Partial Purification and Antiserum Production to the 19 × 50-nm Mushroom Virus Particle

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ABSTRACT

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The 19×50-nm virus particles were purified from infected sporophores collected from mushroom beds exhibiting characteristic disease symptoms. Sporophores were frozen in liquid nitrogen and stored at −20 C. The polyhedral virus particles were removed by differential precipitation with 10% polyethylene glycol and 0.6 M NaCl. Sucrose density-gradient centrifugation resulted in one major peak containing the 19 × 50-nm particles which exhibited an ultraviolet absorbance spectrum characteristic of a nucleoprotein with a 260/280 ratio of 1.4. Antiserum prepared against the virus contained a host-specific component which was removed by absorption with an extract from healthy tissue. The remaining antiserum was specific for the 19 × 50-nm virus particles.

Additional key words: fungal virus, *Agaricus bisporus*.

The viral etiology of LaFrance disease of mushrooms was presented in 1962 (3). Several different sizes of viruslike particles have been observed in infected tissue, however, only three particle types have been consistently associated with the disease: two polyhedral particles 25 and 34 nm in diameter and a bacilliform particle 19×50 nm (2). Several purification schemes have been presented (1, 3, 5) but none of these yielded preparations of sufficient purity for virus-specific antiserum production. This study concentrated on the 19 × 50-nm particle because its distinct morphology facilitated detection by electron microscopic examination and it was easily separated from the polyhedral particles.

MATERIALS AND METHODS

Virus-infected mushroom sporophores were collected from commercial growing houses, frozen in liquid N_2 and stored at −20 C. Healthy tissue of the same strain was obtained from a commercial spawn source, grown for mature sporophores, and similarly frozen and stored.

A previously described purification procedure (5) was modified to obtain a virus preparation for antiserum production. The procedure was carried out at 4 C or in an ice bath. Frozen tissue was homogenized in 0.05 M phosphate buffer pH 7.2 containing 1.0% 2-mercaptoethanol (3 ml/gm frozen tissue) for 2 minutes in a Waring Blendor. This buffer was used throughout the extraction. The extract was centrifuged for 20 minutes at 7,000 rpm (HS-4 Sorvall rotor) followed by a second homogenization of sporophore debris and pooling of the resulting supernatant solutions. The supernatant solution was centrifuged again at 7,000 rpm for 20 minutes and then concentrated by adjusting to 10% polyethylene glycol 6000 (PEG) and 0.6 M NaCl. After slowly stirring for 2.5 hours the precipitate was collected by centrifugation (7,000 rpm, 20 minutes) and resuspended in buffer (1 ml/gm original host material) by stirring for 1 hour. Insoluble material was removed by centrifugation (7,000 rpm, 20 minutes). The supernatant solution was adjusted to 10% PEG, stirred slowly for 2.5 hours, and centrifuged (7,000 rpm, 20 minutes) to remove the precipitate which consisted primarily of host material and the polyhedral virus particles. To collect the bacilliform virus particles the remaining supernatant was adjusted to 0.6 M NaCl and stirred for 2.5 hours. The precipitate was collected by centrifugation at 9,400 rpm for 15 minutes (SS-34 Sorvall rotor), resuspended in buffer by stirring, and the insoluble material was removed by centrifugation. The supernatant solution was concentrated further by ultracentrifugation (27,000 rpm, 90 minutes in Beckman Type-30 rotor), with the resulting pellet being resuspended in buffer overnight and then removing the insoluble material by centrifugation. One milliliter of the resulting supernatant solution containing 18.0 optical density (O.D.) units as measured at 260 nm with a 1-cm path length (uncorrected for light scattering) was layered on a 10-40% sucrose gradient. Gradients were prepared by layering 7-ml aliquots of 10%, 20%, 30%, and 40% sucrose (w/volume of phosphate buffer) in tubes and allowed to diffuse overnight at 4 C. Centrifugation was for 5 hours at 24,000 rpm in an SW 25.1 Beckman rotor. Gradients were fractioned on an ISCO density-gradient fractionator and monitored by absorbance at 254 nm. The absorbing regions of the gradients were collected and

dialyzed overnight against phosphate buffer.

Each of the resulting peaks was examined on a Hitachi HU-11E electron microscope for the presence of virus particles. Preparations were examined on Formvar coated grids and negatively stained with 1.0% phosphotungstic acid, pH 7.0. The peak containing the bacilliform virus particles was used for antiserum production.

