ACTA MYCOLOGICA
Vol. 43 (1): 67–70
2008

# *Cephalotrichum stemonitis* as a biofilm inhabitant in the gold mine in Poland

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Chlebicki A.: *Cephalotrichum stemonitis as a biofilm inhabitant in gold mine in Poland.* Acta Mycol. 43 (1): 67–70, 2008.

*Cephalotrichum stemonitis* and its synanamorph *Echinobotryum atrum* isolated from bacterial biofilm is presented.

**Key words:** mine fungi, *Cephalotrichum*, biofilm, distribution

## INTRODUCTION

Dry-spored synnematous anamorphs from the form-genus *Cephalotrichum* Link are the asexual states of *Microascaceae*. However so far a teleomorph state is known for no species of this genus (Abbott 2000). *Microascaceae* include five genera: *Microascus*, *Kernia*, *Petriella*, *Pseudoalescheria* and *Lophotrichus* (Abbott et al. 2002) as well as eight anamorphic genera containing about 50 species (Abbott 2000). Abbott (2000) analyzed the subunit 18S rDNA for 34 taxa of *Microascaceae*. He obtained *Microascus* clade consisting of *Microascus longirostris*, *M. nidicola*, *M. cirrhosus*, *M. trigonosporus*, *Cephalotrichum stemonitis*, *Wardomyces anomalus*, *Cephalotrichum cylindricum* and *Microascus brevicaulis*. It indicate that the genus *Microascus* can be recognized as hypothetical teleomorph state for *Cephalotrichum stemonitis*. In GenBank there are available 3 sequences of ribosomal RNA genes (5.8S rRNA, 28S rRNA and large subunit rRNA) of *Doratomyces stemonitis* (in fact *Cephalotrichum stemonitis*) with access numbers: AF400852, EF029213 and AJ608983.

Synnemata of *Cephalotrichum* producing chains of powdery conidia are relatively large with 'bootle brush' or "feather" appearance. Ovoid conidia are produced from annelidic conidiogenous cells covering sporogonous area. *Cephalotrichum* synnemata are adapted for dispersion not only by air currents but also by insects (Abbott 2000, 2002). Habitat of *Cephalotrichum* fungi includes soil, compost, wood, wood treated with fungicide, herbaceous stems, oat seeds, decaying plant material and dung, finger nail, sawdust and straw used for growing shiitake, airborne contaminant of wheat-straw agar plate, egg of gypsy moth (*Lymantaria dispar*), roots of potato,

basidiomycete detritus, manore pile, bronchial washing, right feel, leaves of needle-leafed tree (Abbott 2000). *C. stemonitis* was noted in coyote and rat dung, indoor air of honeybee (*Apis mellifera*) overwintering facility, cone of white spruce, sandy soil, decayed wood of white spruce, soil of elm woods, agricultural soil. It is also important colonizer of pPVC buried in soil (Sabev et al. 2006). These authors mentioned it as *Doratomyces* spp. in the article, but in GenBank it is more precisely determined as *D. stemonitis*.

Malloch and Hubart (1987) described unnamed species of *Microascus* from Ramoil Cave. It means that fungi from this group can inhabit also underground environments. Fassatiova (1970) noted *C. stemonitis* on wood in uranium mine in Czech Republic.

## STUDY AREA AND METHODS

Gray biofilms of rock-inhabiting bacteria in Gertruda Adit in a closed gold mine located in Złoty Stok in Lower Silesia were chosen for microbial analysis. This mine posseses constant conditions such as low temperature ca 10º C, darkness, high humidity, high As concentration and other toxic substances (Chlebicki et al. 2005). So far 12 species of fungi were noted in this mine (Chlebicki et al. 2005; Chlebicki, Lorenc 2006). Bacteria with fungi were collected in sterile plastic tubes and refrigeratorated at 10º C. Fungal growth was performed on DRBC, RBC, YMA and PDA media. Inoculated media on Petri dishes were putted in incubator at 10º C. The morphological characters of the living fungi were examined in water and cotton blue in lactophenol using light microscopy (Nikon SMZ 1500, Nikon Labophot 2 and Nikon Eclipse 800). Microphotographs were taken with these microscopes equipped with a digital camera. For scanning electron microscope (SEM) studies mycelium was coated with gold, and photographed using a LEO 1430 VP Zeiss microscope with a working distance of ca 10 mm. Fungus-species nomenclature follows Abbott (2000).

