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Tobacco mosaic virus in Pythium spec.

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Abstract

Cultures of the soil inhabiting fungus *Pythium* spec. were inoculated in vitro with tobacco mosaic virus. Virus could be demonstrated in the mycelia from 4 days on after inoculation. In 15 days old cultures the virus concentration in the mycelium was higher than in the liquid culture medium. It is not yet clear whether the virus only accumulates, or also multiplies in the mycelium. After growth on solid medium infected mycelia still contained virus indicating that the virus is able to persist and possibly also to multiply in the hyphae.

Introduction

The discovery of viruses occurring in hyphae of fungi is of recent date. The possible existence of viruses in mushrooms has already been considered since about 1940, when the 'mummy disease' of mushrooms was attributed to a virus infection (Tucker and Routien, 1942). Hollings (1962) was the first to report the presence of virus particles in cultivated mushrooms affected with the 'die-back disease'. Spherical particles with a diameter of 25 and 29 mμ respectively were demonstrated, besides bacilliform particles of 19×50 mμ. Several other cases of virus-containing fungi have also been reported: Lindberg (1959) described a disease of *Helminthosporium victoriae* Meehan et Murphy of which the unidentified agent was still infectious after dilution to 1:1000, but was inactivated by heating at 50 °C for 10 min.

Most fungus-infecting viruses mentioned in the literature appear to have spherical particles but there are reports on infection of fungi with rod-shaped particles. Dieleman-van Zaayen (1967) detected viruslike rigid rods of 17×350 mμ in apothecia of *Plicaria fulva* Schneider. Infection experiments could not be carried out because it has not yet been possible to cultivate apothecia of *P. fulva* in vitro.

The purpose of the experiments described here, was to determine whether it is possible to infect a fungus culture in vitro with a virus.

Materials and methods

The soil-fungus *Pythium* spec. was chosen because this fungus can easily be cultivated in a liquid medium. The author is indebted to Mrs A.J. van der Plaats-Niterink who will identify the isolate. The mycelium was isolated from *Lepidium sativum* L. and inoculated on a 2% oat meal-water medium in Petri dishes. After 3 days a thick mycelium layer had been formed on the surface of the medium.

This layer was transferred to a 300 ml Erlemeyer containing 100 ml liquid Czapek-Dox medium. This medium contains 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4$. 7 H_2O, 0.01 g $FeSO_4$. 7 H_2O, and 20 g glucose per litre distilled water. The cultures were continuously shaken during the experiment.

A common strain of tobacco mosaic virus (TMV) was chosen as inoculum because of its infectious nature. In studies with fungi a sterile concentrated virus inoculum is needed. This, however, is hard to obtain because a great deal of the virus is retained on the filter in the process of filtration and the virus suspension will be diluted when added to the liquid medium of the fungus culture. Nevertheless a sterile virus suspension was obtained that still produced a few lesions after inoculation on to leaf halves of *Nicotiana tabacum* L. cv. 'Xanthi-NC' in a 1:400 dilution with the liquid Czapek-Dox medium.

To cultures of *P.* spec. in liquid Czapek-Dox medium $\frac{1}{4}$ ml of the sterile TMV-suspension was added. The cultures were shaken for respectively 5, 10, and 15 days. In a preliminary experiment some sterile carborundum was added to some cultures because it might be possible that the hyphae have to be slightly injured before virus can enter the cytoplasm. After shaking each mycelium layer was collected, treated with soap water and rinsed 5 times with distilled water. The mycelium was then ground in a blender or treated with ultrasonic waves in a Kerry 'Vibrason' Cell Disruptor in order to break the cell walls of the hyphae. The homogenized mycelium and the liquid culture medium were tested for the presence of virus by inoculation to leaf halves of 'Xanthi' tobacco.

Results

In a first experiment virus was recovered from both the liquid medium and the mycelium layer, 15 days after inoculation. The liquid media produced 3, 13, and 4 lesions, respectively; the mycelium homogenates 38, 137, and 94 lesions, respectively. Cultures to which sterile carborundum was added did not show any difference with cultures without carborundum. In further experiments carborundum was omitted because it is apparently not necessary for virus entry into the hyphae. The mycelia seemed to contain more virus than the liquid media. It could be possible that the mycelia had externally adsorbed virus from the medium during shaking and that this virus was not inactivated by soap. However, when a concentrated virus solution was added to a control mycelium layer grown in liquid medium without virus, shaken for 5 min and this layer was then treated with soap water and rinsed with water, no virus could be recovered from the homogenized mycelium. This indicates that at any rate virus that might have adhered to the outside of the mycelium was completely inactivated by the soap treatment.

It is unknown at which moment after inoculation virus enters the mycelium. To detect this, cultures were shaken for respectively 5 and 10 days after inoculation. No virus was added to control cultures. Results are shown in Table 1.

