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CONFIRMATION OF TAXONOMIC STATUS OF BLACK YEAST-LIKE FUNGUS BY THREE GENE PHYLOGENY

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Status of black melanin-containing yeast-like fungus *Exophiala alcalophila* isolated from microorganism complex of hermetic damaged in conditions of indoor high humidity in Kiev, Ukraine is proved by combined phylogenetic analysis based on sequences of the internal transcribed spacer 1 (ITS1), the 5.8S gene and the internal transcribed spacer 2 (ITS2) nrDNA, beta-tubulin gene and translation elongation factor 1-alpha gene. Sequences of the mentioned genes of Ukrainian specimen of *E. alcalophila* are for the first time submitted to the GenBank.

Key words: *Exophiala alcalophila*, hermetic, high humidity, microorganism complex, phylogenetic analysis

INTRODUCTION

"Black yeast fungi" is a group of fungi that is quite heterogeneous from taxonomic and phylogenetical point of views, but having in common melanised cell walls and the formation of daughter cells by yeast-like multilateral or polar budding. *Exophiala* is an anamorphic genus defined by annellidic conidiogenesis producing slimy heads of conidia, and a phylogenetical affiliation to the ascomycete order Chaetothyriales (Sterflinger 2006). Nearly all species are characterised and recognisable within the order by their production of budding cells, and the yeast/hypha transition mostly proceeds via torulose hyphae (de Hoog *et al.* 2011). Where they are known, teleomorphs belong to *Capronia*.

Representatives of black yeast genus *Exophiala* are common in nature, on wood decomposing in soil and in water (Sutton *et al.* 1998). Wet cells like

bathrooms, sinks, kitchens and saunas were described as novel niches for adaptation of human pathogens (Hamada 2013, Hamada and Abe 2010, Lian and de Hoog 2010, Nishimura *et al.* 1987, Zalar *et al.* 2011). Bathwater and sludge of bathroom drainpipes are important habitats for members of the genus *Exophiala* (Hamada and Abe 2010, Matos *et al.* 2002, Nishimura *et al.* 1987). They were also recorded on the marble and various rocky substrates, and as members of the communities damaging various building materials, as well as on acrylic hermetic in bathrooms, from drinking water, etc. (Hageskal *et al.* 2006, Kondratyuk 2010).

Some representatives of the genus *Exophiala* are categorized to the Biosafety Level 2 (BSL) risk group, introduced by various international organisations (Gunde-Cimerman and Zalar 2011, Ozerskaya *et al.* 2007). Some taxa of this genus are potential pathogens and opportunists, some species can cause serious illness in healthy, immunocompetent people (de Hoog *et al.* 2000, Kantarcloglu and de Hoog 2004, Rimawi *et al.* 2013). The appearance of human skin infections suggested to be connected with the presence of black yeasts in the rooms, including the bathrooms (i.e. sensu "water systems" rooms) (Lian and de Hoog 2010). These fungi thought to be also associated with many kinds of diseases of wildlife, including fish, marine toads, turtles, shellfish (de Hoog *et al.* 2011).

Considering the ability of black yeast-like fungi of the genus *Exophiala* remain viable and to form different morphological types in the conditions of joint influence a wide range of temperatures, pH and salt content of they are often classified as polyextremotoletant fungi (Döğen *et al.* 2013, Hamada and Abe 2010, Zalar *et al.* 2011). These fungi are rather dangerous, because they can grow at high temperatures and are capable of colonising warm-blooded organisms (Gostincar *et al.* 2011).

Black yeast-like fungus *Exophiala alcalophila* Goto et Sugly was found in microorganism complex of hermetic damaged in conditions of indoor high humidity in Kyiv, Ukraine (Kondratyuk 2010, 2013). It was recorded after results of phylogenetic analysis based on sequences of ITS1/ITS2 (Kondratyuk *et al.* 2013). However, submission ITS1/ITS2 sequence of Ukrainian specimens of this taxon to the GenBank was postponed till sequences of beta-tubulin (BT2), actin (ACT1) and translation elongation factor 1-alfa (TEF-1) gene from the same fungus were obtained.

A recently published paper of de Hoog and colleagues (de Hoog *et al.* 2011) may serve as extremely important handbook in taxonomic revision of this group of black yeast-like fungi, while previous data on morphology and molecular characters of *Exophiala* species were scattered in medical, veterinary and ecological literature (see also Table 1). These authors have provided reliable data on the type collections of major portion of species of the genus

Exophiala, which allow identifying representatives of this genus after multigene phylogeny. There are even two or three gene sequences of the same gene of the same taxon based on the same CBS cultures submitted to the GeneBank by various authors in different time (see also Table 1).

After combined phylogenetical analysis based on ITS, ACT1, BT2 and TEF-1 sequences (after de Hoog *et al.* 2011) *Exophiala alcalophila* belongs to the *Exophiala angulospora* complex, which is positioned in out-position to the SSU *Exophiala salmonis*-clade, type clade of the genus *Exophiala*.

The aim of this paper is to provide complete set of data on molecular identification of Ukrainian specimens of *Exophiala alcalophila*, to present results of combined phylogenetical analysis based on ITS1/ITS2 nrDNA, BT2 and TEF-1 gene sequences and to submit these data to GenBank. To check the position of the Ukrainian specimen all known taxa of the SSU *Exophiala salmonis*-clade (sensu de Hoog *et al.* 2011), as well as taxa of the *Exophiala angulospora* complex for which data on ITS, BT2 and TEF-1 sequences are hitherto available from the GenBank were included in our combined phylogenetical analysis. Unfortunately our attempt to get ACT1 gene sequence from Ukrainian specimen of *Exophiala alcalophila* was unsuccessful; it is why data on this gene were not included in the combined phylogenetical analysis.

