Mycological Progress _####################_
https://doi.org/10.1007/s11557-022-01853-6

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ORIGINAL ARTICLE

Humicolopsis cephalosporioides synthesizes DHN-melanin in its chlamydospores

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Received: 19 July 2022 / Revised: 9 November 2022 / Accepted: 16 November 2022

Abstract

Humicolopsis cephalosporioides is a soil fungus that is associated with *Nothofagus* forests in South America. The aim of this study was to analyze the effect of environmental factors such as temperature, light, and nutrition on chlamydospore differentiation as well as pigment biosynthesis. Temperature did not affect chlamydospore production; it rather altered pigmentation development that also was affected by light. The composition of culture media as well as light modulated chlamydospore differentiation. Microscope observations, spectroscopic analysis as well as culture assays, using melanin inhibitors, suggest that the main pigment of chlamydospores of *H. cephalosporioides* is 1,8 dihydroxynaphthalene (DHN)-melanin–type compound. Furthermore, we found that the genome of *H. cephalosporioides* contains a sequence highly homologous to the *pks* sequences of other fungi that have been associated with the biosynthesis of 1,8 DHN-melanin. All this together suggests that melanization is among the most important features linked to survival of this fungus in the soils of *Nothofagus* forests in sub-Antarctica region and that the ITS, 18S, and 28S rDNA sequences did not provide enough information to delineate the phylogenetic relationships of the fungus within the class *Leotiomycetes*.

Keywords Pigments · Environmental factors · Chlamydospores · Stress · Polyketide synthase

Introduction

Extreme habitats, like the volcanic and shallow soils of South Patagonia (Argentina), where native wood species such as *Nothofagus pumilio* (Poepp & Endl.) Krasser (*Nothofagaceae*) grow, are the best places to look for organisms that survive and/or develop structures in response to environmental stresses. This area is characterized by winters with very low temperatures and extremely short days and quite cold long days in summer, respectively. In this environment, plants and organisms are exposed to abiotic stresses such as low temperature, anoxia, and high UV radiation (Ruisi et al. 2007). All these factors not only alter forest growth but also each component of the ecological niche, including fungi whose

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Section Editor: Ji-Kai Liu

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activity is, compared to bacteria, crucial during the early stages of wood decay in the soils. *Humicolopsis cephalosporioides* is a fungus that was found in the soils of these environments, and although its biological role remains to be studied in detail (Mestre and Fontenla 2021), it has an outstanding capacity to degrade cellulose (Elíades et al. 2015, 2019).

The genus *Humicolopsis* was described by Marchand et al. (1976) and it includes 2 species, *H. cephalosporioides* and *Humicolopsis dimorphospora*. Recently, the phylogenetic position of the latter one was revised and included within the order *Ophiostomatales* (class *Sordariomycetes*) and renamed as *Sporothrix dimorphospora* (Madrid et al. 2010; de Beer et al. 2016). Recently, Ekanayaka et al. (2019a) placed *H. cephalosporioides* in the class *Leotiomycetes*, within a new undescribed order (*Helicogoniales*). Among other phenotypic traits, the fungus differentiates chlamydospores that are characterized by thick cell walls containing dark pigments (Marchand et al. 1976; Damm et al. 2010).

It is well known that fungi synthesize pigments such as carotenoids, melanins, flavins, quinones, azaphilones, and chromenes (Narsing Rao et al. 2017; Melo et al. 2019; Kalra et al. 2020). Such molecules might accumulate both within the mycelium or spores of fungi and additionally might be secreted, which can be seen by the color of colonized substrates (Nitiu et al. 2020). Fungi belonging to *Humicolopsis s. lato* and those phylogenetically related to *Sporothrix* synthesize pigments that differ in color, which might be used as an identification tool. Also, they develop structures like chlamydospores that are hard dark spores most probably due to the presence of polymeric chromophores such as sporopolenin or melanin-like compounds. Probably, they are generated through metabolic pathways that involve PKSs, reductases, and/or oxidative enzymes that catalyze a specific set of reactions that result in polymers (Toledo et al. 2017). As a result of this, they might differ in color, structure, composition and might play a different biological role as well. Polymeric chromophores in fungal propagules mainly have been associated to protection against environmental stresses such as UV-light and/or desiccation. *H. cephalosporioides* has been found living in forest soils in Patagonia (Argentina), which seems to be the natural habitat of the fungus that is characterized by low temperatures and the frequent presence of snow and/or ice. Based on this, we hypothesized that the dark pigments of chlamydospores of *H. cephalosporioides* might play a key role in their survival under such environments. However, in preliminary experiments, we failed to extract pigments from chlamydospores whether we used water or organic solvents, heat, and/or a combination with acids (unpublished results). Therefore, the aim of this work was to study chlamydospore development and pigment biosynthesis as well as how these processes are affected by environmental factors such as temperature, light, and nutrition, since it might be related to the fungus survival in the soils of Southern Patagonia.

