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Highly conserved 5S ribosomal RNA sequences in four rust fungi and atypical 5S rRNA secondary structure in *Microstroma juglandis**

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ABSTRACT

The 5S ribosomal RNA nucleotide sequences of five basidiomycetous fungi, Coleosporium tussilaginis, Gymnosporangium clavariaeforme, Puccinia poarum, Endophyllum sempervivi and Microstroma juglandis were determined. Despite high differentiation in their host spectra the four rust species are highly conserved with respect to their 5S rRNA sequences, which fit with the basidiomycete cluster 5 described by Walker and Doolittle (1). The sequences obtained from the first three rust fungi were proven to be identical while the sequence from Endophyllum sempervivi showed two base substitutions compared with the other rust fungi. The Microstroma juglandis 5S rRNA sequence differs from all other basidiomycete 5S rRNA sequences published so far in respect to its secondary structure which shows an atypical 'CCA' loop in helix D, but it reveals typical basidiomycetous signature nucleotides. Therefore Microstroma juglandis represents a cluster of its own within the Basidiomycetes. A dendrogram was constructed based on Kimura's "Neutral Theory of Molecular Evolution".

INTRODUCTION

When Walker and Doolittle published their first paper on the 5S ribosomal RNA of Basidiomycetes (1) they stimulated a lively discussion on natural relationships in this class of higher fungi. The deep gap between the Rhodosporidium-group and the other Basidiomycetes analyzed by these authors documented both the high diversity within the Basidiomycetes and the age of the higher fungi. In addition, their study fed our hope for a universally applicable tool for classifying even those organisms which show hardly any morphological differentiation. In the meantime, the 5S rRNAs from other basidiomycetous organisms were published (2, 3). Walker and Doolittle tried to allign their results with other criteria such as the septal pore structure, and grouped the Basidiomycetes analyzed so far according to their 5S rRNA into five clusters (3). Huysmans et al. (2) published a secondary structure model for the basidio-

mycetous 5S rRNA. Until now, only a few representatives of the different groups within the Basidiomycetes are characterized.

The rust fungi are considered to be a very old and basic group within the Basidiomycetes. Mainly because of their life cycle, their host spectra and their spore morphology they are grouped into six distinct families (5). Microcyclic life cycle and basidial structure was used to deduce via Herpobasidium filicinum their relationships with the Auriculariales (6-10). Since rust fungi can be cultured, only a few investigations on their systematic position were reported. Here we present 5S rRNA sequences from four rust fungi of different families in order to discuss their relationships among each other as well as their systematic position within the Basidiomycetes.

With respect to its systematic position there were controverses whether Microstroma juglandis is a basidiomycete at all (11-14). Although it was grouped with the Heterobasidiomycetes close to Exobasidium because of its simple pores, holobasidia, type of parasitism on higher plants and yeast-like growth in pure culture, its relationship to fungi of this group still remained unclear (15-18). In this study the 5S rRNAs from Microstroma juglandis was investigated to unveil its affiliation to other Basidiomycetes.

MATERIALS AND METHODS

The following strains were obtained from Dr.R.Bauer, University of Tübingen, as haploid yeasts grown from pycnospores: Coleosporium tussilaginis (Pers.) Lev. strain R.Bauer #776, Gymnosporangium clavariaeforme (Jacq.) DC. strain # 855, Puccinia poarum Niels. strain # 830, and Endophyllum sempervivi (Alb. et Schw.) deBy. strain # 778. The Microstroma juglandis (Bereng.) Sacc. strain PB 4142 was isolated from Juglans regia L. by P.B.

The organisms were grown in shaking cultures using medium I described by Sundström (19). The yeast-like cells were harvested by centrifugation, washed twice in Tris-buffer (10 mM Tris/HCl, pH 7.2, 5mM Mg-acetate, 30 mM KCl), and lysed by passing through a French press. Prior to separation on a 10% polyacrylamide gel containing 0.1% SDS, the lysate was extrac-

ted three times with buffer saturated phenol and precipitated with ethanol. The 5S rRNA was eluted from the gel (20). 3'- and 5'-termini were labelled as described by Krupp and Gross (21). Radioactive RNA was further purified by polyacrylamide gel electrophoresis (20).

Sequences were determined by the enzymatically RNA sequencing method described by Donis-Keller (20) using 3'- and 5'-labelled RNA. In addition to the RNases T1 and U_2 the enzymes Bc (22), Cl3 (23,24) and PhyM (25) were used. In Coleosporium tussilaginis the 5'-endgroup of the 5S rRNA molecule was confirmed by mobility shift analysis (26). Sequencing gels were 12%, 15% and 20% polyacrylamide, 8.3 M urea. The 3'-terminal nucleotide was determined by complete alkaline hydrolysis (50 mM $NaHCO_3/NaCO_3$, pH 9, at 95°C for 1 hour) and by thin layer chromatography.