## RESULTS

***Cephalotrichum stemonitis*** (Pers.) Nees

Magazin Ges. naturf. Freunde, Berlin 3: 20 (1809)

Synonymy: *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm., Mycol. Pap. 86: 70, 1963

*Doratomyces stemonitis* var. *keratinolyticus* (Dominik & Majchr.) Dominik & Majchr. Ekol. Pol., Ser. A 18: 603, 1970 *Echinobotryum atrum* Corda, Sturm's Deutschl. Flora, III (Pilze) 3(12): 51 (1829) *Periconia stemonitis* Pers., Syn. meth. fung. (Göttingen): 687 (1801) *Stysanus stemonitis* (Pers.) Corda, Icon. fung. (Prague) 1: 22 (1837)

Description: *mycelium* creamy-white to dark brown, after 7 days on PDA 17,5-21 mm diam., on RBC 17,0 mm diam., and on DRBC 13-17 mm, all in room temperature (Fig. 1A, B, D). First conidiophores of synanamorph – *Echinonobotryum atrum* – appeared throughout the mycelium after 12 days on PDA. Mycelium growing on RBC after two weeks produced rings of densely distributed synnemata of *C. stemonitis* in central part of the mycelium. Mycelium on PDA formed at the beginning *E.*

*atrum* throughout the surface of dark brown colony and later numerous synnemata of *C. stemonitis*. Mycelium on YMA form brown and irregular colony patches (Fig. 1C). *Synnemata* 190-300 μm long, sterile part 130-210 μm, fertile part 60-120 μm long, (irregular heads) similar to feather (Fig. 2B, C) associated with synanamorph *Echinobotryum atrum* (Fig. 2A), *conidiophores* synnematous, brown, conidiogenous cells ampulliform, percurrent, *conidia s*mooth (Fig. 2D), ovoid with pointed apex and truncate base 7-9,1 x 4,2-6,1 μm, pale brown.

Material examined: on rock bacterial biofilm in Gertruda Adit in gold mine in Złoty Stok, Lower Silesia, Poland, 16 October 2006, coll. A. Chlebicki. First culture obtained on DRBC medium, then transferred to other media.

## DISCUSSION

So far two species of the genus *Cephalotrichum* Link were noted in Poland. Dominik and Majchrowicz (1965) and Dominik (1970) described a new variety *Doratomyces stemonitis* var. *keratinolyticus* (Dominik & Majchr.) Dominik & Majchr. on the basis of specimen isolated from the soil. Conidia of this specimen are something smaller than typical variety, 4-6 x 2.5-4 μm and 'very slightly rough' (Dominik 1970). It resembles *Cephalotrichum microsporum* (Sacc.) P. M. Kirk. Moreover these authors did not mention the presence of *Echinobotryum* synanamorph which is diagnostic character of *Cephalotrichum stemonitis*.

The next species *Cephalotrichum putredinis* (Corda) S. P. Abbott was reported by Dominik (1970) as *Doratomyces albus* (Szilvinyi) Dominik. Unfortunately, these collections are not available for investigation.

Presence of *Cephalotrichum stemonitis* in bacterial biofilm is accidental. However, as indicate informations of Fassatiova (1970) and Malloch and Hubart (1987), such fungi were noted in subterranean environments. Mille-Lindblom (2005), Hogan and Kolter (2002) and Kirkwood (2002) noted mostly antagonistic relation between fungi and bacteria. Fungi were always negatively affected by presence of bacteria. Penetration of fungal hyphae of *Fusarium oxysporum* was not observed where microcolonies of *Pseudomonas* were present, moreover *Pseudomonas* bacteria attached and colonized fungus hyphae (Bolwerk et al. 2003). Also growth of *Cephalotrichum stemonitis* was suppressed by bacteria from the genus *Pseudomonas* isolated from biofilm.