Materials and methods

Fungal material

The strains of *H. cephalosporioides* stored at the culture collection of Spegazzini Institute as LPSC 1155, 1157, 1158, and 1159 were used in this work. They were isolated from soil particles collected in *N. pumilio* forests in Tierra del Fuego, Argentina (54°49′48″S–68°21′35″W), which had been exposed to shelter-wood cut practice 50 years ago (Elíades et al. 2015). Fungal stocks were kept as slant cultures at 4 °C on a malt extract agar (MEA) medium.

DNA extraction, polymerase chain reaction and sequencing

Fungi were cultured on potato dextrose agar (PDA) medium at 25 °C for 7 days and their DNA extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to Medina et al. (2015). The quality and integrity of the DNA were verified by electrophoresis in 0.7 % (w v^{-1}) agarose gels stained with 0.2 μg μL^{-1} ethidium bromide. A *λ-HindIII* DNA ladder marker was used to quantify the extracted DNA, which was stored in a freezer at −20 °C. Standard PCR protocols were used to amplify the internal transcribed spacer (ITS) sequences using primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3', White et al. 1990) and partial sequences of the 18S rDNA small ribosomal subunit (SSU) using primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTCACCTACGGA-3', Nikoh and Fukatsu 2000). In addition, the 28S rDNA large ribosomal subunit (LSU) of isolate LPSC 1155 was amplified using primers LS1 (5'-AGTACCCGCTGAACTTAAG-3') and LR5 (5'-CCTGAGGGAAACTTCG-3', Hausner et al. 1993; Rehner and Samuels 1995). Reactions were performed in a 15-μL final volume containing 50 ng of template DNA, 50 ng of forward and reversed primers, 1.5 μL 10× reaction buffer (500 mmol L^{-1} KCl; 100 mmol L^{-1} Tris-HCl, pH 9.0 a 25 °C; 1% Triton X-100), 1.5 mmol L^{-1} $MgCl_2$, 0.2 mmo L^{-1} dNTPs and 0.5-unit Taq polymerase (Inbio Highway®, Buenos Aires, Argentina). Amplifications were performed in a PTC-0150 MiniCycler (MJ. Research. Watertown, MA, USA) programmed as follows for the amplification of the ITS: 4 min at 94 °C followed by 33 cycles of 45 s at 94 °C, 45 s at 56 °C, and 60 s at 72 °C, followed by final extension for 5 min at 72 °C. The 18S rDNA PCR cycling parameters were denaturation at 94 °C for 5 min followed by 30 cycles, each consisting of 1 min at 94 °C, 45 s at 56 °C, and 150 s at 72 °C, with a final extension at 72 °C for 5 min. The 28S rDNA PCR cycling parameters were: denaturation at 94 °C for 5 min followed by 10 cycles, each consisting of 10 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C; and 25 cycles, each

consisting of 10 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C plus an additional 20 s for each consecutive cycle (Hoy and Jeyaprakash 2005).

The KS domain of isolate LPSC 1155 was amplified by designing primers LC1-F (5′- GAYCCIMGITTYTTYAAYATG-3′) and LC2-R (5′-GTICCIGTICCRTGCATYTC-3′; Bingle et al. 1999). Reaction mixtures contained 1× amplification buffer (Inbio Highway), 2.5 mmol L^{-1} $MgCl_2$ (Inbio Highway), 1.5 μmol L^{-1} of each primer, 200 μmol L^{-1} each deoxynucleoside triphosphate (Inbio Highway), 5 ng of DNA template, and 0.05 U of Taq DNA polymerase (Inbio Highway) in a 15-μL volume. PCR was performed in a PTC-1152 Mini Cycler (MJ Research) programmed as follows: an initial step at 94 °C for 5 min; followed by 35 cycles of a denaturing step at 94 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 1 min; with a final extension at 72 °C for 10 min.

PCR products were resolved by electrophoresis in 1% (w v^{-1}) agarose gel stained with 0.2 μg μL^{-1} ethidium bromide. A 100 to 1000 bp DNA ladder marker (Inbio Highway) was used to estimate the size of the amplicons, that were purified (Sambrook et al. 1989), precipitated, and sequenced at MACROGEN Inc. (Seoul, South Korea). All sequences generated in this study were deposited at the National Center for Biotechnology Information (NCBI) GenBank (Table 1).

