) or somewhat less, typically 40–46 °C (104–115 °F). Starter culture must then be mixed in well, and the mixture must be kept undisturbed and warm for some time, anywhere between 5 and 12 hours. Longer fermentation times produces a more acidic yogurt. The starter culture may be a small amount of live (not sterilized) existing yogurt or commercially available dried starter culture.

Milk with a higher concentration of solids than normal milk may be used; the higher solids content produces a firmer yogurt. Solids can be increased by adding dried milk. The yogurt-making process provides two significant barriers to pathogen growth, heat and acidity (low pH). Both are necessary to ensure a safe product. Acidity alone has been questioned by recent outbreaks of food poisoning by *E. coli O157:H7* that is acid-tolerant. *E. coli O157:H7* is easily destroyed by pasteurization (heating); the initial heating of the milk kills pathogens as well as denaturing proteins. The microorganisms that turn milk into yogurt can tolerate higher temperatures than most pathogens, so that a suitable temperature not only encourages the formation of yogurt, but inhibits pathogenic microorganisms. Once the yogurt has formed it can, if desired, be strained to reduce the whey content and thicken it.

Commercial yogurt

Two types of yogurt are supported by the Codex Alimentarius for import and export.

- Pasteurized yogurt ("heat treated fermented milk") is yogurt pasteurized to kill bacteria.
- Probiotic yogurt (labeled as "live yogurt" or "active yogurt") is yogurt pasteurized to kill bacteria, with *Lactobacillus* added in measured units before packaging
- Yogurt probiotic drink is a drinkable yogurt pasteurized to kill bacteria, with *Lactobacillus* added before packaging.

Under US Food and Drug Administration regulations, milk must be pasteurized *before* it is cultured, and may optionally be heat treated after culturing to increase shelf life. Most commercial yogurts in the United States are not heat treated after culturing, and contain live cultures.

Yogurt with live cultures is more beneficial than pasteurized yogurt for people with lactose malabsorption.

Penecillin

Fermentation biotechnology for penicillin production:

- By fermentation technology penicillin is produced from Penicillium spp. If penicillin fermentation is carried out without addition of side chain precursor, the natural penicillins are produced. But fermentation can be better controlled by adding a side chain precursor to obtain derived penicillin. The synthetic penicillins are produced by enzymatic hydrolysis of 6APA by penicillin acylase enzyme and then addition of desired side chain by chemical means,
- B-lactum thiozolidine ring of penicillin is constructed from l-cystine and l-valine. These two amino acids when combined with L-α-aminoadipic acid (α-AAA) the tripeptide is formed which undergoes two step cyclization process to give isopenicillin.

Regulation of penicillin production:

- The amino acids lysine is synthesized from a pathway that involves L-α-AAA, so that penicillin and lysine share a common but branched biosynthetic pathway. Higher concentration of lysine causes feed back inhibition of homocitrate synthase, an enzyme involved in α-AAA synthesis. Either lysine level should keep low or α-AAA level should added during fermentation.
- Penicillin biosynthesis is affected by Po4—concentration and also shows a distinct catabolic repression by glucose. Therefore, either slowly metabolizable sugars such as lactose is used or fed continuously with glucose with small dose.

Penicillin Production process:

- Penicillin production is previously achieved by surface process ie. Solid state fermentation and surface liquid fermentation. Nowadays a commercial production is carried out by fed batch process
- **Inoculum (Organism): *Penicillium chrysogenum* (improved strain)**

i. Inoculum preparation:

- For inoculum preparation, spore from heavily sporulated working stocks are suspended inwater or non-toxic wetting agensts (sodium sulfonate 1: 10000)
- Theses spore are then added to flask containing wheat bran and nutrient solution for heavy sporulation
- Incubate for 5-7 days at 24C
- Spore are then transferred to seed tank and incubated for 24-48 hours at 24C with aeration and agitation for sufficient mycelial growth
- These mycelia can be used for production fermenter

ii. Production fermentation:

- **Method:** fed-batch or batch

- **Substrate:** glucose, phenoxyacetic acid (fed component used for production of side chain), Corn steep liquor, Additional nitrogen source ie, soyameal, yeast extract, Lactic acid, inorganic ions, growth factors
- **Fermenter:** stirred tank or air lift tank
- **pH**: set at 5.5 t0 6.0 which increased upto 7-7.5 (optimum) due to liberation of NH3 gas and consumption of lactic acid. If pH is 8 or more, CaCO3 or MgCO3 or phosphate buffer is added
- **temperature:** 25-27 C
- **aeration:** 0.5-1 vvm (initially more, latter less O2)
- **agitation:** 120-150 rpm)
- **time:** 3-5 days
- **antiform:** edible oil (0.25%)

iii. Product recovery:

