Heterokaryon Transfer of Viruslike Particles and a Cytoplasmically Inherited Determinant in Ustilago maydis

H. A. Wood and R. F. Bozarth

Associate Virologist and Virologist, respectively, Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers, New York 10701.

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ABSTRACT

Spherical viruslike particles (VLPs) which measured 41 nm in diam, were found in all *Ustilago maydis* strains which carry a cytoplasmically inherited factor for resistance to a killer toxin. Heterokaryon transfer of the VLPs accompanied the transmission of this cytoplasmic factor.

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In 1968, Puhalla (10) reported that some strains of *Ustilago maydis* (DC.) Corda produced a toxin which was lethal to sensitive strains. The toxin was characterized as a low molecular weight protein excreted by the fungus (7). Genetic studies showed that the production of toxin, and insensitivity to toxin, were both controlled by cytoplasmically inherited factors (6). Strains designated P1 contained both factors and therefore produced toxin and were insensitive to it. Strains designated P2 had neither cytoplasmic factor. Strains designated P3, which contained only the toxin-insensitivity factor, were obtained at low frequencies by mating P1 × P2 strains and subsequent haploidization (9).

Preliminary investigations (5) showed that viruslike particles (VLPs) were present in P1 strains of *U. maydis*. Therefore, the possibility that one or both of the cytoplasmic determinants were viral in nature, was investigated in cooperation with P. R. Day and S. Anagnostakis of the Connecticut Agricultural Experiment Station, New Haven, Conn. The P1, P2, P3, and heterokaryon transfer isolates of *U. maydis* described by Day & Anagnostakis (6), were screened for the presence of VLPs.

MATERIALS AND METHODS.—The *U. maydis* isolates were grown in submerged culture on a complex corn-steep medium (11). The cultures were harvested after 3 to 4 days of growth by centrifugation at 4,000 *g* for 20 min. The cells were then frozen and lyophilized. The samples were passed through a Wiley mill (A. H. Thomas Co., Philadelphia, Pa.) using a 60-mesh screen and homogenized in a Waring Blendor with 10 ml of 0.1 M, pH 8.0, phosphate buffer per gram dry weight. The preparations were clarified by centrifugation at 7,000 *g* for 10 min, and partially purified by three cycles of differential centrifugation using high speed centrifugation at 66,000 *g* for 2.5 hr and low speed centrifugation at 6,000 *g* for 10 min.

The final suspension was centrifuged in 100 to 400 mg/ml linear sucrose density gradients (12) at 39,000 rpm in a Spinco SW41 rotor at 5 C for 2 hr, and fractionated with an ISCO density gradient fractionator and ultraviolet analyzer (4). Samples from ultraviolet-absorbing regions of the gradients were collected. Formvar-coated grids with a carbon backing were floated on drops of sample, drained, dipped in distilled water, drained, and then stained with 2% phosphotungstate at pH 7.0 or 1% uranyl acetate. The specimens were examined with a Zeiss EM 9S-2 electron microscope.

RESULTS.—Following differential centrifugation, the preparations contained highly variable amounts of host material. However, the gradient profiles of P1 preparations contained five reproducible peaks in the region of ca. 110 to 160 S, as indicated by the arrows in Fig. 1. Spherical VLPs measuring 41 nm in diameter along with host debris were found only in this region of the gradient (Fig. 2). Similar results were obtained with extracts from P3 strains of *U. maydis*.

Ouchterlony agar double-diffusion tests(1) indicated that these VLPs were not serologically related to those found in *Penicillium chrysogenum* (13) or *P. stoloniferum* (3).

The nucleic acid was extracted from the P1-VLPs and analyzed according to Wood et al. (14). The nucleic acid was double-stranded ribonucleic acid (ds-RNA) as shown by positive orcinol and negative diphenylamine reactions, resistance to pancreatic ribonuclease, and by a sharp hyperchromicity profile with a thermal melting point of 80 C in 0.01 × SSC (0.15 M sodium chloride and 0.015 M sodium citrate) buffer at pH 7.4. Following

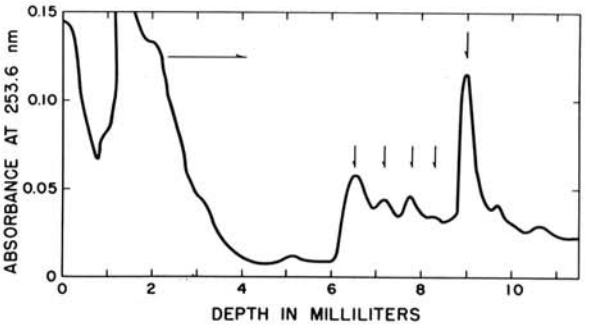

Fig. 1. Absorption profile of viruslike particle (VLP) preparation from *Ustilago maydis* strain P1 following centrifugation on 100-400 mg/ml sucrose density-gradients for 2 hr at 39,000 rpm in a Spinco SW41 rotor. Vertical arrows indicate VLP-containing fractions.

