1. INTRODUCTION

Strategically eliminating damage caused by fungus requires precise identification of the fungal species present (using either conventional or molecular diagnostic techniques). The first step in performing molecular diagnostics on wood-decay fungi is to obtain analyzable fungal DNA.

Five commercially available kits and one CTAB method were tested and analyzed in order to determine effective methods for extracting DNA from field samples. The manual NucleoSpin® Plant II Kit (Macherey-Nagel, MN) yielded the best results, both in terms of the quality and quantity of the fungal DNA extracted from wood samples [1].

Modern diagnostic methods such as chip technology, however, require contamination-free and automated systems that can handle a large sample throughput in a short period of time. When used with appropriate kits for plants, yeasts and bacteria, currently available automated extraction systems yield fungal DNA in smaller quantities and poorer quality than manual extraction methods, thereby limiting the suitability of the former.
To overcome these difficulties, we worked with the manufacturer of the InnuPure® C16 automated extraction system (Analytik Jena, AJ) to adjust and optimize individual work sequences according to both the problem at hand and the type of field samples to be analyzed. Our aim was to achieve results that would be comparable to the preferred manual kit for extracting fungal DNA from wood samples.

2. MATERIAL AND METHODS

Studies were performed on the following field samples: fungal fruiting bodies (category A) and wood with a visible fungal mycelium (category B). The field samples were homogenized and analytical samples were taken (2 x 30 mg per sample) for DNA extraction. 5 ng lambda DNA were added to one analytical sample from each field sample as external quality controls (see table). The samples were then prepared in keeping with the manufacturers’ instructions for manual (NucleoSpin® Plant II Kit, MN) and automatic (innuPREP Plant DNA I Kit – IPC16, AJ) kits. The DNA was dissolved in 100 µL elution buffer. Agarose gel electrophoresis (fig. 3) and spectrophotometric measurements (Biophotometer, Eppendorf) were used for determining the quantity of DNA. The degree of amplification was determined via agarose gel electrophoresis (fig. 4) in order to assess the quality of the lambda DNA and the DNA extracted from the fungus. In addition, the PCR products of *Serpula lacrymans*, both with and without lambda DNA, were analyzed using an LCD array [2] and subjected to a semiquantitative assessment based on the signal strength of the probe (S. lac ITS2, specific for *Serpula lacrymans*) (fig. 5).

3. RESULTS AND DISCUSSION

Figures 3, 4 and 5 and the table below show the assessment results for the four extraction methods with respect to the quantity and quality of the extracted DNA samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control*</th>
<th>Automated DNA extraction by InnuPure® C16 (AJ): innuPREP Plant DNA Kit - IPC16 (AJ)</th>
<th>Manual DNA extraction: NucleoSpin Plant II (MN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>43.32</td>
<td>1.85</td>
</tr>
<tr>
<td>A*</td>
<td>1 µl</td>
<td>30.17</td>
<td>2.18</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>6.66</td>
<td>1.45</td>
</tr>
<tr>
<td>B*</td>
<td>1 µl</td>
<td>7.95</td>
<td>1.44</td>
</tr>
</tbody>
</table>

* External quality control: lambda DNA; stock conc. 5 ng/µl
CUSTOMER APPLICATION

InnuPure® C16

**Fig. 3:** Gel electrophoresis of DNA samples
DNA sample: 5 µL (undiluted)
M: Marker 5 µL (1 kb DNA ladder, Peqlab)

**Fig. 4:** Gel electrophoresis of PCR products
PCR product (5 µL) with duplex primer: CP1/CP4 (specific for lambda DNA; PCR product, top) and ITS1/ITS4 (specific for fungal DNA; PCR product, below)
M = Marker, 5 µL (1 kb DNA ladder, Peqlab), K- negative control, 1K+ positive control (fungal DNA), 2K+ positive control (lambda DNA)

**Fig. 5:** PCR product analysis and identification of *Serpula lacrymans* DNA in the field sample (category B) using an LCD array [2]

The quality and quantity of the extracted fungal DNA were comparably good for all three lysis buffers in the test kit (AJ) (see table and figures 3 – 5). Study results showed the CBV lysis buffer in the test kit (AJ) to be even more suitable than the control kit (MN) for extracting DNA from wood samples, as the former produced the highest signal intensity for the *S. lac* ITS2 probe on the LCD array [2] (see fig. 5).

4. CONCLUSIONS AND OUTLOOK

These findings led to the development of an IHD protocol based on using the innuPREP Plant DNA Kit-IPC16 and the InnuPure® C16 automated extraction system. The protocol was then tested on additional field samples. Whereas the CBV lysis buffer can be used as a universal buffer for extracting fungal DNA from a variety of wood samples, the OTP lysis buffer was particularly suitable for processing plaster and sand samples. Researchers also determined the optimum amount of field sample to use (10 mg for pure fungus, 30 mg for wood samples, up to 100 mg for plaster and sand samples) [2]. The application of automated DNA extraction methods (AJ) opens the door to increasing analytical sensitivity and analyzing large sample volumes in a short time. In conjunction with a novel diagnostic tool based on LCD-array technology [2], this method greatly simplifies analysis of the 50 most important basidiomycetes and soft-rot fungi that destroy wood.
REFERENCES


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