Molecular Characterization and Taxonomic Affinities of Species of the White Rot Fungus *Ganoderma*

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The systematic affinities of *Ganoderma* have largely been resolved in the extensive publications of Moncalvo and coworkers (Moncalvo *et al.,* 1995a, b; Hseu *et al.,* 1996). The present communication adds further sequences of the ITS1 region of *Ganoderma* isolates from Poland and corrects some of the classifications of *Ganoderma* species. The sequence data indicate that *G. australe* and *G. adspersum* are different species. Both morphological and molecular data are in accord with an interspecific separation of *G. pfeifferi* and *G. resinaceum.* The ITS1 region is particularly suited for the taxonomic segregation of *Ganoderma* by molecular methods.

Introduction

The polypore fungus *Ganoderma* is widespread worldwide. Some *Ganoderma* isolates are used in folk medicine, particularly in Eastern Asia (Jong and Birmingham, 1992). More importantly, species differ in their capability of wood-degradation, can cause diseases in trees and are therefore of commercial interest (Adaskaveg *et al.,* 1990). Variations in lignin and polysaccharide degradations can occur between isolates of the same species which appears to be pathogenic at one location whereas it is saprophytic at the next. Morphological criteria of the fruit bodies and of cell cultures does not allow to unambiguously differentiate species in many instances and particularly not to separate pathogenic isolates from the others. The whole genus *Ganoderma* was considered being a taxonomic chaos (Ryvarden and Gilbertson, 1993). A collaboration between the Katowice and Cologne laboratories was, therefore, started to resolve taxomic uncertainties by examining molecular traits. In the course of this study, an extensive description of the genus *Ganoderma* combining both molecular and morphological criteria has appeared (Moncalvo *et al.,* 1995a,b). The present communication, therefore, presents some additional characters of fungi of the genus *Ganoderma* which supplement to the impressive set of data already published (Moncalvo *et al.,* 1995a, b; Hseu *et al.,* 1996).

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Materials and methods

Fungi used.

Ganoderma adspersum (S. Schulzer 1878) Donk 1969 fruit bodies were collected from the trunks of several living trees of *Quercus rubra* L. in the park near the river Odra at PL-Raciborz, Silesia. *G. pfeifferi* (Bresadola in Patouillard 1889) fruit bodies came from the trunk of one living beech-tree (*Fagus sylvatica* L.) at Bobrek near Oswieçim/ South Poland and *G. resinaceum* (Boudier in Patouillard 1889) was picked from a dead trunk of *Acer platanoides* L. near the house of the president of the Silesian University in Katowice. The source of the isolate of *G. lucidum* (Fr.) P. Karst. was originally M. Zang of the Herbarium of Cryptogams at Kumming, China (culture F 1827), and the agar gel plates were transferred via the Institute of Systematical Botany in D-Tübingen and then *via* Katowice to the Botanical Institute in D-Köln. Mycelia of the fungi were grown in a liquid medium containing 5% yeast extract, and genomic DNA was

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isolated according to Raeder and Broda (1985). The primers ITS2 and ITS5 (White *et al.*, 1990) were used for amplifying DNA encompassing part of the 5.8S rRNA, ITS1 region and part of the 18S-rRNA. Amplification was performed in a total vol of 50 µl containing 2U Taq-DNA-polymerase (Promega, Madison), 5 µl Taq polymerase reaction buffer (Promega), 4 µl 25 mM $MgCl_2$, 4 µl DNTP-Mix (2.5 mM each, Boehringer, D-Mannheim), 1 µl of each of the two primers and 1 µl of the genomic DNA (100–500 ng). The reactions were performed in 36 cycles with the following conditions: 30 s denaturation at 92 °C, 30 s annealing at 52 °C and 60 s elongation at 72 °C.

PCR products were cloned into the pGEM-T-Easy Vector (Promega) following the manufacturer's protocol and transformed into competent *E. coli* XL1 Blue by the heat shock method (Sambrock *et al.*, 1989). Sequencing was done on an ABI 310 sequencer using the ABI PRISM dye terminator cycle sequencing reaction kit (Perkin Elmer, Foster City, USA). Sequence data were compared with the NCBI databank using the BLASTN program (Altschul *et al.*, 1997). Restriction enzymes were purchased from MBI Fermentas and digests were separated on 2% agarose gels.