MATERIAL AND METHODS

Strains and culture conditions

Standard microbiological methods of microorganism cultivation in agar nutrient media (Malt extract agar (Merck, Germany, containing peptone); Malt extract agar (Heimedia, India, without peptone); Nutrient agar (Sigma); Czapek-Dox agar (CZD); Sabouraud agar (HiMedia); Potato dextrose agar (PDA); Synthetic medium SNA, depleted in nutrients (i.e. synthetischer nährstoffarmer agar), which were prepared in accordance with specifications, set out in Samson *et al.* (2004); and liquid media as Potato-glucose broth containing 2% glucose (PGB) (Samson *et al.* 2004); Glucose-yeast-peptone medium (GPY, Sigma, USA); and Meat-peptone broth (MPB)) were used. Furthermore the following original and modified media, i.e.: MPB with 2% glucose; 10% sucrose solution; Potato broth without glucose; PGB with 2% and 10% glucose; GPY (containing 10% glucose, 1% peptone, 1% yeast extract); and GP (glucose-peptone medium containing 10% glucose, and 1% peptone) were also used within these studies.

Photographing fungal specimens performed using microscope Carl Zeiss Primo Star company (Germany) and Camera Scope Tek, m. Etrek DCM-510, at magnification ×400. The length and width of the cells was measured by the

morphometric computer program AxioVision 4.8 (Carl Zeiss). Morphometric data (length and width of cells), the intensity of budding were calculated with the usage of arithmetic mean and standard deviation using Statistica 12. The reliability of differences was determined using Student *t*-test, significance level $r \leq 0.05$. To understand the morphological features of *Exophiala alcalophila* additionally used scanning electron microscopy (SEM JSM-6060 LA, Japan).

Ukrainian strain from hermetic has been isolated from microorganism complex in conditions of indoor high humidity in Kyiv, Ukraine in 2010 by a pour-plate method. Sampling location was black outgrowth on surface of hermetic layer between bath-cabin and wall of bathroom of private flat in Kyiv city (Kondratyuk 2010, 2013). Strains were maintained on MEA (2% Malt Extract Agar) or PDA (Potato-Dextrose Agar) slants at 4 °C.

Culture obtained and supported in "Institute of Biology" Educational and Scientific Centre of Taras Shevchenko National University of Kyiv (FCKU 304).

Identification of pure cultures after morphological characters was done with applying traditional manuals (de Hoog *et al.* 2000, 2011, Sutton *et al.* 1998, 2001). Data on morphology of colonies, conidiogenous structures were compared with recent publications (de Hoog *et al.* 2011, Lian and de Hoog 2010).

Prior to analysis, small pieces from mature colonies were suspended in 4.5 ml sterile water to obtain conidial suspensions. Aliquots of 0.5 mL were plated on liquid media in culture plates and incubated at 24 °C for 2–4 weeks. Physiological data on cultures studied were obtained using automated microbiological analyser Vitek 2 Compact (BioMerieux, France). Appropriate special ID-cards (YST-cards – for yeasts and filamentous fungi) were used. The special medium Phenol red dextrose broth (HiMedia) was additionally applied for detection of the ability to ferment glucose, as well as Nitrate broth (HiMedia) for the capacity to restore nitrates in nitrites.

Genomic DNA was isolated using the NucleoSpin Plant II DNA extraction kit according to the manufacturer's protocol for DNA from fungi (Macherey-Nagel, Düren, Germany). The nuclear ribosomal RNA gene region including the internal transcribed spacers 1 and 2 and the 5.8S subunit (ITS) were amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990). Amplification was performed using CFX 96 Real-Time PCR Detection System (BioRad). The fluorescent dye SYBR Green was used to stain DNA.

Sequences derived in this study were lodged at GenBank KX793104–KX793106.

DNA extraction and amplification methods were outlined by de Hoog *et al.* (2011). Nuclear DNA was isolated from fungus mycelium cultivated on PDA with the usage of Ultra Clean TM Microbial DNA isolation Kit (MoBio-Laboratories, inc. Solana Beach, CA, USA). Fragments of rDNA were ampli-

fied using the universal primers V9G and LS266 for rDNA ITS, Ef1-728F and Ef1-986R for TEF-1, Bt2a and Bt2b for BT2 for ITS, TEF and BT consequently (de Hoog *et al.* 2011).

Alignment and phylogenetical reconstruction

For genealogical concordance analysis, three genes ITS, TEF-1 and BT2 were first analysed separately. Alignment was performed automatically and adjusted iteratively by hand with BioNumerics v.4.61. MP, ME and LP analysis implemented in PAUP v. 4.0b10 were used. Consequently taxa of the SSU-based *Exophiala salmonis*-clade (sensu de Hoog *et al.* 2011) were included in a combined ML analysis based on ITS, TEF-1 and BT2 gene sequences. Strains analysed are listed in Table 1.

