

## Transmission of PsV-F and PsV-S Mycoviruses during Conidiogenesis of *Penicillium stoloniferum*

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### SUMMARY

Levels of both fast and slow *Penicillium stoloniferum* virus (PsV-F and PsV-S) were evaluated in single conidial isolates of *P. stoloniferum* NRRL5267. Approximately 7% (3/43) of a random population of conidia from a culture of *P. stoloniferum* contained no PsV-F, while other conidia produced cultures with various levels of PsV-F. The greatest variation in PsV-F levels was observed in single conidial isolates from cultures that contained low to intermediate levels of virus, i.e. 11 to 25  $E_{260}^{1\text{cm}}$  units per g dry weight of mycelium. The least variation was seen in isolates from a culture with a high PsV-F level (37  $E_{260}$  units per g dry weight of mycelium). Two of the original 43 single conidial isolates contained no detectable PsV-F or PsV-S. Of the second-generation single conidial isolates from an original PsV-S<sup>+</sup> PsV-F<sup>-</sup> isolate, 6% failed to show detectable PsV-S.

PsV-F levels of cultures remained constant throughout a series of transfers when the inoculum was a mixture of conidia and mycelium. The presence of PsV-F at different levels, or its complete absence, did not affect fungal biomass, conidium size, morphology or viability. The results are discussed in terms of how conidiogenesis may influence the transmission or persistence of PsV-F and PsV-S during morphogenesis.

### INTRODUCTION

The presence of polyhedral virus-like particles in *Penicillium stoloniferum* Thom was first noted by Ellis & Kleinschmidt (1967). Further investigations revealed two electrophoretically and serologically distinct double-stranded RNA viruses associated with *P. stoloniferum* NRRL5267 (Banks *et al.*, 1968; Buck & Kempson-Jones, 1973). The virus with the higher electrophoretic mobility (PsV-F) contains three distinct molecular weight classes of double-stranded RNA whereas the slower virus (PsV-S) contains two double-stranded RNA classes (Bozarth, Wood & Mandelbrot, 1971).

PsV-F and PsV-S have been isolated and characterized from mycelia (Detroy, Lillehoj & Hesseltine, 1974) and from conidia of *P. stoloniferum* (Sansing *et al.*, 1973). Using *in vivo* fluorescent-antibody techniques, Adler & MacKenzie (1972) observed that both viruses occur in the cytoplasm of the hyphae and are not compartmentalized. Viruses have also been seen throughout the cytoplasm in thin sections of conidia of *P. stoloniferum* (Hooper *et al.*, 1972). Recently, PsV-F<sup>-</sup> isolates of *P. stoloniferum* were obtained from conidia irradiated with ultraviolet (u.v.) light (Still, Detroy & Hesseltine, 1975).

To understand the distribution of virus within cultures of *P. stoloniferum* and how certain distributions might influence virus levels in single conidial (SC) isolates, we analysed the PsV-F and PsV-S levels in 43 SC isolates and in second-generation SC isolates derived from four of these original cultures.

## METHODS

*Organism and growth conditions.* The virus-containing strain of *Penicillium stoloniferum* Thom NRRL5267 (ATCC14586) was from the ARS Culture Collection, Northern Regional Research Center. Using a micromanipulator, 43 random conidia were selected from a suspension prepared from a 7-day-old M40Y slant [containing (% w/v): sucrose, 40; malt, 2; yeast extract, 0.5; and agar, 2]. Single conidia were placed on slants of potato/sucrose agar (containing infusion from 200 g potatoes l<sup>-1</sup>, 2 % sucrose and 2 % agar) and incubated at 24 °C. All subsequent mass transfers and SC isolates were grown on slants of M40Y and incubated at 28 °C. Cultures for virus analyses were grown by inoculating 75 ml YED medium (containing 2 % yeast extract and 5 % dextrose) in a 300 ml Erlenmeyer flask with 1 ml of a suspension of 10<sup>7</sup> conidia in 0.01 % (v/v) Triton X. The cultures were incubated at 28 °C on a Brunswick shaker at 250 rev. min<sup>-1</sup> for 72 h.

*Preparation and measurement of PsV-F and PsV-S.* Mycelia were harvested by vacuum filtration and 1 g (wet weight) samples were transferred to 75 ml glass Bronwill mechanical cell homogenizer flasks containing 50 ml 0.1 M-phosphate buffer pH 7.2 and 45 g of 1 mm glass beads. The samples were homogenized for 3 min at 4000 rev. min<sup>-1</sup> under a stream of CO<sub>2</sub> at 0 °C. The homogenates were centrifuged at 8000 g for 10 min to remove cell debris, and the resulting supernatant fluid was centrifuged at 105000 g for 2 h. The pellet was resuspended in 1 ml phosphate buffer, centrifuged at 8000 g for 10 min, and the resulting supernatant fluid was then analysed for virus. All analyses were based on duplicate cultures.

