Chapter 4

Cladophialophora psammophila, a novel species of Chaetothyriales with a potential use in the bioremediation of volatile aromatic hydrocarbons

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Abstract

Cladophialophora is a genus of asexual black yeast-like fungi with one-celled, hydrophobic conidia which is predicted to have teleomorphs in the ascomycete genus Capronia, a member of the order Chaetothyriales. Cladophialophora species are relatively frequently involved in human disease ranging from mild cutaneous lesions to cerebral abscesses. Although the natural niche outside humans is unknown for most opportunistic Cladophialophora species, the fungi concerned are rarely isolated from environmental samples such as dead plant material, rotten wood, or soil. The objective of the present paper is to describe a novel species of Cladophialophora which was isolated from soil polluted with benzene, toluene, ethylbenzene, and xylene (BTEX). It proved to be able to grow on toluene and other related alkylbenzenes as its sole carbon and energy source. This strain is of interest for the complete biodegradation of toluene and other related xenobiotics under growth limiting conditions, particularly in air biofilters, dry and/or acidic soil. A preliminary genetic analysis using MLST and AFLP showed that this fungus was closely related to the pathogenic species C. bantiana, sharing a C. bantiana-specific intron in SSU rDNA. However, it was unable to grow at 40 °C and proved to be non-virulent in mice. The clear phylogenetic and ecophysiological delimitation of the species is fundamental to prevent biohazard in engineered bioremediation applications.

Key words: Cladophialophora, black yeasts, air biofilters, ITS rDNA, AFLP, in vitro antifungal activity, pathogenicity, neurotropism.

Introduction

Cladophialophora is a genus of asexual black yeast-like fungi with one-celled, hydrophobic conidia arranged in long, branched, usually coherent chains and with nearly unpigmented conidial scars. The genus was initially erected to accommodate a species exhibiting Phialophora-like conidiogenous cells in addition to conidial chains (Borelli 1980), but thus far this feature is expressed in only a few strains of C. carrionii and thus has limited diagnostic value. Judging from multigene phylogeny (nucLSU, nucSSU, RPB1) Cladophialophora is predicted to have teleomorphs in the ascomycete genus Capronia, a member of the order Chaetothyriales in the family Herpotrichiellaceae (Badali et al. 2008).

Cladophialophora species are relatively frequently encountered in human disease, infections ranging from mild cutaneous lesions to cerebral abscesses (Li et al. 2009, de Hoog et al. 2007, Badali et al. 2009). At present the genus contains seven species proven to be involved in human disease (de Hoog et al. 2007, Badali et al. 2008). Among the most virulent species is the neurotrope Cladophialophora bantiana, which is potentially able to cause fatal infections in otherwise healthy individuals (Horré & de Hoog 1999, Kantarcioglu & de Hoog 1999). Cladophialophora carrionii and C. samoënis are the main etiologic agents of the mutilating skin disorder chromoblastomycosis. Also this type of chronic infection occurs primarily in immunocompetent individuals (Yeguez-Rodriguez et al. 1992, de Hoog et al. 2007). The natural niche outside humans is unknown for most opportunistic Cladophialophora species. In contrast to frequently expressed opinions (Revankar 2007), listing black yeasts as common saprobes, the fungi concerned are rarely isolated from environmental samples such as dead plant material, rotten wood, or soil. A number of plant-associated Cladophialophora species has been described (Crous et al. 2007), but most of these are located at the phylogenetic base of the Chaetothyriales, remote from the human opportunists (Badali et al. 2008).

For recovery of herpotrichiellaceous fungi, selective isolation methods are required, e.g., the use of high temperature (Sudhadham et al. 2008), a mouse vector (Gezuele et al. 1972), extraction
via mineral oil (Vicente et al. 2008) or enrichment on volatile aromatic hydrocarbons (Zhao et al. 2010). The success of the latter method, enabling isolation of black yeasts where previously direct plating had failed, has supported the hypothesis that herpotrichiellaceous black yeasts are potent degraders of aromatic hydrocarbons. Previously, the alkylbenzene enrichment has been applied in the biofiltration of air polluted with volatile aromatic compounds (Kennes & Veiga 2004, Cox et al. 1993, Weber et al. 1995).

