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

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Z. Naturforsch. 54c, 314–318 (1999); received January 25, 1999

Polycope Fungi, White Rot, Molecular Systematics, ITS1 Region, Ganoderma

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 taxonomic 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).

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 a living beech-tree (Fagus sylvatica L.) at Bobrek near Oswiecim/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 MgCl₂, 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 (Sambrook 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 _Alu I, HaeIII, HinfI, HpaI_ 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>
<thead>
<tr>
<th>Species</th>
<th>G. ad PL</th>
<th>G. ad 351.71</th>
<th>G. luc China</th>
<th>G. pfeif PL</th>
<th>G. pfeif 745.84</th>
<th>G. resin PL</th>
<th>G. resin 194.76</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. ad PL</td>
<td>X</td>
<td>16</td>
<td>16</td>
<td>9</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>G. luc China</td>
<td>16</td>
<td>15</td>
<td>X</td>
<td>33</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>G. pfeif PL</td>
<td>9</td>
<td>12</td>
<td>33</td>
<td>X</td>
<td>29</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>G. resin PL</td>
<td>15</td>
<td>15</td>
<td>1</td>
<td>27</td>
<td>3</td>
<td>X</td>
<td>2</td>
</tr>
</tbody>
</table>

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 Oswiecim, Poland; _G. pfeif 745.84_ = _G. pfeifferi_ CBS 745.84, sequence data from Moncalvo et al. (1995a); _G. resin PL_ = _Ganoderma resinaceum_ from Katowice, Poland; _G. resin 194.76_ = _Ganoderma resinaceum_ CBS 194.76, sequence data from Moncalvo et al. (1995a). For other details see Materials and Methods.
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 Moncalvo 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-

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**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 Oswieim, Poland; AdMCBS = _Ganoderma adspersum_ CBS 351.71, sequence data from Moncalvo et al. (1995a); PFPL* = _G. pfeifferi_ PL from Oswieim, Poland.
Table II: Morphological differences in selected *Ganoderma* species.

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

* 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. Sokol).

** Context = inside part of the fruitbody of fungi.

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 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.

**Acknowledgement**

This work was kindly supported by a grant from the BMBF (project no 0310733 of BEO, Forschungszentrum Jülich. The authors are indebted to U. Hildebrandt and Kerstin Nawrath, Cologne, for participating in some of the experiments.