Samples of the virus preparation were layered on to polyacrylamide gels [containing 2.4 % (w/v) acrylamide, 0.15 % (w/v) bisacrylamide and 0.5 % (w/v) agarose] in TAE-SDS buffer, pH 7.0 [containing 0.09 M-Tris, 0.07 M-sodium acetate, 0.002 M-EDTA and 0.5 % (w/w) sodium dodecyl sulphate] prepared essentially as described by Loening (1967). Electrophoresis was performed for 2 h at 7 mA per tube at 25 °C. A Gilford linear transport system was used to scan the gels at 260 nm. A PsV-F standard curve was prepared because the area under the PsV-F curve shows a linear relationship to the amount of PsV-F applied between 0.03 and 0.25  $E_{260}^{1\text{cm}}$  units of standard PsV-F.

The presence or absence of PsV-S was determined by comparing the gel electrophoretograms with that of a standard preparation containing both viruses. Levels of PsV-S were estimated in  $E_{260}$  units compared with known quantities of PsV-F.

## RESULTS

As indicated in Fig. 1, PsV-F was the preponderant virus in *P. stoloniferum* NRRL5267. PsV-S occurred at low titres and showed instability. Analyses of vegetative tissue generally indicated a 10- to 20-fold difference between PsV-F and PsV-S levels, as in Fig. 1.

Preliminary analyses of the original 43 SC isolates disclosed six that were of particular interest because of their differing titres of PsV-F and PsV-S (Fig. 2). The other 37 SC isolates contained 20 to 29  $E_{260}$  units of PsV-F virus per flask, and various levels of PsV-S. In isolates SC-7 and SC-10, neither PsV-F nor PsV-S was detected by gel electrophoresis. Isolate SC-19 contained no PsV-F, but 0.1 to 0.4  $E_{260}$  units of PsV-S per flask were detected. Isolate SC-30 contained 25  $E_{260}$  units of PsV-F per flask and very low levels of PsV-S, while SC-37 and SC-40 contained 45 and 15  $E_{260}$  units of PsV-F per flask, respectively, and 0.1 to 0.5  $E_{260}$  units of PsV-S per flask.

Isolates SC-19, 30, 37 and 40 were selected as representatives for further conidial selection because of their different titres of PsV-F (0 to 50  $E_{260}$  units per flask) and PsV-S (0 to 0.5  $E_{260}$

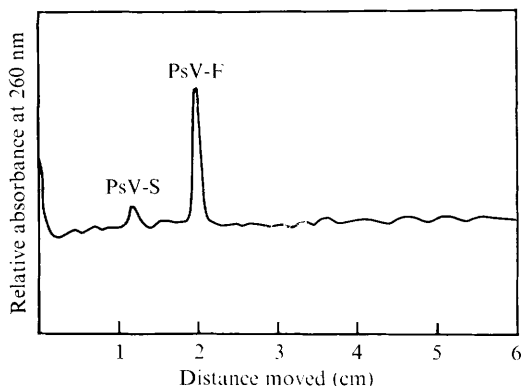


Fig. 1. Electrophoretogram of PsV-S and PsV-F from *P. stoloniferum* tissue.

units per flask). A number of conidia (35 to 43) were selected randomly from each representative isolate. Figure 2 indicates the genealogy of the conidia and their PsV-F and PsV-S status.

All of the 35 SC isolates from SC-19 that were investigated were devoid of PsV-F. However, all but two isolates (SC-19-31 and SC-19-34) contained PsV-S. Analyses of 36 SC isolates from SC-30, which had an intermediate PsV-F titre, revealed 34 with intermediate levels of PsV-F and variable contents of PsV-S, one (SC-30-11) with a high level of PsV-F and detectable PsV-S, and one (SC-30-12) with no PsV-F but detectable PsV-S. The 34 SC isolates from SC-37 had high to intermediate PsV-F levels and variable levels of PsV-S. SC isolates from SC-40 had a wide range of PsV-F titres as shown by SC-40-30 (high), SC-40-49 (low) and SC-40-43 (none).

