First find of *Cadophora antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano in the Arctic

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Abstract

Cadophora antarctica Rodr.-Andrade, Stchigel, Mac Cormack & Cano was isolated from spoil tip of coal mine in the Arctic, on the territory of the Svalbard archipelago, and is represented by strain IVA-206. Macro- and micromorphology of the isolate were examined along with partial sequences of Internal transcribed spacer rDNA region (ITS1-5.8S-ITS2) and D1/D2 region of 28S rDNA (LSU). The isolate *C. antarctica* IVA-206 had a number of features that distinguished it from the strain *C. antarctica* CBS 143035 from Antarctica. Colonies of Arctic strain had darker pigmentation, ramoconidia and conidia were larger, and the optimal growth temperature was higher. As a result of our study, we first discovered the microfungi *C. antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano in the Arctic. Our study shows that *C. antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano is a bipolar species found in both the Arctic and Antarctic region.

Key words: microfungi, Svalbard, Arctic, *Cadophora antarctica*, bipolar species, spoil tips, coal mines

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Introduction

The genus *Cadophora* was described in 1927 by Lagerberg and Melin with the type species *C. fastigiata* Lagerb. & Melin, as a dark-colored hyphomycete that produce single phialides with distinct hyaline collarettes (Lagerberg et al. 1927). Conan transferred eight species of *Cadophora* to the genus *Phialophora* based on the similar morphology of phialide in 1937 (Conant 1937). Just recently, a change in the systematics of phialophora-like anamorphic species based on morphology was proposed by (Gams 2000). The change was confirmed by the data based on molecular studies later by Harrington and McNew (2003). It turned out that the genus *Cadophora* belongs to the order Helotiales (Leotiomycetes), and the genus *Phialophora* belongs to the order Chaetothryiales (Eurotiomycetes). To the date, according to the database Index Fungorum, the genus *Cadophora* had 22 species [1].

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Microfungi of the genus *Cadophora* are distributed worldwide and occupy various ecological niches. Most species of the genus *Cadophora* are plant parasites or endophytes (Walsh et al. 2018), wood destroyers (Travadon et al. 2015), and soil inhabitants (Domsch et al. 2007). Some species of the genus *Cadophora* are psychrotrophs. Thus, *C. malorum*, *C. luteo-olivacea* and *C. fastigiata* were found both in Antarctica (Blanchette et al. 2004, Arenz and Blanchette 2009) and in the Arctic (Kirtsideli et al. 2014, Bubnova and Nikitin 2017). However, these three species are cosmopolitan and distributed in different regions of the Earth (Lagerberg et al. 1927, Gams 2000, Harrington and McNew 2003, Domsch et al. 2007, Navarrete et al. 2011). Therefore, they are neither bipolar species with a separate distribution of the habitat zone in both polar zones (Wirtw et al. 2008), nor endemic species for regions of high latitudes.

In 2017, a new species *Cadophora antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano (Crous et al. 2017) was described. The fungus was isolated from diesel-contaminated soils on the King George Island (Antarctica, South Shetlands archipelago, near to Carlini's Argentinean scientific base, 62°14'17"S, 58°40' 02"W). Until recently, there was no evidence of the discovery of this microfungi in other habitats.

It is generally accepted that the ecological similarity of the Polar Regions leads to the convergence of the mycobiota of the Arctic and Antarctic and a bipolar distribution of species (*e.g.* Ricklefs 2004). On the other hand, the vast distance separating the two polar regions limits the spread of microfungi and should minimize the number of identical species at the poles (Morlon et al. 2008). However, recent metagenomic studies have shown that Antarctic and Arctic soil micromycete communities show a high percentage of similarity (Cox et al. 2016). In polar regions, in contrast to the temperate and tropical ones, relatively small endemism and prevalence of fungi with widespread habitats are observed.

Thus, as a result of our study, we first discovered the microfungi *Cadophora antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano in the Arctic, on the territory of the Svalbard archipelago. Arctic strain of this species was isolated from spoil tip of coal mine near Barentsburg. Also in this work, we have identified a number of features that distinguish the Arctic and Antarctic morphotypes.