Results and Discussion

Ganoderma has been sampled worldwide, but not so much in Europe, particularly not in its Eastern part. Out of the seven species occurring in Europe (Domanski *et al.*, 1973; Ryvarden and Gilbertson, 1993), fruit bodies of five were collected, the DNA was isolated, and their ITS1 region were amplified by PCR and sequenced. In two cases (*G. applanatum* and *G. carnosum*), sequences did not show homologies to published ITS1 sequences of *Ganoderma* species (Moncalvo *et al.* 1995a), indicating that foreign DNA inside the fruit bodies must have been amplified. The sequences of the remaining three and of one *G. lucidum* isolate are given in Fig 1. The differences in the sequences between the *Ganoderma* species *adspersum*, *lucidum, pfeifferi* and *resinaceum* were 9–33% and thus high enough to allow differentiation on a species level (Table I). It had already been noted that sequences of the ITS1 region are particularly suited for taxonomic purposes (Moncalvo *et al.*, 1995a, b). Within species, as documented for *G. adspersum* and *resinaceum*, the differences between sequences of Polish (this study) and of word-wide (Moncalvo *et al.*, 1995a) samples are under 2% which unlikely allows intraspecies differentiation immediately. However, the ITS1 region shows highest variations in its middle part (Fig. 1) and is amenable to digestion by the restriction enzymes *AluI, HaeIII, HinfI, HpaII* or *TaqI*. Restriction provides digests of characteristic sizes in the case of *G. adspersum, pfeifferi* and *resinaceum* (not documented). PCR amplification followed by restriction enzyme analysis might be a tool to differentiate between species since ITS-

Table I: Sequence divergence in the ITS1 region of different *Ganoderma* isolates.

Species	*G. ad* PL	*G. ad* 351.71	*G. luc* China	*G. pfeif* PL	*G.p feif* 745.84	*G. resi* PL	*G. resi* 194.76
G. ad PL	X	1	16	9	15	15	15
G. luc China	16	15	X	33	5	1	2
G. pfeif PL	9	12	33	X	29	27	27
G. resi PL	15	15	1	27	3	X	2

Data are given in % of the total number of bases of the ITS-region.
Abbreviations: G. ad PL = *Ganoderma adspersum* from Raciborz, Poland; *G. ad* 351.71 = *Ganoderma adspersum* CBS 351.71, sequence data from Moncalvo *et al.* (1995a); *G. luc* China, isolate originally from China and sequenced in Cologne; *G. pfeif* PL = *G. pfeifferi* from Oswieçim, Poland; *G. pfeif* 745.84 = *G. pfeifferi* CBS 745.84, sequence data from Moncalvo *et al.* (1995a); *G. resi* PL = *Ganoderma resinaceum* from Katowice, Poland; *G. resi* 194.76 = *Ganoderma resinaceum* CBS 194.76, sequence data from Moncalvo *et al.* (1995a). For other details see Materials and Methods.