Sequence alignments and phylogenetic analyses

The taxonomic position of the isolates was assessed by means of a maximum-likelihood (ML) analysis using the 5.5S sequence, and 18S and 28S rDNA partial sequences. The taxon sampling criteria included all *Leotiomycetes* type strains available in GenBank by May 23rd, 2021, provided their 5.5S, 18S, and 28S rDNA sequence coverage was ≥ 90% relative to LPSC 1155 (Table 2). In addition, the *Sordariomycetes Beauveria caledonica* ARSEF 2567 and *Trichoderma reesei* DSM 768 were selected as outgroups. Multiple sequence alignments were performed for each gene separately using MAFFT version 1.4.0 (Katoh and Standley 2013).

Table 1 GenBank accession numbers for the DNA sequences of the *H. cephalosporioides* LPSC isolates obtained in this study

Isolate	GenBank accession number			
	18S	ITS	28S	PKS KS domain
LPSC_1155	KY065158	KY065162	MZ566634	AGH27151
LPSC_1158	KY065159	KY065164	NA	NA
LPSC_1159	KY065160	KY065163	NA	NA
LPSC_1157	KY065161	KY065165	NA	NA

NA, not available

Alignments were first manually edited, and then automatically-edited using Gblocks version 0.91b (Talavera and Castresana 2007), setting the minimum length of a block to a value of 2 and leaving all the other options by default. The multiple sequence alignments were concatenated, and the best substitution model for the resulting matrix was selected by jModelTest version 2.1.10 (Darriba et al. 2012) using the Akaike Information Criterion (AIC, Akaike 1973). The ML analysis was carried out in PhyML version 3.1 (Guindon and Gascuel 2003). The statistical support of the nodes was estimated through 1000 bootstrap replicates. The sequence alignment is provided in Supplementary Information 1.

The phylogenetic analysis of the deduced KS domains was conducted using MEGA (version 5.01). The analysis included amino acid sequences for KS domains of PKS proteins from 33 ascomycetous fungi (Table 3), including the KS domain of a PKS from *H. cephalosporioides* LPSC 1155. *Streptomyces avermitilis* was used as outgroup (Varga et al. 2003). The sequences were aligned using the ClustalW algorithm (version 1.83; Thompson et al. 1994). The alignment was visually checked and manually optimized. Phylogenetic analysis was performed by the neighbor-joining method. Clade stability was assessed based on 1000 bootstrap replicates. The sequence alignment is provided in Supplementary Material 2.

Microscopical analysis of isolate LPSC 1155

Mycelium, conidia and chlamydospores collected from 21-day-old PDA cultures of isolate LPSC 1155 were examined with an Olympus CX41 UC-MAD3 light microscope (LM). Samples for in situ detection of melanin in cell walls were treated according to Kayatz et al. (2001) and observed with an epifluorescence microscope Olympus ®BX-51, equipped with an Olympus E-330 digital camera. Material from 21-day-old cultures of isolate LPSC 1155 growing on PDA (control) and PDA supplemented with tricyclazole at 100 ppm was processed according to Bárcena et al. (2015) and observed with LM and transmission electron one (TEM).

Biomass production, extraction, and characterization of chlamydospore pigments

H. cephalosporioides LPSC 1155 was grown on PDA at 25°C for 21 days. The biomass produced by each individual culture consisted mainly in a mass of chlamydospores, as inferred by the intense darkness of the differentiated colonies. It was pooled to make a composite sample that was processed according to Bárcena et al. (2018) and Llorente et al. (2012). The extracted pigments were dissolved in 0.1 M NaOH to a final concentration of 0.1 mg mL^{-1} and the UV-visible absorption spectrum was measured. The electron spin resonance (ESR) spectrum of pigments either in dry state within the fungal

Table 2 GenBank accession numbers of the DNA sequences used in the multi-locus phylogenetic analysis

