

Identification of Relevant Wood Decay Fungi using the Novel Mycotype® Basidio^{QS} Microarray Detection Kit

Daniel Müller*, Caroline Hiller, Dana Tusche, Werner Brabetz

Biotype Diagnostic GmbH, Moritzburger Weg 67, D-01109 Dresden

Natalya Rangno, Kordula Jacobs, Wolfram Scheiding

Institut für Holztechnologie Dresden gGmbH (IHD), Zellescher Weg 24, D-01217 Dresden

*Correspondence to: d.mueller@biotype.de Tel.: +49 351 8838 124, Fax: +49 351 8838 435

Background

Wood-rotting fungi (Basidiomycetes) cause often extensive damage on load-bearing constructions of buildings. According to the German norm DIN 68800-4, there is an explicit identification of dry rot fungi required for the assessment and elimination of the fungi decay. To date, the determination of dry rot fungi is made either by macroscopic, microscopic or molecular biological examinations. Thereby occur frequently difficulties in differentiation of morphological characteristics (visual analysis), in examination of mixed samples (PCR-analysis, direct sequencing), or with reference to time exposure (pure cultures). Using Mycotype® Basidio^{QS} it is possible to perform, without long-term expertise, a precise and reliable determination of dry rot fungi – particularly with mixed samples – within just 6 hours.

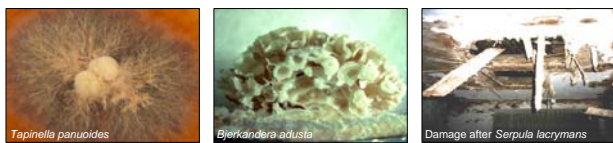


Fig. 1: Illustration of fungi *Tapinella panuoides* and *Bjerkandera adusta* as well as floor damage caused by *Serpula lacrymans*

Method

The identification of fungi is based on the detection of an individual DNA sequence. Within the ribosomal DNA region the internal transcribed spacer regions ITS I and ITS II are highly variable and thus well suited for species and genus specific probes. The conserved coding rDNA genes encode the primer binding regions.

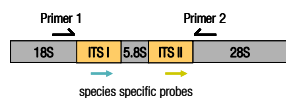


Fig. 2: ITS (internal transcribed spacer) target region. PCR primers anneal within the conserved rDNA genes 18S and 28S. Species specific probes of the ALR bind to ITS I or ITS II.

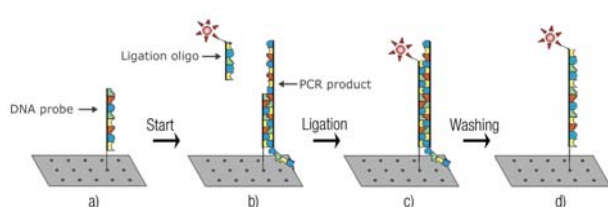


Fig. 3: Arrayed ligation reaction. Species specific captor molecules (DNA probes) are immobilised on a planar surface (a). Complementary target sequences, previously PCR amplified (PCR products), bind to these single stranded DNA probes (b). Once both the DNA probe and the ligation oligonucleotide are complementary to the target sequence (PCR product), a joining between the DNA probe and the ligation oligonucleotide occurs (c). Thereby a thermophilic ligase catalyses a phosphodiester bond between the 3'-hydroxyl end of the DNA probe and the 5'-phosphate end of the ligation oligonucleotide. In the subsequent washing step with high temperatures the target sequences and the unbound ligation oligonucleotides are removed from the probe (d). However, the covalently and specifically joined ligation oligonucleotides stay linked to the DNA probes and can be detected by fluorescence measurement.

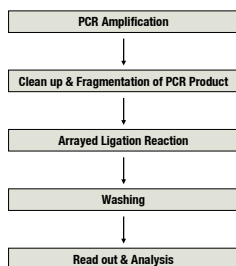


Fig. 2: Workflow. Starting from an isolated DNA sample, the target rDNA region is amplified in one PCR approach. The PCR product is purified and afterwards fragmented through UDG (Uracil-DNA glycosylase). Performing the ALR chip reaction. Washing of microarrays. Detection of fluorescence signals by microarray scanner and software-supported analysis.

