Double-Stranded Ribonucleic Acid in Viruses of Penicillium citrinum

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The nucleic acid content of two viral populations in a strain of Penicillium citrinum is shown to be double-stranded ribonucleic acid, resolved through polyacrylamide gel electrophoresis into 10 size groups ranging from 1.17 to 3.98 megadaltons.

In a previous paper, we described a strain of Penicillium citrinum Thom that divides at high frequency to form small patches of white asporogenous mycelium that seem to be associated with necrotic areas when observed from the bottom of the petri dish (6, 14). We detected in this unstable strain the presence of two icosahedral virus-like particles distinguished by their diameters as Pcit-1 (30 nm) and Pcit-2 (20 nm). These particles were observed by electron microscopy in negatively stained preparations performed on crude viral extracts and in thin sections of chemically fixed hyphal fragments.

In the present work we present biochemical evidence for the presence of double-stranded ribonucleic acid (dsRNA). The dsRNA from P. citrinum particles is compared with dsRNA from viruses in two other fungi, Penicillium chrysogenum and Saccharomyces cerevisiae.

MATERIALS AND METHODS

Strains. A strain of P. citrinum described previously by us (14) and the NRRL 1951 (ATCC 9480) strain of P. chrysogenum were used.

Media and growth conditions. P. citrinum was grown in potato dextrose broth, pH 7.0 (4). P. chrysogenum was grown in a minimal medium described by Lemke and Ness (11).

Virus purification. Mycelia, after 5 days of incubation on a rotary shaker at 25°C, were washed and suspended in 0.1 M phosphate buffer, pH 7.0. The mycelia were blended at high speed (Waring blender) to obtain a more homogeneous preparation and then passed three times through a Manton-Gaulin homogenizer (Gaulin Corp.) at a pressure of 600 kg/cm². This suspension was maintained at low temperature (>5°C). The suspension was clarified by the addition of an equal volume of chloroform (2), and the resulting emulsion was shaken for 5 min and finally centrifuged at 480 x g for 10 min. The upper aqueous layer was mixed with polyethylene glycol (6,000 molecular weight), dextrose sulfate (500 molecular weight), and NaCl at final concentrations of 69, 2, and 17.5%, respectively. This material was mixed in a separatory funnel and allowed to stand overnight in the cold. The turbid bottom layer was removed and centrifuged at 480 x g for 10 min. The resultant interface, containing virus-like particles and cellular debris, was suspended in 15 to 20 ml of 1% dextran sulfate solution, and 0.15 ml of 3 M KCl was added per ml of suspension. The mixture was kept at 4°C for 2 h and then centrifuged at 480 x g for 10 min (1). The supernatant, containing virus-like particles, was ultracentrifuged at 78,000 x g for 2.5 h at 4°C. The pellet was stored and used for nucleic acid extraction.

Nucleic acid extraction and purification. Pellets containing viruses were suspended in 0.02 M phosphate buffer (pH 7.0), and an equal volume of phenol reagent was added. The phenol reagent was prepared by adding 0.4 ml of phenol to 1.5 ml of a 3% solution of sodium dodecyl sulfate in 0.02 M phosphate–0.01 M ethylenediaminetetraacetic acid (EDTA) buffer, pH 7.0. After 10 min of gentle shaking, the suspension was centrifuged at 12,000 x g for 30 min. Nucleic acid in the supernatant was precipitated with 2 volumes of cold ethanol (8).

In extraction from whole mycelia, cells were harvested by centrifugation and washed with saline-EDTA (0.15 M NaCl plus 0.1 M EDTA, pH 8). They were broken in a Manton-Gaulin homogenizer, placed in saline-EDTA plus 1% sodium dodecyl sulfate, and incubated for 10 min at 60°C. The suspension was adjusted to 1 M with 2 M NaClO₄ and shaken twice with an equal volume of chloroform-phenol (1:1, vol/vol) and once with chloroform. Phases were separated by centrifugation (5,000 x g), and the nucleic acid in the top aqueous phase was precipitated with 2 volumes of cold ethanol. The precipitate was collected by low-speed centrifugation (5,000 x g) and dissolved in 1 x SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). The solution was brought to 2 M with LiCl. Most of the ribosomal RNA precipitated under these conditions (3) and was removed by low-speed centrifugation (5,000 x g). Following this treatment, residual nucleic acid was precipitated with ethanol.