Species name	ID[a]	18S	ITS	28S
Pilidium acerinum	266073	NG_061022 (Rossman et al. 2004)	NR_119500 (Schoch et al. 2014)	MH870939 (Vu et al. 2019)
Xeropilidium dennisii	1792682	NG_070636 (Pärtel et al. 2017)	NR_171225 (Pärtel et al. 2017)	KX090824 (Pärtel et al. 2017)
Sarcotrochila longispora	1659226	NG_064860 (Gernandt et al. 2001)	NR_138393 (Crous et al. 2014)	NG_066161 (Crous et al. 2014)
Synchaetomella acerina	1282988	NG_065600 (Crous et al. 2012)	NR_111811 (Schoch et al. 2014)	NG_042747 (Crous et al. 2012)
Brahmaculus moonlighticus	2834542	MK248054 (Johnston et al. 2021)	MK248036 (Johnston et al. 2021)	MK248011 (Johnston et al. 2021)
Chlorociboria novae-zelandiae	2834546	JN939875 (Schoch et al. 2012)	JN943456 (Schoch et al. 2012)	JN939940 (Schoch et al. 2012)
Corniculariella rhamni	2716939	MT214941 (Li et al. 2020)	NR_169718 (Li et al. 2020)	MT183457 (Li et al. 2020)
Dermea persica	2482816	MH104721 (Mehrabi et al. 2018)	NR_160616 (Mehrabi et al. 2018)	MH104720 (Mehrabi et al. 2018)
Duebenia subcompta	2544957	MK585058 (Ekanayaka et al. 2019a)	NR_163784 (Ekanayaka et al. 2019a)	MK592002 (Ekanayaka et al. 2019a)
Neodermea rossica	2717006	MT214969 (Li et al. 2020)	NR_169720 (Li et al. 2020)	MT183493 (Li et al. 2020)
Neofabraea malicorticis	108569	AY544706 (Lutzoni et al. 2004)	KR859085 (Chen et al. 2016)	AY544662 (Lutzoni et al. 2004)
Neofabraea brunneipila	2544961	MK585060 (Ekanayaka et al. 2019a)	MK584984 (Ekanayaka et al. 2019a)	MK592004 (Ekanayaka et al. 2019a)
Pezicula italica	2716949	MT214976 (Li et al. 2020)	NR_170044 (Li et al. 2020)	MT183505 (Li et al. 2020)
Phlyctema coronillae	2717616	MT214988 (Li et al. 2020)	MT449717 (Li et al. 2020)	MT449705 (Li et al. 2020)
Neobulgaria alba	1461596	HM116781 (Johnston et al. 2010)	NR_137054 (Johnston et al. 2010)	HM116782 (Johnston et al. 2010)
Bloxamia cyatheicola	1924116	NG_065079 (Guatimosim et al. 2016)	NR_153617 (Guatimosim et al. 2016)	NG_058691 (Guatimosim et al. 2016)
Helicodendron microsporum	1692299	NG_063050 (Sri-indrasutdhi et al. 2015)	NR_137974 (Sri-indrasutdhi et al. 2015)	NG_058155 (Sri-indrasutdhi et al. 2015)
Hymenoscyphus waikaia	1443587	KC164671 (Johnston and Park 2013)	NR_137111 (Johnston and Park 2013)	KC164641 (Johnston and Park 2013)
Hymenoscyphus ohakune	1443589	MH682221 (Johnston and Park, unpublished)	NR_137109 (Johnston and Park 2013)	MH682241 (Johnston and Park, unpublished)
Mycofalcella calcarata	1398240	NG_065767 (Baschien, unpublished)	NR_154165 (Baschien et al. 2013)	NG_060298 (Baschien et al. 2013)
Zymochalara cyatheae	1924123	NG_061228 (Guatimosim et al. 2016)	NR_154509 (Guatimosim et al. 2016)	NG_059652 (Guatimosim et al. 2016)
Zymochalara lygodii	1924124	NG_062415 (Guatimosim et al. 2016)	NR_154510 (Guatimosim et al. 2016)	NG_059653 (Guatimosim et al. 2016)
Blastosporium persicolor	2478831	NG_065765 (Zheng et al. 2019)	NR_163764 (Zheng et al. 2019)	MH992517 (Zheng et al. 2019)
Cadophora lacrimiformis	2544952	MK585020 (Ekanayaka et al. 2019a)	NR_163787 (Ekanayaka et al. 2019a)	MK591959 (Ekanayaka et al. 2019a)
Graphium rubrum	67626	NG_063388 (Okada et al. 2000)	NR_145268 (Harrington et al. 2001)	MH866974 (Vu et al. 2019)
Infundichalara microchona	1056133	HQ609486 (Réblová et al. 2011)	MH860844 (Vu et al. 2019)	NG_067269 (Réblová et al. 2011)

Table 2 (continued)

