NEW VIRUSES ISOLATED FROM A CULTURE OF A FUNGUS OF THE GENUS Penicillium

G. A. Velikodvorskaya, A. F. Bobkova, T. S. Maksimova, S. M. Klimenko, and T. I. Tikhonenko

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Three types of virus particles were found in a culture of the fungus Penicillium brevi compactum, strain no. 192. One of the particles was identical with the virus-like particles described previously and containing double-helical RNA, while the other two (called PB-1 and PB-2) are new types of DNA-containing phages. Phages PB-1 and PB-2 are infectious for a number of strains of Escherichia coli: the first produces lysis of E. coli B, E. coli C, E. coli K12, and E. coli CK, while the second produces lysis of E. coli CK only.

Recent work has shown that virus-like particles are present in certain cultures of fungi of the genera Penicillium and Aspergillus [4-7]. These particles contained double-helical RNA and were capable of inducing interferon synthesis in animals and in tissue cultures.

The authors have found three types of virus particles in specimens of fungi of the species Penicillium brevi compactum isolated in the Institute for the Search for New Antibiotics: type 1 consisted of small polygonal particles 300 Å in diameter, morphologically similar to those described previously [4, 7], while the other two types of virus particles, described as PB-1 and PB-2, were new viruses with an unusually broad spectrum of action and universality. Both viruses were found to be infectious for several strains of Escherichia coli.

TABLE 1. Results of Titration of Mycelial Preparations from Penicillium strain no. 192 on E. coli C and E. coli CK

Time of cultivation of fungus	Infectivity per gram biomass		Infectious titer in culture fluid	
	E. coli C	E. coli CK	E. coli CK	E. coli C
6 h	$1 \cdot 10^2$	$3 \cdot 10^2$	0	0
17 h	$8{,}5 \cdot 10^2$	$2{,}6 \cdot 10^3$	0	0
1 day	$1{,}2 \cdot 10^3$	$4 \cdot 10^3$	0	0
2 days	$5 \cdot 10^4$	$1 \cdot 10^5$	0	0
3 "	$1 \cdot 10^5$	$2 \cdot 10^5$	0	0
4 "	$2 \cdot 10^6$	$9 \cdot 10^6$	0	0
5 "	$9 \cdot 10^6$	$7 \cdot 10^7$	0	0
6 "	$9{,}1 \cdot 10^6$	$6{,}9 \cdot 10^7$	0	0
7 "	$1{,}5 \cdot 10^3$	$5 \cdot 10^3$	0	0
Spores	$1 \cdot 10^3$	$5 \cdot 10^3$	0	0

The object of the present investigation was to study the properties of these viruses.

EXPERIMENTAL METHOD

The method of isolation and growth of fungi of the genus Penicillium, strain No. 192, was described previously [1]. To obtain the viruses, the mycelium from cultures of the fungus after incubation for different times (6 h to 7 days) and spores formed during growth of the fungus on solid medium were used. During its growth, seedings of the fungus were taken twice a day on Hottinger's agar in order to look for a bacterial flora. The mycelium was filtered from culture fluid on a Büchner funnel (paper filter), weighed, and ground with powdered glass. Viruses were extracted from the ground mycelium with 0.1 M NaCl in 0.05 M phosphate buffer, pH 7.0. The viruses were then precipitated with 30% ammonium sul-

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. Institute for the Search for New Antibiotics, Academy of Medical Sciences of the USSR. Department of Virology, Faculty of Biology and Soil Science, M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 5, pp. 90-93, May, 1972. Original article submitted May 31, 1971.

TABLE 2. Concentration and Purification of Virus PB-2 on Column with DEAE-cellulose

Preparation	Volume	Titer (in particles/ml)	E_{280}	Protn. by Lowry's method (mg/ml)	E_{260}/ plaque dose	Protein per plaque dose (in mg)	Yield %
Original phage suspension	2 liters	$1 \cdot 10^{11}$	20,0	2,9	$2 \cdot 10^{-10}$	$2{,}9 \cdot 10^{-8}$	—
Fluid draining out	2 liters	$2 \cdot 10^{6}$	3,9	—	—	—	—
Washings	1 liter	$1 \cdot 10^{9}$	2,5	—	—	—	—
Eluate	110 ml	$1{,}5 \cdot 10^{12}$	30,0	3,1	$2 \cdot 10^{-11}$	$2 \cdot 10^{-9}$	80