RESULTS

Cultural morphological characters

Brownish black colonies of *Exophiala alcalophila* to 20–25 ± 1 mm in diam. were observed after 7–10 days at +26±2 °C on MEA (Figs 1, 3–5, 8–12), PDA (Figs 6–7), and Sabouraud agar (Figs 2, 13). The mucilage-like colonies with oily glance were observed in Sabouraud agar.

Formation of true hyphae was observed on MEA medium, PDA, CZD, and SNA media. Cell clusters and cell chains were formed on PDA medium, and dominance of almost spherical (to 3–6 μm diam.) or oval (2.5–3 × 3–5.8 μm) cells was found on Sabouraud agar.

Fig. 1. General habit of *Exophiala alcalophila* FCKU 304 colony on MEA medium

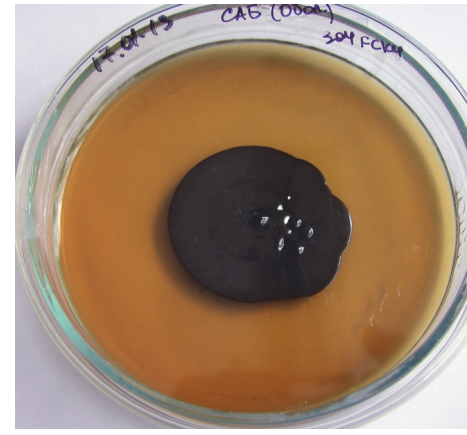


Fig. 2. General habit of *Exophiala alcalophila* FCKU 304 colony on Sabouraud agar

Table 1
List of taxa and sequences used in phylogenetical analysis

Taxon	References	Country (specimen)	ITS	BT2	TEF-1
Exophiala alcalophila 1	de Hoog *et al.* (2011)	Japan (CBS 520.82)	JF747041	JN128771	JN112423
Exophiala alcalophila 2	de Hoog *et al.* (2011)	Japan (CBS 521.82)	JF747042	JN128772	JN112424
Exophiala alcalophila 3	de Hoog *et al.* (2011)	Brazil (CBS 118722)	JF747043	–	–
Exophiala alcalophila 4	de Hoog *et al.* (2011)	Denmark (CBS 122256)	JF747044	JN128773	JN112425
Exophiala alcalophila	**this paper**	**Ukraine (FCKU 304)**	**KX793104**	**KX793105**	**KX793106**
Exophiala angulospora 1	de Hoog *et al.* (2011)	Japan (CBS 482.92)	JF747046	JN128780	JN112426
Exophiala angulospora 2	de Hoog *et al.* (2011)	Germany (CBS 109906)	JF747047	JN128777	JN112428
Exophiala angulospora 3	de Hoog *et al.* (2011)	Germany (CBS 109905)	JF747048	JN128778	JN112429
Exophiala angulospora 4	de Hoog *et al.* (2011)	Russia (CBS 121503)	JF747049	JN128779	–
Exophiala angulospora 5	de Hoog *et al.* (2011)	USA (CBS 119911)	JF747050	JN128784	JN112430
Exophiala angulospora 6	de Hoog *et al.* (2011)	The Netherlands (CBS 441.92)	JF747051	JN128785	JN112431
Exophiala angulospora 7	de Hoog *et al.* (2011)	Denmark (CBS 122264)	JF747052	JN128786	JN112432
Exophiala angulospora 8	de Hoog *et al.* (2011)	Germany (CBS 146.93)	JF747053	JN128787	JN112433
Exophiala brunnea	de Hoog *et al.* (2011)	South Africa (CBS 587.66)	JF747062	JN128783	JN112442
Exophiala cancerae 1	de Hoog *et al.* (2011)	Brazil (CBS 120532)	JF747063	JN128746	JN112443
Exophiala cancerae 2	de Hoog *et al.* (2011)	Brazil (CBS 120420)	JF747064	JN128800	JN112444
Exophiala cancerae 3	de Hoog *et al.* (2011)	The Netherlands (CBS 117491)	JF747066	JN128799	JN112446
Exophiala castellanii 1	de Hoog *et al.* (2011)	Denmark (CBS 122325)	JF747068	JN128763	JN112447
Exophiala castellanii 2	de Hoog *et al.* (2011)	Denmark (CBS 122265)	JF747069	JN128749	JN112448
Exophiala castellanii 3	de Hoog *et al.* (2011)	Sri Lanka (CBS 158.58)	JF747070	JN128766	JN112449
Exophiala castellanii 4	de Hoog *et al.* (2011)	Germany (CBS 109915)	JF747073	JN128764	JN112450
Exophiala castellanii 5	de Hoog *et al.* (2011)	Germany (CBS 121496)	JF747074	JN128768	JN112451
Exophiala equina 1	de Hoog *et al.* (2011)	The Netherlands (CBS 121501)	JF747077	JN128806	JN112453
Exophiala equina 2	de Hoog *et al.* (2011)	The Netherlands (CBS 120278)	JF747079	JN128803	JN112454
Exophiala equina 3	de Hoog *et al.* (2011)	The Netherlands (CBS 121283)	JF747087	JN128809	JN112456
Exophiala equina 4	de Hoog *et al.* (2011)	Canada (CBS 515.76)	JF747102	JN128819	JN112468
Exophiala equina 5	de Hoog *et al.* (2011)	Germany (CBS 109913)	JF747145	JN128817	JN112507
Exophiala halophila 1	de Hoog *et al.* (2011)	USA (CBS 121512)	JF747108	JN128774	JN112473
Exophiala halophila 2	de Hoog *et al.* (2011)	Germany (CBS 121499)	JF747109	JN128775	JN112474