The evaluation of sequence differences is first of all based on the number of differing nucleotides between each two sequences compared. From these numbers the corresponding K_{nuc}-values and their standard errors were calculated as introduced by Motoo Kimura (27,28) and used for comparing 5S rRNA sequences by Kumazaki et al. (29). Different lengths and gaps of the 5S rRNA sequences were regarded as transversions, as described by the former authors. In the case of varying 3'-ends in a sequence the prevailing form was used for our calculations. A dendrogram was constructed from the K_{nuc}-values based on the average-linkage-method as described in Kumazaki et al. (l.c.), including the indication of the standard error which was calculated as described by Kimura (27,28). For the dendrogram all known sequences of that cluster were used as published by former authors (1-3).

RESULTS

The 5S rRNA sequences from the three rust fungi Coleosporium tussilaginis, Gymnosporangium clavariaeforme and Puccinia poarum were found to be identical. Therefore only one 5S rRNA sequence appears in figure 1 for the three identical molecules together with the deviating sequences from Endophyllum sempervivi and Microstroma juglandis. In Endophyllum sempervivi the

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                  10        20        30        40        50
(1,2,3)  AUCCACGGCCAUAGGACCUUGAAAACACCGCAUCCCGUCCGAUCUGCGCAGUUAACCAG
(4)      AUCCACGGCCAUAGGACCCUGAAAGCACCGCAUCCCGUCCGAUCUGCGCAGUUAACCAG
(5)      AUCCACGGCCAUAGGACACAGAAAACAUCGCAUCCCGUCCGAUCUGCGCAAUCAAGCUG

         60        70        80        90        100
(1,2,3)  GGUGCCGCCUAGUUAGUACCACGGUGGGGGACCACGCGGGAAUCCUA---GGUGCUGUGGUU
(4)      GGUGCCGCCUAGUUAGUACCACGGUGGGGGACCACGCGGGAAUCCUA---GGUGCUGUGGUU(U)
(5)      UGUACCGCCCAGUCAGUACCGGAGUGGGGGACCAUCCGGGAAUCCUGCCAGGUGCUGUGGUU
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Figure 1: 5S ribosomal RNA sequences from (1) Coleosporium tussilaginis, (2) Puccinia poarum, (3) Gymnosporangium clavariaeforme, (4) Endophyllum sempervivi and (5) Microstroma juglandis

3'-end was found to be heterogenous, showing a 'U' in position 119 in about 40% of the molecules while in the other molecules the sequence ended in position 118. Walker and Doolittle's 5S rRNA sequence from Filobasidium floriforme (1) was found to be identical with that from Endophyllum sempervivi.

The sequences shown here fit the secondary structure model of the 5S rRNA of Basidiomycetes as proposed by Huysmans et al. (2). Microstroma juglandis shows an atypical 'CCA' loop in helix D.

The dendrogram presented in figure 2 shows the relative positions between the organisms based on their 5S rRNAs. For comparison other basidiomycetous 5S rRNA sequences worked out by Huysmans et al. (2) and by Walker and Doolittle (1,3) have been included; sequences from each of the five clusters of the last authors have been selected. In this comparison, the sequences of the four rust fungi appear among the organisms listed in cluster 5, while the 5S rRNA from Microstroma juglandis does not fit with any of the present clusters.

DISCUSSION

The 5S ribosomal RNA nucleotide sequences from Coleosporium tussilaginis, Puccinia poarum and Gymnosporangium clavariaeforme were found to be identical, and to differ only in two positions from that of Endophyllum sempervivi. Although the rust fungi have many features in common, like transversely septate metabasidia and simple septal pores with septal walls

tapering towards the central opening (30), this result was not to be expected because the fungi used for this comparison differ in some important respects: a) In the genus Coleosporium the basidia do not develop according to the usual basidial type (31). b) In the genus Gymnosporangium the dicaryotic state is parasitizing on gymnosperms, while all other rust fungi parasitizing on gymnosperms, including Coleosporium tussilaginis, are growing there in their haplophase. c) Our selection of rust fungi covers quite diverse host spectra, containing species parasitizing on gymnosperms and angiosperms (Gymnosporangium clavariaeforme and Coleosporium tussilaginis), on dicots and monocots (Puccinia poarum), and a microcyclic rust on dicots (Endophyllum sempervivi). Therefore, because of their high diversity in respect to host spectra, life cycles, spore morphology and basidial type, the rust fungi were by many authors assumed to represent quite an old group (5-10). Thus, the 5S rRNA sequences from various rust fungi were expected to show significant differences rather than being similar, as high differences were found within other basidiomycetous groups like the smut fungi, where analyses revealed that more than one third of the nucleotides differ between Ustilago violacea and Tilletia controversa (1). Therefore, we assume that all rust fungi are very much consistent in respect to their 5S rRNA, in contrast to their host spectra and their life cycles.