- harvest broth from fermenter tank by filtration (rotary vacuum filtration)
- chill to 5-10 C (because penicillin is highly reactive and destroyed by alkali and enzyme)
- acidify filtrate to pH 2.0-2.5 with H2SO4 (to convert penicillin to its anionic form)
- extract penicillin from aqueous filtrate into butyl acetate or amyl acetate (at this very low pH as soon as possible in centrifugal counter current extractor)
- discard aqueous fraction
- allow the organic solvent to pass through charcoal to remove impurities and extract penicillin from butyl acetate to 2% aqueous phosphate buffer at pH 7.5
- acidify the aq. Fraction to pH 2-2.5 with mineral acid and re-extract penicillin into fresh butyl acetate (it concentrated up to 80-100 times)
- add potassium acetate to the solvent extract in a crystallization tank to crystallize as potassium salt
- recover crystal in filter centrifuge
- sterilization
- further processing
- packaging

Application of penicillin:

- clinical uses of penicillin:
- naturally effective antibiotics against gram + bacteria
- used for treatment of bacterial endocarditis

Streptomycin

Streptomycin is an antibiotic medication used to treat a number of bacterial infections. This includes tuberculosis Mycobacterium avium complex endocarditis, brucellosis, *Burkholderia* infection, plague, tularemia, and rat bite fever. For active tuberculosis it is often given together with isoniazid, rifampicin, and pyrazinamide. It is given by injection into a vein or muscle

Common side effects include vertigo, vomiting, numbness of the face, fever, and rash. Use during pregnancy may result in permanent deafness in the developing baby. Use appears to be safe while breastfeeding. It is not recommended in people with myasthenia gravis or other neuromuscular disorders.[4] Streptomycin is an amino

glycoside. It works by blocking the ability of 30S ribosomal subunits to make proteins, which results in bacterial death.

Streptomycin was discovered in 1943 from *Streptomyces griseus*. It is on the World Health Organization's List of Essential Medicines. The World Health Organization classifies it as critically important for human medicine.

Mechanism of action

Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome irreversibly, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the mRNA strand. This results in an unstable ribosomal-mRNA complex, leading to a frame shift mutation and defective protein synthesis; leading to cell death. Humans have ribosomes which are structurally different from those in bacteria, so the drug does not have this effect in human cells. At low concentrations, however, streptomycin only inhibits growth of the bacteria by inducing prokaryotic ribosomes to misread mRNA. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria, and is therefore a useful broad-spectrum antibiotic.

Streptomycin is obtained by inoculating a sterilized nutrient medium with a microorganism (Sterptomycis griseus) capable of producing the same and aerobically fermenting the culture for a suitable period of time,

One common material of this description well known to the trade contains about one-quarter mineral ash, about one-half carbohydrate, about one-eighth protein, and is unusually rich in usually about 48-96 hours. The microorganism vitamin B complex. commonly employed is Actinomyces. S.griseus, which is seeded in the medium amount of fermentation solubles in a medium in amount of about five per cent of the total volume containing no other protein, The medium commonly 1y to produce streptomycin in high yield.

About 100 liters, of liquid culture medium was prepared comprising the following ingredients: 1000 grams dextrose; 250 grams ammonium sulfate; 500 grams sodium chloride; 100 grams potassium hydrogen phosphate; 25 grams MgSOq-7Hz0; 100 grams fermentation solubles; and sufficient tap water to make up about 100 liters. In a separate container 400 grams of calcium carbonate was suspended in about 1500 milliliters of water. The medium and the calcium carbonate suspension were sterilized separately by autoclaving for 30 minutes at about 15 pounds steam pressure. The medium was cooled, the calcium carbonate suspension was added there to, and seeded with about 5 liters of a three-day-old culture of Actinomuces ariseus grown on a suitable medium.

The incubation was carried out at 22-24 degrees centigrade and the brew was vigorously agitated and aerated by the passage of air there through at a rate of about one cubic foot per liter per hour. After 70 hours the fermentation was stopped. The product contained about 14 grams of streptomycin averaging 500 units per milligram as tested by current assay.

Uses

Medication

- Infective endocarditis: An infection of the endocardium caused by enterococcus; used when the organism is not sensitive to gentamicin
- Tuberculosis: Used in combination with other antibiotics. For active tuberculosis it is often given together with isoniazid, rifampicin, and pyrazinamide. It is not the first-line treatment, except in medically under-served

populations where the cost of more expensive treatments is prohibitive. It may be useful in cases where resistance to other drugs is identified.