Table 1 (continued)

Taxon	References	Country (specimen)	ITS	BT2	TEF-1
Exophiala jeanselmei 1	Prenafeta-Boldu *et al.* (2006)	unknown (CBS 528.76)	AY857530	–	EF551531
Exophiala jeanselmei 1	Zeng *et al.* (unpubl.)	unknown (CBS 528.76)	–	EF551502	EF551531
Exophiala jeanselmei 2	Woo *et al.* (2013)	England (CBS 677.76)	JN625228	JN625233	–
Exophiala jeanselmei 2	Zeng *et al.* (unpubl.)	England (CBS 677.76)	–	–	EF551532
Exophiala jeanselmei 3	Vitale and de Hoog (2002)	Uruguay (CBS 507.90)	AY156963	–	–
Exophiala jeanselmei 3	Zeng *et al.* (unpubl.)	Uruguay (CBS 507.90)	–	EF551501	EF551530
Exophiala lecanii-corni 1	Lian and de Hoog (2010)	The Netherlands (CBS 124176)	GQ426959	–	–
Exophiala lecanii-corni 2	Lian and de Hoog (2010)	The Netherlands (CBS 124177)	GQ426960	–	–
Exophiala lecanii-corni 3	Lian and de Hoog (2010)	The Netherlands (CBS 124188)	GQ426975	–	–
Exophiala lecanii-corni 4	Lian and de Hoog (2010)	Austria (CBS 124193)	GQ426980	–	–
Exophiala lecanii-corni 4	Borman *et al.* (unpubl.)	England (NCPF 7903)	–	–	LT594727
Exophiala mesophila 1	de Hoog *et al.* (2011)	USA (CBS 121508)	JF747117	JN128756	JN112481
Exophiala mesophila 2	de Hoog *et al.* (2011)	USA (CBS 120910)	JF747118	JN128757	JN112482
Exophiala mesophila 3	de Hoog *et al.* (2011)	USA (CBS 121507)	JF747120	JN128759	JN112484
Exophiala mesophila 4	de Hoog *et al.* (2011)	USA (CBS 121511)	JF747122	JN128751	JN112485
Exophiala mesophila 5	de Hoog *et al.* (2011)	The Netherlands (CBS 121509)	JF747116	JN128762	–
Exophiala nigra	Zeng and de Hoog (2008)	Russia (CBS 546.82)	EF551550	–	–
Exophiala oligosperma 1	Woo *et al.* (2013)	USA (CBS 658.76)	JN625230	JN625235	JN625245
Exophiala oligosperma 1	Prenafeta-Boldu *et al.* (2006)	USA (CBS 658.76)	AY857532	–	–
Exophiala oligosperma 2	de Hoog *et al.* (2003)	Germany (CBS 725.88)	AY163551	–	–
Exophiala oligosperma 2	Attili-Angelis *et al.* (2014)	Germany (CBS 725.88)	–	KF928550	–
Exophiala oligosperma 2	Zeng *et al.* (unpubl.)	Germany (CBS 725.88)	–	EF551508	EF551534
Exophiala oligosperma 3	Lian and de Hoog (2010)	The Netherlands	DQ426977	–	–
Exophiala oligosperma 4	Attili-Angelis *et al.* (2014)	The Netherlands (CBS 637.69)	KF928423	KF928551	
Exophiala opportunistica 1	de Hoog *et al.* (2011)	Germany (CBS 109811)	JF747123	JN128792	JN112486
Exophiala opportunistica 2	de Hoog *et al.* (2011)	Germany (CBS 122269)	JF747124	JN128795	JN112487
Exophiala opportunistica 3	de Hoog *et al.* (2011)	Germany (CBS 122268)	JF747125	JN128794	JN112488
Exophiala pisciphila 1	de Hoog *et al.* (2011)	Germany (CBS 101610)	JF747130	–	JN112492
Exophiala pisciphila 2	de Hoog *et al.* (2011)	USA (CBS 537.73)	JF747131	JN128788	JN112493
Exophiala pisciphila 3	de Hoog *et al.* (2011)	Germany (CBS 119913)	JF747132	–	JN112494

Table 1 (continued)

Taxon	References	Country (specimen)	ITS	BT2	TEF-1
Exophiala pisciphila 4	de Hoog *et al.* (2011)	Germany (CBS 119914)	JF747133	JN128791	JN112495
Exophiala pisciphila 5	de Hoog *et al.* (2011)	Germany (CBS 121500)	JF747134	JN128789	JN112496
Exophiala psychrophila 1	de Hoog *et al.* (2011)	Norway (CBS 191.87)	JF747135	JN128798	JN112497
Exophiala psychrophila 2	de Hoog *et al.* (2011)	Ireland (CBS 256.92)	JF747136	–	JN112498
Exophiala salmonis 1	de Hoog *et al.* (2011)	Canada (CBS 157.67)	JF747137	JN128747	JN112499
Exophiala salmonis 2	de Hoog *et al.* (2011)	The Netherlands (CBS 120274)	JF747138	JN128802	JN112500
Exophiala salmonis 3	de Hoog *et al.* (2011)	The Netherlands (CBS 110371)	JF747139	JN128748	JN112501
Exophiala xenobiotica 1	de Hoog *et al.* (2006)	The Netherlands (CBS 117675)	DQ182590	DQ182574	DQ182582
Exophiala xenobiotica 2	de Hoog *et al.* (2006)	The Netherlands (CBS 117641)	DQ182591	DQ182575	DQ182583
Exophiala xenobiotica 3	de Hoog *et al.* (2006)	The Netherlands (CBS 117676)	DQ182592	DQ182576	DQ182584
Exophiala xenobiotica 4	de Hoog *et al.* (2006)	The Netherlands (CBS 117642)	DQ182593	DQ182577	DQ182585
Exophiala xenobiotica 5	Yanagihara *et al.* (unpubl.)	Japan (KMU 8770)	LC018823	LC018835	LC018831