The objective of the present paper is to describe a novel species of Cladophialophora, based on the strain ATCC MYA-2335 (CBS 110553) which was isolated from soil polluted with benzene, toluene, ethylbenzene, and xylene (collectively known as BTEX). It proved to be able to grow on toluene and other related alkylbenzenes as its sole carbon and energy source (Prenafeta-Boldú et al. 2001). This strain is of interest for the complete biodegradation of toluene and other related xenobiotics under growth limiting conditions, particularly in air biofilters, dry and/or acidic soil (Prenafeta-Boldú et al. 2004, Prenafeta-Boldú et al. 2008). A preliminary genetic analysis showed that this fungus was closely related, perhaps even conspecific, to the pathogenic species C. bantiana (Prenafeta-Boldú et al. 2006). A clear phylogenetic and ecophysiological delimitation of the species is therefore fundamental to prevent biohazard in engineered bioremediation applications.

Materials and Methods

Fungal strain

The isolate used in this study was obtained from the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (Table 1). Stock cultures were maintained on slants of 2 % malt-extract agar (MEA, Difco) and oatmeal agar (OA, Difco) and incubated at 24 °C for two weeks (Gams et al. 2007). The studied strain (CBS 110553) had been isolated from soil in a former gasoline station polluted with BTEX hydrocarbons, which was being treated by means of bioventing (Bennekom, The Netherlands). Soil texture was homogeneously sandy, and a sample was taken from about 2 m deep into the unsaturated zone of the contamination plume. About 10 g of soil were enriched in a solid state-like cultivation batch system that used perlite humidified with a mineral nutrient medium as carrier material (Prenafeta-Boldú et al. 2001). Toluene was supplied via the gas phase as the only carbon and energy source and the incubation was performed at 30 ºC under a relatively low water activity (0.9). Intense dematiaceous growth was observed on the top perlite layer after 5 weeks of incubation. The predominant fungal strain was isolated and purified by re-suspending colonized perlite granules and plating serial dilutions on mineral medium agar plates incubated under a toluene atmosphere.

Morphological and physiological characterization

The isolate was cultured on 2 % MEA and OA and incubated at 24 °C in the dark for two weeks (Gams et al. 2007). Provisional identification was based on macroscopic and microscopic morphology. Microscopic observations were made with slide culture techniques using potato dextrose agar (PDA, Difco) or OA because these media readily induce sporulation and suppress growth of aerial hyphae (de Hoog et al. 2000). Mounts of two-week-old slide cultures were made in lactic acid or lactophenol cotton blue and light micrographs were taken using a Nikon Eclipse 80i microscope with a Nikon digital sight DS-Fi1 camera. Cardinal growth temperatures were determined by incubating MEA culture plates in the dark for 2 weeks at temperatures ranging from 21 to 40 ºC at intervals of 3 ºC (Crous et al. 1996). Experiments consisted of three simultaneous replicates for each isolate; the entire procedure was repeated once.
<table>
<thead>
<tr>
<th>Taxon name</th>
<th>CBS no.</th>
<th>Other reference</th>
<th>GenBank number</th>
<th>Source</th>
<th>Geography</th>
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<td>EU103995, EU140584</td>
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<td>dH 12535, IHM 1727</td>
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<td>dH 12939</td>
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<td>dH 11474</td>
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<td>CDCB-5680; dH 10680 (T)</td>
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<td>dH 11588</td>
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<td>dH 11601</td>
<td>FJ385272, EU137258, -</td>
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<td>ATCC 56280; dH 15405 (T)</td>
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<td>ISO 13F</td>
<td>FJ385275, -</td>
<td>Litter, vegetable cover/soil</td>
<td>Brazil, Paraná, Curitiba</td>
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<td><em>Cladophialophora arxii</em></td>
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<td>(T)</td>
<td>EU103986, EU140593, -</td>
<td>Tracheal abscess, male</td>
<td>Germany</td>
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</table>
Molecular characterization

