Viruslike Particles in Penicillium brevi-compactum and P. stoloniferum Hyphae and Spores

G. R. Hooper, H. A. Wood, R. Myers, and R. F. Bozarth

First and third authors, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48823; second and fourth authors, Boyce Thompson Institute for Plant Research, Yonkers, New York 10701. Present address of senior author: Department of Biological Sciences, California State Polytechnic College, San Luis Obispo 93401.

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ABSTRACT

Viruslike particles were observed in thin sections of hyphae and spores of *Penicillium brevi-compactum* and *P. stoloniferum*. The particles in *P. brevi-compactum* had an average diameter of 36 nm, and occurred throughout the cytoplasm. The particles observed in *P. stoloniferum* were present in high numbers, and had an average diameter of 26 nm. In both cases, particles were most evident in cultures 10 or more days old.

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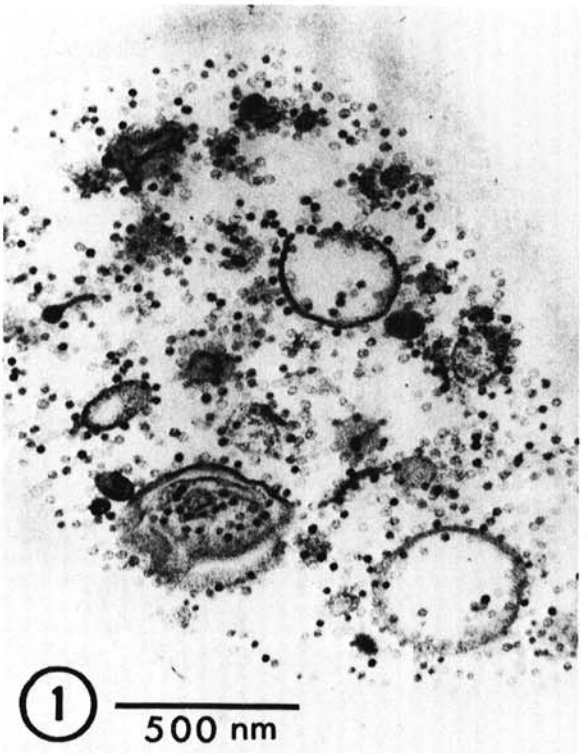
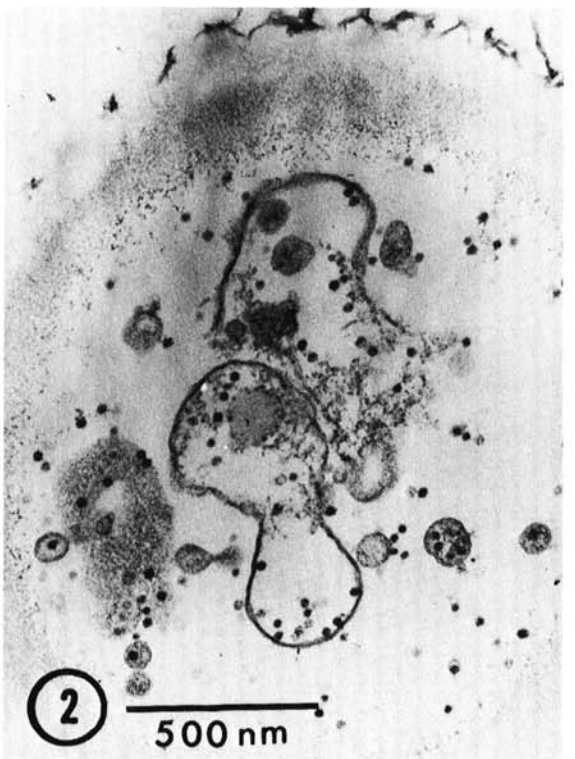
The presence of viruslike particles (VLP) in the mycelium of *Agaricus bisporus* (Lange) Sing. (2) and *Peziza ostracoderma* Korf (3) has been established by electron microscopy. Since *Penicillium* species which contain VLP can be propagated readily under defined conditions, in situ studies were initiated to determine the VLP distribution in mycelium and spores. Furthermore, ultrathin section electron microscopy


1
500 nm


2
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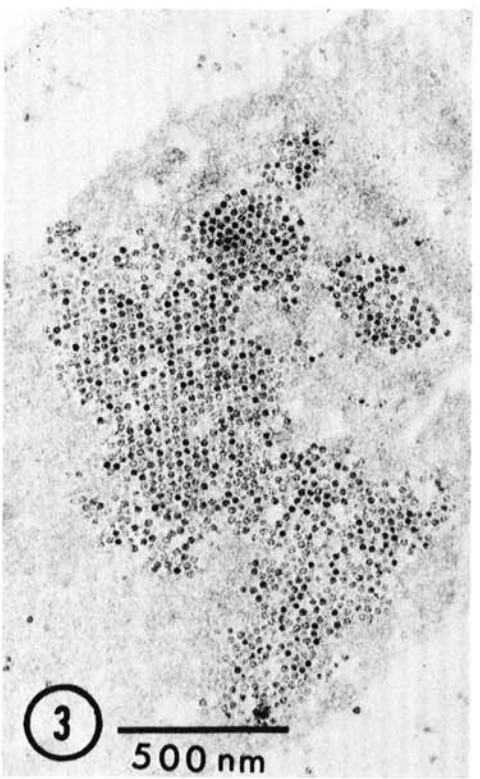
3
500 nm


