Cystobasidiopsis nirenbergiae, a new agaricostilbomycete (Pucciniomycotina)

Robert BAUER\textsuperscript{a,}*, Berthold METZLER\textsuperscript{b,1}, Dominik BEGEROW\textsuperscript{c}, Franz OBERWINKLER\textsuperscript{a}

\textsuperscript{a}Universität Tübingen, Botanisches Institut, Lehrstuhl Spezielle Botanik und Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen, Germany
\textsuperscript{b}Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Mikrobiologie, D-14191 Berlin, Germany
\textsuperscript{c}Ruhr-Universität Bochum, Geobotanik, ND 03/174, Universitätsstraße 150, D-44801 Bochum, Germany

A B S T R A C T

A new genus, Cystobasidiopsis, and a new species, Cystobasidiopsis nirenbergiae, are described for a fungus isolated from an arable loess soil in Ahlum near Braunschweig, Niedersachsen, Germany. An integrated analysis of morphological, ecological, ultrastructural and molecular data indicates that the new species belongs to the Chionosphaeraceae within the Agaricostilbales. Relevant characteristics of the new species are discussed and compared with those of related taxa.

© 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

The Agaricostilbales represent one of the pucciniomycotinous lineages (Weiß et al. 2004; Bauer et al. 2006). This order contains the teleomorphic genera Agaricostilbum, Chionosphaera, Kondoa, Mycogloea (\textit{pro parte}) and Stilbum (Bauer et al. 2006; for the systematic position of Stilbum see Oberwinkler & Bauer 1989; Aime et al. 2006). All are dimorphic, producing a yeast or yeast-like phase in the haploid state. Morphologically and ecologically, however, the members of Agaricostilbales possess a high degree of divergence. Thus, Chionosphaera is holobasidioid, whereas the other teleomorphic members are phragmobasidioid. In addition, in contrast with the other Agaricostilbales the basidia of Kondoa are ballistosporic (Fonseca et al. 2000), and the basidial cells in Agaricostilbum bud in a yeast-like manner (Bauer et al. 1992). Furthermore, the species of Agaricostilbum, Chionosphaera and Stilbum develop stipitate, capitulate basidiocarps (Oberwinkler & Bandoni 1982). The basidiocarps of Mycogloea are minute and are normally found in association with sporocarps of ascomycetes (Bandoni 1995, 1998). Distinctive features of Mycogloea are the deciduous basidia that easily detach from the probasidia. The auricularioid basidial stage of Kondoa is known only from cultures (Fonseca et al. 2000).

\textsuperscript{5} Part 235 in the series Studies in Heterobasidiomycetes from Botanical Institute, University of Tübingen.

\* Corresponding author. Tel.: +49 7071 2973212; fax: +49 7071 295344.
E-mail address: robert.bauer@uni-tuebingen.de

\textsuperscript{1} Present address: Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Abt. Waldschutz, Wonnhaldestr. 4, D-79100 Freiburg, Germany.

0953-7562/$ – see front matter © 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycres.2009.05.003
Agaricostilbum species have frequently been found on dead material of palms, whereas Stilbum vulgarum has been collected from heterogeneous substrates. Chionsphaeria species have sometimes been suspected to be mycoparasitic due to their frequent association with other fungi (e.g., Kirschner et al. 2001).

Phylogenetic analyses have revealed a high diversity of fungi in the soil (e.g., Schadt et al. 2003; Jumpponen & Johnson 2005). However, isolation of previously unknown taxa of heterobasidiomycetes from soil has only sporadically done (e.g., Metzler et al. 1989). Here we describe a new agaricostilbomycete isolated from an arable loess soil in Ahlum near Braunschweig, Niedersachsen, Germany. Relevant characteristics of the isolate are discussed and compared with those of related taxa.

Materials and methods

Isolation

The fungus was isolated by Sauthoff et al. (1994) in the frame of a large scale screening for the fungal flora of an arable loess soil (Ahlum, near Braunschweig, Niedersachsen, Germany). The soil samples were homogenized by shaking and sieving under sterile conditions, and then soil particles of 0.5–0.63 mm diameter (corresponding weight ca. 0.4 mg) were placed individually on SNA-Agar (SNA), malt extract agar (MEA), 5 % carrot juice agar, and NaCl malt-peptone agar (corresponding weight ca. 0.4 mg) were placed individually on SNA-Agar (SNA). The fungus was subsequently placed on synthetic nutrient agar (SNA) at room temperature for 5 min, and washed with distilled water.

