Plant Virus Isolation, Purification and Characterization

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

Purified viral preparations help in:
- Study of physico-chemical properties of the virus
- Virus morphology (Shape & size)
Isolation

- First step in purification and characterization of plant viruses
  - Choice of host:
    - Propagative host
    - Assay host: LL
  - Preparation of sap extract
    - Use of additives or stabilizing agents
- Substances protecting against phenolics:
  - cysteine hydrochloride, sodium sulphite: prevent action of phenol oxidases.
  - PVP-polyvinyl pyrrolidine, PEG- Polyethylene glycol: reduces binding of virus with phenols
- Additives that removes plant protein and ribosomes
  - Mg bentonite- reduces contamination of virus extract with nucleases and ribosomes (mainly 19s protein)
  - Charcoal: adsorb host pigments
  - NaEDTA-ethylene diamine tetracetic acid @ 0.01M, ph 7.4
- Enzymes:
  - eg. Pectinase is used to degrade mucilage in sap of cocoa leaves prior to precipitation of CSSC, Trypsin -TuMv
- Detergents & other additives
  - Non-toxic detergents like Triton X-100 or Tween 80 – used in extraction medium help in release of virus particles from cell components
Purification

- **Clarification:**
  - removal of host constituents only
  - Extracting medium supplemented with anatioxidanats or reducing agents (2-mercaptaethanol, thioglycolic acid, Sodium sulphite) & chelating agents (EDTA-ethylene diamine tetracetic acid)
    - Potyviruses in alkaline medium
    - Isometric viruses in acidic medium
  - **Centrifugation at 1000 to 10,000 rpm for 5-15 min.**
  - The host constituents settle as pellet no the virus particles
Concentration

- Concentration: commonly used method
  - High speed centrifugation or
  - Ultra centrifugation

- Done for 1-2 hrs at 35,000 to 60,000 rpm

- In this aqueous phase is discarded and pellet containing virus particles is resuspended in buffer.

- To increase the purity of the virus preparation the suspension may be subjected to
  - alternate cycles of low and high speed centrifugation called differential centrifugation
Final purification

- **Density gradient centrifugation (rate zonal centrifugation):**
  - Involves high speed centrifugation of 50,000 to 70,000 rpm
  - Uses some dense substances sucrose/ CsCl2 to create different densities
- Components of virus suspension are separated according to size, shape and density (Sedimentation coefficient)
- **Testing of purity**
- **Other methods of final purification are**
  - Gel electrophoresis
  - Gel chromatography
- **Storage of purified preparations**
  - At -20°C by adding few drops of chlorobutanol, sodium azide, etc. to prevent growth of microbes and stabilize the virus
  - In liquid nitrogen by adding equal vol. of glycerol in final preparation.
  - As lyophilized
Virus concentration

- US absorption spectrum
  - Virus concentration in the purified preparations analyzed by measuring the absorption spectrum of the virus particles at 260/280 nm ratio under UV spectrophotometer.
  - Values of $A_{\text{min}}/A_{\text{max}}$, $A_{260}/A_{280}$ calculated to know the approximate percentage of nucleo-protein by using data processor yielding spectral curves (absorbance vs. wavelength).
  - The UV-absorption of the purified virus preparations show optical density (OD) value of
    - 0.29 to 0.31 at 260nm and
    - 0.861 to 1.013 at 280nm.
Methods of Purification

- Method vary from virus to virus or even strains
- For isometric viruses
- For rod shaped viruses
- For flexuous viruses
  - Carbon tetra chloride based method
  - N-butanol based method
  - Calcium phosphate based method
  - Cavileer buffer based method