Although virus levels varied from isolate to isolate, the biomass of all cultures was approximately the same, as was the viability of all isolates except culture 40 which exhibited

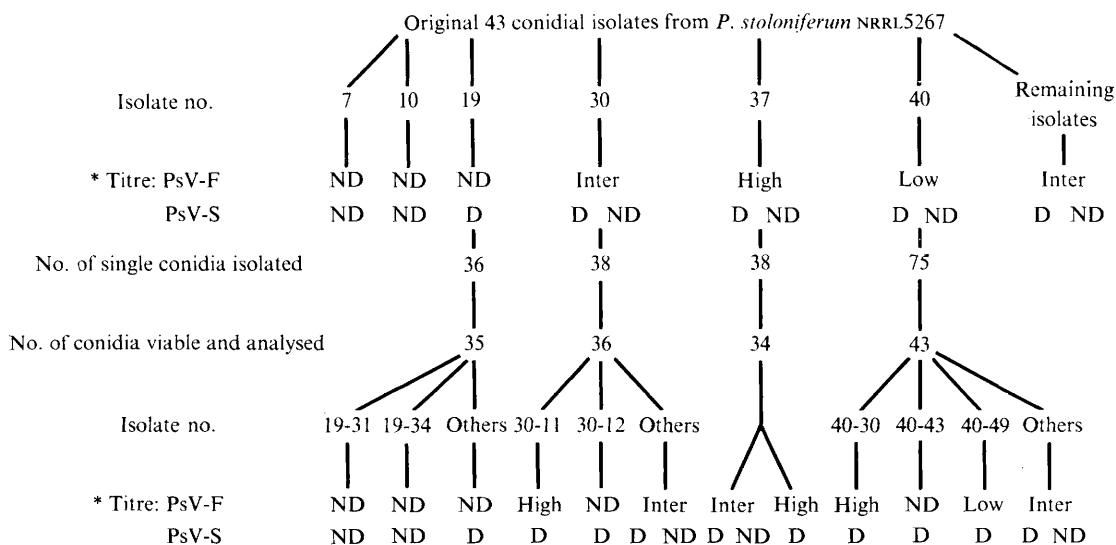


Fig. 2. Genealogy of conidial isolates of *P. stoloniferum*.

\* Range of titre (in  $E_{260}$  units per flask): ND, none detected; D, detectable, 0.1 to 0.9; Low, 1 to 19; Inter(mediate), 20 to 29; High, 30 to 90.

Table 1. *Growth characteristics of single conidial isolates*

Isolate	Conidial viability (%)	Biomass of SC isolate (g dry wt per flask)	
		Original	2nd generation
SC-19	97	0.953	1.008
SC-30	92	0.962	0.806
SC-37	92	0.962	0.958
SC-40	57	0.973	1.003

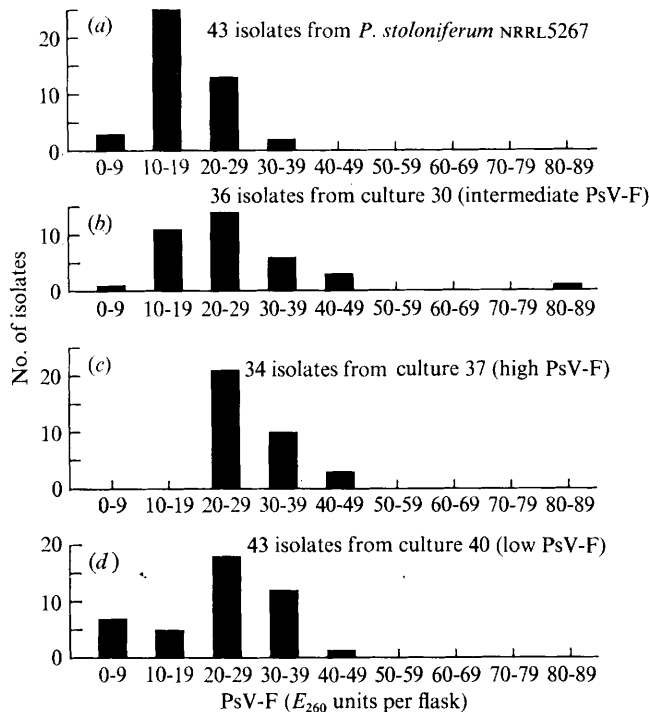


Fig. 3. Histograms of PsV-F levels in: (a) SC isolates from the original *P. stoloniferum* NRRL5267 culture; (b, c, d) SC isolates from three SC isolates derived from the original *P. stoloniferum* culture.

low viability (Table 1). No cultural or morphological differences were observed in the original 43 SC isolates.

