Recovery of Genomic DNA from a Fungus *(Sclerotinia homoeocarpa)* with High Polysaccharide Content

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Restriction fragment-length polymorphism (RFLP) analysis and polymerase chain reaction (PCR)-based fingerprinting techniques (e.g., random amplified polymorphic DNA analysis [RAPD] or RAPD-PCR) have become important tools for studying genome organization, molecular evolution and population genetics of fungi (7). A common problem encountered in such studies is difficulty in the isolation of pure, high-molecular-weight DNA from the fungal cultures because of the copurification of high amounts of polysaccharides. High-molecular-weight polysaccharides interfere with restriction endonuclease digestion, ligation and PCR (2,3,10). Various methods have been described for the purification of fungal DNA (1,4,5,8). Most methods begin with freezing of the tissue sample with liquid nitrogen and pulverizing with a mortar and pestle, followed by extractions with phenol-chloroform or chloroform-isooamy alcohol and finally precipitation with a hexadecyltrimethylammonium bromide (CTAB) solution low in salt or in the presence of sodium dodecyl sulfate (SDS) and high salt to remove contaminating proteins, hydrates, polysaccharides and other debris.

In our studies with the plant pathogenic fungus *Sclerotinia homoeocarpa* (the causative agent of dollar spot disease in turf grass), which produces high amounts of polysaccharides in culture, it was difficult to recover high yields of pure fungal DNA using standard extraction methods. Repeated extractions of the frozen, pulverized mycelium with phenol-chloroform or chloroform-isooamy alcohol could not completely remove the polysaccharides. Thus, the DNA preparations remained viscous, and low yields of DNA resulted.

Attempts at using alternative protocols for genomic DNA purification designed to handle high-polysaccharide-content problems (6,9,11) failed to yield good-quality DNA. Thus, a modified procedure was developed, which resulted in an almost complete removal of contaminating polysaccharides from the genomic DNA preparations of *S. homoeocarpa*. As an alternative to phenol-chloroform/chloroform-isoamyl alcohol extractions or CTAB precipitations, the protocol described below relies on: (i) the incubation of dry fungal mycelium in benzyl chloride (11); (ii) the selective elution of nucleic acids from a pellet consisting of DNA, RNA and polysaccharides with repeated TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA) extractions based on the differential solubility of nucleic acids and polysaccharides in aqueous media (9); and (iii) the preferential precipitation of polysaccharides from the DNA extract using 0.35 volumes of ethanol (6).

Cultures of *S. homoeocarpa* were grown in potato-dextrose broth without shaking for 8–10 days. Mycelium was harvested by vacuum filtration, washed with 1× phosphate-buffered saline (PBS) and sterile water, lyophilized immediately and stored in Seal-a-Meal® bags at 4°C. Lyophilized mycelium (250 mg) was resuspended in 5 mL extraction buffer (100 mM Tris-Cl, pH 9.0, 40 mM EDTA), 1 mL 10% SDS and 3 mL benzyl chloride. Suspensions were vortex mixed and incubated at 50°C for 30 min with repeated mixing followed by the addition of 3 mL of 3 M sodium acetate, pH 5.0. Samples were incubated on ice for 60 min instead of the 15 min called for in the original protocol (11), because it took this long for the aqueous phase to become clear, non-viscous and easy to pipet. Samples were then centrifuged at 16,000× g for 15 min and the supernatant was collected. Isopropanol (2.5 volumes) was added to the aqueous phase and quickly mixed. The resulting floating pellet contained DNA, RNA and polysaccharides. This was spooned out and transferred into multiple 1.5-ml microcentrifuge tubes. Nucleic acids were eluted from the pellet with repeated (three to four) extractions (5 min each at 65°C followed by centrifugation at 16,000× g for 1 min in a microcentrifuge) using 500 μL of TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA). The extracted aqueous phases were pooled (1.5–2.0 mL total), and any remaining contaminating polysaccharides were eliminated through precipitation by the slow addition of 0.35 volumes of 100% ethanol with rapid mixing, followed immediately by centrifugation at 9000× g for 5 min. The addition of 0.35 volumes of ethanol (optimum amount), followed by incubation of samples on ice for 15–20 min, was found effective for the removal of polysaccharides from plant DNA preparations (6). However, in the present protocol, centrifugation was performed immediately after addition of ethanol because longer incubation times resulted in the low yields of DNA possibly due to co-precipitation with the polysaccharides. Nucleic acids were precipitated from the supernatant with 0.1 volumes of 7.5 M ammonium acetate and 0.65 volumes of 100% ethanol followed by incubation at -20°C for 30 min. The supernatant was then centrifuged at 16,000× g for 5 min.
and the resulting pellet was dissolved in 400 μL TE. Twenty microliters of RNase A (10 mg/mL) were added, and the samples incubated for 60 min at 37°C. The DNA was re-precipitated with 0.1 volumes of 7.5 M ammonium acetate and 2 volumes 100% ethanol followed by incubation for 30 min at -20°C. After centrifugation 6000x g for 5 min, the pellet was washed with 70% ethanol, vacuum-dried and resuspended in modified TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA).

By using this procedure, high-quality, polysaccharide-free genomic DNA was isolated from twenty-six different isolates of the fungus S. homoeocarpa. The quantities of DNA obtained were in the range of 24–104 μg per gram dry weight of tissue depending on the fungal isolate. The quality of DNA was verified based on susceptibility to restriction endonuclease digestion (Figure 1) and the ability to serve as a substrate for PCR amplification (Figure 2).

The method presented may be suitable for the isolation of pure DNA from other fungi that tend to have a high polysaccharide content. The DNA is released with minimal mechanical shearing by the benzyl chloride, which destroys cell walls of plants, fungi or bacteria by reacting with the -OH residues of polysaccharides (11). Benzyl chloride also extracts proteins and other cell debris from the aqueous phase (11). Subsequent TE extractions removed most of the remaining polysaccharides (80%). Elimination of the final low levels of contaminating polysaccharides was possible using ethanol precipitation (0.35 volumes) followed by immediate centrifugation. A combination of these three steps in the present procedure was found effective for almost complete removal of polysaccharides from genomic DNA preparations.

REFERENCES


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