ISOLATION AND PARTIAL CHARACTERIZATION OF A VIRUS-LIKE PARTICLE AND ITS GENOME ASSOCIATED WITH ENDOTHIA PARASITICA STRAIN 43

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ABSTRACT.--A *virus-like particle (VLP) infecting Endothia parasitica strain 43 has been partially purified by polyethylene glycol (PEG) precipitation, differential centrifugation and isopycnic density gradient centrifugation in cesium chloride (CsCl) and cesium sulphate (Cs2SO4). The buoyant density of the particle was determined to be 1.207 and 1.177 g/cc respectively. A sedimentation (S) value of 164 was calculated for the particle. Examined under the electron microscope, the VLP's were pleiomorphic in morphology with a diameter of 100 nm. The particles possessed an ultraviolet absorption profile characteristic of nucleoproteins. The viral genome was extracted, partially purified and found to consist of two segments of double-stranded ribonucleic acid (dsRNA). The molecular weights of the two components were resolved by polyacrylamide gel electrophoresis to be 6.76 and 6.02 x 10^6 daltons. The dsRNA possessed an S value of 34, a buoyant density of 1.597 g/cc in Cs2SO4, and upon thermal denaturation a 30 percent shift in absorbance was observed with a Tm value of 89.3 C. Partially purified preparations from a virulent strain lacked the VLP's and any detectable dsRNA.*

Since the first report of a virus in a fungus (Gandy and Hollings 1962), viruses have been observed in over 100 fungal species and in some 60 genera of fungi (Saksena and Lemke 1976). Due to the unusual characteristics and nature of the hypovirulent strains of *Endothia parasitica*, the cause of chestnut blight, the possibility of involvement of a virus was investigated.

To examine for the presence of a fungal virus, two different strains of *E. parasitica* were tested. One was a hypovirulent strain, 43; the other an American virulent, strain 671-b, which served as a control. The cultures were maintained on Difco potato dextrose agar and grown in liquid glucose-yeast extract (GYE) for subsequent virus extractions.

A modified method (Dodds 1978) was developed to allow the quick and simple isolation of the virus-like particles (VLP's). Fifty to 100 grams of freshly harvested mycelia were homogenized in 0.1 M sodium acetate buffer, pH 5.0. Following centrifugation, the supernatant was adjusted to 10 percent PEG-6000 and 0.3 M sodium chloride and incubated at 4 C. The VLP's were pelleted and subjected to two rounds of differential centrifugation, followed by a final purification by isopycnic density gradient centrifugation.

The partially purified and the purified VLP's showed a typical nucleoprotein profile with peaks at 260 and 280 and unusual peaks at 293, and 269 nm. These same absorbancy peaks are not observed when the virulent strain was tested in the same experiment.

Virus-like particles were observed to be somewhat pleiomorphic in morphology when negatively stained in 2 percent phosphotungstic acid (PTA) and examined under the electron microscope. The particles had a diameter of 100 nm (Figure 1) and closely resembled the virus associated with a disease of cultivated mushrooms (Lesemann and Koenig 1977). No virus particles were detected in preparations from the virulent strain. To examine the possibility of the particle being membrane bound, the VLP's were treated with chloroform and reexamined under the electron microscope. No particles were observed suggesting the presence of a lipid membrane, possibly involved as an integral part of the virus structure.

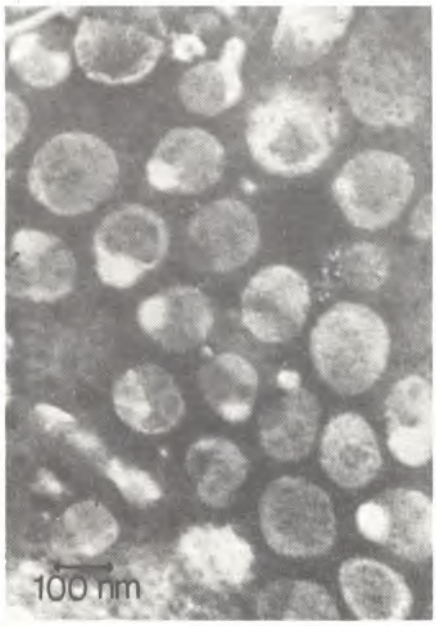

Figure 1. Electron micrographs of the partially purified VLP's stained with 2 percent PTA. Magnification of the particles is 165,000x and the bar represents 100 nm.

The buoyant density of the particle was determined by banding in cesium chloride (CsC1) and cesium sulphate (Cs_2SO4). The particles banded at 1.207 and 1.177 g/cc respectively. To determine the sedimentation value, the virus particle was banded in a linear 5 percent to 50 percent sucrose gradient and with Squash Mosaic Virus 118 S, 95 S and 57 S RNA as markers. A value of 164 S was determined. The low density and the pleiomorphic appearance of the particle supports the contention that the particle is lipid bound.

The nucleic acid of the particle was extracted by a single phase phenol-chloroform-sodium dodecyl sulfate (SDS) treatment. The nucleic acid had a typical uv absorption profile with a maxima at 260 nm and a minima at 236 nm.

The nucleic acid was negative with diphenylamine reaction, and was positive with orcinol and was resolved into two major segments by polyacrylamide gel electrophoresis (Figure 2, lanes C and D). These segments were resistant to RNAase in high salt concentration and to DNAase, but were sensitive to RNAase in low salt. Upon thermal denaturation, a 30 percent hyperchromic shift was 6bserved indicating that the nucleic acid of the particle is dsRNA.