The rust fungi were considered to be a basic group of the Basidiomycetes with resemblances to the Ascomycetes because they also show simple septal pores, bipolar mating system, rudimental sexual organs and asexual fructification structures (10). The comparison of the 5S rRNA sequences shown in figure 1 with sequences from Ascomycetes (32-39) does not confirm any specific relationship of Uredinales with ascomycetous fungi.

Within the Basidiomycetes, the rust fungi seem to fit best with Walker and Doolittle's cluster 5 as shown in figure 2. Walker and Doolittle pointed out (1,3) that cluster 5 contains only organisms which also show dolipores. Now it becomes apparent that the 5S rRNA sequence does not in all cases parallel the septal pore type, since the rust fungi do not share the dolipore type with the other organisms in cluster 5.

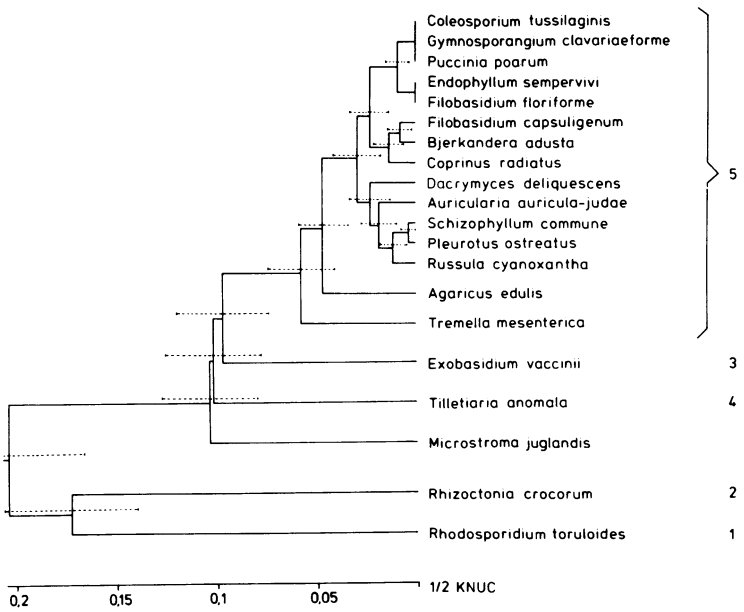

Figure 2: Relations between basidiomycetous fungi based on their 5S rRNA nucleotide sequences; dashed lines indicate range of standard error. Numbers on the right side correspond to Walker and Doolittle's clusters 1-5 (3).

When Walker and Doolittle established their cluster 5, this cluster was already difficult to understand because it harbours organisms like Auricularia auricula-judae from the Heterobasidiomycetes as well as Russula cyanoxantha from Homobasidiomycetes. This situation becomes now even more confusing, as we see that the 5S rRNAs from the rust fungi appear to be most similar to such molecules from the holobasidial organisms Filobasidium capsuligenum, Bjerkandera adusta and Coprinus radiatus, or even identical in Endophyllum sempervivum and Filobasidium floriforme. These results are in contrast to studies based on morphological characteristics. We think that this congruence with respect to the 5S rRNA occurs by chance. Thus the 5S rRNA sequences do not support the other data which indicate that the Hetero- and Homobasidiomycetes are two distinct groups within the Basidiomycetes. We suppose that the 5S rRNA nucleotide sequences are not as reliable a systematic tool

inside cluster 5 because of the low degree of differentiation in this cluster as they are within the other clusters and for discriminating clusters from each other.

The 5S rRNA from Microstroma juglandis is unique in two respects: First, it does not fit with any of the five clusters established by Walker and Doolittle (3), as can be seen in figure 2. Second, it shows a unique 'CCA' loop in helix D of De Wachter et al.'s secondary structure model (4). In clusters 1 and 2, in position 107 a 'U' is inserted which corresponds to the 'CCA' loop. In the dinoflagellate Crypthecodinium cohnii at the same position a 'UUA' loop was found (40) without showing any further congruences in the 5S rRNAs from Crypthecodinium cohnii and Microstroma juglandis. Former suggestions to the effect that this organism might be related to the Exobasidiales (15-18) could not be confirmed. Whether there are any relations to the Cryptobasidiales, as assumed by Oberwinkler (17), remains to be proven. So far the 5S rRNA from Microstroma juglandis represents a cluster of its own within the Basidiomycetes.

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