Quantitative PCR Assay for Proving Dry Rot by Way of the Implemented Molecular Vitality Test

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Initial Situation and Objective

The most dangerous and, yet by far, most frequent fungal wood destroyer in buildings in Central Europe is dry rot Serpula lacrymans (Wulfen: Fr.) Schroeter apud Cohn (Fig. 1).

Fig. 1: Genuine dry rot infestation of a balcony substructure

Due to its highly destructive potential, special restructuring measures are prescribed for this fungus, which involve clearly higher costs than compared to regular restructuring caused by other fungi (Grosser et al. 2003). Inevitably, proof or exclusion of infestation by S. lacrymans is one of the most important tasks of wood preservation experts within the scope of building refurbishment and evaluation.

Fungal determination within the scope of damage assessment first requires a visual on-site inspection by an expert. If this does not result in conclusive findings, standard laboratory investigations need to be performed (DIN 68800-4:2012). These may involve macroscopic and microscopic analyses, cultivation tests or forms of biological proof, DNA analyses in particular (Gros ser et al . 2013).

The majority of molecular-biological evidencing systems for wood-destructive fungi are based on the specific amplification of rDNA-ITS sequences by means of conventional PCR and subsequent gel electrophoresis (e.g., Jacobs et al. 2010, Schmidt and Moreth 2006, Schmidt and Moreth 1999). Also real-time PCR procedures are increasingly being used (Jacobs et. al 2013). Moreover, the DNA chip technology has been in use at the IHD since 2010, applying species-specific rDNA-ITS probes (Rangno et al. 2010, Jacobs et al. 2010).

The competence and experience of providers of PCR-based diagnostics in the field of wood and building preservation is widely varied or partly non-existent. The "inhouse methods" in use vary strongly, and there is no universal standard. This results in the necessity of evaluating and standardising available methods. Besides, rapid progress in molecular biology provides numerous approaches towards the further development and enhancement of DNA-based diagnostic methods, e.g., for proof of the respective harmful organisms being alive or dead.

Due to the disadvantages and the potential of improvement of existing diagnostic measures, a two-year R&D project was initiated, co-funded by the German Federal Ministry of Economic Affairs and Energy (BMWi).

The aim of the project was to develop a quantitative PCR assay for proving genuine dry rot as well as its closest relative, wild dry rot (S. himantioides) on the basis of new molecular markers and the implementation of proof of the fungi being alive or dead. At the same time, the sensitivity and specificity of the proof was envisaged to be higher than that of conventional PCR.

Results

At first, several genomic DNA areas were investigated and evaluated with a view to their suitability as differentiation markers as well as to the quantification of the target organisms.

As a result, it was shown that both a repetitive marker identified from whole-genome sequences (360-fold occurring repeat motif) and a mitochondrial marker also derived from inherent sequences are principally suitable for the differentiation and quantification of genuine dry rot. Both approaches are of interest for commercial use, but require a comprehensive sequence data basis for developing and validating proof assays.

Therefore, the core DNA markers based on the betatubulin gene, which was also developed as part of the project, and the modified markers from the rDNA-RTS region proved to be favourites for the assay design. Especially the combination of both systems showed a high potential for differentiation, not only regarding genuine dry rot, but also concerning other dry rot in buildings. A probe-based multiplex qPCR assay was developed on their basis and validated in a field test.

The assay was supplemented by implementing extraction and amplification control and universal fungal proof (PAN fungus probe). Apart from the classical application of genomic DNA in graded dilutions, the use of artificial single-strand template oligonucleotide constructs also stood the test.

For the molecular-biological vitality determination or the live/dead proof, two of the betatubulin-gene-derived splice PCR primer combinations were successfully tested on the live and dead material. The specific range of application and the framework conditions for respective diagnostics are yet subject to investigation, but the principally verified functionality is promising for commercial use.

Within the scope of validation, a verification limit of 100 fg of genomic DNA or 1.5 spores/ml was proven in an extraction formulation (corresponding to 15 spores/ml) for the multiplex-probe assay at 95 % reliability. Furthermore, the reproducibility of diagnostic findings was demonstrated on three different qPCR devices (StepOne by Applied Biosystems, Q-Tower by Analytik Jena and Piko-Real by Thermofisher). The reliability, specificity and sensitivity of the assay were eventually confirmed in a field test on twenty timbers from practice, damaged by fungi.

Conclusion

The project results can be applied immediately to the development or further development of diagnostic products for the laboratory proof of fungi, especially the real-time PCR kit for genuine and wild dry rot as well as a DNA extraction kit for higher fungi and fungus-infested timber. Moreover, the solutions found can, regarding their marker structure and assay setup, be transferred to other destructive organisms.

Literature

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