Three injections of the virus preparation mixed with equal amounts of complete Freund's adjuvant were administered subcutaneously to rabbits at weekly intervals. The fourth week an intravenous booster was given. Serum was collected and the antibody component reacting with healthy tissue was removed by incubating the serum with equal amounts of an extract from healthy tissue prepared in the same manner as the extract from infected tissue used to produce the antiserum. Serological tests were performed in gel diffusion plates (0.8% agarose, 0.85% sodium azide in 0.85% NaCl). Virus samples were placed in the outer wells 6 hours prior to adding antiserum to the center well.

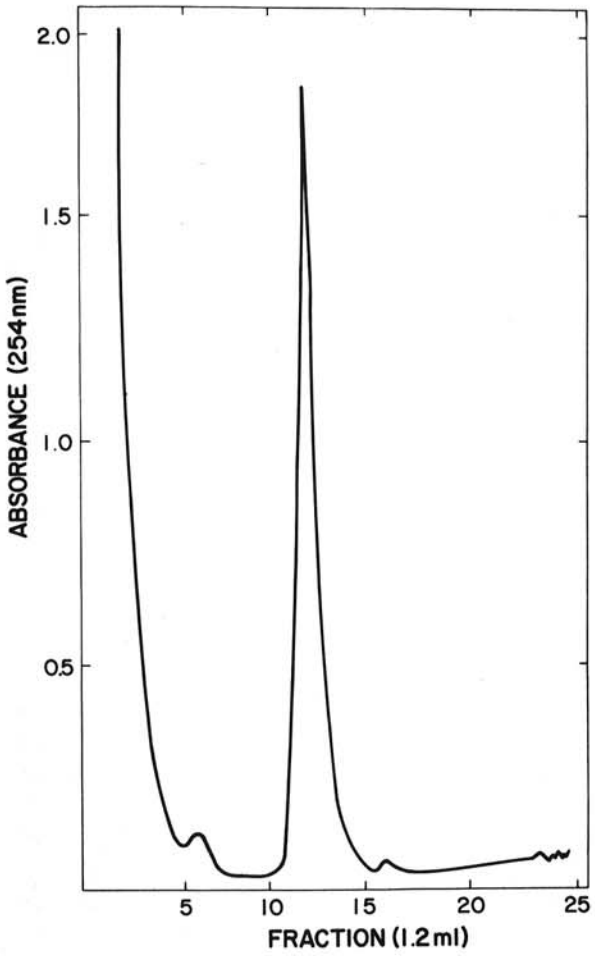

Fig. 1. Absorption profile of bacilliform virus preparation from mushrooms *(Agaricus bisporus)* layered on 10-40% sucrose density gradient after 5 hours of centrifugation in a Beckman SW 25.1 rotor at 24,000 rpm.

RESULTS

Density-gradient centrifugation of the concentrated virus preparation resulted in two ultraviolet (UV)-absorbing regions near the center of the gradient (Fig. 1). The peak consisting of fractions 10-15 contained the 19× 50-nm particles. This was followed by a much smaller peak which contained spherical virus particles not removed by the differential precipitation. When healthy tissue was processed by the same procedure, no UV-absorbing regions were observed nor were any virus particles. Following dialysis in 0.05 M phosphate buffer pH 7.2, the preparation containing the 19 × 50-nm particles gave an ultraviolet absorbance spectrum characteristic of a nucleoprotein with a 260/280 ratio of 1.4.

Serum collected from rabbits sensitized with the bacilliform particles and tested against extracts from healthy and virus-infected sporophores resulted in two precipitin bands on gel diffusion plates. One band from a rapidly diffusing component was common to both healthy and infected extracts. The second band was present only when virus-infected tissue was used and was presumed to be due to a reaction between the antibody and the slowly migrating virus particles. Serum incubated with an extract from healthy material prior to testing by gel diffusion precipitated only the slow-diffusing component.

DISCUSSION

This procedure enabled us consistently to extract the 19 × 50-nm particle in relatively high yield compared to other procedures (1, 3, 5) and to obtain preparations of sufficient purity for antiserum production. The absence of a bioassay for the mushroom virus has made it necessary to use other techniques to detect the presence of this virus. Mushroom strains are currently indexed for virus by electron microscopic examination of tissue sonicates (4) and by subculturing from spawn or sporophores onto potato-dextrose yeast agar and comparing growth with that of healthy cultures of the same strain (5). Neither of these two methods is virus-specific and both lack the sensitivity necessary to assay spawn and mycelial cultures. Furthermore, the culture test requires a minimum of 3 weeks for radial growth differences to be significantly different and known healthy cultures of all strains are not available. This antiserum has enabled us to detect the presence of the 19× 50-nm virus particles in crude extracts made from only a few grams of mushroom tissue.

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