- Plague (*Yersinia pestis*): Has historically been used as the first-line treatment. However streptomycin is approved for this purpose only by the US Food and Drug Administration.
- In veterinary medicine, streptomycin is the first-line antibiotic for use against gram negative bacteria in large animals (horses, cattle, sheep, etc.). It is commonly combined with procaine penicillin for intramuscular injection.
- Tularemia infections have been treated mostly with streptomycin.[9]

Streptomycin is traditionally given intramuscularly, and in many nations is only licensed to be administered intramuscularly, though in some regions the drug may also be administered intravenously.

Fungicide

Streptomycin also is used as a fungicide, to combat the growth of bacteria beyond human applications. Streptomycin controls bacterial diseases of certain fruit, vegetables, seed, and ornamental crops. A major use is in the control of fire blight on apple and pear trees. As in medical applications, extensive use can be associated with the development of resistant strains. Streptomycin could potentially be used to control cyano bacterial blooms in ornamental ponds and aquaria.[10] While some antibacterial antibiotics are inhibitory to certain eukaryotes, this seems not to be the case for streptomycin, especially in the case of anti-fungal activity.

Cell culture

Streptomycin, in combination with penicillin, is used in a standard antibiotic cocktail to prevent bacterial infection in cell culture.

Protein purification

When purifying protein from a biological extract, streptomycin sulfate is sometimes added as a means of removing nucleic acids. Since it binds to ribosomes and precipitates out of solution, it serves as a method for removing rRNA, mRNA, and even DNA if the extract is from a prokaryote.

Side effects

The most concerning side effects, as with other amino glycosides, are kidney toxicity and ear toxicity. Transient or permanent deafness may result. The vestibular portion of cranial nerve VIII (the vestibulocochlear nerve) can be affected, resulting in tinnitus, vertigo, ataxia, kidney toxicity, and can potentially interfere with diagnosis of kidney malfunction

Common side effects include vertigo, vomiting, numbness of the face, fever, and rash. Fever and rashes may result from persistent use.

Use is not recommended during pregnancy. Congenital deafness has been reported in children whose mothers received streptomycin during pregnancy. Use appears to be okay while breastfeeding.

It is not recommended in people with myasthenia gravis.

Biopesticides

What are Biopesticides?

Biopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) Certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides.

- Classes of biopesticides
- Advantages of using biopesticides
- How EPA (Environment protection agency) encourages the development and use of biopesticides

Classes of Biopesticides

Biopesticides fall into three major classes:

1. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances that interfere with mating, such as insect sex pheromones, as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, EPA has established a special committee to make such decisions.
2. Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds and other fungi that kill specific insects.
3. The most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis, or Bt. Each strain of this bacterium produces a different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.
4. Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium,

manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.

What are the advantages of using biopesticides?

- Biopesticides are usually inherently less toxic than conventional pesticides.
- Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals.
- Biopesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
- When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the use of conventional pesticides, while crop yields remain high.

To use biopesticides effectively (and safely), however, users need to know a great deal about managing pests and must carefully follow all label directions.

Trichoderma Viride -Biopesticide Application in Agriculture

Trichoderma viride is a high-efficiency organic biological agent. It produces antibiotics, nutrient competition, parasitic, cell-wall degradation, enzymes, and induced plant resistance mechanisms, which have antagonism effect on a variety of plant pathogenic fungi. Protection and treatment can effectively control soil spread fungus diseases with dual effect**.**

Use Trichoderma viride products, which can enhance the survival rate of seedling and transplant, keep the seedlings robust growth. It can also be used to prevent grey mold.Features of Trichoderma viride:

1, Through the protection and treatment, can effectively prevent and control root rot, cataplexy, blight, wilt, verticillium wilt, anthrax, and other soil-borne diseases;

2, Improve the soil, break the knot, improve the soil permeability and oxygen supply of the root system;

3. Promote root growth, make crop growth more vigorous and increase crop yield, etc.

Mechanism of Action of Trichoderma viride

1. **Competitive effect**: Trichoderma viride has a fast growth and reproduction rate, and can quickly absorb and utilize nutrients, water, space and oxygen in the soil, thus worsening the living environment of plant pathogens;

2. **Antibiosis**: It secretes antibacterial substances to inhibit the growth of plant pathogens;

3. **Symbiosis and resistance induction**: the co-growth of Trichoderma viride and plant roots activates the plant's internal defense system and improves the plant's disease resistance;

4. **Heavy parasitism**: Trichoderma viride mycelium will grow, twine and Pierce along the mycelium of plant pathogens and absorb the nutrients in the mycelium of plant pathogens, leading to the death of plant pathogens;

5. **Promote plant growth**:

1) Increase plant growth and yield.