Figure 3 shows the distribution of PsV-F levels both in SC isolates of the original *P. stoloniferum* culture and in second-generation SC isolates from three of the original ones. The PsV-F levels ( $E_{260}$  units per flask) of SC-37, 30 and 40 were high (35), intermediate (26) and low (10), respectively. The histograms demonstrate that the PsV-F profile of a series of SC isolates from a particular culture reflects the PsV-F level of that culture. All cultures showed considerable variation in PsV-F levels of SC isolates; the greatest variation was in SC isolates from cultures that contained low to intermediate levels of virus, i.e. SC-40 and 30, and the least, in isolates from culture 37 which had a high level of PsV-F. PsV-S, when detected, was low in all SC isolates examined.

Although cultures derived from single conidia varied from one to another in PsV-F level,

mass transfer of conidia permitted maintenance of stock cultures of SC isolates in which PsV-F levels remained constant.

#### DISCUSSION

The similarity of the biomass of the isolates shows that the presence of PsV-F and/or PsV-S at different levels or its absence does not alter the growth of the fungus. Also, the presence of PsV-F or PsV-S produced no detectable morphological changes in the conidia or the vegetative culture. Furthermore, conidia from isolates with either high levels of PsV-F or PsV-S or no viruses were equally viable.

Mass transfer of a large population of conidia ( $10^6$  to  $10^7$ ) or mycelial fragments from a particular isolate eliminates variability and results in a culture with virus levels similar to that of the original SC isolate.

Virus-like particles have not been observed in the rapidly extending tips of actively growing hyphae of virus-infected cultures (Hooper *et al.*, 1972; Border *et al.*, 1972), whereas older hyphae contain many virus particles. This characteristic was used in obtaining virus-free subcultures of a virus-containing strain of *Agaricus bisporus* by hyphal-tip isolations (Hollings & Stone, 1971). If a similar condition prevails in the developing penicillus, the variation in both PsV-F and PsV-S levels shown by the different SC isolates may be explained by the process of conidiogenesis in *Penicillium* (Fletcher, 1971; Cole & Kendrick, 1969; Zachariah & Fitz-James, 1967).

Phialides, the sporogenous cells from which the catenate conidia are produced in basipetal succession, arise asynchronously at the apex of their subtending conidiophore or metula. On a suitable medium, conidium production begins as soon as the first phialide is formed. The protoplast of a conidium initial in the expanding tip of a phialide is continuous with that of the phialide. Shortly before the developing conidium emerges, the two protoplasts are separated by a thin perforate septum that permits continued transfer of cytoplasm and nutrients. Within minutes after the conidium bursts through the tip of the phialide, its cytoplasmic connection with the phialide is severed by plugging of the pore in the thickening septum. Should this separation occur before the infecting viruses advance into the developing conidium, a virus-free conidium may result. Subsequent conidia from the same phialide, or from other phialides in the same penicillus, might well contain different concentrations of functional or non-functional viruses.

Obviously, this theory may be tested by isolation of conidia in sequence from a single phialide. Various techniques have been applied in efforts to obtain a preparation suitable for this operation; to date, none has succeeded.

Our results demonstrate that 7% of the original SC isolates did not contain PsV-F and 5% harboured no virus particles whatsoever. Subsequent analysis of a large number of SC isolates derived from original SC isolates with high and intermediate PsV-F levels, showed that 5 to 10% contained only PsV-S or no viruses. Analyses of SC isolates derived from a strain containing only PsV-S revealed that 6% failed to show detectable PsV-S.

This loss of ability to support PsV-F or PsV-S replication suggests that the conidiation process may be involved. Lack of transmission of mature replicating virus particles to developing conidia might explain the variation in virus level or complete absence of detectable virus in SC isolates.

The PsV-S and PsV-F strains obtained by SC isolation should be genetically compatible with the PsV-F-containing strain from which they were derived and, therefore, useful in forming heterokaryons for mycovirus infection experiments. Lhoas (1971) obtained limited infection through heterokaryosis, a process that depends on compatibility between donor and recipient strains.

Volterra *et al.* (1975) reported that the distribution of two viruses of *Penicillium citrinum* differed in sporogenic and asporogenic segregants of the culture. We have also noted asporogenic segregants in isolates of *P. stoloniferum*. However, our preliminary investigation does not suggest that the presence of PsV-F or PsV-S at different levels, or their absence, accounts for these segregants. The demonstration of PsV-F<sup>-</sup> and PsV-S<sup>-</sup> conidia in non-irradiated cultures of *P. stoloniferum* may rule out replication of these viruses by the host genome as suggested by Still *et al.* (1975) who isolated u.v. mutants lacking PsV-F, but harbouring PsV-S.

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