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Biologia. — *Inorganic salts as inducers of sporification in a strain of* Penicillium citrinum *affected by two virus-like particles.* Nota di LAURA VOLTERRA (*), ANTONIO CASSONE (**), MARIA LUISA BRUZZONE (*), LAURA GUBBINI e LAURA DE MAGISTRIS, presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — È stato rinvenuto un ceppo di *Penicillium citrinum* affetto da due particelle simil–virali, a simmetria icosaedrica, distinguibili per dimensioni (*Pci*–1 del diametro di circa 30 nm e *Pci*–2 del diametro di circa 20 nm). Questo ceppo emette settori di micelio bianco asporigeno ricchi del virus *Pci*–2, mentre nel ceppo sporificato abbonda il virus *Pci*–1. Nel presente lavoro si descrive l'azione sporogenica, sul segregante privo di conidi, di alcuni elementi aggiunti nel terreno di coltura sotto forma di cloruri. Dai risultati ottenuti sembra esistere, almeno nel sistema in esame, una correlazione tra la sporificazione e la distribuzione delle due particelle simil–virali.

For last few years the attention of some investigators has been focused upon fungal viruses (for a review see Lemke and Nash, 1974). Random sampling of fungus cultures has indicated that at least 10-15 % of the fungal species contain mycoviruses (Bozarth, 1972). With improved techniques it might be demonstrated that the actual percentage of virus-containing fungi is much higher than demonstrated so far.

The presence of mycoviruses could give a new dimension to mycology because these viruses must influence in some manner the metabolism of fungal cells. However, we speak, generally, of virus-like particles, because, in spite of the fact that they possess a number of structural viral characteristics, a clear, well defined replicative cycle has not so far been demonstrated.

In a previous publication (Battaglia *et al.*, 1973) it has been described that conidia of *Penicillium citrinum* Thom poured on Petri dishes, segregated, with a high frequency (4,75%), sectors of white aerial mycelium lacking conidia. The results of a previous research (Volterra *et al.*, 1975, in press) showed that the two characters, sporogenic and asporogenic mycelia, were associated with a different distribution of two viral populations. While the sporogenic strain is rich in virus-like particles *Pci*-1 with a diameter of about 30 nm, the asporogenic strain shows a predominance of virus-like particles *Pci*-2 with a diameter of about 20 nm.

To confirm and extend our previous results, we describe in this paper the effects of some inorganic salts on the induction of sporification and on the viral distribution.

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Materials and methods

Organism: A previously described strain of *P. citrinum* (Borrè *et al.* 1970), was used throughout this study. From this strain, a clone segregating sectors of white asporogenic mycelium has been isolated. The two isolates, were cultivated on potato dextrose agar and the sporification was induced on Czapek Dox medium, modified according to Clutterbuck *et al.* (1907), to which we added the chlorides of the elements to be tested, at a concentration shown in Table I.

Virus extraction: Virus-like particles were extracted from mycelium after 2-3 days of incubation in potato dextrose brot. The mycelia were filtered and washed with phosphate buffer 0,1 M pH = 7. They were homogenized in an Omni Mixer and pressed in Mounting Gouling at the pressure of 4.000 Kg/m^2. The lysate was clarified with the addition of an equal volume of chloroform (Alauni and Jacquemin, 1972). The resulting emulsion was mechanically shaked for 5′ and then centrifuged at 480 g for 10′. The upper watery layer was mixed with polyethileneglycol 6.000 MW (PEG-6.000), dextrane sulphate 500 MW (DS-500), NaCl, at a final concentration in the solution of 69 %, 2 %, 17,5 % respectively. The material was mixed in a separating funnel, this being allowed to stand in the cold overnight. The turbid bottom layer was removed and centrifuged at 480 g for 10′. The interfacies so obtained containing virus-like particles and cellular debris, was suspended in 15–20 ml of DS-500 solution 1 % and 0,15 ml of a 3 M KCl solution per ml of suspension was added. The mixture was mantained at 4 °C for 2 hours, then centrifuged at 480 g for 10′ (Per-Ake, 1968); and the supernatant was recentrifuged at 78.000 g for 2,30 hours at 4 °C. The final pellet was suspended in few milliliters of phosphate buffer 0,1 M pH = 7.