Material and Methods

Samples were taken in August 2018 from spoil tip of coal mine No. 1-5 near the village of Barentsburg (78° 03' 51" N, 14° 11' 09" E), Svalbard archipelago. The spoil tip of coal mine from which sampling was carried out was at the formation stage (exploited).

The method of serial dilutions with spread on agar plates was used to isolate fungi cultures (Goldman and Green 2015). Single spore isolation was used to obtain pure culture. Inoculations were prepared from spore suspensions made in a 0.2% agar and 0.05% Tween 80 solution and agar plates were inoculated as described by Samson et al. (2014). The strain was designated as IVA-206.

The pure culture was grown on CZ medium without antibiotics at 20°C for 14 days for molecular analysis. DNA was extracted by using a DiamondDNA Plant kit (ABT, Russia, Barnaul) according to the manufacturer's instructions. Internal transcribed spacer rDNA region (ITS1-5.8S-ITS2) (White et al. 1990) and D1/D2 region of 28S rDNA (LSU) (O'Donnell, 1993) were used as a phylogenetic markers. Internal transcribed spacer rDNA region

(ITS1-5.8S-ITS2) was amplified using the PCR-primers ITS1 (5′- TCC-GTA-GGT-GAA-CCT-TGC-GG -3′) and ITS4 (5′- TCC-TCC-GCT-TAT-TGA-TAT-GC -3′). D1/D2 region of 28S rDNA (LSU) was amplified using the PCR-primers NL-1 (5′- GCATATCAATAAGCGGAGGAAAAG- 3′) and NL-4 (5′- GGTCCGTGTTTCAA GACGG -3′). At the end of amplification, the samples were detected by agarose gel electrophoretic method; sequencing of the obtained DNA fragments was carried out in the commercial organization BioBeagle (St. Petersburg) using the Sanger method. Sequences were proofread and edited using BioEdit version 7.1.9. Newly generated sequences were compared to the available sequences in the GenBank database (NCBI) by using BLAST instrument [2].

The isolate was cultivated on Czapek agar (CZ) (Raper and Thom 1949), malt extract agar (MEA) (Samson et al. 2010) for follow-up morphological observations. The isolate was inoculated on 11-cm Petri dishes and incubated for 21 days at 2, 4, 8, 12, 15, 18, 21, 25, 27 and 30°C. Color determination was performed according to the ISCC-NBS Centroid Color Charts (Kelly 1964), according to the recommendations of Nováková et al. (2012).

For micro-morphological examination, microscopy by Carl Zeiss AxioImager A1 was used.

Statistical processing (medium size) was performed using the statistical software package MS Excel 2007.

Results and Discussion

Our study of mycobiota of coal mine spoil tips in the Svalbard archipelago resulted in the isolation of strain IVA-206. BLAST analysis of the partial LSU gene sequence showed 100% similarity of the isolate IVA-206 **(MT362720)** and *Cadophora antarctica* CBS 143035 (= FMR 16056) **(MG385663)**. BLAST analysis of the ITS region showed 99% similarity of the isolate IVA-206 **(MN833351)** and *Cadophora antarctica* CBS 143035 (= FMR 16056) **(MG385664)**.

The observed micro- and macromorphology of isolate IVA-206 also corresponded to the micro- and macromorphology of *Cadophora antarctica* (Crous et al. 2017). Therefore, molecular and morphological data led to the conclusion that the obtained isolate IVA-206 belongs to the species *Cadophora antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano.

Micromorphology. Mycelium consists of hyaline to olive-brown, smooth to verrucous, thin- to thick-walled. Hyphae are anastomosing 2–4 μm wide. Conidiophores are mainly reduced to a short chain of ramoconidia on a scar, laterally or terminally disposed on curved hyphae, simple, poorly developed, stalked, up to 200 μm long, up to 4 μm broad. Ramoconidia are brown or dark brown, sometimes irregularly colored, with one side darker than the opposite side, holoblastic, aseptate, in longitudinal chains to six, smooth- and thick-walled, ovoid-, cylindrical-, lemon-, flask-shaped, 6–16 × 4–8 μm. Conidia are brown to dark brown, irregularly colored, holoblastic, aseptate, disposed in long, simple or branchy chains, smooth- and thick-walled, mostly broadly lens-shaped but irregularly due to one side being more oblate than the other side, 4–6 × 4–8 μm.

