Isolation and identification of a new species of the genus *Bjerkandera*, *Bjerkandera paranensis*

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Introduction

During a selection program aiming the isolation of new potential lignin degraders, an isolate (CCMI 1070) from decaying wood collected in Iguaçu Natural Park, Brazil, was identified as very promising. Further investigation provided a detailed characterization of its ligninolytic enzymes, leading to the first full description of a versatile peroxidase in *Bjerkandera* (Moreira *et al.* 2005, 2006).

Materials and Methods

For the identification protocol of the aforementioned *Bjerkandera* sp. strain (CCMI 1070) to the species level, morphological characterization was carried out and carpophore production was achieved *in vitro*. Mycelium growth rates, vegetative compatibility and RAPD fingerprints were assessed for *Bjerkandera* type strain CCMI 1070, as well as for other known strains belonging to genus *Bjerkandera*. DNA sequences from ribosomal nucSSU, mitSSU and ITS nrDNA were determined and a multi-locus phylogeny constructed by a Bayesian MCMC approach. A method based in a multiplex PCR approach targeting mitSSU rDNA was also developed, which allowed the reliable molecular identification of *B. paranensis* isolates.

Results and Discussion

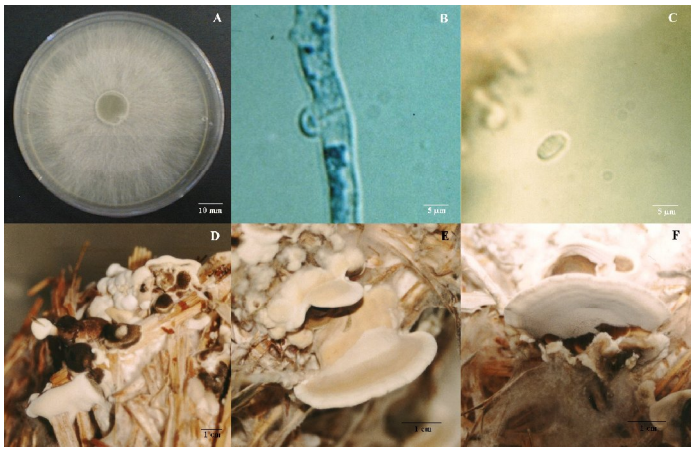

Figure 1. Morphological characteristics of *Bjerkandera* sp. CCMI 1070. (A) Colony grown at 30 ºC on PDA, reaching 35 mm in diameter by 4 d; (B) Clamped, septate, dikaryotic hyphae, after staining with lactophenol cotton-blue; (C) Basidiospore from carpophore slice; (D) Primordia grown on straw; (E and F) Carpophores, grown on straw.

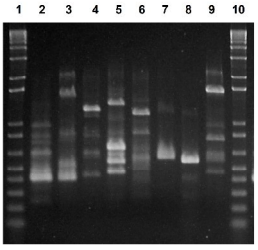

Figure 2. RAPD fingerprints of *Bjerkandera* strains using primers M13 (lanes 2-5) and (GACA)3 (lanes 6-9). Lanes: 1, 10 — 1 kb Plus DNA ladder (GibcoBRL); 2, 6 —*Bjerkandera* sp. CCMI 1070; 3, 7 — *Bjerkandera* sp. BOS55; 4, 8 — *Bjerkandera adusta* DAOM 215869; 5, 9 — *Bjerkandera fumosa* CBS 100982.

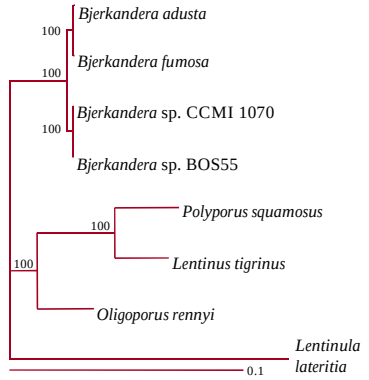

Figure 3. Phylogenetic relationships among *Bjerkandera* species and other polypore species based on combined data from ITS nrDNA, mitSSU and nucSSU rDNA sequences. *Lentinula lateritia*, belonging to euagarics, was chosen as outgroup. This phylogeny resulted from a 50% majority rule consensus, based on 60 000 trees from Bayesian MCMC tree sampling. Branch support by Bayesian posterior probabilities is shown at nodes.

Bjerkandera sp. CCMI 1070 highly resembles *Bjerkandera* sp. BOS55 in terms of phenotypic characters (Fig 1.), viz. macroscopic and microscopic basidiomata observation and growth rate. The later phenotypic characteristic clearly distinguishes the group formed by CCMI 1070 and BOS55 from *B. adusta* and *B. fumosa*. The uniqueness of RAPD fingerprints (Fig 2.) is in agreement with the vegetative compatibility tests (data not shown), and the closer similarity between strains CCMI 1070 and BOS55 supports the claim for a new species associated with these isolates. The phylogeny (Fig 3.) obtained Bayesian MCMC approach allows distinction of two separate clusters: one with *Bjerkandera* sp. CCMI 1070 and BOS55, and the other comprising *B. adusta* and *B. fumosa*. The high level of similarity within these groups is remarkable, supported by 100% posterior probability values.

Conclusions

The combination of differences in phenotypic characters and DNA sequences fully justifies our proposal for a new species. As a consequence, a new species should be created, able to allocate strains CCMI 1070 and BOS55. Taking in account that the geographic origin of strain CCMI 1070 is reliably established, the name of *Bjerkandera paranensis* is proposed — and strain CCMI 1070 is chosen as typus. A method based in a multiplex PCR approach targeting mitSSU rDNA was also developed, based on a 5nt indel detected in the mitSSU rDNA sequence, which allows the reliable molecular identification of *B. paranensis* isolates.

References

Moreira, P.R. *et al.* 2005. Molecular characterization of versatile peroxidase from a *Bjerkandera* strain. Journal of Biotechnology, 118: 339-352.
Moreira, P.R. *et al.* 2006. Purification, kinetics and spectral characterisation of a new versatile peroxidase from a *Bjerkandera* sp. isolate. Enzyme and Microbial Technology, 38: 28-33.

Acknowledgments

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