Double-stranded RNA and Virus-like Particles in *Endothia parasitica*

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ABSTRACT. — Strains of *Endothia parasitica* were screened for the presence of double-stranded (ds) RNA. All virulent and physiologically normal strains lacked dsRNA. Many physiologically abnormal strains contained dsRNA and this included all strains designated hypovirulent. Hypovirulent strains contained one, two or three distinct size classes of dsRNA with estimated molecular weights ranging between 3.0 and 3.4 x 10^6. Double-stranded RNA from hypovirulent strains was transmitted by anastomosis to dsRNA free virulent strains which became hypovirulent. A French hypovirulent strain contained three dsRNA components and these were all associated with an unusual pleiomorphic club-shaped virus-like particle (VLP) which could be purified from mycelial pads. The VLP had a sedimentation coefficient between 115 and 190 S and a density of 1.28 in cesium chloride. A particle of this kind could not be purified by the same methods from an American dsRNA containing hypovirulent isolate.

Several observations on the nature of hypovirulence in *Endothia parasitica* (Murr.) P. J. & H. W. And., suggest that a fungal virus may be involved in the phenomenon. Hypovirulent strains are debilitated. Most of them have lowered pathogenicity and sporulate poorly on chestnut, and they are often abnormal in culture (Elliston, 1979). These abnormalities are controlled by cytoplasmic factors and not by nuclear genes. They can be transmitted by anastomosis to virulent strains which become hypovirulent (Van Alfen *et al.*, 1975). Hypovirulent strains are unstable when cultured and this is especially true when strains derived from single conidia are examined. A notable observation is that normal virulent strains can develop from single conidia of many hypovirulent strains (Grente, 1969).

A method was adapted for the rapid analysis of multiple samples of *E. parasitica* for the presence of double-stranded RNA (Day *et al.*, 1977), since this is the genetic information of most fungal viruses (Lemke, 1976). The method used cellulose powder for the purification of dsRNA and polyacrylamide gel electrophoresis for its analysis.

Strains from 28 mass mycelial isolates of *E. parasitica* from France, Italy and North America were tested for the presence of dsRNA by this method. None was found in any of the 15 pathogenic wild-type strains. Double-stranded RNA was detected in the remaining strains. These included a French hypovirulent B strain and a French hypovirulent JR strain (Grente, 1969). Five Italian white strains and six American orange strains, four from Michigan and two from Virginia, also contained dsRNA and, with one exception, none of these was as virulent as any of the 15 dsRNA free isolates (Elliston, 1979). Six American strains that had been converted to hypovirulent after being paired in chestnut with a French hypovirulent strain also contained dsRNA.

The quality of dsRNA in hypovirulent strains was variable. The number of major components detected by polyacrylamide gel electrophoresis ranged between one and three from strain to strain. Minor

components were also detected. Estimated molecular weights for the major dsRNA components ranged from 3.0 to 3.3 x 10^6. The 3.3 x 10^6 component appeared to be common to all hypovirulent strains including those containing a single dsRNA component.

The French hypovirulent B strain mentioned above (strain 3) has been the subject of a careful search for the presence of virus-like particles (VLP) (Dodds, 1977). Preliminary nonelaborate virus purification involved two cycles of differential centrifugation of unclarified extracts from mycelial pads mechanically disrupted in 0.01M Tris buffer, pH 7.0. The final samples contained dsRNA characteristic of the strain but lacked detectable icosohedral VLP's of the type commonly found in fungi. More elaborate virus purifications, which involved two cycles of polyethylene glycol (M. Wt. 6,000) precipitation of extracts clarified at 5 C by overnight incubation in 0.1M acetate buffer pH 5.0, demonstrated that the three dsRNA components found in this strain were associated quantitatively with an unusual club-shaped VLP (Fig. 1). It was somewhat pleiomorphic when negatively stained in neutral 2 percent phosphotungstic acid and had dimensions of about 100 nm. It resembled closely the VLP associated with a disease of mushrooms (Lesemann and Koenig, 1977). It sedimented as a broad band in rate zonal sucrose density gradient centrifugation with a sedimentation coefficient between 115 and 190 S. It was banded by equilibrium density gradient centrifugation in both sucrose (density = 1.21) and cesium chloride (density = 1.28). The low density and the pleiomorphic appearance suggest that the VLP is membrane bound. A French virulent strain (strain 2), which induces cankers in chestnut that can be cured by the hypovirulent strain contained no dsRNA and preparations purified from it did not contain the pleiomorphic VLP's described above.

