ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Volume LVI 35 Number 2, 2008

APPLICATION OF MOLECULAR GENETIC METHODS FOR IDENTIFICATION OF WOOD-DECAYING FUNGI IN WOOD CONSTRUCTIONS

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Received: November 5, 2007

Abstract

BOBEKOVÁ, E., TOMŠOVSKÝ, M., HORÁČEK, P.: *Application of molecular genetic methods for identification of wood-decaying fungi in wood constructions*. Acta univ. agric. et silvic. Mendel. Brun., 2008, LVI, No. 2, pp. 281–284

The aim of the paper is to evaluate the utilization of molecular biology methods for detection of wood decaying fungi directly from decomposed wood using a commercial DNA extraction kit developed for soil substrates (PowerSoil™ DNA isolation kit). The experiment based on dry rot fungus (*Serpula lacrymans*) detection from inoculated wooden pieces under laboratory conditions was followed by field detection of wood-decaying fungi from wood structures on building constructions. Fungal DNA was identified using the PCR–based methods including species-specific PCR and sequencing of amplified ITS region of ribosomal DNA.

wood, fungi, *Serpula lacrymans, Donkioporia expansa, Amylostereum areolatum, Cladosporium oxysporium,* DNA, PCR

Damages caused by wood-decaying fungi (mainly Basidiomycetes) are frequently found on wooden constructions in buildings. Successful sanitation of these damages requires foremost identification of causal fungal organisms. The identification of important wood decaying fungi is in majority of cases carried out visually, according to macroscopic features of their basidiocarps, surface mycelium fans or wood-decay patterns (Weiss et al., 2000). However sometimes only mycelium or rotten wood is apparent without recognizable characters of the fungus (Schmidt et Moreth, 2000). That is why methods of molecular biology were introduced.

The choice of a target part of DNA is crucial for fungal identification. The suitable part of DNA should include sufficient amount of polymorphisms to distinguish closely related fungal species. The most frequently analyzed DNA region for fungal identification is ITS region of nuclear ribosomal DNA (White et al., 1990) and ITS sequences of most important fungal species are accessible for comparison in internet databases.

Due to rather high expenses for DNA sequencing, species-specific primers have been designed for detection of the most important wood-decaying fungi on wooden constructions, eg. *Serpula lacrymans, Coniophora puteana, Gloeophyllum sepiarium* (Schmidt et Moreth, 2000; Moreth et Schmidt, 2000). In case of species-specific primers the PCR is successful only when the specific DNA is present. Species-specific primers are mostly developed on the basis of ITS sequence.

The most difficult part of DNA analysis is the extraction of fungal DNA from wood substrates due to presence of numerous polysaccharides and polyphenolic compounds inhibiting PCR (Bodles et al, 2006; Green et al, 1999; Högberg et Land, 2004; Jasalavich et al, 2000). Commercial companies produce many kits available for DNA extraction from various substrates but according to authors' information there is no kit developed for DNA extraction from wood substrates.

The aim of our work was to test the possible utilization of PCR-based methods in wood-workers' practice using PowerSoil™ DNA extraction kit (MoBio, USA) developed for DNA extraction from soil and other environmental samples rich in polyphenolic compounds.

MATERIAL AND METHODS

Laboratory experiment

A strain of dry rot fungus (*Serpula lacrymans* (Wulfen) J. Schröt.) were obtained from the Mendel University of Agriculture and Forestry at Brno, Faculty of Forestry and Wood Technology, Department of Forest Protection and Wildlife Management culture collection (MUAF, strain number 824). The strain was maintained on Malt extract agar (Himedia) in Kolle flask and incubated at 18 °C in darkness for two weeks. Then, spruce wood blocks (40×20×20 mm) were placed on medium with grown mycelium (Fig. 1.). and incubated for 5, 8, 12, 15 and 19 days respectively. After cultivation, the grown mycelium was removed from wood blocks, clean blocks were washed in ethanol and mechanically broken with a homogenizer (Fritsch, fraction 1 mm) (Fig. 2). The fungal DNA was then isolated from sawdust; using PowerSoil™ DNA isolation kit (Mo-Bio, USA) according to the manufacturer's instructions.

Field detection

The laboratory experiment was by several identifications of fungal mycelia or rotted wood collected by 1st author during the field observation.