Mycelia were grown on 2 % (MEA) plates for 2 weeks at 24 °C. A sterile blade was used to scrape off the mycelium from the surface of the plate and transferred to a 2 ml Eppendorf tube containing 400 µL TEX-buffer (Tris 1.2 % w/v, Na-EDTA 0.38 % w/v, pH 9.0) and glass beads (Sigma G9143). The fungal materials were homogenized by Mobio vortex for 5–10 min. Subsequently 120 µL SDS 10 % and 10 µl proteinase K were added and incubated for 30 min at 65 °C, the mixture was vortexed for 3 min. After addition of 120 µl of 5 M NaCl and 1/10 vol CTAB 10 % (cetyltrimethylammonium bromide) buffer, the material was incubated for 60 min at 55 °C. Subsequently 700 µl SEVAG (24:1, chloroform: isoamylalcohol) was slowly mixed, incubated for 30 min on ice water and centrifuged for 10 min at 14 000 r.p.m. The supernatant was transferred to a new tube with 225 µl of 5 M NH₄-acetate, mixed carefully by inverting, incubated for 30 min on ice water, and centrifuged again for 10 min at 14 000 r.p.m. The supernatant was transferred to another tube with 0.55 vol isopropanol and centrifuged for 5 min at 14 000 r.p.m. Finally, the pellet was washed with 1000 µl ice cold 70 % ethanol. After drying at room temperature, it was re-suspended in 100 µl TE buffer (Tris 0.12 % w/v, Na-EDTA 0.04 % w/v) plus 1.5 µl RNAse 20 U/mL and incubated for 15–30 min at 37 °C. DNA extracts were stored at −20°C until use (Gerrits van den Ende et al. 1999).

Three genes were amplified for phylogenetic characterization of the new species: the small subunit of the nuclear ribosomal RNA gene (nucSSU), the large subunit of the nuclear ribosomal RNA gene (nucLSU), and the largest subunit genes of RNA polymerase I (RPB1). For multilocus sequencing typing (MLST), the internal transcribed spacer region (ITS rDNA), the translation elongation factor 1 alpha (EF1-α), and the partial beta tubulin (TUB) genes were amplified. The primers used for amplification and sequencing were described previously (Badali et al. 2008, Gueidan et al. 2008). PCR reactions were performed on a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA) in 50 µL volumes containing 25 ng of template DNA, 5 µl reaction buffer (0.1 M Tris-HCl, pH 8.0, 0.5 M KCl, 15 mM MgCl₂, 0.1 % gelatine, 1 % Triton X-100), 0.2 mM of each dNTP and 2.0 U Taq DNA polymerase (ITK Diagnostics, Leiden, The Netherlands). Amplification of those genes were performed with cycles of 2 min at 94 °C for primary denaturation, followed by 35 cycles at 94 °C (45 s), 52 °C (30 s) and 72 °C (120 s), with a final 7 min extension step at 72 °C. Annealing temperatures used to amplify SSU, LSU, RPB1, ITS, EF1-α and TUB genes were 55 °C, 52 °C, 52 °C, 52 °C, 50 °C and 58 °C, respectively. Amplicons were purified using
QIAquick PCR purification Kit (Cat. No 28104, U.K.). Sequencing was performed as follows: 95 °C for 1 min, followed by 30 cycles consisting of 95 °C for 10 s, 50 °C for 5 s and 60 °C for 2 min. Reactions were purified with Sephadex G-50 fine (GE Healthcare Bio-Sciences, Uppsala, Sweden) and sequencing was done on an ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Sequence data obtained in this study were adjusted using the SeqMan of Lasergene software (DNAStar Inc., Madison, Wisconsin, U.S.A.).