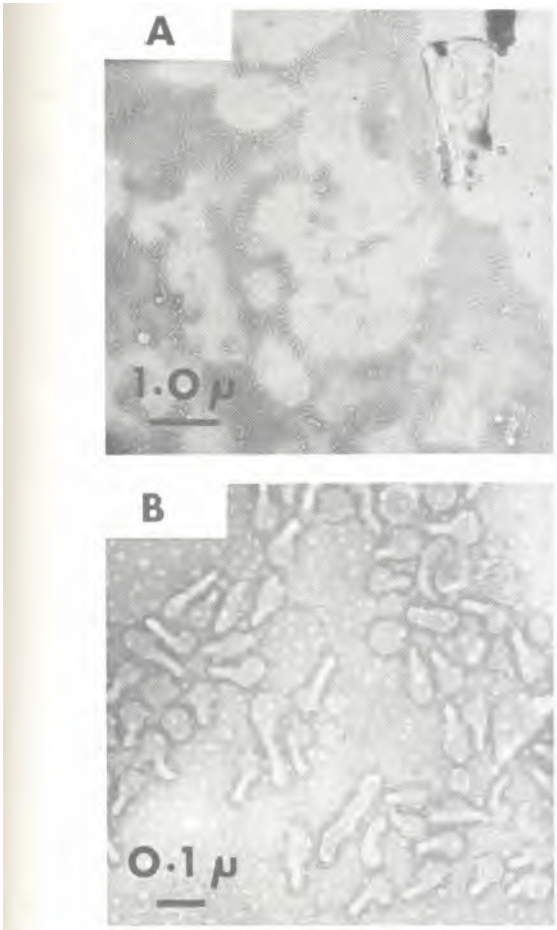

Figure 1. Club-shaped, virus-like particles purifie from a French hypovirulent B strain and negativel stained in 2 percent phosphotungstic acid pH 7.0 (A) Low magnification to show particle frequency (B) Higher magnification to show particle detail.

Extracts from an American hypovirulent strain (strain 60) have also been purified by the procedure that yields the pleiomorphic bodies from the French hypovirulent strain. No such bodies were in the product, nor could any of the dsRNA characteristic of the American strain be detected in it. The dsRNA was, however, isolated from the material precipitated by the clarification step. This suggests that the dsRNA in the various hypovirulent strains is associated with at least two different types of VLP's, or strains of the same VLP; and, they cannot all be purified by the same method.

The cytoplasmic genetic determinants for hypovirulence could obviously be part of the viral dsRNA genome. The best available transmission data to shed light on this possibility is as follows. A dsRNA containing hypovirulent strain (strain 9) was the starting point. It contained one class of dsRNA molecules with an estimated molecular weight of 3.3 x 10^6 and was a white French-derived American strain with a complex pedigree. The source of both hypovirulence and dsRNA was the white French strain (strain 3) which, as described previously, contains three dsRNA components. The hypovirulent strain was paired in chestnut with an orange virulent strain (strain 6) which carries a nuclear genetic marker (methionine requirement) but which lacks dsRNA. The large canker expected from the virulent strain did not develop and from the smaller arrested canker, a white methionine requiring hypovirulent strain (strain 14) was isolated which contained the 3.15 x 10^6 dsRNA component (Van Alfen *et al.,* 1975; Day *et al.,* 1977).

It has been demonstrated that two cytoplasmic factors, hypovirulence and dsRNA, are transmitted coincidentally following fungal anastomosis. This, together with the observation that all hypovirulent strains contain dsRNA, is strong correlative evidence that viral dsRNA is involved in hypovirulence. Further correlative evidence can be found in an analysis of single conidial strains isolated from three white hypovirulent Italian strains all of which contain a single dsRNA component. In each case single spore strains, which were white and hypovirulent like the parent strain in culture, contained the dsRNA component characteristic of the parent

strains while single spore strains that were orange did not (Day *et al.*, 1977).

While the above correlative studies appear to equate dsRNA with the cytoplasmic factor responsible for hypovirulence, there could be other unanalyzed cytoplasmic factors involved. These can hardly be common or able to operate on their own, however, since we have found no example of a hypovirulent strain that lacked dsRNA. Until it is possible to directly transmit purified VLP's or viral dsRNA to dsRNA free strains, the question of other cytoplasmic factors will remain unanswered.

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