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## *Cephalotrichum stemonitis* zasiedlający bakteryjny biofilm w kopalni złota w Polsce

Streszczenie

Praca zawiera opis grzyba *Cephalotrichum stemonitis* i jego synanamorfy *Echinobotryum atrum* wyizolowanych z bakteryjnego biofilmu z Sztolni Gertrudy w Kopalni Złota w Złotym Stoku. Pierwsza informacja o tym gatunku podana z Polski przez T. Dominika i I. Majchrowicz jest niezbyt dokładna. Diagnostyczną cechą gatunku *C. stemionitis* jest obecność synanamorfy *E. atrum* o czym wymienieni autorzy nie wspominają. Podano również podstawowe dane o siedliskach i ekologii tego gatunku. Izolacja tego rzadkiego gatunku grzyba z bakteryjnego biofilmu nie była dotychczas notowana.

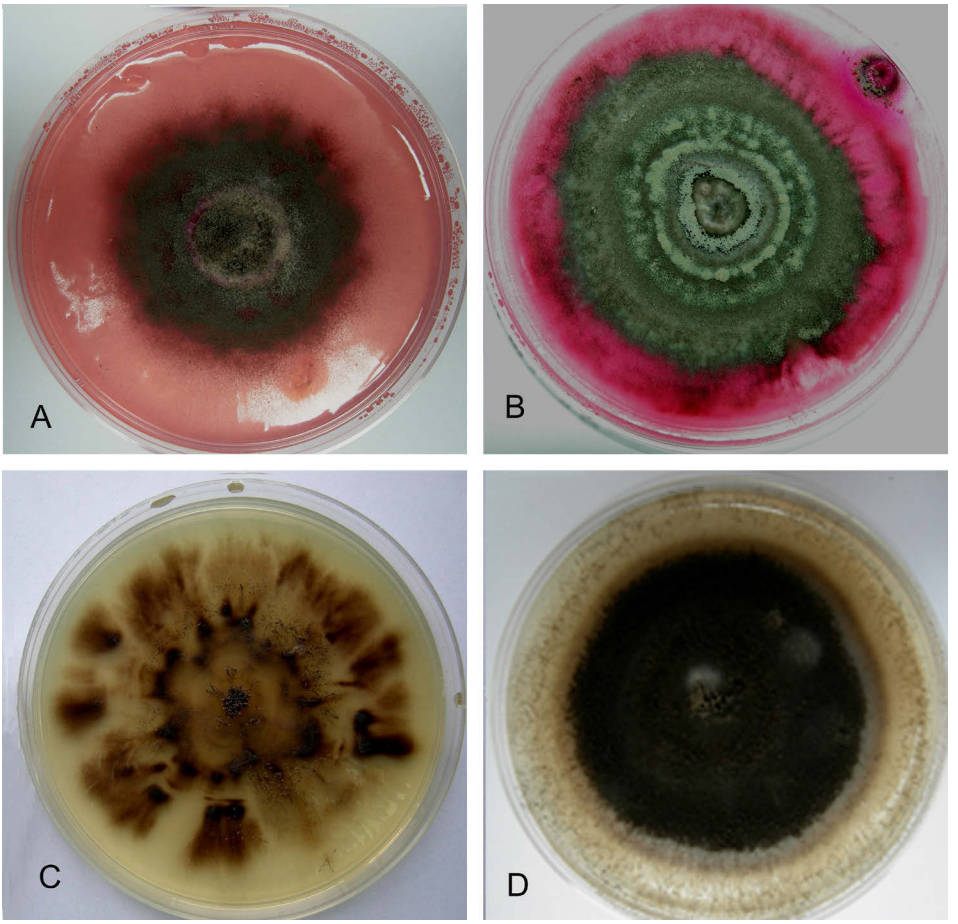


Fig. 1. Colonies of *Cephalotrichum stemonitis* on different media: A – on RBC; B – on DRBC; C – on YMA; D – on PDA.