Alignments of DNA sequences were done manually for each gene using MacClade 4.08 (Maddison et al. 2003) with the help of amino acid sequences for protein coding loci. Ambiguous regions and introns were excluded from the alignments. The program RAxML-VI-HPC v.7.0.0 (Stamatakis et al. 2008), as implemented on the Cipres portal v. 1.10, was used for the tree search and the bootstrap analysis (GTRMIX model of molecular evolution and 500 bootstrap replicates). Bootstrap values equal or greater than 70 % were considered significant (Hillis et al. 1993). Data were analysed as Clade II (Badali et al. 2008). The Bantiana-clade comprised 36 taxa. Phylogenetic reconstructions and bootstrap values were first obtained for each locus separately using RAxML (as described above). The congruence between loci was assessed using a 70 % reciprocal bootstrap criterion (Mason-Gamer et al. 1996). The loci were then combined and analysed using RAxML (as described above). The phylogenetic trees were edited using Tree View v. 1.6.6. AFLP analysis was performed as described previously (Badali et al. 2010).
**Virulence testing**

The virulence of the new species was compared with that of three reference strains of *Cladophialophora bantiana* and a strictly non-pathogenic strain of *C. minourae* using a murine model, which was previously described by Mariné *et al.* (2009) determining the mortality rates of the infected animals. Briefly, six-week-old OF-1 male mice (ten per group) (Charles River, Criffa SA, Barcelona, Spain) weighing 28-30 g were used. Animals were housed five per cage in standard boxes with corncob redding and free access to food and water. Conditions were approved by the Animal Welfare committee of the Medical School of the University Rovira i Virgili. The isolates tested were *Cladophialophora* sp. CBS 110553, *C. bantiana* (CBS 110013, CBS 110009 and CBS 173.52 as ex-type strain), and *C. minourae* (CBS 556.83). They were stored on corn meal agar (CMA, 30 g corn and 15 g agar per litre) slants covered with paraffin oil, and prior to the study they were cultured on PDA for 5–8 days at 30 °C. Inocula were prepared by flooding the surface of the agar plate with sterile saline, scraping the sporulating mycelium with a culture loop, and

![Fig. 2. Amplified fragments length polymorphism (AFLP) profile of *Cladophialophora psammophila* (CBS 110553) in relation to that of reference strains of *Cladophialophora bantiana* (CBS 173.52) and *Cladophialophora minourae* (CBS 556.83).](image)

![Fig. 3. Survival rates of OF-1 mice infected intravenously with 1x10^7 CFU/animal. T = type strain.](image)

<table>
<thead>
<tr>
<th>Name</th>
<th>CBS no</th>
<th>AmB</th>
<th>FLU</th>
<th>ITC</th>
<th>VOR</th>
<th>POS</th>
<th>ISA</th>
<th>CAS</th>
<th>ANI</th>
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<tbody>
<tr>
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<td>173.52 (T)</td>
<td>0.25</td>
<td>16</td>
<td>0.016</td>
<td>0.125</td>
<td>0.016</td>
<td>0.031</td>
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<td>0.125</td>
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<td>32</td>
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<td>0.25</td>
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<td><em>C. minourae</em></td>
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<td>32</td>
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<td>0.031</td>
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<td>1</td>
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</table>

**Table. 2. Minimum inhibitory (azoles) and effective (echinocandins) concentrations in mg/L of different antifungal drugs against *Cladophialophora psammophila* (CBS 110553) and other *Cladophialophora* reference strains**
Fig. 4. *Cladophilophora psammophila* (CBS 110553). A–B. Culture on MEA and PDA) produced velvety to hairy, powdery and olivaceous-black with a wide well-defined margin. C. Hyphal coil. D–G. Conidiophores laterally or terminally on undifferentiated hyphae, ellipsoidal to fusiform or sub-cylindrical. F. One-celled and long conidia, produced in long, strongly coherent chains. Scale bars = 10 µm.
Fig. 5. Line drawing of microscopic morphology of *Cladophialophora psammophila* (CBS 110553). Scale bar = 10 µm.
drowning up the resultant suspension with a sterile Pasteur pipette. Suspensions were filtered once through sterile gauze to remove hyphae. Conidial suspensions were adjusted after counting with a haemocytometer. The viability of these inocula was verified by plating dilutions of the suspension on PDA plates. Infection was established intravenously with 0.2 ml of inoculum via the lateral tail vein. Inocula administered were $2 \times 10^7$ conidia/animal. Preliminary experiments with several reference strains of *C. bantiana* demonstrated that this concentration produced infections with subsequent 100 % mortality within 20 days of inoculation (data not shown).