I. Identification of wood decaying fungi from mycelium grown on wood constructions. In two objects (Náměšť nad Oslavou castle, Czech Republic, – the beam; family house in Očová, Slovakia – the wall beam) mycelium of fungi was found. Mycelium was scrapped off and stored in microtube and in laboratory under –20 °C. The DNA was isolated by using Power Soil™ DNA isolation kit (Mo-Bio, USA) according to the manufacturer's instructions.
II. Dry rot fungus (*Serpula lacrymans*) was found directly in the floor of family house in Banská Štiavnica town (Slovakia; Sládkovičova street). The house construction was affected by dry rot fungus – sporocarps and numerous mycelium fans were seen on many places. The affected beam in the ground floor of the house was chosen for the experiment. Four samples of decayed wood were removed (in one-meter interval) for laboratory detection.
III. Identification of wood-decaying fungi in pillar construction of Funicular Centre (Brno, Milénova street; Czech Republic). The construction of pillar from fir wood was affected by wood-decaying fungi (reduced strength properties, visible sporocarps). The pillars were most seriously affected in contact with ground, in place with the highest moisture. The Funicular Centre was divided into two parts. In the first one, consisting of thirteen pillars yellow-red gill polypore (*Gloeophyllum sepiarium*) was identified according to the morphology of basidiocarps. In the second part, consisting of three pillars the wood-decaying fungus could not be identified due to absence of fruit bodies. Therefore, sampling was done using Pressler increment borer.

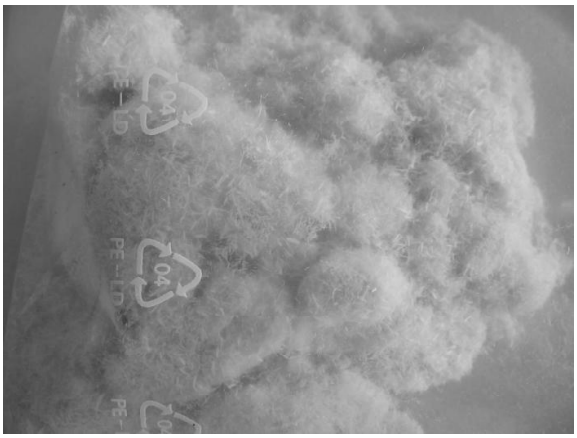
Obtained wood samples were washed in ethanol and mechanically broken with a homogenizer (Fritsch, Germany). The fungal DNA was then isolated from resulted sawdust (size fraction = 1 mm) using PowerSoil™ DNA isolation kit (Mo-Bio, USA) according to the manufacturer's instructions.

PCR ANALYSIS

For the identification of unknown fungi from collected field samples the ITS region of ribosomal DNA was amplified using the primer pair specific for fungi ITS1F/ITS4 (Gardes and Bruns, 1993). In case of laboratory experiment focused on detection of *Serpula lacrymans* and field detection No. II the species-specific primer combination ITS1/SL was used instead of the above one (according to Schmidt and Moreth, 2000).

The DNA was amplified with PCR, using the Mastercycler® ep thermocycler (Eppendorf, Germany). The PCR reaction was carried out in a 25 µl reaction volume; the mixture for the PCR contained 50 ng of DNA, 20 pmol of each primer, 0.2 mM dNTP's, and 1U of DynaZyme™ polymerase with the appropriate

1: *Spruce pieces on fungal culture*



2: *Disintegrated spruce wood pieces*

buffer (Finnzymes, Finland). The PCR protocol involved an initial denaturing step of 3 min at 94 °C, annealing of 50 °C at 30 s and extension of 30 s at 72 °C, then 33 cycles of denaturation of 30 s at 94 °C, annealing of 50 °C at 30 s and extension of 30 s at 72 °C and final extension step of 72 °C for 5 min.

The PCR products were analysed by horizontal electrophoresis (Omni-Bio, the Czech Republic) in 1% DNA agarose gel. DNA was visualized by UV transillumination and photographed. The PCR products were purified by NucleoSpin Extract II (Macherey-Nagel, Germany) prior to sequencing.

Sequences were determined with an ABI PRISM 3100 Avant DNA sequencer (Applied Biosystems) at the Department of Animal Morphology, Physiology and Genetics, Faculty of Agriculture, Mendel University, Brno using the ABI PRISM BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems). All samples were sequenced with the primers used in the PCR. The obtained sequences were compared to the data deposited in the NCBI database online (http://www.ncbi.nlm.nih.gov) using the BLAST program accessible at the web page.

RESULTS

In case of the laboratory experiment the DNA of *Serpula lacrymans* was detected in all samples. The DNA extraction kit seems to be suitable for DNA extraction from desintegrated wood and the PCR-based method is sufficiently sensitive for *Serpula lacrymans* detection after relativelly short time cultivation (5 days).

