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Detection of double-stranded RNA in *Mucor ramannianus*

Abstract

Double-stranded RNA (dsRNA) molecules have been found in numerous fungal species and are considered to be indicators of the presence of virus-like particles (VLPs) in the cells (Hollings 1978 Adv. Virus Res. 22:1-53; Buck ed., 1986 Fungal Virology, CRC Press, Boca Raton, FL). Such mycoviruses have been found mainly from studies of the nucleic acids of different strains. In this respect, fungal species belonging to the Zygomycetes are rarely screened. We have investigated the nucleic acids of 30 *Mucor* strains representing ten different species. The presence of dsRNA molecules was detected in only one of the two *Mucor ramannianus* strains examined.

Detection of double-stranded RNA in *Mucor ramannianus*

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Strains were cultivated in liquid medium (Pontecorvo et al. 1953 Adv. Genet. 5:141-238) in shake cultures for 2 days at 25 C. Mycelia were harvested, washed with distilled water and lyophilized overnight. Dry mycelia were pulverized and nucleic acids were isolated as described earlier (Leach et al. 1986 FGN 33:32-33) by the rapid lithium chloride procedure, except that the RNase treatment was omitted. Nucleic acids were resuspended in TE buffer and analyzed by electrophoresis on a 0.8% agarose gel stained with 5 ug/ml ethidium bromide.

Electrophoretic separation of the nucleic acids purified from *M. ramannianus* NRRL 1296 revealed four distinct extra bands, which moved faster than the chromosomal DNA (Fig. 1). The molecular weights corresponding to these bands were approximately 2.79, 2.70, 1.94 and 1.83 x 10⁶. They were determined by using dsRNA species of known size as molecular weight markers, harbored by an *Aspergillus niger* strain (Buck et al. 1973 Trans. Biochem. Soc. 1:1138-1140). The nuclease digestion tests confirmed the dsRNA nature of these four newly observed bands. They disappeared after RNase treatment at low ionic strength (0.1 x SSC), but remained mostly undigested after such treatment at high ionic strength (2 x SSC). Similarly, they were insensitive to S1 nuclease digestion.

These results suggest the presence of dsRNAs in this *M. ramannianus* strain. This might mean that mycovirus particles are present, but their existence requires further proof.

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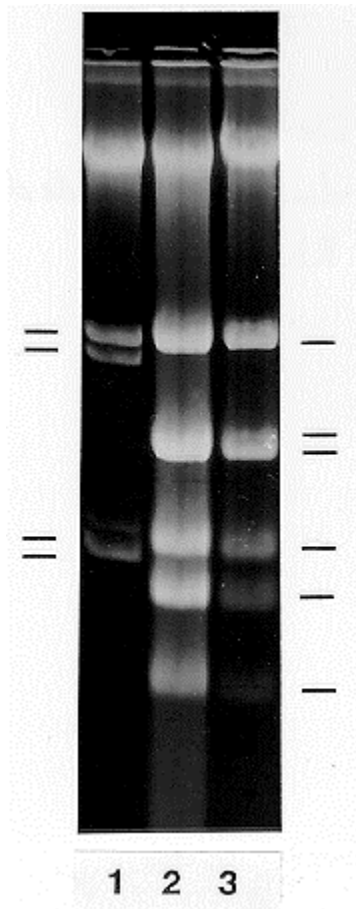


Figure 1. Agarose gel electrophoresis of the nucleic acid preparations.

Lane 1: Nucleic acid preparation from *M. ramannianus* NRRL 1296.

Lane 2-3: Nucleic acid preparations from *Aspergillus niger* No. 1003. Molecular weights of detected double-stranded RNA species: 2.76, (2.31, 2.24), 1.87, 1.70 and 1.40 x 10⁶.