Electron microscopy: The extract was directly stained with a solution of DMSO (dimethylsulfoxide) + phosphotungstic acid (PTA) 1,5 %, pH = 7.0 (Battaglia *et al.*, 1973) on carbon coated grids and then observed under Siemens IAEM operating at 60 or 80 KV.

Results

Table I shows the results of our experiments which indicate that the chlorides of platinum, copper, cobalt, aluminum, palladium, stannous give a striking inhibition of the growth at the reported concentrations; while rubidium and manganese influenced mildly the growth, barium did not inhibit growth at all. It is difficult quantitatively to describe this phenomenon of inhibition. However, it is possible to obtain easily a gradient of toxicity for some substances, as indicated in Table I. Salt concentrations used in our

experiments are largely under the coefficients of solubility characteristic for every examined substance. The sporification of the asporogenic variant was induced by the following salts only: RbCl, $BaCl_2 \cdot 2\ H_2O$, $FeCl_2 \cdot 4\ H_2O$, $NiCl_2 \cdot 6\ H_2O$ and $MnCl_2$.

TABLE I.

Concentration and maximum limit of inhibition of chlorides as sporification inductors on an asporogenic strain of P. *citrinum.*

Categories	Chlorides	Concentrations mg/ml	Maximum limit of inhibition
Very poisons	K_2PtCl_4, $CuCl_2$, $CoCl_2 \cdot 6\ H_2O$	0,01–0,05	0,05
Moderately poisons . .	$AlCl_3$, $PdCl_2$, $SnCl_2 \cdot 2\ H_2O$	0,01–0,05–0,1	0,1
Slightly poisons. . . .	$CdCl_2 \cdot 2\ \frac{1}{2}\ H_2O$, $ZnCl_2$, $FeCl_2 \cdot 4\ H_2O$, $NiCl_2 \cdot 6\ H_2O$	0,01–0,05–0,1	
Very slightly poisons .	RbCl, $MnCl_2$	0,05–0,1–0,5–1,0	
Non poisons	$BaCl_2 \cdot 2\ H_2O$	0,05–0,1–0,5–1,0–1,5	

Revertants were observed at light microscope and their conidiophores were compared to those typical of the sporogenic parental strain. The results of these observations are summarized in Table II.

TABLE II.

Comparison between conidiophores of parental and revertant strains. Dimensions are related in (length X *width).*

	Sporogenic strain	Asporogenic strain	Revertant strains				
			Ba^{++}	Fe^{++}	Mn^{++}	Ni^{++}	Rb^{+}
Metulae . .	10×1,6	—	8×1,6	12×3,2	6,6×2,2	5,6×3,2	12×1,6
Sterigmata .	7×1,6	—	5,6×1,6	5,6×1,6	7,4×2,2	4,0×2,4	8×1,6
Conidia . .	4	—	3	4	4	4	3

Extracts from revertants showed the typical distribution of the two viral populations present in the sporified parental strain. In fact, we have noted

that *Pci*-1 represented the majority of the population, as shown in Plate I, figs. 3, 4, 5 e 6 (Compare with Plate I, figs. 1, 2 which illustrates the situation in the parental strain).

Discussion

The results reported above have shown that a correlation between sporification and distribution of the two virus-like particles exist. When the asporogenic strain reverts to the sporogenic one, the mycoviruses *Pci*-2 decrease in number, while the virus-like particles *Pci*-1 increase in number . However, the activity of the salts found as inducers of sporification can not be fully explained owing to the lack of information on their effects on the fungal metabolism.