In contrast, when the same method was applied to wooden samples from infected indoor wood construction (field detection no. II), only one sample of four showed positive result. The negative results of PCR reactions employing both (universal and species-specific) primer pairs were caused by degraded DNA template (observed on 2% agarose gel).

The two samples of unknown fungi obtained as mycelium found on wooden construction (field detection no. I) were identified according to sequences of ITS region of ribosomal DNA. Both samples have 100% identity to a DNA sequences deposited in the NCBI database. The sample from Náměšť nad Oslavou has identical ITS sequences to *Donkioporia expansa* (accession number AJ249501). This basidiomycete species is commonly detected on wood constructions (Moreth and Schmidt, 2000). The culture of *D. expansa* was successfuly isolated from the infected wood and it is deposited in the MUAF culture collection (strain No. 850). The ITS sequence of sample from Očová is identical to *Cladosporium oxysporium* (accession number AJ300332). Genus *Cladosporium* is not a wood-decaying basidiomycete but this mold (Ascomycetes) commonly occurs in indoor environment.

The DNA sample of an unknown fungus obtained from the wood of partially decayed pillar at the Funicular Centre (field detection no. III) was also amplified succesfully. The ITS sequence of the sample has 100% identity to *Amylostereum areolatum* (NCBI accession number AF454428). The detected basidiomycete species is a common decayer of coniferous wood in Central-European forests and it may occur on poorly impregnated wood.

DISCUSSION

The identification of wood-decaying fungi from detected mycelia and from infected wood samples was generally successful. The PowerSoil™ DNA isolation kit seems to be a suitable tool for DNA extraction from desintegrated wood substrates.

The successful detection of *Serpula lacrymans* from artificially inoculated wooden blocks confirmed suitability of the method. In contrast, the fungal DNA detection from wooden constructions in the buildings apparently atacted by the fungus was not successful in all cases. The reason of dubious result caused by partially degraded DNA may be explained as insufficient sampling or as local perishing of the fungus. While decayed wood can be visible for decades, the fungus may have died due to humidity fluctuations or some chemical treatment applied several years ago.

CONCLUSION

In general, methods of molecular biology can be added to set of methods enabling to detect and identify wood-decaying fungi on wooden constructions. The crutial problem of these methods is extraction of DNA from infected wood. The PowerSoil™ DNA extraction kit applied in this study can be applicable in isolation of fungal DNA from wood. Nevertheless, the results were not fully favorable in case of older samples of decayed wood without visible mycelium. Nevertheless, other DNA extraction protocols should be checked for comparison to find out more suitable and cost-effective method of DNA isolation from wood.

SOUHRN

Použití metod molekulární genetiky pro identifikaci dřevních hub v dřevěných konstrukcích

Cílem tohoto článku je zhodnotit možné použití molekulárně biologických metod pro detekci dřevních hub přímo z rozloženého dřeva při použití komerčního kitu pro izolaci DNA vyvinutého pro půdní substrát. Experiment založený na identifikaci dřevomorky domácí (*Serpula lacrymans*) z naočkovaných dřevěných špalíčků v laboratorních podmínkách byl následován terénní detekcí dřevních hub přímo ze dřevěných konstrukcí. Houbová DNA byla identifikována při použití metod založe-

ných na polymerázové řetězové reakci (PCR) zahrnující druhově specifickou PCR a sekvenaci namnožené ITS oblasti ribozomální DNA.
Výsledky ukázaly, že použitý kit (PowerSoil™ DNA extraction kit), vyvinutý výrobcem na izolaci z půdy, je poměrně vhodný na izolaci DNA i ze dřeva – v případě laboratorního experimentu se podařilo detekovat dřevomorku se 100 % úspěšností. Na druhou stranu, v případě starších vzorků zetlelého dřeva bez viditelného mycelia (dům v Banské Štiavnici) byla úspěšnost detekce nižší. U jiných vzorků z terénu (zámek v Náměšti nad Oslavou, lanové centrum Brno-Lesná) byla pomocí sekvenace ITS oblasti detekována přítomnost druhů dřevních hub *Donkioporia expansa* a *Amylostereum areolatum*, zatímco v případě domu v Očové se podařilo odhalit pouze přítomnost plísně *Cladosporium oxysporium*.

dřevo, houby, *Serpula lacrymans, Donkioporia expansa, Amylostereum areolatum, Cladosporium oxysporium,* DNA, PCR

AKNOWLEDGEMENTS

We thank MSM 6215648902 and the Grant Agency of the Czech Republic, project No. 526/03/HO36.

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