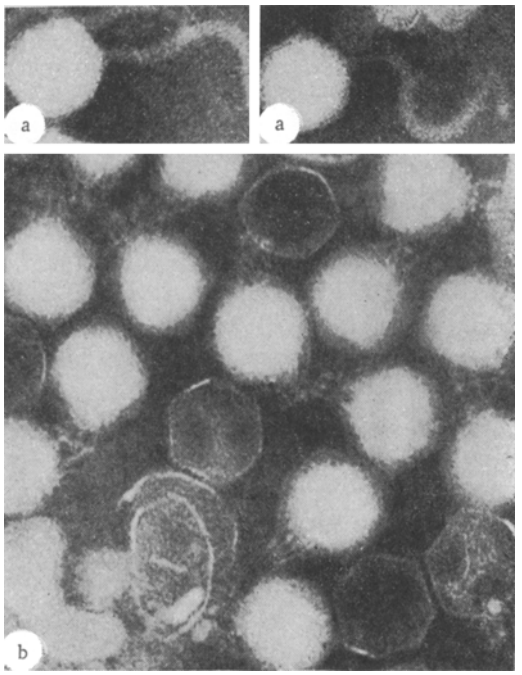

Fig. 1. Viruses PB-1 (a) and PB-2 (b), 400,000×.

fate. Ability to produce lysis of E. coli was determined in the original culture fluid and in the eluate from the ground mycelium. Spores of the fungus were treated in the same way. Pure lines of viruses PB-1 and PB-2 were obtained from an isolated plaque [3] by plating out on layers of agar: PB-1 on E. coli C, PB-2 on E. coli CK.

Viruses PB-1 and PB-2 were grown on bacteria in a 5-liter flask (2 liters of medium) on a shaker at 37°C. The bacteria were grown on Kellenberger's synthetic medium to a titer of 1×10^8 particles/ml, after which they were infected with virus with a multiplicity of infection of 1×10^5. Lysis began after 5 h. The viruses were concentrated and purified, in principle, by the scheme described previously [2] on a column with fibrous DEAE-cellulose. The eluting solution was 0.5 M NaCl in 0.05 M phosphate buffer, pH 7.0.

After chromatography the virus suspension was centrifuged at 70,000 g for 40 min, and the viruses were further purified in a preformed Cs_2SO_4 gradient, followed by spinning in a 3 × 5 bucket rotor on a type UPR-1 centrifuge at 35,000 rpm and at 4°C for 14 h. Nucleic acids were isolated by phenolic deproteinization [8]. For electron microscopy the viral suspension was applied to a grid with a positively charged formvar-carbon supporting film and with 2% potassium phosphotungstate, pH 7.0.

EXPERIMENTAL RESULTS

Investigation of mycelium obtained from cultures of the fungus after different periods of incubation showed that in the early stages of development of the fungus viruses titratable on E. coli CK and E. coli C could be found in the ground mycelium. As Table 1 shows, during subsequent incubation of the fungus the titer of virus rose gradually (by four to five orders of magnitude) to reach a maximum by the fifth to sixth day. By the seventh day there was a marked decrease in infectious activity. This was probably due to inactivation or degradation of the virus particles through autolysis of the fungal culture. No virus infectious to the bacteria could be found in the culture fluid. So far as spores formed during growth of the fungus on solid medium are concerned, after destruction they showed fairly high phage activity (Table 1). If the culture of fungus was grown in the presence of neomycin (250 mg/ml), an antibiotic with broad spectrum of action, extract of the ground mycelium also showed a marked lytic action on E. coli CK and E. coli C. At no stage of development of the fungal culture did seeding of the bacterial flora give positive results. The results cannot therefore be attributed to trival contamination. Most likely in this case virus particles with an unusually broad spectrum of action and universality developed inside the mycelium.

By seeding viruses from individual plaques it was possible to obtain pure lines of the two viruses: virus PB-1 produced lysis of E. coli CK, B, C, and K-12, while virus PB-2 produced lysis of E. coli CK only. Growth of both viruses on Kellenberger's medium led as a rule to the formation of suspensions with a titer

of $1\text{-}3 \times 10^{11}$ particles/ml. The results of chromatography of PB-2 virus on DEAE-cellulose are given in Table 2. The yield of virus was 80% in a mean concentration of 15 times. The index of purity with respect of nucleic acid was taken conventionally as the absorption at 260 nm [2]. The degree of purity by this criterion was 90% (calculated per plaque dose), while the purity as regards protein (Lowry's method) was 93%. During preparative centrifugation in a Cs_2SO_4 density gradient both viruses were located in the zone of 1.38 g/cm^3. Nucleic acid isolated from both viruses gave a positive Ceriotti's reaction and a negative reaction with orcine. It can accordingly be concluded that the nucleic acid of PB-1 and PB-2 is of the deoxyribose type.

Viruses PB-1 and PB-2 were investigated electron-microscopically (Fig. 1). Virus PB-1 has a polygonal head about 450 Å in diameter and a flexible, noncontracting tail (Fig. 1a), while virus PB-2 is a polygonal particle with a head measuring 520-540 Å in diameter (Fig. 1b).

The culture of the fungus Penicillium brevi compactum, strain No. 192 thus contains at least three types of virus particles. Of these, type 1 is identical with the virus-like particles containing double-helical RNA described previously, while the other two (called PB-1 and PB-2) are new types of viruses containing DNA. Viruses PB-1 and PB-2 are infectious for several strains of E. coli. The first of these viruses produces lysis of E. coli B, E. coli C, E. coli K-12, and E. coli CK, but the second produces lysis only of E. coli CK.

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