2) Increase the systemic disease resistance of plants.

3) Increase root growth and tolerance to drought.

4) Increase the absorption of nutrients and the effective use of fertilizers

5) Improve the light and efficiency of plants.

6) Increase the rate and percentage of seed germination.

Attentions of using Trichoderma viride

1.Trichoderma viride is a microbial fungus, mainly saprophytic, which can grow only when the soil has a certain humidity and temperature.

2. It should be used in the soil, and avoid spraying on the stems and leaves;

3. Avoid using chemical pesticides with strong acids and bases as much as possible;

4, Trichoderma itself is harmless to crops, continuous use is better.

Bacillus sp as biopesticides

Bacillus thuringiensis (*Bt*) is a rod shaped, gram-positive bacteria that forms a spore and is found in the soil. Classification of this bacteria includes Bacteria (domain); Eubacteria (kingdom); Firmicutes (phylum); Bacilli (class); Bacillales (order); Bacillaceae (family). *Bt* was isolated in 1901 and named in 1911 Microbial disruptors of insect midgut membranes. *Bt* is toxic to caterpillars, some fly larvae, and some beetle larvae but not toxic to other organisms. *Bt* var. kurstaki is toxic to lepidopteran (butterfly, skipper, and moth) larvae; *Bt* var. aizawai is toxic to wax moth larvae; *Bt* var. israelensis is toxic to mosquito, midge, fungus gnats, and blackfly larvae; *Bt* var. galleriae is toxic to larvae of May or June beetles (white grubs); *Bt* var. tenebrionis is toxic to Colorado potato beetle, elm leaf beetle, and willow leaf beetle larvae. However, *Bt* var. tenebrionis does not kill all leaf beetles.

Bt strains are very specific to the insects they kill. Therefore, identification of the injurious insect is very important. The correct strain must be applied to susceptible insects. Applications of *Bt* to insects that are not susceptible will be ineffective. *Bt* is most effective against young larvae and usually does not kill adults or other stages of an insect. Insects must eat *Bt* for it to be effective, and good coverage is important. Some insects do not eat the outside of the plant part they attack and applications of *Bt* on the surface of the plant are ineffective against them. For example, the pecan nut case bearer bites the outside of nutlets and spits it out. This insect eats the inside of nutlets and does not eat the *Bt*. *Bt* as a biopesticide applied to plants is not systemic or translaminar and does not kill on contact. It is not toxic to beneficial and is listed as an organic insecticide.

Bt is rapidly deactivated by ultraviolet radiation. Applications made in the evening, on cloudy, or on rainy days last longer. However, heavy rains wash *Bt* off the plant. Applications become inactivated in one to a few days and may need to be reapplied in 3 to 7 days. Applications for leaf beetles may be effective for only one day. Applications of *Bt* do not result in continuous management of insects by reproduction of bacterial cells, and *Bt* is applied similar to chemical insecticides. Once a solution of *Bt* is prepared it should be used immediately; especially, if the water used to make the solution has a pH greater than 7 (basic).

The effectiveness of *Bt* may be reduced after two or three years of storage. Dry formulations last longer than liquid formulations. *Bt* products should be stored out of sunlight and in cool, dry conditions.

A crystalline toxin and spore is usually produced by *Bt* cells. The toxin is called a delta endotoxin. *Bt* products usually contain the toxin and spores (environmental resistant stage of the bacterium) but some products do not contain spores. Spores may become bacterial cells inside the insect. Once the insect eats the *Bt* the delta endotoxin is activated in the insect's gut by enzymes and alkaline (basic) conditions of the gut. A specific pH is required to activate the endotoxin. The endotoxin disrupts the cell walls of the gut. Bacterial cells enter the body of the insect. Infected insects stop feeding in a few hours and die in a few hours to weeks (frequently 2-3 days). Different strains of *Bt* have different endotoxin and kill different insects. The endotoxin is not activated in the gut of humans.

In summary, *Bt* is a microbial biopesticide that is very specific to certain insects. It causes insects to stop feeding in a few hours and usually kills insects in a few days. It must be eaten and kills larvae. It does not last long on the plant, may require frequent applications, is considered organic, and is not toxic to beneficial.

Bio-pesticides (Trichoderma)

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