Only $MnCl_2$ was studied extensively for its action on fungi. From these studies it has resulted that $MnCl_2$ reduces chromosomic damages induced by mutagens (Morpurgo and Sermonti, 1961); moreover, it has also shown to affect cytoplasmic inheritance (Sermonti and Morpurgo, 1959). According to Cochrane (1968) manganese deficiency has several physiological effects, including a pronounced decrease in sporulation. The role of this metal in activation of enzymes, especially those of citric acid cycle, suggests a role for it in metabolism of this cycle; manganese concentration also affects the concentration of other enzymes in the cell (Cochrane, 1958).

Iron, aside from the known role as part of iron containing metabolites (catalase, cytochrome, etc.), is also important in fungal sporification and in production of antibiotics like penicillin, citrinin, etc. (Cochrane, 1958).

Rubidium can replace potasium and, like potasium, it can stimulate glycolisis in blocked systems (Cochrane, 1958).

In conclusion, even if we ignore the mechanisms of the action of the tested elements, we can suggest that they may be involved in metabolic reactions, somehow related to the sporification and directly might modify the virus-cell relationship inducing a mixing of the two viral populations proper of the asporogenic strain, in favour of the larger particles *Pci*-1.

The results summarized in Table II are of some interest. The configuration of penicillia in the revertants can be morphologically altered in the relation metulae/sterigmate. For instance, in the case of revertant obtained with nickel, the hypha is short and thick as compared to that of the parental sporogenic strain. On the contrary, in that obtained with the rubidium we can observe an elongation of the penicillium. The viral populations, however, is present in these strains too, with abundance of the *Pci*-1 virus. This fact excludes the possibility of a contamination.

This strongly suggests that, at least in the case of *P. citrinum*, there exists a correlation between viruses and sporification, a correlation which includes other sporification systems like those in bacteria (Gould and Hurst, 1969). It should be noted that three isolates of *Sclerotium cepivorum* Berk of Mycelia

sterilia (fungi unable to sporificate) have shown the presence of a virus-like particles (Spire, 1971; Lepierre *et al.*, 1971).

Furthermore it seems that the morphology of the conidiophores might be modified in the revertants. The inability by some laboratory fungal strains to sporify spontaneously might be the results of particular virus-host relations (Grikoraki, 1925; Jinks, 1959), and the tollerance of the host might have a genetic origin (Koltin *et al.*, 1973). A nuclear control has been observed and confirmed in the "killer" factors of *Saccharomyces cerevisiae* Hansen and *Ustilago maydis* D.C. (Bevan *et al.*, 1973; Herring and Bevan, 1974; Day and Anagnostakis, 1973) and in the production of lytic plaques of *Schyzophyllum comune* Fr. (Koltin *et al.*, 1973). These results have been confirmed by the fact that recently many cases of latent viruses have been found. Only when the host-virus relation is altered in a certain way, the morphologic and physiologic alterations are manifested by the host.

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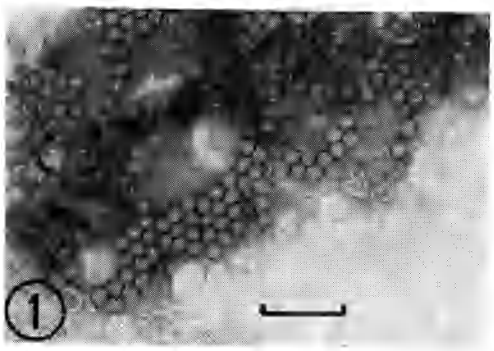
EXPLANATION OF PLATE I

All the samples are negatively stained with phosphotungstic acid (PTA) 1,5%+dimethylsulfoxide (DMSO), pH=7.0. The bar indicates 80 nm.

Fig. 1. – *Pci*-2 virus-like particles (VLP) extracted from the asporogenic strain of *P. citrinum*.

Fig. 2. – *Pci*-1 VLP extracted from the sporogenic strain.