Microscopy

Living material was examined with a Zeiss Axiosplan microscope using bright field, phase contrast, or Nomarski interference contrast optics. The ultrastructure was studied with a Zeiss EM 109 transmission electron microscope at 80 kV. Samples were fixed overnight with 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature. Following six transfers in 0.1 M sodium cacodylate buffer, samples were postfixed in 1 % osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1 % aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, using 10 min changes at 25 %, 50 %, 70 %, 95 %, and 3 times in 100 % acetone. Samples were embedded in Spurr’s plastic and sectioned with a diamond knife. Serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and washed with distilled water.

Molecular analyses

DNA of the isolate (Cystobasidiopsis nirenbergiae) was isolated from culture, ITS and partial LSU rDNA was amplified as described earlier (Begerow et al. 1997, 2001; Stoll et al. 2005). To obtain a hypothesis on the phylogenetical position of the isolate (C. nirenbergiae), we analysed a data set containing the sequence of the isolate and all sequences of species belonging to the Chionsphaeriaceae available at GenBank (redundant sequences were removed) together with a representative assortment of species covering most of the classes of Pucciniomycotina (taxonomical concept after Bauer et al. 2006) (GenBank accession numbers are given in Fig 3). The alignment was built with MAFFT 3.85 (Katoh et al. 2002) using the accurate and interactive refinement method. After trimming of the ends, the LSU alignment consisted of 570 bp. Phylogenetic analyses were carried out using RAxML (Stamatakis et al. 2008) and PAUP* 4.0b10 (Swofford 2001). Modeltest 3.0 (Posada & Crandall 1998) was carried out to determine a model of DNA substitution that fits the data set. GTR + I + G was selected from the Akaike information criterion for the LSU alignment (base frequencies: \( \pi_A = 0.1804, \pi_C = 0.2158, \pi_G = 0.3313, \pi_T = 0.2725 \); substitution rates: \( A/C = 1.6353, A/G = 4.1346, A/T = 2.2066, C/G = 0.8178, C/T = 5.5234, G/T = 1.0000 \); gamma shape parameter = 0.6200; proportion of invariant sites = 0.1584). Neighbour-joining analysis was done using genetic distances according to the specified substitution model. RAxML was conducted with the default settings on the webinterface (number of categories = 25, model = GTR+GAMMA, initial rearrangement setting = 10) using the rapid bootstrap analysis and the search for best-scoring ML tree in one program run (Stamatakis et al. 2008). The genbank accession numbers are given in Fig 3.

Results

Growth on all agar media tested was very slow. On malt extract agar at room temperature colonies stopped growing at a diameter of ca. 15 mm. At 37 °C no growth was observed within 8 d, at 10 °C the colonies grew 1 mm. No aerial mycelium was formed.

The mycelium was white, very sparse on SNA, more compact on MEA, 5 % carrot juice agar or MYP. The hyphae were very thin (ca. 1–3 μm). All structures were hyaline and mostly thin walled. Occasionally thickening of the wall to ca. 250 nm could be observed. Clamps were present only at the base of the probasidia (Fig 1A). The hyphal cells were dikaryotic. On MEA synnemata were formed. Within a three month period no carpophores were obtained.

Within two weeks, spheroid probasidia (5–6 μm in diam.) formed at the end of lateral branches, scattered on the mycelium (Fig 1A). Karyogamy took place in the probasidium. Subsequently, a stipe, cylindrical to allantoid metabasidium (stipe 2–3 × 2–10 μm, metabasidium 4–5 × 15–17 μm) developed from each probasidium. During the growing phase of the metabasidium the cytoplasm of the probasidium and, subsequently, also of the stipe migrated in the metabasidium which became separated from the stipe by the formation of a retraction septum. At this stage the probasidium and stipes were empty. However, the metabasidium remained attached with their stipes and probasidia (Fig 1A). Meiosis began in the unseptate metabasidium (Fig 2A). During meiosis, the metabasidium became four-celled by transverse septation. Subsequently, the basidial
cells gave rise to mononucleate basidiospores, emptying during this process (Fig 1). Basidiospores were ovoid to spheroid (ca. 4–6 × 4–6 μm in diam.), formed on minute sterigmata of max. 0.5 μm in length (Figs 1 and 2B). Basidiospores were not forcibly discharged and usually remained on the old basidia. The spore wall was very thick, up to ca. 600 nm. Basidiospores were able to conjugate while connected with the basidium (Fig 2C). Conjugated basidiospores germinated with hyphae (Fig 2D).

The septa had simple pores with more or less rounded pore lips (Fig 2E). The pores were not associated with microbodies, cystosomes or other organelles. Occasionally, however, they were plugged with amorphous, electron-opaque material.

At prophase I the spindle pole body (SPB) consisted of a pair of discs, connected at their inner margin by a cylindrical, large middle piece. In a longitudinal section through the SPB, the discs were nearly perpendicular to the nuclear envelope. Consequently, they were in close proximity to the nuclear envelope only at their edges (Fig 2F).

