DETECTION OF RNA DEPENDENT-RNA POLYMERASE ACTIVITY IN CRUDE EXTRACTS OF VIRUS-LIKE PARTICLES IN ENDOTHIA PARASITICA STRAIN EP-43

Katherine Harper-Morris

Division of Plant and Soil Sciences
West Virginia University
Morgantown, WV 26506

ABSTRACT.--The RNA dependent-RNA polymerase activity has been detected in crude virus-like particle extracts of 14-day-old shake cultures of EP-43 grown in glucose yeast extract agar. Extracts were made by homogenizing mycelium in 1M sodium acetate (pH 5.0) in a bead-beater. The homogenate was subjected to a low speed spin. The supernatant was made 0.3M NaC1 and PEG 8000 was added at the rate of 10 g/1,000 ml. The precipitate was collected and subjected to one round of differential centrifugation (10,000x g for 30 minutes and 27,000x g for 90 minutes). The RNA polymerase assay mixture contained ATP, GTP, CTP, ^{3}H-UTP, magnesium ions, EDTA and crude extract, all buffered in TRIS-HC1 to pH 7.9. Incorporation of ^{3}H-UNP into TCA-insoluble RnA was shown to be actinomycin D insensitive suggesting that polymerase activity was not DNA dependent. Similar experiments done with *Endothia parasitica* strain 671B also detected incorporation of label into RNA, but to a lesser extent.