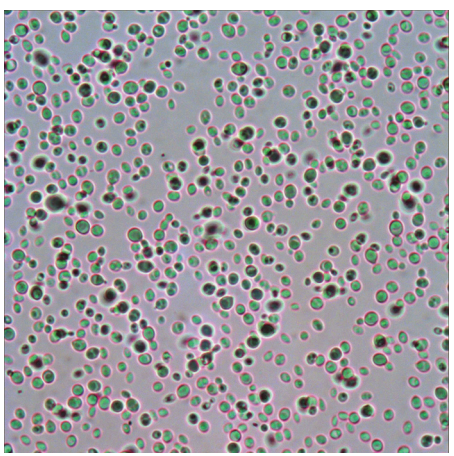
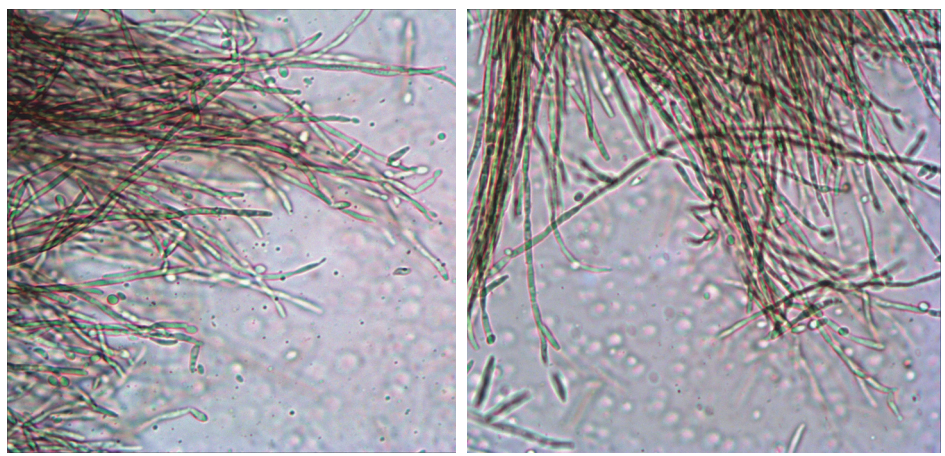
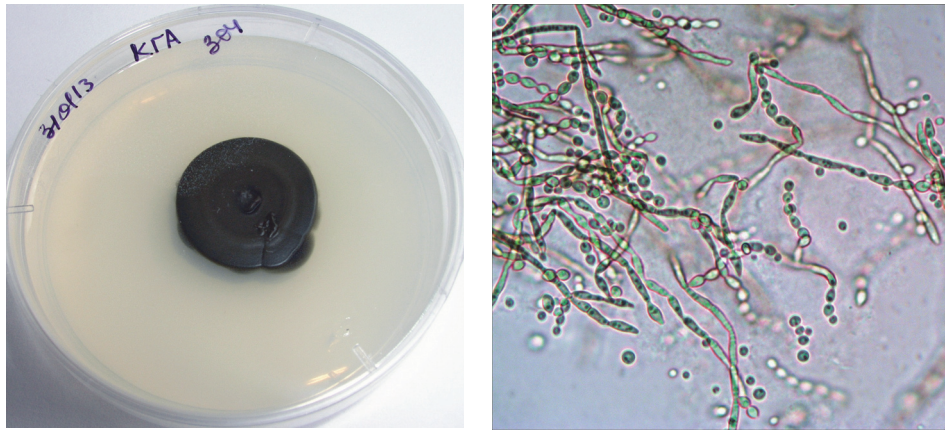


Fig. 3. Cells from centre of colony of *Exophiala alcalophila* FCKU 304 on MEA medium



Figs 4–5. Hyphae from peripheral portion of colony of *Exophiala alcalophila* FCKU 304 on MEA medium

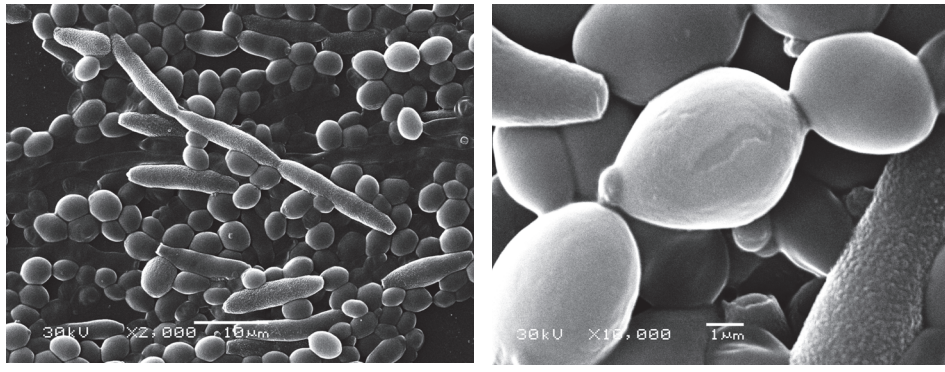


Figs 6–7. General habit of *Exophiala alcalophila* FCKU 304 colony on PDA medium (left); conidial apparatus, conidia, budding cells and torulose hyphae of *Exophiala alcalophila* FCKU 304 colony on PDA medium (7th day of cultivation) (right)