The phylogenetic analyses resulted in a grouping with highly supported terminal branches. Using the ascomycetes as outgroup, the higher taxa and groups appearing in the phylogenetic tree were congruent to the classes discussed in Bauer et al. (2006). The arrangement of some classes differed in the backbone between the neighbour-joining and maximum-likelihood analyses. However, none of these differences were supported by bootstrap values higher than 50 % and similar arrangements have been published already (Weiß et al. 2004; Bauer et al. 2006; Aime et al. 2006). The new isolate clustered within the Agaricostilbomycetes (Fig 3) where it was located in a statistically well supported clade (ML/NL: 95 %/86 %) with Sporobolomyces spp., Mycogloea nipponica, Chionosphaera apobasidialis and Kurtzmanomyces spp., representing the Chionosphaeraceae sensu Bauer et al. (2006) of the Agaricostilbales. The neighbour-joining analysis clustered the new isolate together with Sporobolomyces lactophilus (AY512889).

**Taxonomy**

*Cystobasidiopsis* R. Bauer, B. Metzler, Begerow & Oberw., gen. nov.

MycoBank no: MB 512850.

Etym.: Referring to the morphological similarities with *Cystobasidium*.

Fungi Agaricostilbalium sensu Bauer et al. (2006) probasidiis basidia stipitata transverse septata eferentibus, basidiosporis sessilibus.


*Cystobasidiopsis nirenbergiae* R. Bauer, B. Metzler, Begerow & Oberw., sp. nov.

MycoBank no.: MB 512851.

Etym.: In honour to the excellent German mycologist Helgard I. Nirenberg, expert in phytopathology and in biodiversity of soil fungi.

---

Fig 1 – Line drawings of different stages of basidial development in *Cystobasidiopsis nirenbergiae*. Bar = 10 μm. (A) Young stages of basidial development. Right: young probasidium (p) at the top of a hypha. Left: probasidium (p) with stipe (s) and young, still unseptate metabasidium (m). Note that clamp connections are present only at the base of the probasidia (p). (B) Two probasidia (p) with stipes (s) and transversely septate metabasidia (m). Note that clamp connections are present only at the base of the probasidia (p). Bottom: plasma of the probasidium (p) and stipe (s) has migrated into the metabasidium (m) which gets transversely septate. Note the sessile initials of basidiospores (b). Upper left: collapsing metabasidium (m) during basidiospore (b) ripening.
Carposomata nulla. Hyphae hyalinae, ca. 1–3 μm in diam., tenuitunicatae vel raro muris moderate incrassatis, non fibulatae. Septorum pori simplices, minutissimi, ca. 40 nm in diam. Probasidia globosa, ca. 5–6 μm, hyalina, tenuitunicata, basaliter fibulata. Metabasida stipitata et cylindracea vel allantoidea, ca. 4–5 × 15–17 μm, mature divisa sunt in cellulas quattuor, sterigmatibus minimis. Basidiosporae globosae vel oviformae, ca. 4–5 × 5–6 μm, hyalinae, crassitunicatae, sessiles ad sterigmatibus minimis (ca. 0.5 μm), gasteromycetum modo non abjectae.

Typus and deposits: Living cultures are deposited in BBA (BBA 65452), DSMZ (DSM 22580) and the TUB culture collection (TUB F580). Dried specimen (holotypus) has been deposited in the TUB herbarium (TUB 019163).

No fruiting bodies are formed. Hyphae are hyaline, ca. 1–3 μm in diameter, mostly thin walled. The septa are not provided with clamps except at the base of the probasidia. The septal pores are small (ca. 40 nm) and simple. The spheroid, thin walled probasidia (ca. 5–6 μm in diam.) form at the end of lateral branches. Metabasidia are stipitate and cylindroid to allantoid, ca. 4–5 × 15–17 μm. Septa are formed between stipes and metabasidia. The latter become four-celled by transverse septation. The four basidiospores are ovoid to spheroid (c. 4–5 × 5–6 μm in diam.), formed on minute sterigmata of max. 0.5 μm in length. They are not forcibly discharged and usually remain on the old basidia. The sporal wall is very thick, up to ca. 600 nm. ITS rDNA sequence is deposited in genbank (GQ180106).

Distribution: Known only from an arable loess soil located in Ahlum near Braunschweig, Niedersachsen, Germany.

Discussion

Our molecular phylogenetic analyses demonstrate that the isolate is a member of the Agaricostilbales sensu Bauer et al.