Nucleic acid assays. All nucleic acid preparations were assayed for extinction ratio, absorbancy at 260 nm/absorbancy at 280 nm (1.8 to 2.0). Samples were then analyzed by 2.5% polyacrylamide gel electrophoresis. Gel polymerization was carried out by adding 0.86 ml of 10% ammonium persulfate and 0.086 ml of
N,N,N',N'-tetramethylenediamine to 100 ml of the stock solution, 2.6% acrylamide−0.13% methylenebisacrylamide (12). The electrophoresis buffer was 0.04 M tris(hydroxymethyl)aminomethane−0.02 M sodium acetate−2 mM EDTA, pH 7.8. Gels 8 to 10 cm long were developed for 4 to 5 h at 5 mA/gel and stained with 0.01% toluidine blue. After being destained with distilled water, they were scanned in a Beckman Acta III spectrophotometer electrophoretic system at 546 nm. The samples loaded onto gels were 10 to 20 μl in volume and contained 5 to 20 μg of nucleic acids and sucrose at a final concentration of 10%.

Resistance to pancreatic ribonuclease (RNase) A (type I-A, Sigma Chemical Co.) was tested by treating the nucleic acid samples with RNase at various concentrations (0.1 to 10 μg/ml) in 1× SSC at 37°C for 30 min.

Deoxyribonuclease (DNase) treatments were performed by incubating the samples in 0.1 M acetate buffer containing 5 mM MgSO4 (pH 5.0) and 0.2 mg of DNase I (Worthington Biochemicals Corp.) per ml at 25°C for 30 min. The reactions were stopped by adding phenol to the incubating solutions, and sucrose was added before electrophoresis.

RESULTS AND DISCUSSION

Nucleic acids both from viral preparations and from mycelia of P. citrinum were analyzed by acrylamide gel electrophoresis. For comparison, we also analyzed dsRNA from P. chrysogenum strain NRRL 1951, a strain in which the presence of virus-like particles containing three molecular-weight species of dsRNA is well established. All nucleic acid samples were analyzed by 2.6% polyacrylamide gel electrophoresis. In the experiments with P. chrysogenum, we confirmed the presence of three dsRNA species irrespective of the source of nucleic acid. In extractions from the mycelium, we observed also a large, slow-moving peak, characterized as deoxyribonucleic acid (DNA) by virtue of sensitivity to DNase treatment.

Figure 1 shows the electrophoretic profiles of P. citrinum total nucleic acids and viral nucleic acids. Both profiles exhibited 10 peaks, roughly divided into two groups. In the total nucleic acid profile, a large peak of DNA was evident near the top of the gel; all other peaks were DNase resistant. No new bands were apparent on gels when either twice or half as much nucleic acid was analyzed.

The 10 bands were tested for their resistance to pancreatic RNase A. Results of these tests on P. citrinum and P. chrysogenum were in agreement. Nucleic acid peaks were resistant to RNase treatment only at a high molar salt concentration (1× SSC). This is good evidence for the RNA character of the nucleic acids of P. citrinum virus-like particles.

To determine the molecular weights of these dsRNA molecules, we subjected them to electro-
agreement with a recent finding that the relationship between log molecular weight and electrophoretic mobility in polyacrylamide gels is nonlinear for dsRNA (7) (Fig. 3). The molecular sizes ranged approximately from 3.98 to 1.17 megadaltons.

In future studies, we shall verify how the 10 peaks found in our strain of *P. citrinum* are differentially distributed between the two viral populations. By electron microscopy, we have observed a certain degree of polymorphism among viruses of the same diameter. This polymorphism could be attributed to differences in nucleic acid contents. To confirm this hypothesis, we are carrying out experiments on the asporogenic strain of *P. citrinum* and on a series of strains in which we have induced spore formation with several known substances. The latter have already given results in *P. chrysogenum* (10), reducing the number of lytic plaques, and on *S. cerevisiae*, affecting the P₂ particles and the killer factor (9, 13).

ACKNOWLEDGMENTS

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LITERATURE CITED


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Macromolecule Synthesis of *Escherichia coli* BB at a Lower or Transient Growth State

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Volume 34, number 6, page 751, column 1, paragraph 1, line 18: "... and proposed the self-contained model..." should read "... and proposed the self-consistent model..."

Page 752, column 1, paragraph 3, line 5: "... two plates with a stainless-steel top and bottom." should read "... two stainless-steel plates for the top and bottom."

Page 754, column 1, line 3: "*E. coli* Bu" should read "*E. coli* B u-.

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Volume 34, number 6, page 811, column 1, line 14 from bottom: "...0.1 M phosphate buffer, 7.0." should read "...0.1 M phosphate buffer, pH 7.0."

Page 811, column 1, line 9 from bottom: "...at low temperature (>5°C)." should read "...at low temperature (<5°C)."

Page 811, column 1, line 2 from bottom: "...final concentrations of 69, 2, and 17.5%..." should read "...final concentrations of 69, 2, and 17.5%..."

Page 812, column 1, line 16: "...in 1× SSC at 37°C..." should read "...in 1× SSC and 0.1× SSC at 37°C..."

Page 813, Literature Cited: Reference 6 should read "Borre, E.,..."