NOTES

Presence of Two Virus-Like Particles in *Penicillium citrinum*

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Two icosahedral virus-like particles (28 and 19 nm in diameter, respectively) have been detected in sporogenic and asporogenic segregants of a strain of *Penicillium citrinum*. The distribution of the two particles differed among the two segregants.

Virus-like particles have been detected and physicochemically characterized in various species of *Penicillium* (12). As reported previously, such particles are present in a wild strain of *Penicillium citrinum* Thom (3, 5, 15). This strain segregates spontaneously and at high frequency for small patches of white asporogenic mycelium which, in turn, may redevelop into normally green, sporogenic mycelium. We have been able to detect in this unstable strain the presence of two virus-like particles which can be distinguished by their dimensions and distribution in these two mycelial types.

The organism was grown at 24°C on potato-dextrose broth or on potato-dextrose agar, pH 7.0. Extracts were made from both mycelial types after 24 and 48 h of growth (1, 2) and were negatively stained with 2% (wt/vol) phosphotungstic acid (pH 7.2) containing dimethyl sulfoxide (13) and examined with a Siemens IA electron microscope operating at 60/80 kV.

In these preparations two distinct virus-like particles were observed, one (Pcit-1) ranging from 26 to 28 nm in diameter and the other (Pcit-2) 17 to 19 nm. Both particles were clearly seen as icosahedrons and sometimes were observed as empty, single-capsid structures (Fig. 1b and 2b).

Particles Pcit-1 and Pcit-2 differed in their relative distribution among the two types of mycelial segregants, in that Pcit-1 was predominantly seen in extracts from sporogenic mycelia (Fig. 2b) whereas Pcit-2 was more abundant in similarly prepared extracts of white, asporogenic mycelia (Fig. 1b). It should be stressed, however, that both particle types were present in all mycelia.

These findings from negatively stained preparations were confirmed by observations of virus-like particles in thin sections of chemically fixed hyphal fragments prepared according to methods described previously (8). The particles were found normally associated with, or enclosed within, membranous structures in parts of the hyphae with prominent vacuolization or full degeneration (Fig. 1a and 2a). In the virus-infected hyphae of *P. citrinum* membrane whorls, tubule formations and filamentous structures of unknown origin were frequently seen as well.

The association of virus particles in fungi with membrane systems has been noted by other investigators (17, 21; see also review 12) and might indicate an attempt of the fungus to delimit a potentially lytic virus into separate bodies.

Two serologically distinct viral particles have been identified in *Pencillium stoloniferum* Thom (6, 18, 20) and three components of a single virus have been recognized in *Penicillium chrysogenum* Thom on the basis of their content of different double-stranded ribonucleic acid molecules (14). However, these particles, in contrast to those present in *P. citrinum*, are uniform in size. Pcit-2, moreover, appears to be the smallest virus-like particles detected so far in fungi.

The two virus-like particles present in *P. citrinum*, if indeed viral in nature, may contain different, perhaps complementary, genomic determinants, and for either particle to replicate both particles have to infect the same hypha. There are precedents for multicomponent and interdependent viral systems in fungi and else-
**FIG. 1.** (a) Transverse section of a degenerated hypha of an asporogenic (white) segregant of *Penicillium citrinum* showing a large accumulation of virus-like particles enclosed within a membrane. Particles were fixed with tris(hydroxymethyl)aminomethane-1-aziridinyl-phosphine oxide plus glutaraldehyde plus osmium (8). The bar indicates 200 nm. (b) Negative staining of the asporogenic mycelial extract showing numerous icosahedral particles, 17 to 19 nm, some appearing as empty capsids. Other particles show a core-like granule. The arrow points to a larger empty particle (compare with Fig. 2b). Particles were stained with 2% (wt/vol) phosphotungstic acid (pH 7.2) plus dimethyl sulfoxide. The bar indicates 50 nm.

**FIG. 2.** (a) Longitudinal section of a hypha of a sporogenic (green) segregant of *Penicillium citrinum*. Virus-like particles for the most part are enclosed in a membrane. Tris(hydroxymethyl)aminomethane-1-aziridinyl-phosphine oxide glutaraldehyde plus osmium fixation. The bar indicates 200 nm. (b) Negative staining of the sporogenic mycelial extract showing virus-like particles, 26 to 28 nm. Note the presence of empty capsid and the presence of a core-like structure in some particles (compare with Fig. 1a). Phosphotungstic acid plus dimethyl sulfoxide preparation. The bar corresponds to 50 nm.
where (10, 16, 18). If this is in fact the case with the viruses of *P. citrinum*, the dissimilar distribution of particles in the two mycelial types, which show profound differences in sporulation efficiency, antibiotic production, and growth rate (3, 15), suggests that these particles in disproportionate combination may somehow control fungal metabolism and differentiation. Some authors have already suggested a relationship between mycoviruses and extrachromosomal genetic factors (4, 7, 11, 19) and we are currently investigating this prospect in *P. citrinum*. Preliminary experiments of curing have shown that cycloheximide promotes the reversion of the asporogenic mycelium into the sporogenic one with its own typical properties. In *Saccharomyces cerevisiae* Meyen ex Hansen, cycloheximide has been shown to be an effective curing agent of the "killer" determinant, an extrachromosomal genetic factor (9) that recent studies have strongly related to the presence of double-stranded ribonucleic acid containing virus-like particles (10).

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