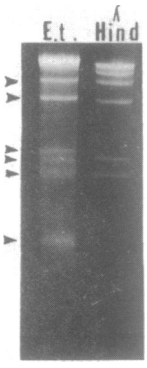
Volume 16 Number 2 1988 Nucleic Acids Research

A Sephadex column procedure for DNA isolation is also useful for detecting dsRNA

Alberto B.Livore, Peter Grubb[1], Clint W.Magill and Jane M.Magill[1]

Departments of Plant Pathology and Microbiology, [1]Biochemistry and Biophysics, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843, USA
Submitted November 18, 1987

Agarose gel electrophoresis of DNA isolated from certain strains of Exserohilum turcicum (formerly Helminthosporium turcicum) gave 6 extra ethidium stained bands below the high molecular weight band of undigested DNA. As the extra bands were not digested by restriction endonucleases, further experiments were undertaken to determine the nature of the bands. The bands did not appear if extracts were first treated with RNase A which had been treated to eliminate DNase (1). Further, the bands when stained with acridine orange (2) fluoresced green on UV exposure, while low molecular weight material fluoresced red. The material in the bands could also be recovered by binding to cellulose fibers (Whatman CF-11) in the presence of 15% ethanol (3). These results lead to the conclusion that the bands (arrows in E.t. lane) are double stranded RNA. The occurrence of dsRNA mycoviruses or of dsRNA inclusions in fungi where viruses cannot be detected is quite common (4). In some cases the number and size of the dsRNA species present can be used to identify specific fungal strains and may be related to pathogenicity (5). In other cases, including E. turcicum, the existence of dsRNA inclusions has been implicated by serological techniques, though no dsRNA has been isolated (6). We feel that the simplicity and efficiency .of the procedure will make it extremely useful to others interested in locating and characterizing dsRNA from fungi and perhaps from other organisms as well especially when isolation of DNA is also desirable. The procedure is basically that for DNA isolation reported by Biel and Parrish (7). In brief, mycelium is frozen in liquid nitrogen in a prechilled mortar, and ground to a fine powder. The powder is suspended (approximately 0.5g/ml) in 5x TE buffer, pH 8.0 (1x TE is 10 mM Tris.HCl, 1mM EDTA) which also contains 3% SDS, and proteinase K is added to 250 ug/ml. Following incubation for 20 min at 65°C, the lysate is extracted in an equal volume of Tris saturated phenol: chloroform: isoamyl alcohol (25:24:1). The suspension is mixed thoroughly and centrifuged for 20 min at 15,000 xg (4°C) to separate the phases. The aqueous phase is placed directly on a small Sephadex G 50 column in a 10cc syringe hydrated and equilibrated in TE. Eight 0.5 ml samples are collected in microfuge tubes and the nucleic acids precipitated by the addition of 50 ul of 1M NaAc, and 0.5 ml of isopropanol. Following 15 min centrifugation in a microfuge, the samples are dried, and dissolved in 40 ul of TE. A sample from each fraction is then subjected to electrophoresis in a 0.8% agarose minigel, stained with ethidium bromide, and examined under UV light to identify fractions containing DNA and/or dsRNA.



References: 1) Maniatis, T, Fritsch, EF, and Sambrook, J, (1982) Molecular Cloning. Cold Spring Harbor. 2) McMaster, GK, and Carmichael, GC, (1977) PNAS 74, 4835-4838. 3) Morris, TJ, and Dodds, JA, (1979) Phytopathology 69, 354-858. 4) Buck, KW, (1986) Fungal Virology, pp. 1-84. CRC Press, Inc, Boca Raton. 5) Van Alfen, NK (1986) In Buck, KW (ed), op cit pp 143-162. 6) Moffitt, EM, and Lister, RM, (1975) Phytopathology 65, 851-859. 7) Biel, SW, and Parrish, FW, (1986) Anal. Biochem. 154, 21-25.