The yeast phase of *Exophiala alcalophila* found to be dominated on the MEA and PDA media during the first week, while hyphal morphotype is observed at the edges of colony later. However, it should be emphasised that the dominance of any morphotype (yeast or mycelium) of *Exophiala alcalophila* is very unstable characters. The structure of colonies on solid media is quite different from those in liquid ones (MEA, PDA and Sabouraud agar).

It is very characteristically radiating / radially ramified on SNA medium (Fig. 14), and more compact and loosing lustre and edges becoming somewhat irregular in other solid media (i.e.: CZD, on CZD with low-carbon content (0.1%)) (Fig. 15).

Thus, in contrast to data of de Hoog *et al.* (2011), where *Exophiala alcalophila* formed hyphal structures only on MEA medium, formation of hyphal



Figs 8–9. Budding cells and conidia of *Exophiala alcalophila* FCKU 304 on MEA medium

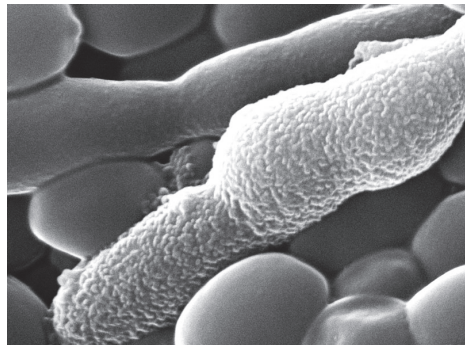


Fig. 10. Torulose hyphae of *Exophiala alcalophila* FCKU 304 on MEA medium

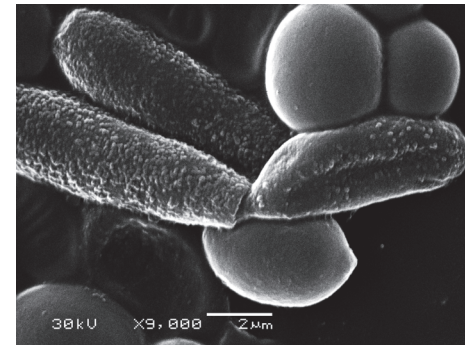


Fig. 11. Terminal conidia formation of *Exophiala alcalophila* FCKU 304 on MEA medium

morphotype in our case was observed also on PDA, MPB, CZD and CZD with low-carbon (0.1%) content, as well as under influence of biocides and under influence of plant essential oils (i.e.: *Abies*, *Foeniculum*, *Juniperus*, *Lavandula*, *Melaleuca*, *Mentha*, *Origanum*, *Pelargonium*, *Pinus*, *Syzygium* (*Caryophyllus*) and *Thymus*), as well as polyhexamethylene guanidine (Kondratyuk and Kalinichenko 2014, 2015).

Exophiala alcalophila found to be well developed on liquid media tested. Morphological exchanges of *Exophiala alcalophila* (i.e. formation of septate hyphae, moniliform annelophores widened at the basis, with urn-like (attenuated) ends were observed on MPB. Exchange of medium colour (medium becoming dark grey almost black colour probably owing to abundant synthesis of melanin) additionally to slight distraction was found in conditions of *E. alcalophila* growth on low pH (to 2–3.5) of liquid media GPY, GP and 10%

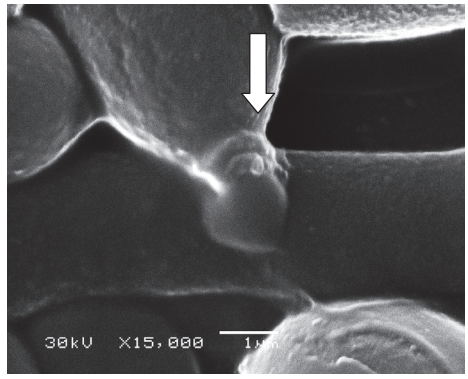
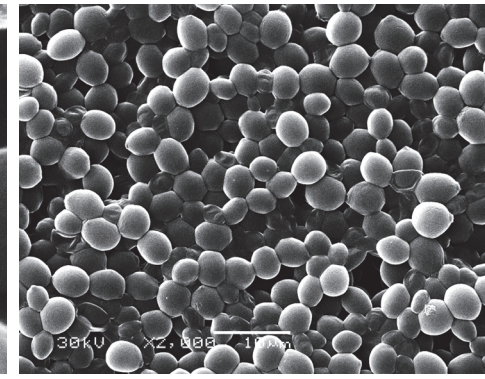


Fig. 12. Intercalary conidia formation in *Exophiala alcalophila* FCKU 304 (on MEA medium); arrow shows short annelation zone



Figs 13. Budding cells of *Exophiala alcalophila* FCKU 304 on Sabouraud agar

solution of dextrose. Examined specimen of *E. alcalophila* weakly fermentates D-glucose with minor gas formation. It shows ability to assimilation of LeuA, GLYLa, 2KGa, and XLTa, as well as urease formation. *E. alcalophila* assimilate (assimilates) nitrates (nitrate converts to nitrite). No growth of *E. alcalophila* at +37 °C was observed.