---

Fig 2 – Ultrastructure of Cystobasidiopsis nirenbergiae. (A) Prophase I nucleus in a young metabasidium showing a synaptosomal complex (arrowhead). Bar = 0.5 μm. (B) Section through a transversely septate metabasidium during basidiospore ripening. Note the minute sterigma (arrowhead). Bar = 2 μm. (C) Section through two basidiospores (b) showing the conjugation (arrow). Note that at least one basidiospore is still connected with the metabasidium (m). Bar = 1 μm. (D) Section through a germinating basidiospore (b). Note that the basidiospore germinates with a hypha (arrowhead). Conjugated basidiospore not sectioned. Bar = 2 μm. (E) Median section though a simple pore. Note that the pore is not associated with microbodies or other organelles. Bar = 0.1 μm. (F) Longitudinally sectioned prophase I spindle pole body showing two discoidal elements (arrowheads) connected by a middle piece. Note that the discs are in a more upright position in respect to the nuclear envelope. Bar = 0.2 μm.
Fig 3 – Phylogenetic hypothesis based on maximum-likelihood analysis of Pucciniomycotina with a special emphasis on Agaricostilbomycetes. The topology with the best likelihood was rooted with three species of Ascomycota. Numbers on branches are bootstrap values of maximum-likelihood and neighbour-joining analyses (ML/NJ). Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. The taxonomical concept applied corresponds to Bauer et al. (2006).
Mycogloea in the incapacity of the basidial cells to germinate by budding lack of basidiocarps, in the presence of distinct probasidia, accommodate the isolate in the (2006) Cystobasidiopsis nirenbergiae high for the sidiomycetes are devoid of microbodies, cystosomes (and phase is reduced to the basidial cells and young basidiospores, dia. In addition, in contrast with the isolate, the from the probasidia, whereas the of ascomycetes with deciduous basidia that easily detach cies form minute basidiocarps in association with sporocarps tosporic basidia without probasidia in culture. These differ- cies with Figs 1 and 2 in 2000 et al. (2006) Bauer et al. 2000, the septal pore apparatus in the isolate are devoid of microbodies, cystosomes (Weiß et al. 2004; Bauer et al. 2006) or other organelles. Within the Agaricostilbales, the isolate appears within a well supported clade representing the Chionosphaeraceae sensu Bauer et al. (2006). These molecular phylogenetic data correlate well with the morphological characteristics. Thus, the teleomorphic members of the Chionosphaeraceae (Chionosphaera, Stil- lum, Mycogloea nipponica) are characterized by having gastertoid basidia with simultaneous basidiospore production per basidium (Oberwinkler & Bandoni 1982; Bandoni 1998; Bauer et al. 2006). The isolate shares these characteristics with the other teleomorphic members of the Chionosphaeraceae.

In interpreting this phylogenetic indication, despite of some morphological similarities the fungus is not closely related to Cystobasidium (Roberts 1999). The similarities include for all the formation of distinct probasidia and transversely septate metabasidium. The isolate differs from Cystobasidium in the gasteroid mode of spore release, in the incapacity of the basidiospores to germinate by budding, in the lack of tremelloid haustorial cells and in the absence of cystosomes at the septal pores (see Weiß et al. 2004; Bauer et al. 2006; and the references therein). These differences clearly separate the isolate and Cystobasidium.

Within the Agaricostilbales, the isolate is unique in the absence of a yeast phase. Obviously, in this fungus the haploid phase is reduced to the basidial cells and young basidiospores, which become dikaryotic by conjugation. Beyond it, the isolate differs from Agaricostilbum, Stilbum and Chionosphaera (Oberwinkler & Bauer 1989) for all in the lack of basidiocarps, in the presence of distinct probasidia, in the incapacity of the basidial cells to germinate by budding and in the formation of phragmobasidia (Chionosphaera). These differences separate the isolate and these genera.

Morphology of the isolate somewhat resembles that of Mycogloea (Bandoni 1995, 1998) and Kondoa (Fonseca et al. 2000). It differs from the species of both genera by the formation of basidial stipes, connecting the probasidia with the metabasidium. In addition, in contrast with the isolate, the Mycogloea species form minute basidiocarps in association with sporocarps of ascomycetes with deciduous basidia that easily detach from the probasidia, whereas the Kondoa species develop ballisto- sporic basidia without probasidia in culture. These differ- ences separate the isolate from Mycogloea and Kondoa.

In summary, the isolate cannot be ascribed to any genus of the Agaricostilbales. Accordingly, a new genus is necessary to accommodate the isolate in the Agaricostilbales.

Acknowledgements

We are grateful to Heidi Anders and Marlene Goedecke/BBA Berlin, M. Wagner-Eha, J. Götzte, J. Aubry and F. Albrecht for technical assistance, M. Weiß for critically reading the manuscript and a part of the Latin diagnoses, and the Deutsche Forschungsgemeinschaft and the German Federal Ministry for Research and Technology for financial support.

REFERENCES


Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Darlem 295: 143–166.