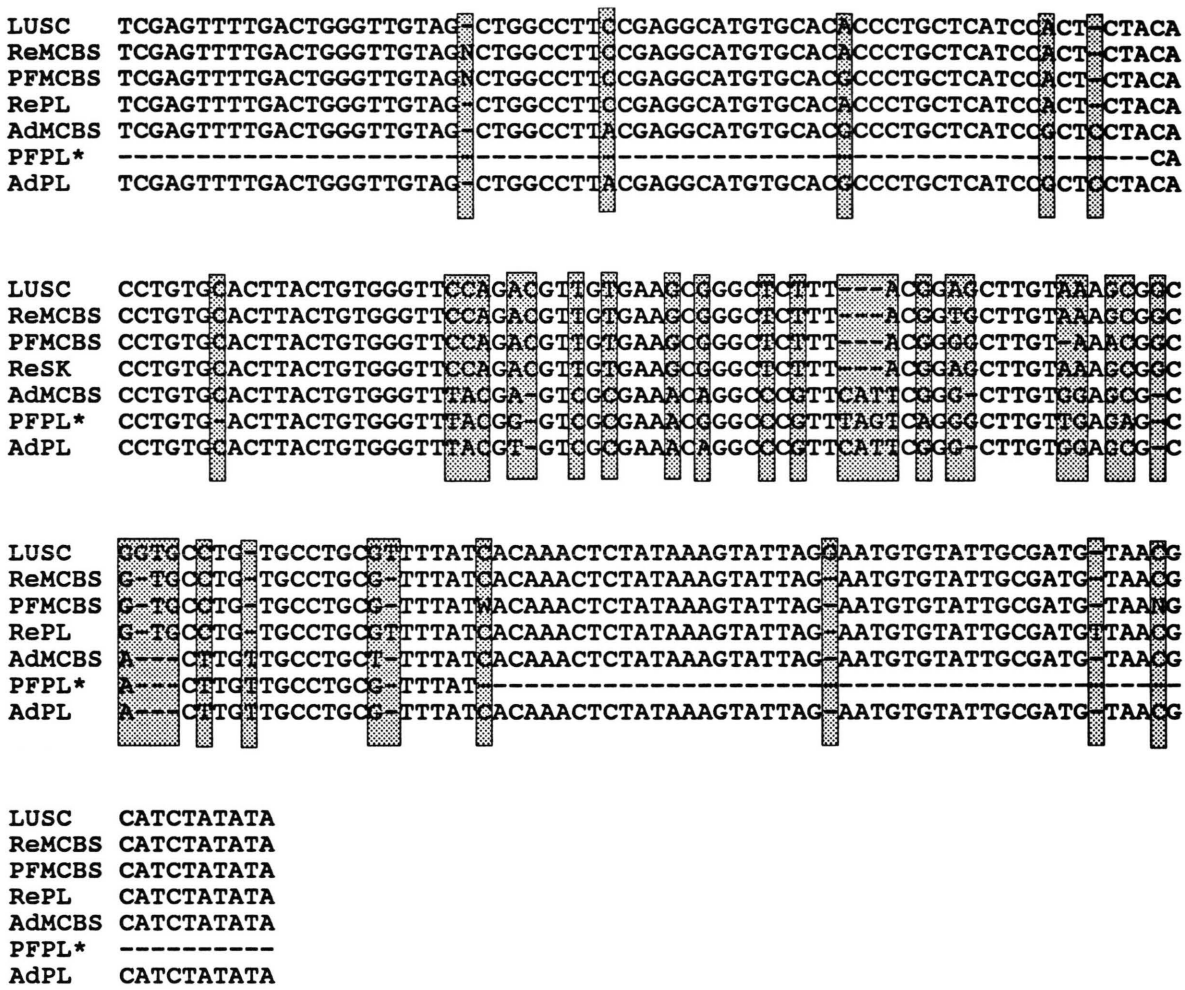

Fig. 1. The DNA sequences of the ITS1 region of seven *Ganoderma* isolates.

Abbreviations: LUSC = isolate originally from China and sequenced in Cologne; ReMCBS = *Ganoderma resinaceum* CBS 194.76, sequence data from Moncalvo *et al.* (1995a); PFMCBS = *G. pfeifferi* CBS 745.84, sequence data from Moncalvo *et al.* (1995a); RePL = *G. pfeifferi* from Oswieçim, Poland; AdMCBS = *Ganoderma adspersum* CBS 351.71, sequence data from Moncalvo *et al.* (1995a); PFPL* = *G. pfeif* PL = *G. pfeifferi* from Oswieçim, Poland.

amplificates alone are sometimes not sufficient (Hseu *et al.*, 1996). The alternative method (random amplified polymorphic DNA PCR = RAPD) is probably to fine to allow species identification (Hseu *et al.*, 1996) but is possibly useful for differentiating between pathogenic and saprophytic forms within species.