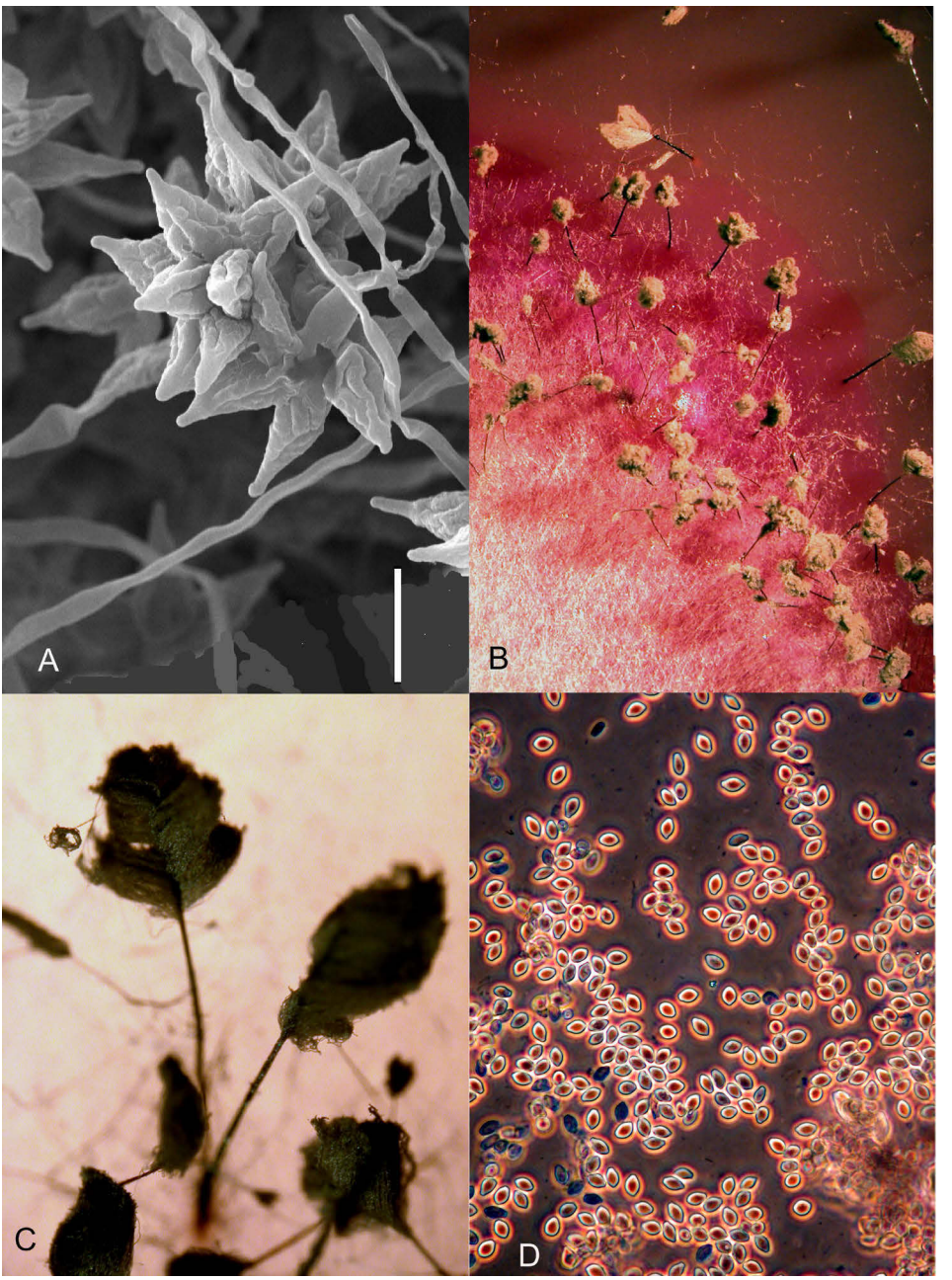


Fig. 2. Morphology of *Cephalotrichum stemonitis* and its synanamorph – *Echinobotryum atrum*: A – conidia of *E. atrum*; scale bar = 10 $\mu$m; B – synnemata of *C. stemonitis* on DRBC medium; C – synnemata of *C. stemonitis* similar to feather; D – conidies of *C. stemonitis*.