**Antifungal susceptibility**

*In vitro* antifungal susceptibilities of different drugs against CBS 110553 and reference strains (*C. bantiana* and *C. minourae* which are a neurotropic pathogen and a saprobe, respectively) were performed by microbroth dilution as described in the Clinical and Laboratory Standards Institute (CLSI) document M38-A2. Minimum inhibitory concentrations (MIC) for amphotericin B (AmB, Bristol-Myers Squib, Woerden, The Netherlands); fluconazole (FLU, Pfizer Central Research Sandwich, UK); itraconazole (ITC, Janssen Research Foundation, Beerse, Belgium); voriconazole (VOR, Pfizer); posaconazole (POS, Schering-Plough, Kenilworth, USA); isavuconazole (ISA, Basilea, Basel, Switzerland), and minimum effective concentrations (MEC) for caspofungin (CAS, Merck Sharp & Dohme, Haarlem, The Netherlands) and anidulafungin (ANI, Pfizer) were determined. As per the CLSI guidelines, stock solutions of the drugs were prepared in the appropriate solvent. The drugs were diluted in the standard RPMI-1640 medium (Sigma Chemical Co.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) with L-glutamine without bicarbonate to yield two times their concentrations were as follows: amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin 0.016–16 mg/ml; fluconazole 0.063–64 mg/ml; isavuconazole 0.002–2 mg/ml and anidulafungin 0.008–8 mg/ml. Plates were stored at –70 °C until they were used. Methods for sporulation and preparation of conidial suspensions were those of Badali *et al.* (2010). *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality controls.
**Results**

Using the phylogenetic markers including nucSSU, nucLSU and RPB1, *Cladophialophora* species are predicted to belong to at least four different lineages. Most of the human-opportunistic species belong to two well supported clades within the *Herpotrichiellaceae* (Badali et al. 2008). Strain CBS 110553 was nested in lineage one corresponding to the family *Herpotrichiellaceae* (Tree not shown) that contained the pathogenic *Cladophialophora* species (*C. carrionii*, *C. boppii*, *C. bantiana*, *C. arxii*, *C. immunda*, *C. devriesii*, *C. saturnica*, *C. emmonsii* and *C. mycetomatis*) as well as the pathogenic genus *Fonsecaea* (*F. pedrosoi* and *F. monophora*) and *Phialophora* (*P. verrucosa* and *P. mericana*). Phylogenetic reconstructions were first performed for each gene separately (ITS: 495 characters, EF1-α: 133 characters, TUB: 355 characters). Topological conflicts were detected only within the species *C. saturnica*, the incongruence involving only below species-level relationships, and therefore – in agreement with the genealogical recognition species concept (Taylor et al. 2000) – the conflicts were ignored and the three loci were combined. In this multilocus analysis, *Cladophialophora mycetomatis*, an agent of true mycetoma was taken as an outgroup (Fig. 1). This analysis showed that CBS 110553, originating from hydrocarbon polluted soil was phylogenetically distinct from other species of *Cladophialophora*, and a sister taxon (85 % bootstrap support) of the pathogenic species *C. bantiana*. Hence, it is described below as a novel species of *Cladophialophora*.

Survival rates of mice experimentally infected with strains of *Cladophialophora* are shown in Fig. 3. All animals infected with three different strains of *C. bantiana* died between days 12 and 17; differences between strains were insignificant. Animals infected with *C. psammophila* and *C. minourae* survived and remained alive until the end of the experiment.