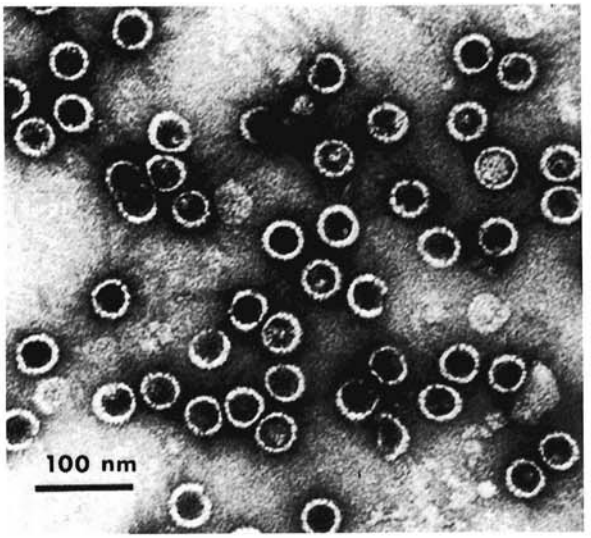

Fig. 2. Electron micrograph of viruslike particles from *Ustilago maydis* (P1) stained with 1% uranyl acetate.

polyacrylamide gel electrophoresis with *P. chrysogenum* VLP-ds-RNAs (13) as molecular weight markers, six components were observed (Fig. 3) with approximate molecular weights of 2.87, 2.52, 0.93, 0.49, 0.44, and 0.06 $\times 10^6$ daltons for components one to six, respectively.

In six different P2 isolates studied, VLPs were not detected by electron microscopy of pellet or gradient fractions. When one of these P2 isolates was converted by heterokaryon transfer to a P1 phenotype, as described by Day & Anagnostakis (6), transmission of VLPs from the P1 to the P2 genotype strain occurred. Transmission of VLPs from a toxin-insensitive strain (P1) to a toxin-sensitive strain (P2) was accompanied by transmission of the cytoplasmically inherited toxin production and insensitivity factor.

In similar mating experiments, approximately 1% of the progeny had P3 phenotypes. In these isolates, the VLPs and insensitivity factor (but not the toxin production factor) were present.

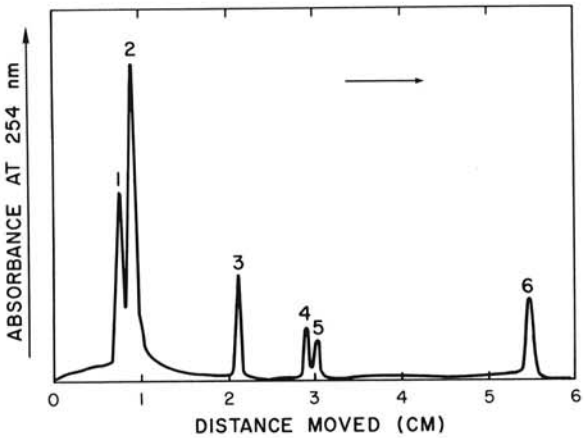

Fig. 3. Electrophoretogram of viruslike particle RNA from P1 strain of *Ustilago maydis* following fractionation by polyacrylamide gel electrophoresis for 2 hr at 6 mA/tube in 2.4% gels.

VLPs were found only in isolates of *U. maydis* which contain the cytoplasmically inherited toxin insensitivity factor. The transfer of VLPs during mating experiments resulted in either P1 or P3 phenotypes, both of which contain the insensitivity factor. Therefore, there was a positive correlation between the presence of VLPs and this cytoplasmic factor. However, it could not be determined whether the VLPs (i) merely accompany the independent transfer of the factor, (ii) contain the genetic information for insensitivity, or (iii) influence the biochemical events which confer toxin insensitivity.

DISCUSSION.—Since the VLPs have been transmitted and shown to possess the properties of a virus, these VLPs can be considered a mycovirus. Recently, Berry & Bevan (2) reported the presence of a high-molecular-weight ds-RNA in killer, neutral, and some sensitive strains of *Saccharomyces cerevisiae*. This RNA may be viral in origin; however, no correlation with ds-RNA and a cytoplasmic determinant in yeast was found.

Similar heterokaryon transfer of a mycovirus in *P. stoloniferum* was reported by Lhoas (9). The transfer was not associated with any experimentally determined alteration in host properties. As suggested by Hollings & Stone (8), many cytoplasmically inherited characteristics of fungi may be viral in nature.

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