The dimensional characteristics of the isolated strain IVA-206, in general, correspond to the description of *Cadophora antarctica* (Crous et al. 2017). The exceptions are sizes of ramoconidia and conidia. Ramoconidia and conidia of the strain isolated by us were, on average, 1.5 times larger than that of the Antarctic strain. In *Cadophora antarctica* from the Antarctic, the size of ramoconidia was 5–13 × 2–4 μm and the size of conidia was 4–5 × 3–4 μm. Micromorphology is shown in Fig. 1c.

Culture characteristics. Colonies cultivated on MEA after 14 days of cultivation at 21°C reached a diameter of 38–44 mm, velvety, non-zonate, brownish black (#28201c); exudates absent; sporulation abundant; reverse black (#222222). Colonies on CZ after 14 days of cultivation at 21°C reached a diameter of 34–37 mm, velvety, non-zonate, brownish black (#28201c); exudates absent; sporulation abundant; reverse black (#222222). Cultivation at different temperature conditions did not affect the color and structure of the colonies. Colonies are shown in Fig. 1a, b.

The colonies of the strain IVA-206 on MEA were darker than the CBS 143035 strain from the Antarctic, and also did not have zonal coloration.

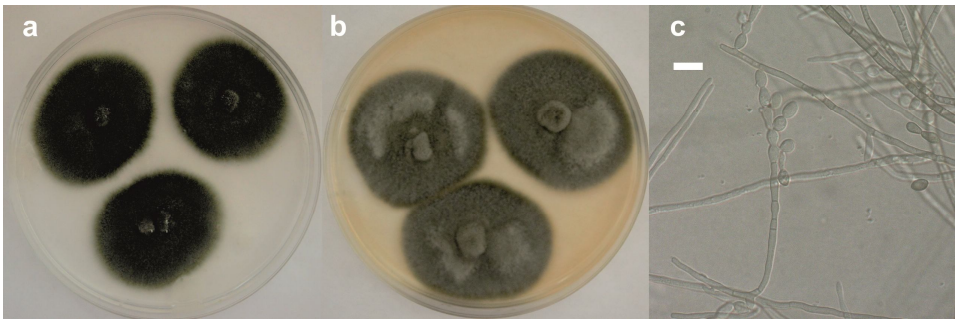

Fig. 1. *Cadophora antarctica* morphology in 14-day-old cultures: a – CZ, b – MEA, c – conidiophores and conidia. Scale bars = 10 μm.

The strain *Cadophora antarctica* IVA-206 grows over a fairly wide temperature range (min. 2°C, max 27°C). This temperature range is not different from the range of the CBS 143035 strain from the Antarctic (5-25°C). However, the Arctic and Antarctic strains differed quite strongly in the optimal growth temperature: 21°C and 15°C, respectively.

The isolate *Cadophora antarctica* IVA-206 that had been isolated from the spoil tip of coal mine of the Svalbard archipelago had a number of features that distinguished it from the strain *Cadophora antarctica* CBS 143035 from the South Shetland archipelago. IVA-206 colonies had darker pigmentation, ramoconidia and conidia were larger, and the optimal growth temperature was higher. It is likely that these differences in the phenotype are caused by milder environmental conditions for the Arctic strain, which is especially confirmed by a higher optimal growth temperature.

Thus, *Cadophora antarctica* Rodr.-Andrade, Stchigel, Mac Cormack & Cano is a bipolar species found in both the Arctic and Antarctic.

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Web sources / Other sources

[1] database Index Fungorum
www.indexfungorum.org/Names/fungic.asp

[2] GenBank database (NCBI) by using BLAST instrument
http://blast.ncbi.nlm.nih.gov/Blast.cgi