4
500 nm

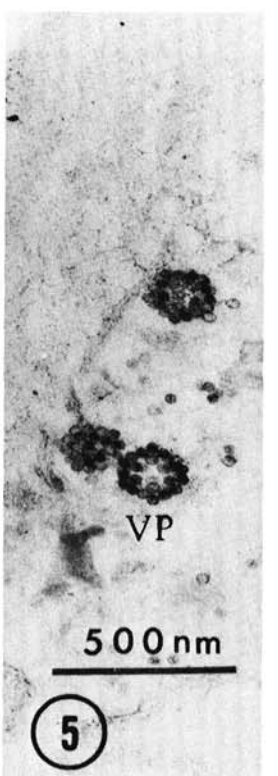
VP
500nm
5

Fig. 1-5. **1)** Cross-sectioned hyphae from a 10-day-old culture of *Penicillium stoloniferum*. Viruslike particles and some membranes are all that remain in the hyphae. **2)** *P. stoloniferum* spore from 10-day-old culture with viruslike particles and remnants of cytoplasmic organelles evident. **3)** Crystalline mass of viruslike particles in 7-day-old *P. stoloniferum* hyphal culture. **4)** Portion of hyphal cytoplasm of 5-day-old *P. brevi-compactum* culture. Electron-dense, viruslike particles at arrows; smaller particles in cytoplasm are presumed ribosomes. **5)** Viruslike particles in 10-day-old *P. brevi-compactum* hyphal culture. Most particles are poorly preserved. Note clustered arrangement of particles (VP).

of VLP-infected mycelia was expected to provide information regarding the replication of double-stranded ribonucleic acid-containing VLP and host responses to VLP.

VLP-containing and "cured" cultures of *P. brevi-compactum* (9) and a VLP-containing culture of *P. stoloniferum* (ATCC No. 14586) (5) were grown in shake culture on a complex corn steep medium (8). Mycelia were harvested at 1-, 3-, 7-, 10-, and 14-day intervals and fixed for 1 hr in a mixture of 3% acrolein and 3% glutaraldehyde (1:1) in 0.1 M cacodylate buffer at pH 6.8. The samples were then washed and postfixed for 5-15 min in 2% osmium tetroxide, dehydrated in an ethanol series containing 5% uranyl acetate in all except the last step (100% ethanol), and embedded in epoxy resins (7). Thin sections were stained with alcoholic uranyl acetate and aqueous lead citrate and examined in Zeiss EM 9A or Philips 300 electron microscopes.

Hyphae and spores from 1- to 7-day-old cultures of *P. stoloniferum* contained dense cytoplasm and it was not possible to distinguish, with certainty, VLP from cellular constituents. However, after 7 days' growth, most of the cells contained few cytoplasmic constituents and VLP were easily discernible (Fig. 1). The particles measured approximately 26 nm in diam and were scattered throughout the cell. Spores from the same age cultures showed a similar distribution of VLP (Fig. 2). Occasionally, paracrystalline arrays were found in hyphal and spore sections (Fig. 3). Particles also were observed in the embedding medium adjacent to lysed hyphae. The inability to find *P. stoloniferum* VLP until after 7 days' growth was apparently due to difficulties in distinguishing the VLP in the dense, ribosome-rich cytoplasm, since 1 mg of VLP/g dry weight mycelium can be purified from similarly grown 3-day-old cultures (1).

The *P. brevi-compactum* VLP measured approximately 36 nm in diam, and were occasionally discerned in 3-day-old hyphal sections (Fig. 4). Particles of similar morphology were evident in 10-day-old cultures, and tended to aggregate into clusters or rings (Fig. 5). Some particles appear to be electron-transparent, and suggest empty protein shells; however, no evidence for such particles was found in purified preparations of the VLP (2).

The 26- and 36-nm diam of the VLP from *P. stoloniferum* (1) and *P. brevi-compactum* (9), respectively, are smaller than those reported from negatively stained purified preparations (34 and 40 nm, respectively). A similar size difference has been reported with sections of infected tissue and purified preparations of the tobacco necrosis and southern bean mosaic viruses (4). The differences probably arise from the preparative procedures and from the fact that maximum diameters are found only when particles are sectioned through their centers.

Sections of "cured" *P. brevi-compactum* mycelia or spores from various age cultures did not contain VLP. Aside from the lack of VLP, the "cured" isolate appeared cytologically similar to the VLP-containing isolate of *P. brevi-compactum*. The growth and morphological characteristics of these isolates are also identical (9). In transmission studies with *P. stoloniferum* VLP, Lhoas (6) also found no differences in morphology or growth rates before and after infection.

Further investigations to develop techniques by which the synthesis of VLP in fungi and any resulting cellular aberrations can be observed are in progress.

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