From combined phylogenetical analysis based on ITS, BT2 and TE1-1 sequences (Fig. 16), as well as from separate ITS, BT2 and TEF-1 analyses (not shown) Ukrainian specimen of *Exophiala* belongs to the *Exophiala alcalophila* branch with the highest level of bootstrap support. Our data confirm the previous results of de Hoog *et al.* (2011) that *Exophiala alcalophila* belongs to the *Exophiala angulospora* complex, which is positioned in out-position to the SSU *Exophiala salmonis*-clade. After our data the *Exophiala angulospora* complex includes *E. angulospora*, *E. alcalophila*, and *E. halophila*, as well as *E. jeanselmei*, *E. oligosperma*, *E. xenobiotica*, and *E. nigra*. The *Exophiala salmonis*-clade was presented by *E. salmonis*, *E. equina*, *E. pisciphila*, *E. psychrophila*, *E. opportunistica*, *E. cancerae*, and *E. brunnea* in our study. Furthermore *Exophiala lecanii-cornii* found to be in separate out-position to the *Exophiala salmonis*-clade and to the *Exophiala angulospora* complex if *E. mesophila* and *E. castellanii* are selected as out-group (Fig. 16).

CONCLUSIONS

Thus, status of black melanin-containing yeast-like fungus *Exophiala alcalophila* isolated from microorganism complex of hermetic damaged in conditions of indoor high humidity in Kiev, Ukraine, recently (newly) recorded for

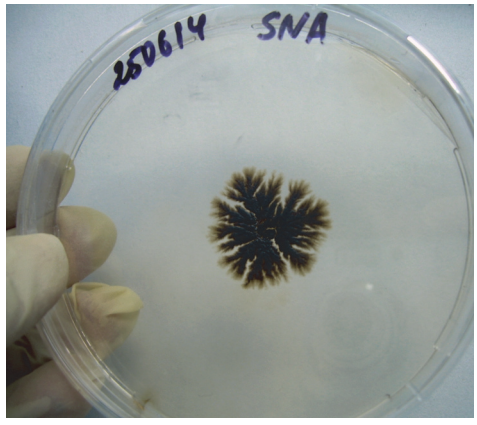


Fig. 14. Radiating / radially ramified colony of *Exophiala alcalophila* FCKU 304 on solid SNA medium

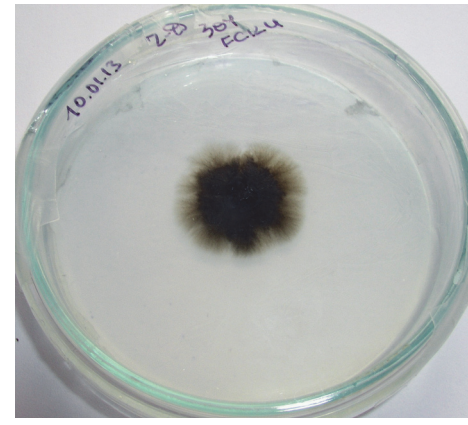


Fig. 15. General habit of colony of *Exophiala alcalophila* FCKU 304 on solid CZD medium

Ukraine, is proved by combined phylogenetical analysis based on sequences of the internal transcribed spacer 1 (ITS1), the 5.8S gene and the internal transcribed spacer 2 (ITS2) nrDNA, beta-tubulin gene and translation elongation factor 1-alpha gene. Sequences of the mentioned genes of Ukrainian specimen are for the first time submitted to GenBank.