Table 2 summarizes the MIC values of eight antifungal drugs against three highly neurotropic isolates (*C. bantiana*) and two non-pathogenic species (CBS 110553 and *C. minourae*). There was a uniform pattern of low MICs for itraconazole, posaconazole and isavuconazole. The highest MICs were for fluconazole followed by amphotericin B, the echinocandins and voriconazole.

### Cladophialophora psammophila

*Cladophialophora psammophila* Badali, Prenafeta-Boldú, Guarro & de Hoog, **sp. nov.** MycoBank MB 515525. Figs 4, 5

Colonies moderately expanding, with a daily growth rate of 2.1 mm (1.5 mm at 37 °C). Colonies velvety to hairy, powdery when sporulating, olivaceous-black with a wide well-defined margin which is darker than the colony centre; reverse olivaceous-black (Figs 4A–B).

Microscopy on MEA: Fertile hyphae septate, ascending to erect, smooth- and thin-walled, hyaline to pale olivaceous, guttulate, branched, 2–3 μm wide, forming hyphal strands and hyphal coils.
Conidiophores laterally or terminally on undifferentiated hyphae, pale olivaceous, smooth- and thin-walled, ellipsoidal, fusiform or sub-cylindrical, 6.5–10.0 × 2.0–3.5 µm, erect. Conidia one-celled, produced in long, strongly coherent, poorly branched chains (Figs 4C-H, 5). Teleomorph unknown.

**Physiology:** Cardinal temperatures: optimal development at 27–30 °C, with growth abilities between 9–37 °C. No growth at 40 °C (Fig. 6).

**Specimen examined:** The Netherlands, Bennekom, isolated from sandy soil in a former gasoline station polluted with BTEX hydrocarbons (benzene, toluene, ethylbenzene, and xylene), CBS H-20384 (Holotype) = CBS 110553 (ex-type culture).

**Discussion**

The new species *C. psammophila* morphologically resembles *C. bantiana*, which is a virulent neurotropic agent of fatal cerebral phaeohyphomycosis in humans, causing granulomatous abscesses in the brain and having a maximum growth temperature of 42 °C. The multiple sequences analyzed in this study deviate sufficiently from those of *C. psammophila* to be sure that the two species can be considered to represent separate though highly related species. Members of *C. bantiana* are unique by consistently containing a distinctive 558-bp intron beginning at position 1768 in the small subunit of the ribosomal DNA gene (Gerrits van den Ende et al. 1999). Interestingly, this intron is also present in *C. psammophila*, underlining the close kinship of the two species. *Cladophialophora bantiana* has a worldwide distribution, but until now it could be isolated from the environment only by using a mammal vector (Conti-Diaz et al. 1977, Dixon et al. 1980). The natural niche of this organism remains unknown and it is presumably introduced via inhalation as the main infection route. In contrast, *C. psammophila* was isolated by hydrocarbon enrichment from a location where hydrocarbon pollution was prevalent. It does not grow at temperatures above 37 °C. Pathogenicity tests on a murine model indicated that *C. psammophila* is non-virulent, whereas *C. bantiana* kills mice within two weeks. Both *Cladophialophora* species, although at short phylogenetic distance, thus exhibit striking ecological differences. The ecophysiological divergence between *C. bantiana* and the newly described *C. psammophila* is puzzling. Future genome comparisons will serve to reveal the essential virulence factors involved in the pathogenicity of the Bio-Safety Level-3 species *C. bantiana*.

Phylogenetically the genus *Cladophialophora* – morphologically consistently characterised by poorly or profusely branched chains of dry, rather strongly coherent, moderately melanized conidia – is polyphyletic within the order Chaetothyriales (Badali et al. 2008). In part the genus is closely related to members of genera that are consistently associated with human infection, such as *Exophiala*, *Fonsecaea* and *Rhinocladiella* (lineage 1 in combined phylogenetic reconstructions (nucSSU, nucLSU and RPB1) of Chaetothyriales published by of Badali et al. 2008). This part of the tree comprises a number of *Capronia* teleomorphs and may therefore be regarded to represent the family Herpotrichiellaceae. This family also encompasses several species that have been related to the metabolism of monoaromatic hydrocarbons and other related aromatic substrates, both in polluted and natural environments. Consequently, these organisms are of interest in biotechnological applications for the bioremediation of hydrocarbon pollution. The distantly related *Cladophialophora* species have been described as host-specific plant-associates (Crous et al. 2007) and are unlikely to belong to the Chaetothyriales. No association to aromatic substrates has ever been reported in this latter group.