Five days after inoculation the virus can be recovered from the mycelium in 4 out of 5 cases but the concentration is less than in the first experiment. After 10 days of culture the virus had penetrated all mycelia tested. The next question was whether the virus will enter the mycelia within 5 days after inoculation.

In a following experiment mycelium was shaken for 1, 2, 3 and 4 days, respectively,

Table 1. Number of local lesions produced on two leaf halves of *Nicotiana tabacum* L. 'Xanthi-NC' by inoculation with liquid culture medium and homogenized mycelium of *Pythium* spec. grown in presence of TMV.

Days of culture	Inoculum	Culture number					Control
		1	2	3	4	5	
5	medium	2	4	50	3	29	0
5	mycelium	10	50	8	5	0	0
10	medium	70	50	9	27	56	0
10	mycelium	25	37	25	42	30	0

Tabel 1. Aantal lokale lesies op twee bladhelften van Nicotiana tabacum L. 'Xanthi-NC' veroorzaakt door inoculatie met cultuurvloeistof en gehomogeniseerd mycelium van Pythium spec. gekweekt in medium met TMV.

after virus inoculation. Up to 3 days no virus could be recovered. After 4 days, however, virus was present in the mycelium in a few cases.

From the first experiment on the impression was made that the mycelium accumulates virus or that the virus is able to multiply inside the fungus tissue. To check these possibilities, parts of mycelia that had been shaken for 10 days with virus and tested for virus (Tabel 2,A) were removed and transferred to liquid medium without virus and shaken for another 10 days. Then parts of mycelia were disinfected with soap and tested for virus (Table 2,B). Other parts were removed and placed on a solid maize

Table 2. Number of local lesions produced on two leaf halves of *Nicotiana tobacum* L. 'Xanthi-NC' by inoculation with liquid culture medium and homogenized mycelium of *Pythium* spec. grown successively with and without TMV.

	Days of culture	Inoculum	Culture number					Control
			1	2	3	4	5	
A	10 days + TMV after growth on liquid medium	medium	3	1	2	5	2	0
		mycelium	16	25	20	10	17	0
B	10 days — TMV after growth on liquid medium	medium	3	1	5	11	5	0
		mycelium	2	2	3	3	3	0
C	10 days — TMV after growth on solid medium	medium	0	0	0	0	0	0
		mycelium	4	4	5	0	6	0

Tabel 2. Aantal lokale lesies op twee bladhelften van Nicotiana tabacum L. 'Xanthi-NC' veroorzaakt door inoculatie met cultuurvloeistof en gehomogeniseerd mycelium van Pythium spec. achtereenvolgens gekweekt in medium met en zonder TMV.

meal agar medium in a Petri dish at one side. When the fungus had covered the whole surface of the medium in 7 days, mycelium was collected at the remote other side and transferred to liquid culture medium. After 10 days of shaking the mycelia were tested for virus (Tabel 2,C).

From Table 2 it appears that after 10 days of shaking in liquid medium with virus the mycelium contained virus in a moderate concentration. Transfer of the mycelium into liquid culture without virus lowers the virus concentration but virus can still be recovered from both mycelium and medium. The cultures shaken for 10 and 15 days are rather old and contain many dead hyphae. Virus may be released from them. After growth on solid medium followed by 10 days of shaking in liquid medium, virus is still present in the mycelia in 4 out of 5 cases. No virus could be detected in the media.

Discussion

The presence of virus in mycelium grown on solid medium points to a persistance of virus within the mycelium. It is known that the concentration of virus in plant tissue cultures is always low and 30 to 40 times lower than in the leaves of a plant (Kassanis, 1957). There is little information about virus concentration in fungus cells but in general the virus content seems to be low. The virus contents in fungi may also be low because of the rapid mycelium growth. Virus particles might lag behind the growing hyphal tips.

From our results it became evident that it is possible to introduce virus in a fungus culture in vitro. It is not yet clear whether the virus is able to multiply within the fungus cells but further studies are in progress.

Samenvatting

Tabaksmozaïekvirus in Pythium spec.

Cultures van de bodemschimmel *Pythium* spec. werden in vitro geïnoculeerd met tabaksmozaïekvirus. In enkele gevallen kon al 4 dagen na inoculatie virus in mycelium worden aangetoond. In 15 dagen oude cultures bevatte het mycelium meer virus dan de cultuurvloeistof. Het is nog niet bekend of het virus zich slechts ophoopt of zich ook vermeerdert in het mycelium. Na groei op een agarmedium gedurende 7 dagen bevatte het mycelium nog virus, wat er op zou wijzen dat het virus in staat is zich te handhaven in de hyfen en zich daarin wellicht ook te vermeerderen.

References

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