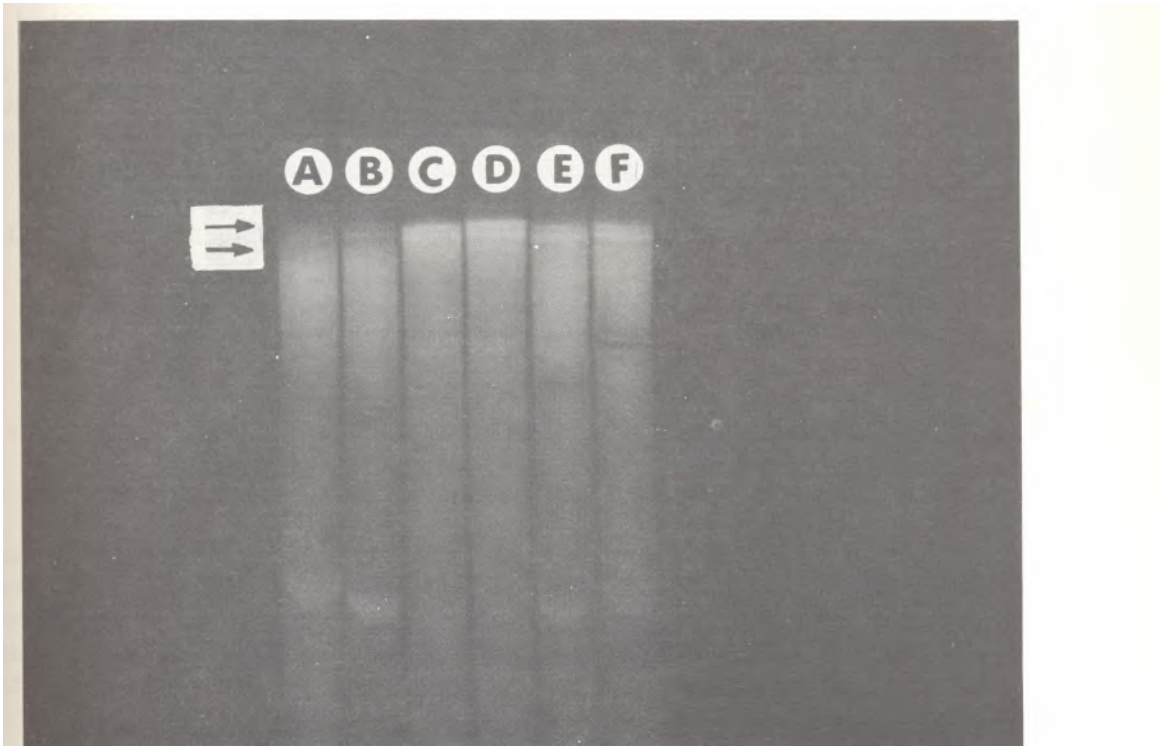

Figure 2. Polyacrylamide gel electrophoresis of the dsRNA extracted from the fungal mycelia, lanes A and B; from the viral particle, lanes C and D; both sources of dsRNA coelectrophoresed, lanes E and F. Note the same banding pattern obtained.

The dsRNA was extracted from the fungal mycelium of strain 43 directly. Two different extractions protocols were tested in the isolation procedure. In the first protocol, a modified method (Franklin 1966) of a phenol-SDS extraction was used, followed by Whatman CF-11 column chromatography. The isolated dsRNA demonstrated a typical uv absorption for nucleic acids and a 260/280 ratio of greater than 1.80 was consistently obtained. Total yield of the dsRNA was relatively low ranging between 1.2 to 1.6 pg/g of tissue.

The second isolation procedure (Morris and Dodds 1979) was modified to increase the dsRNA yield from strain 43. The method employed the direct addition of CF-11 cellulose (treated with tRNA) to the nucleic acid solution. The dsRNA was eluted and repassed through a second column of CF-11 cellulose. Significantly higher yields of dsRNA were obtained in the range of 4.8 to 5.4 ug/g of fungal tissue.

DsRNA isolated by either protocol was resolved by gel electrophoresis into two major components (Figure 2, lanes A and B). Molecular weight estimations of the isolated dsRNA were based on the migration patterns of other dsRNA species with known molecular weights and determined to be 6.76 and 6.02 x 10^6 daltons. The dsRNA was observed to be resistant to DNAase and RNAase in high salt while sensitive to RNAase in low salt. The dsRNA's extracted from the virus particle and the fungal mycelia were coelectrophoresed (Figure 2, lanes E and F) and were observed to have the identical banding patterns indicating the dsRNA isolated from either the particle or the mycelia are the same. Both sources of dsRNA demonstrated a buoyant density of 1.597 g/cc in Cs2SO4, again a good indication of the double-stranded nature of the RNA, and had an S value of 34. The dsRNA isolated from the fungal mycelia was heat denatured and a 30 percent increase in the absorbance was detected with a Tm value of 89.3 C. These results are consistent with the results of the dsRNA extracted from the particle and clearly indicate the RNA was double-stranded.

Conclusions

The VLP isolated from *E. parasitica* strain 43 could be classified as a mycovirus; the particle possesses a genome of dsRNA, having definite size and shape. This report marks the first instance of a VLP infecting *E. parasitica* to be termed as a mycovirus.

The dsRNA extracted from the mycovirus and from the fungal mycelia are exactly the same. Experiments using gel electrophoresis, nucleases, molecular weight estimations, density gradient centrifugation and thermal denaturation confirmed this nature. The dsRNA associated with the hypovirulent strains of *E. parasitica* has been assigned the responsibility as the factors determining the hypovirulence nature of these strains.

The dsRNA components can be isolated in a particle that is lipid bound. The particle was isolates intact and can be referred to as a virus-like particle or a mycovirus. The work presented that the true nature of the hypovirulence phenomenon may reside in this virus particle.

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