Enrichment on toluene as the sole sources of carbon and energy was a key factor for the isolation
Cladophialophora psammophila, with a potential use in the bioremediation of volatile aromatic hydrocarbons

of the strain CBS 110553. The physiological and metabolic characterization of axenic cultures demonstrated that assimilation of aromatic hydrocarbons was limited to certain alkylbenzenes, such as toluene and ethylbenzene (Prenafeta-Boldú et al. 2001). This biodegradation pattern was confirmed in biodegradation assays of complex BTEX mixtures simulating gasoline pollution, in which only toluene and ethylbenzene were mineralized, while the xylene isomers were co-metabolized and benzene remained undegraded throughout the experiment (Prenafeta Boldú et al. 2002). Alkylbenzene degradation proceeded through the initial oxidation of the alkyl side chain, rather than the aromatic ring (Prenafeta Boldú et al. 2001). Growth of C. psammophila in liquid cultures under agitation was predominantly yeast-like. Furthermore, enzymological studies on the related Cladophialophora saturnica CBS 114326, initially misidentified as a Cladosporium sphaerospermum, showed the involvement of a cytochrome P450 monooxygenase in the initial oxidation of the alkyl group (Luykx et al. 2003). The exceptional metabolic and physiological capacities of CBS 110553 have been investigated in relation to the biofiltration of waste gases polluted with volatile aromatic hydrocarbons and for the bioremediation of BTEX pollution in soil microcosms (Prenafeta-Boldu et al. 2004 and 2008).

The striking close phylogenetic proximity of strain CBS 110553 to the notorious human pathogen C. bantiana has put its applicability in bioremediation into question due to potential biohazard (Prenafeta-Boldu et al. 2006). Cladophialophora bantiana has regularly been recognized in human disease by its high mortality rate despite the application of combined surgery and antifungal therapy, and has therefore been considered as one of the most dangerous pathogenic fungi known to date (de Hoog et al. 2003). The majority of reported CNS infections caused by C. bantiana were found to be brain abscesses with no predisposing factors or immunodeficiency (Horré & de Hoog 1999). The pathogenesis may be haematogenous spread from an initial, presumably subclinical, focus which may be pulmonary, subcutaneous or intestinal (Li et al. 2009). It remains unclear why this fungus preferentially causes CNS disease in immunocompetent individuals. It has been hypothesized that, in addition to other virulence factors that are common in herpotrichiellaceous fungi, such as thermotolerance, dimorphic yeast-like growth, wall melanization, etc., the tendency to infect central nervous tissue in mammals is also related to the capacity of metabolizing aromatic compounds, which is also conspicuous in the Herpotrichiellaceae (Prenafeta-Boldu et al. 2006). Being primary neurotropic, a characteristic from just a few species in the Herpotrichiellaceae, these fungi must also possess other, as yet undiscovered attributes conferring infective capacity towards the brain.

An association between metabolism of aromatic hydrocarbons and opportunistic pathogenicity has also been observed in Cladophialophora immunda and C. saturnica, as well as in the related genus Exophiala, particularly E. oligosperma and E. xenobiotica. Representatives have been reported from opportunistic human infections and from hydrocarbon polluted sites (Badali et al. 2008, de Hoog et al. 2003, Vicente et al. 2008). However, infection patterns of these species tend to be coincidental, and are mostly limited to skin and nails. Cladophialophora psammophila thus far has never been reported from clinical cases, and virulence test on the strain CBS 110553 indicated that the new species is, in principle, non-pathogenic.

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