G. pfeifferi and *G. resinaceum* are classified as different species because of several morphological criteria (Table II). However, nucleotide divergence in the ITS1 region between the two species was only <2% (Table II in Moncalvo *et al.* 1995a). In contrast, sequencing of part of the ITS1 region of the Polish samples gave a 27% divergence between *G. pfeifferi* and *G. resinaceum* and even 29% between the Polish *G. pfeifferi* and the *G. pfeifferi* isolate CAS 74584 of Monalvo *et al.* (1995a). By contrast, the differences in the sequences between the Polish *G. resinaceum* and the *G. pfeifferi* isolate CAS 74584 was only 3% (Table I). This casts some doubts about the *G. pfeifferi* isolate CAS 74584. In our hands, sequence data obtained for *G. pfeifferi* and *G. resinaceum* match with the morphological dif-

Table II: Morphological differences in selected *Ganoderma species.*

Taxonomic character	*G. lucidum*	*G. resinaceum*	*G. pfeifferi*	*G. adspersum*
Life span of basidiocarps	annual	annual*	perennial	perennial
Crust of the pileus	hymeniderm	hymeniderm	characoderm	anamixoderm
Stem form	stipitate	stipitate with a short thick stem	sessile	sessile
Colour of the context**	creamy white, becoming dark purple brown with the age in some portions	greyish brown	reddish brown	reddish brown
Tubes	1 layer	1 layer	many layers in many years old specimen	many layers in many years old specimen
Parasite of	roots and the base of trunk of trees	base and lower parts of trunk of trees	trunk of trees	trunk of trees

* According to Ryvarden and Gilbertson (European Polypores, Part 1: *Abortiporus-Lindtneria*, Synopsis fungorum, Oslo, 66, p. 279, 1993) basidiocarps of *G. resinaceum* are perennial which is in contrast to own experience (S. Sokoł).
** Context = inside part of the fruitbody of fungi.

ferentiation, thus these are apparently not conspecific.

In addition, *G. pfeifferi* was placed into the *G. lucidum* complex by Moncalvo *et al.* (1995a, b). The morphological characteristics of the *G. pfeifferi* isolate CBS 747.84 from the Netherlands are unknown to us and Moncalvo *et al.* (1995a, b) probably had access only to mycelian cultures and not to fruit bodies. The morphological characterization of *G. pfeifferi* and of *G. adspersum, resinaceum* and *lucidum* are given in Table II. The morphological criteria listed in Table II and the sequence data (Fig. 1) clearly indicate that *G. pfeifferi* belongs to the *applanatum* and not to the *lucidum* complex.

Another amendment concerns *G. australe.* In the D2 region of the 25S rDNA, *G. australe* and *G. adspersum* share an identical sequence in the data matrix submitted to parsimony analysis (Fig. 4 in Moncalvo *et al.,* 1995a). However, in the ITS1 and 2 regions, the percentage of nucleotide divergence between *G. australe* and *G. adspersum* is 12% (Table II in Moncalvo *et al.,* 1995a). As regard to the Polish *G. adspersum* isolate, the sequence comparison of the ITS1 region (Fig. 1) reveals 8 % divergence to *G. australe* and only 2% to the *G. adspersum* sequenced by Moncalvo *et al.* (1995a). Thus *G. australe* and *G. adspersum* are two different species.

The *Ganoderma lucidum* culture originally came from China, but its stations on the way to Cologne can hardly be tracked back anymore. *G. resinaceum* fruit bodies were collected in Silesia, and tissue cultures were made from the fruit bodies. DNA interspanning part of the 5.8S-rRNA region – ITS1 region and part of the 18S-rRNA of both cultures was amplified and sequenced twice with identical results. As revealed by the BLASTN program, the sequences of the Chinese *G. lucidum* and of *G. resinaceum* were nearly 100% identical but the nearest fit to the next of the published *G. lucidum* sequences was only 94%. Thus the culture from China sequenced in Cologne apparently belongs to *G. resinaceum.* This is an example where sequencing can help to identify the true nature of an isolate. As stated (Moncalvo *et al.,* 1995b), *G. lucidum* and *resinaceum* can easily be mixed up.

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