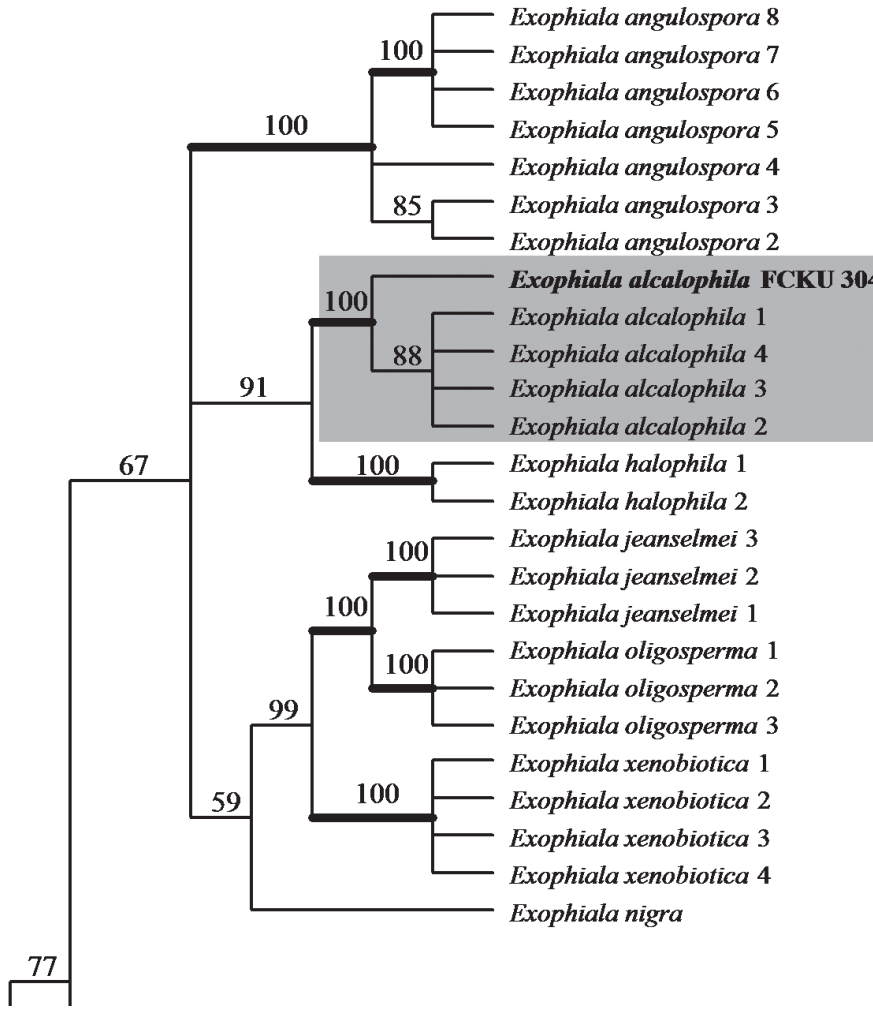


Fig. 16. Phylogeny of the SSU-based *Exophiala salmonis*-clade (sensu de Hoog *et al.* 2011) obtained from a combined ML analysis based on ITS, TE-1 and BT2 gene sequences. Supported branches are drawn in bold. The tree was rooted with *Exophiala mesophila* and *E. castellanii*

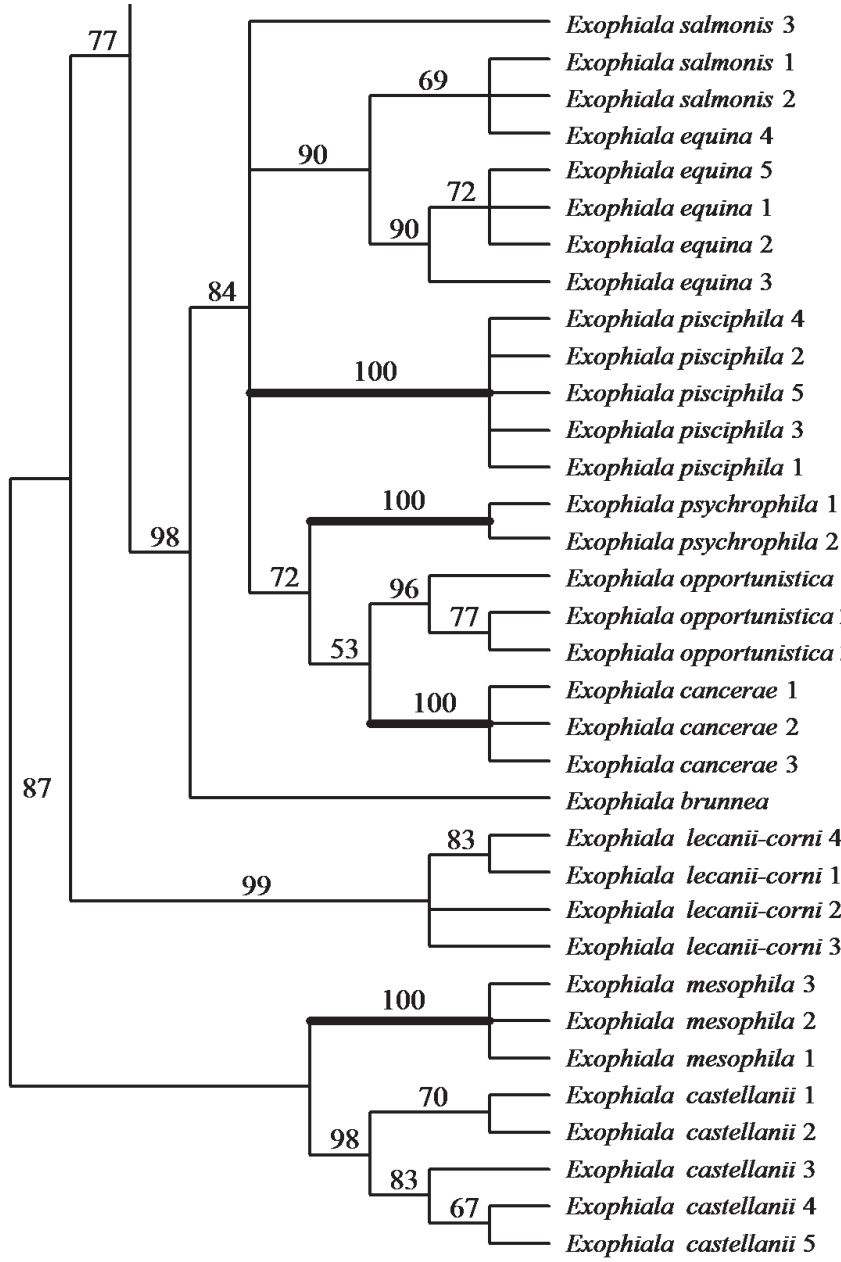


Fig. 16 (continued). Phylogeny of the SSU-based *Exophiala salmonis*-clade (sensu de Hoog *et al.* 2011) obtained from a combined ML analysis based on ITS, TE-1 and BT2 gene sequences. Supported branches are drawn in bold. The tree was rooted with *Exophiala mesophila* and *E. castellanii*

*

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