Improved Method for Isolation of DNA from Slow-Growing Basidiomycetes Such as *Armillaria mellea*


Interest in identification and characterization of fungi is increasing because of the expanding impact of fungi on biotechnological, industrial and medical systems. Fungal taxonomy, traditionally based on comparative morphology, has at times led to confusion when apparently insignificant and continuously variable morphological criteria are used to describe new taxa and to reclassify existing taxa (4). Comparative nucleic acid studies are becoming more widely used in the systematics of fungi, including Basidiomycetes (1–3,6). Existing methods of DNA isolation (7–10) have proved to be unsatisfactory with slow-growing, darkly pigmented Basidiomycetes such as species of *Armillaria, Paxillus, Rhizopogon, Collybia, Marasmius* and *Suillus*. Using *Armillaria mellea* sensu lato as the test species, we have approached the parallel problems of slow growth and poor DNA yields by developing an airlift culture system that decreases the amount of moribund mycelium produced, and by adopting a DNA extraction procedure using cetyltrimethylammonium bromide (CTAB) to remove contaminating pigments and polysaccharides. This isolation method was successfully applied to a broad range of fungi, both laboratory-grown mycelium and basidiocarp samples collected from nature.

Fungal cultures were grown at 20°C on modified Melin-Norkrans medium (MMN) containing ammonium tartrate (0.25 g/L), KH₂PO₄ (0.5 g/L), MgSO₄·7H₂O (0.15 g/L), CaCl₂ (0.05 g/L), FeCl₃ (0.0012 g/L), NaCl (0.025 g/L), glucose (15 g/L), malt extract (3 g/L), yeast extract (0.5 g/L), soy peptone (0.5 g/L), biotin (5 µg/L) and thiamine (100 µg/L) on 1.5% agar. The colonized agar was cut into thin strips and transferred to 300 mL of MMN broth in a 2.8-L Fernbach flask, which was incubated at 25°C for two weeks. The standing cultures were transferred to 2-L Erlenmeyer flasks containing 1.6 L of MMN, which were sparged with air at a flow rate of 300 mL/min for two weeks at 25°C. Mycelia were then harvested by filtration, washed with water, frozen in liquid nitrogen and lyophilized. The dry mycelium was ground to a fine powder (mycelial fragment lengths of 10–100 µm) and used for DNA isolation.

To 10 g of powdered mycelium, 100–150 mL of extraction buffer, preheated to 60°C, were added. Extraction buffer consisted of 2% CTAB, 10 mM EDTA, 0.7 M NaCl, 0.05 M Tris-HCl (pH 8.0 at 25°C) and 0.05% β-mercap-
toethanol. The samples were incubated for 1–2 h at 60°C and vigorously shaken every 30 min. The suspension was centrifuged at 20,000× g for 20 min at 20°C, and the DNA-containing supernatant fluid was mixed with 0.13 volume of chloroform-isooamyl (24:1) alcohol. The mixture was shaken for 10 min at 40 rpm on a reciprocal shaker and was centrifuged at 20,000× g for 15 min. The DNA-containing aqueous upper phase was carefully removed and gently mixed with an equal volume of 1% CTAB, 10 mM EDTA and 50 mM Tris-HCl (pH 8.0). The resulting solution was placed on ice and centrifuged at 20,000× g for 60 min to precipitate RNA, which was removed by centrifugation at 20,000× g for 10 min at 4°C. The DNA concentration was determined spectrophotometrically with measurement of hypochromicity to correct for impurities, as described by Johnson (5).

### Table 1. Effect of Growth Conditions on DNA Yield from Mycelia

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<th>Agrocybe acericola</th>
<th>Armillaria mellea</th>
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<tr>
<td></td>
<td>Airlift Culture</td>
<td>Rotary Culture</td>
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<tr>
<td>Mycelial Dry wt/g</td>
<td>5 days 10 days 15 days</td>
<td>7 days 12 days 20 days</td>
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<tr>
<td>DNA μg/g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 7 13</td>
<td>5 8 13</td>
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<tr>
<td>DNA Purity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95 95 97</td>
<td>98 94 96</td>
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<th>Agrocybe acericola</th>
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<tr>
<td></td>
<td>5 days ALC &gt; RC</td>
<td>P &lt;0.01</td>
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<td>10 days ALC &gt; RC</td>
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<td>15 days ALC &gt; RC</td>
<td>P &lt;0.01</td>
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<sup>a</sup>DNA yield based on dry weight of fungus and corrected for purity, using a 260-nm extinction coefficient of 20 mL mg/cm².

<sup>b</sup>% purity determined from hypochromicity. % purity = 2.5× (A<sub>260</sub> denatured - A<sub>260</sub> native) ÷ A<sub>260</sub> native × 100%.

<sup>c</sup>Student t test

Figure 1. Evaluation of DNA purity through restriction endonuclease digestion and agarose gel electrophoresis. Lane 1, λ DNA digested with HindIII with all remaining digestions catalyzed by Sau3A; lane 2, λ DNA digested with Sau3A; lane 3, undigested Cryptococcus neoformans (H99) preparation I; lane 4, digested C. neoformans (H99) preparation I; lane 5, digested C. neoformans (H99) preparation II; lane 6, undigested Montagnea aerenaria (VT 1747); lane 7, digested M. aerenaria (VT 1747); lane 8, undigested Armillaria mellea (VT 1863); lane 9, digested A. mellea (VT 1863); lane 10, undigested A. mellea (VT 1865); lane 11, digested A. mellea (1865); lane 12, undigested A. mellea (VT 1847); and lane 13, digested A. mellea (VT 1847).
The effect of the cultivation method on fungal growth and DNA yield was determined. No difference was seen in the growth rate, measured as mycelial dry weight end of the gel. All DNA samples collected from nature.

The efficacy of the described DNA extraction method was determined by applying it to 11 species of Basidiomycetes, 2 Zygomycetes and 1 Chytridiomycete. In all cases, DNA of high purity (>85%) was obtained in good yield (150–340 µg/g biomass) as shown in Table 2.

To look at the suitability of DNA for hybridization studies, DNA was purified from 40 isolates of Armillaria and used in cross-hybridization studies to examine the extent of genetic variation within this species complex. These results will be published elsewhere. To establish that DNA isolated by the described method is of sufficient quality for other hybridization methods, such as restriction fragment length polymorphisms (RFLP), we examined the susceptibility of samples of DNA to digestion by restriction endonucleases. DNA samples from 5 Basidiomycete strains were incubated with Sau3A (1 U per µg DNA) for several hours and then analyzed by electrophoresis in 0.8% agarose gels (Figure 1). The undigested samples all ranged in size from 10 to 50 kbp and showed no RNA contamination at the low molecular weight end of the gel. All DNA samples were extensively digested by Sau3A, showing some banding patterns that probably represented multiple copy ribosomal RNA genes.

REFERENCES

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