Figg. 3, 4, 5, 6. – Viral extracts from revertants, rispectively obtained with the chlorides of rubidium, barium, iron and nickel.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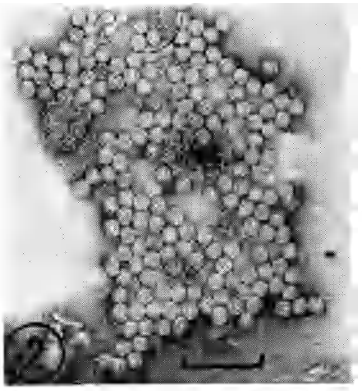
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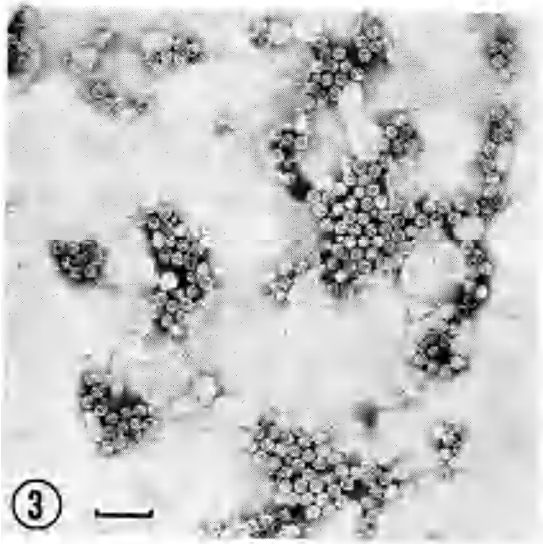
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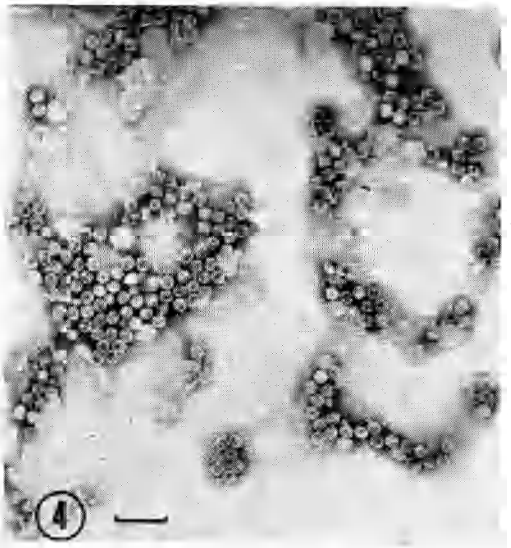
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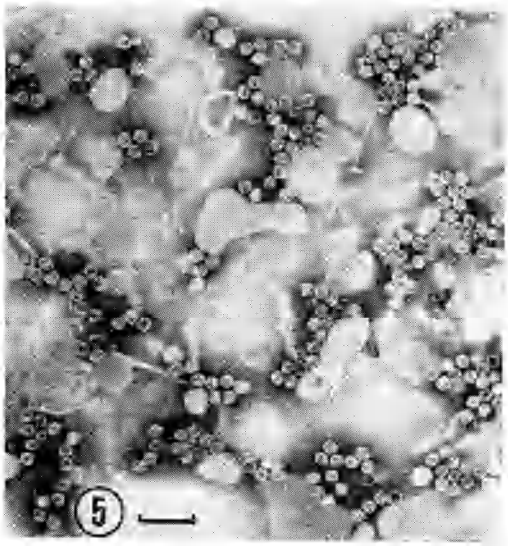
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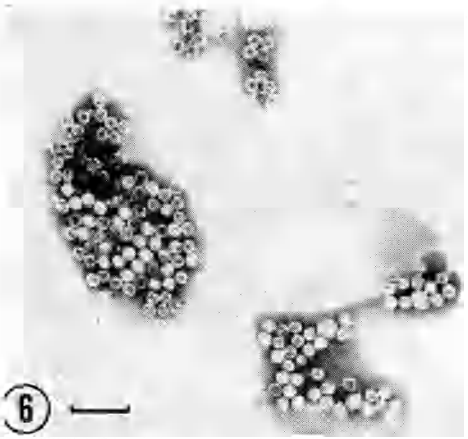
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