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Unit 1 : Nature and Properties of viruses

Module Name : Isolation and Purification of viruses.

Module Number : 5

Name of the Presenter: Dr.Rasika Desai Gaokar

Notes

- **Isolation of Viruses:**
- Viruses require a living host cell for replication.
- Many viruses can be isolated as a result of their ability to form visible zones called plaque in layers of host cell.
- If a layer of cells is inoculated with the amount of virus to infect a small proportion of cells, than plaques may formed where the cells are killed or altered by virus infection.
- Each plaque is formed when infection spreads rapidly from the infected cell to the surrounding cell.

- Many viruses can be isolated as a result of their ability to form discrete visible zones (plaques) in layers of host cells. If a confluent layer of cells is inoculated with virus at a concentration so that only a small proportion of the cells is infected, then plaques may form where areas of cells are killed or altered by the virus infection.
- Each plaque is formed when infection spreads radially from an infected cell to surrounding cells.
- Plaques can be formed by many animal viruses in monolayers if the cells are overlaid with agarose gel to maintain the progeny virus in a discrete zone

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- Plaques can also be formed by phages in lawns of bacterial growth . It is generally assumed that a plaque is the result of the infection of a cell by a single virion.
- If this is the case then all virus produced from virus in the plaque should be a clone, in other words it should be genetically identical. This clone can be referred to as an isolate, and if it is distinct from all other isolates it can be referred to as a strain.
- This is analogous to the derivation of a bacterial strain from a colony on an agar plate. There is a possibility that a plaque might be derived from two or more virions so, to increase the probability that a genetically pure strain of virus has been obtained, material from a plaque can be inoculated onto further monolayers and virus can be derived from an individual plaque. The virus is said to have been plaque purified.

- **PURIFICATION OF VIRUSES**
- Viruses can be purified in numerous ways. The methods for purification include:
- 1. Centrifugation
- 2.Precipitation
- 3.Chromatography
- 4.Nanofiltration
- Centrifugation:
- Centrifugation at different speeds separates virus particles and host components of different densities. Low speed 500-10,000 rpm centrifugation, is used in the initial stages of clarifying the crude infective plant extract to sediment the gross host material.
- High speed centrifugation at speeds of 30,000 rpm is used at later stages to get relatively pure virus, devoid at most of the host components.
- **TYPES OF CENTRIFUGATION**

- Differential centrifugation
  Involves alternating low speed centrifugation after which most of the virus is still in the supernatant and then higher speed centrifugation after which the virus is seen in the pellet.
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- A crude preparation of virus containing host debris is subjected to low speed/short time centrifugation followed by high speed long time centrifugation.
- This cycle is repeated to obtain high degree of purity of the virus.
- The final pellet obtained contains the purified virus.
- 2) Density Gradient Centrifugation
- Involves centrifuging particles such as virion and cell debris in a solution of increasing concentration.
- eg : In a sucrose gradient the solutes used have high solubility
- The most commonly used is sucrose some times cesium chloride can also be used.
- There are two major categories of density gradient centrifugation
- a) Rate zonal Centrifugation
- b) Equilibrium / isopycnic centrifugation
- A) In Rate zonal centrifugation a particle moves\ through the gradient
- at a rate determined by its sedimentation coefficient ,a value that
- depends principally on its size, forming sharp bends.
- B) In equilibrium centrifugation a concentration of solute is selected
- to ensure that the density at the bottom of the gradient is greater
- than that of particles to be purified. A particle/molecule
- suspended in a gradient moves to a point where the gradient
- density is same as its density and therefore forms separate bench debris
- and virus, thus viruses can be purified using density gradient

- centrifugation.
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CHROMATOGRAPHY

- Chromatography is useful for purifying both enveloped and non-enveloped viruses. Most viruses are enveloped which mean that they have their nucleic acid (DNA and RNA) covered in a protein cover called the capsid which further has a membrane envelope on it.
- Examples of enveloped viruses are the chickenpox virus and the influenza virus. Non-enveloped viruses do not have the envelope.
- Examples of these non-enveloped viruses are parvovirus and adeno virus. Non-enveloped viruses are not impacted by heat, drying or acids while enveloped viruses can be affected by these.
- The level of purification varies from one virus to the next. Pore size has an impact on how much of the virus is removed and so does the kind of resin, protein solution, and buffer. It is also more difficult to remove smaller viruses fully with this method.
- Some columns use calcium phosphate, usually at pH7. Elution rates can be changed by altering the pH level and impacting the phosphate concentration.
- An example of a virus that can be treated with this method is the influenza virus. Purity can be improved by 30 to 100 fold. Further improvement can be made by employing an additional chromatography column which can improve the concentration by 10 to 30 fold.
- **PRECIPITATION**
- This is a method where a salt such as ammonium sulphate is added to a protein solution to saturation levels until the virus precipitates. The amount of salt added to achieve this needs to be noted carefully.
- Initially sufficient ammonium sulphate is added to raise its concentration to a level just below that which will precipitate the virus.
- After any precipitated contaminants are removed more ammonium sulphate is added and the precipitated virus is removed by

centrifugation.This method purifies the protein. It can be used with both enveloped and non-enveloped viruses.

- Other factors can also affect such as the temperature, pH, protein concentrations, and precipitation agents.
- **NANOFILTRATION**
- Nanofiltration is a membrane separation process which involves the diffusion of a solution through a membrane with a very small pore size. This method can be used for enveloped viruses and non-enveloped viruses. It is particularly good for obtaining small particles.
- It has been more frequently used as a purification for plasma products that contain viruses such as hepatitis B and hepatitis C. It is viewed as a good method of separating out any viruses in the plasma and helping to prevent them entering human plasma stocks.