Conclusions

Mycotype® Basidio^{QS} provides the possibility to unambiguously detect and differentiate the 27 most relevant wood-rotting fungi within 4 hours upon DNA extraction. By using a two-step assay, comprising the PCR amplification of the ITS region with Basidiomycete specific primers and the ALR with species and genus specific DNA probes, a high analytical specificity for fungal detection is approved. Especially, the simultaneous detection of several species in mixed samples was successfully demonstrated and gives a competitive advantage over conventional diagnostics. The robustness and quality of Mycotype® Basidio^{QS} is guaranteed not only by the extensive validation, but also by the certified development, production and sales of all Biotype Diagnostic products according to DIN EN ISO 9001:2000. This reliable shows that the Mycotype® Basidio^{QS} Microarray Detection Kit is suitable for the identification and differentiation of wood decay fungi in routine diagnostics.

Results and Discussion

First of all, 121 Basidiomycetes (laboratory strains, isolates, practice samples) were clearly and reliably assigned to a particular fungal species by morphological methods. On the basis of the obtained ITS sequences, specific DNA probes were designed and coupled by amino linkers to the polymeric coating of the microarray slide. Through several rounds of redesigning specific and functional DNA probes were evaluated.

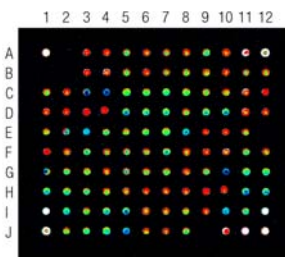


Fig. 5: Functional Analysis of Mycotype® Basidio^{QS}. A DNA mixture of 27 fungi was analyzed to determine the functionality of DNA probes. All DNA probes show the expected results. DNA probe layout: positive control: A1, A12, I1, I12, J1, J11, J12; negative control: A2, B1; empty spots: B2, E12, J9; fungi specific probes: all remaining positions.

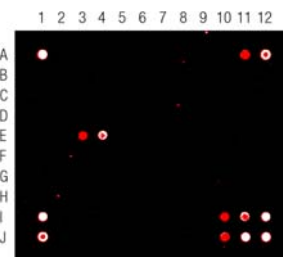


Fig. 6: Specificity Analysis of Mycotype® Basidio^{QS} for *Serpula lacrymans*. Of all fungi specific DNA probes only the *S. lacrymans* specific probes are positive (E3, E4, I10, I11). DNA probe layout: positive control: A1, A12, I1, I12, J1, J11, J12; negative control: A2, B1; empty spots: B2, E12, J9; fungi specific probes: all remaining positions.

To validate the newly developed Mycotype® Basidio^{QS} Microarray Detection Kit extensive studies have been conducted. The analytical sensitivity was determined to 10 pg fungal genomic DNA. For *Serpula lacrymans* (this fungi is subject to report in some federal states) the detection limit was even appointed to 100 fg gDNA, which is equivalent to 8 genome copies.

In practice, wooden constructions are often affected by several different fungi species. Therefore, DNAs from up to four different fungi (*S. lacrymans*, *Coniophora marmorata*, *Gloeophyllum trabeum*, *Daedalea quercina*) were assorted in different proportions and analyzed. It turned out that all co-occurring species of fungi can be identified and differentiated in a single step.

In further experiments, the method proved to be very robust. Thus, various parameters, such as PCR template volume, total PCR/ALR volume, annealing/hybridization temperature, quantity of enzymes, different types of devices were diversified, whereby in all cases a correct result was achieved.

The universal validity of this diagnostic tool was further shown by a multicenter study with 8 national assessor diagnostic institutes. This field test evinced that the Mycotype® Basidio^{QS} Kit can be successfully used by every laboratory person even without long-term expertise of wood-destroying Basidiomycetes, to perform a precise and reliable determination of dry rot fungi.

For straightforward analysis of the microarray experiments, we developed a user-friendly software that imports the scanned image file, analyses the signal intensities and presents the results in a clearly arranged style.

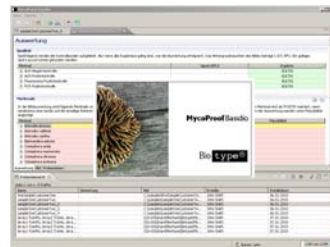


Fig. 7: MycoProf Basidio. User-friendly and easy to use software for complete analysis of signal intensities and output of results in an automated one step process.