**Plant Virus Isolation, Purification and Characterization**

The isolation, purification, and characterization of plant viruses are essential steps in studying their biology, ecology, and interactions with host plants.

Purification is the process of separating the virus particles from host constituents and other chemicals present in sap

There are no generally applicable rules for virus purification. Procedures that are effective for one virus may not work with another apparently similar virus. Even different strains of the same virus may require different procedures for effective isolation.

1. Isolation of Plant Viruses:

a. Sample Collection:

Collect symptomatic plant material (leaves, stems, etc.) showing signs of viral infection.

Include both infected and healthy control samples for comparison.

b. Preparation of Extracts:

Grind plant material in a buffer to create a crude sap extract.

Common extraction buffers include phosphate buffers or buffers containing polyvinylpyrrolidone (PVP) to reduce plant-derived contaminants.

c. Centrifugation:

Centrifuge the crude extract to separate larger debris from the virus-containing supernatant.

d. Filtration:

Filter the supernatant through a series of filters (e.g., 0.22 μm) to remove plant cell debris and other particles.

e. Inoculation:

Inoculate the filtered sap onto indicator plants that are susceptible to the virus being studied.

Observe the development of symptoms to confirm the presence of the virus

Purified viral preparations help in

1- Study of physico-chemical properties of the virus

2- Virus morphology (Shape & size)

Detailed protocols for isolation procedures for a number of viruses are given in numerous publications including

1. Assay Host

This is best done with a local lesion host. Great accuracy usually is not necessary in the preliminary assays, but reliability and rapid development of lesions are a great advantage. If no local lesion host is available, then assays must be done using a systemic host.

2. Propagation Host

The choice of host plant for propagating a virus may be of critical importance for its successful isolation. Various points have to be considered in the choice of a propagating host:

● The host plant should be easy to grow, preferably from seed. However, care should be taken that there is no seed-transmitted virus such as SoMV, which is highly transmitted in seed of *Chenopodium spp*

● The virus should reach a high concentration in the host.

● The host should not contain high amounts of substances

●The host plant constituents should be easily separable From the virus during purification. In practice, species of *Chenopodium, Cucumis, Nicotiana, Petunia, Phaseolus, and Vigna* have been found suitable for the propagation of a large number of viruses.

**3. Extraction Medium**

Once infected plant cells are broken, and the contents released and mixed, the virus particles find themselves in an environment that is abnormal. Thus, it is often necessary to use an artificial extraction medium designed to preserve the virus **particles in an infectious, intact, and unaggregated** state during the various stages of isolation.

The main factors to be considered in developing a suitable medium are as follows.

a. pH and Buffer System

b. Metal Ions and Ionic Strength

Some viruses require the presence of divalent metal ions (Ca2+ or Mg2+) for the preservation of infectivity and even for the maintenance of structural integrity Ionic strength may be important.

C. Reducing Agents and Substances Protecting Against Phenolic Compounds.

Reducing agents such as ascorbic acid, cysteine hydrochloride,

d. Additives That Remove Plant Proteins and Ribosome Many viruses lose infectivity fairly rapidly in vitro.

e. Enzymes have been added to the initial extract for various purposes. Thus, used pectinase to degrade mucilage in extracted sap. Cfsv f. Detergents and Other Additives Nonionic detergents such as Triton X-100 or Tween 80 are often used in the initial extraction medium to assist in release of virus particles from insoluble cell components and to dissociate cellular. Membranes that may contaminate or occlude virus particles. However, detergents should not be used with enveloped viruses.

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**4. Extraction Procedure**

Freezing of the plant tissue, say by liquid N2, before extraction enables disruption of vascular tissues releasing phloem-limited viruses and facilitates subsequent removal

of host materials.For some viruses, however, freezing may have a deleterious effect.

**5. Preliminary Isolation of the Virus**

a. Clarification of the Extract

In the crude extract, the virus is mixed with a variety of cell constituents that lie in the same broad size range as the virus and that may have properties that are similar in some respects. These particles include ribosomes, Rubisco (fraction

I) proteins from chloroplasts, which has a tendency to aggregate, phytoferritin, membrane fragments, and fragments of broken chloroplasts. Also present are unbroken cells, cell wall fragments, all the smaller soluble proteins of the cell, and low-molecular-weight solutes.

b. Concentration of the Virus and Removal of Low-Molecular-Weight Materials High- Speed Sedimentation.

**6. Further Purification of the Virus Preparation**

Virus preparations taken through one step of purification and concentration will still contain some low- and high-molecular- weight host materials. Further purification steps can remove more of these. The procedure to be used will depend very much on the stability of the virus, the scale of the preparation,

and the purpose for which the preparation is required.

a. Density Gradient Centrifugation

One of the most useful procedures for further purification and for assay, particularly of less stable viruses, is density gradient centrifugation.

b. Gel Filtration.

Filtration through agar gel or Sephadex may offer a useful step for further purification of viruses that are unstable to the pelleting involved in high-speed centrifugation. However, such a step will dilute the virus.

**7. Storage of Purified Viruses**

Storage of purified preparations of many plant viruses for more than a few days may present a problem. It is often best to avoid long-term storage by using the preparations as soon as possible after they are made. Under the best of conditions, most viruses except TMV lose infectivity on storage at 4°C in solution or as crystalline preparations under ammonium sulfate. Such storage allows fungi and bacteria to grow and contaminate preparations with extraneous antigens and enzymes. Addition of low concentrations of sodium azide, thymol, or EDTA will prevent growth of microorganisms but EDTA may affect virus structure. Preparations may be stored in liquid form at low temperature by the addition of an equal volume of glycerol.