Species name	ID[a]	18S	ITS	28S
Leptodontidium boreale	205359	NG_065505 (Sogonov et al. 2005)	NR_145270 (Sogonov et al. 2005)	NG_067409 (Vu et al. 2019)
Mycochaetophora gentianae	1187133	NG_064831 (Nekoduka et al. 2010)	NR_121201 (Schoch et al. 2014)	AB496937 (Nekoduka et al. 2010)
Rhexocercosporidium carotae	194552	AF487897 (Shoemaker et al. 2002)	MH858647 (Vu et al. 2019)	AB469688 (Nekoduka et al. 2010)
Rhexocercosporidium microsporum	2719221	MK585027 (Ekanayaka et al. 2019a)	NR_163776 (Ekanayaka et al. 2019a)	MK591966 (Ekanayaka et al. 2019a)
Hyaloscypha variabilis	2482757	AY762619 (Hambleton and Sigler 2014)	NR_121313 (Schoch et al. 2014)	MH018944 (Fehrer et al. 2019)
Lachnopsis catarinensis	1924117	NG_061227 (Guatimosim et al. 2016)	NR_154125 (Guatimosim et al. 2016)	NG_059651 (Guatimosim et al. 2016)
Proliferodiscus chiangraiensis	2544962	MK585037 (Ekanayaka et al. 2019b)	NR_164304 (Ekanayaka et al. 2019b)	MK591985 (Ekanayaka et al. 2019b)
Unguiculella globosa	2544967	MK585044 (Ekanayaka et al. 2019a)	NR_163778 (Ekanayaka et al. 2019a)	MK591972 (Ekanayaka et al. 2019a)
Acephala applanata	327282	KT259197 (Schoch, unpublished)	NR_119482 (Schoch et al. 2014)	KT225544 (Schoch, unpublished)
Loramyces macrosporus	380016	NG_062693 (Spatafora et al. 2006)	NR_138379 (Han et al. 2014)	MT026502 (Han et al. 2014)
Neomollisia gelatinosa	2544971	MK585021 (Ekanayaka et al. 2019a)	NR_163788 (Ekanayaka et al. 2019a)	MK591960 (Ekanayaka et al. 2019a)
Pulvinata tomentosa	2544973	MK585026 (Ekanayaka et al. 2019a)	NR_163775 (Ekanayaka et al. 2019a)	MK591965 (Ekanayaka et al. 2019a)
Entimomentora hyalina	2488986	MK185670 (Untereiner et al. 2019)	NR_121472 (Schoch et al. 2014)	GU727562 (Bogale et al. 2010)
Pleuroascus nicholsonii	95330	AF096182 (Suh and Blackwell 1999)	NR_156627 (Starodumova et al., unpublished)	MF375777 (Starodumova et al., unpublished)
Venustampulla parva	2656788	NG_067688 (Untereiner et al. 2019)	NR_165893 (Vu et al. 2019)	MK185690 (Untereiner et al. 2019)
Dicephalospora sessilis	2544956	MK585047 (Ekanayaka et al. 2019b)	NR_163779 (Ekanayaka et al. 2019a)	MK591974 (Ekanayaka et al. 2019a)
Poculum pseudosydowianum	1450545	LC434561 (Johnston et al. 2019)	NR_171204 (Hosoya et al. 2014)	AB926136 (Hosoya et al., unpublished)
Mitrula brevispora	329405	AY789292 (Wang et al. 2005)	AY789294 (Wang et al. 2005)	AY789293 (Wang et al. 2005)
Srinivasanomyces kangrensis	2511729	MN121705 (Singh and Rana, unpublished)	NR_169691 (Hyde et al. 2020)	MK478470 (Hyde et al. 2020)
Lauriomyces cylindricus	2022202	KX649955 (Somrithipol et al. 2017)	NR_155326 (Somrithipol et al. 2017)	KX649966 (Somrithipol et al. 2017)
Lauriomyces ellipticus	2022203	KX649960 (Somrithipol et al. 2017)	NR_155327 (Somrithipol et al. 2017)	NG_060342 (Somrithipol et al. 2017)
Byssoascus striatosporus	37242	AJ315170 (Cano et al. 2002)	NR_111040 (Schoch et al. 2014)	MH870573 (Vu et al. 2019)
Myxotrichum deflexum	78138	NG_065476 (Sugiyama et al. 1999)	NR_156338 (Martin-Sanchez et al. 2016)	MH869596 (Vu et al. 2019)
Pseudeurotium zonatum	95332	AF096183 (Suh and Blackwell 1999)	NR_111127 (Schoch et al. 2014)	DQ470988 (Spatafora et al. 2006)
Pseudeurotium hygrophilum	205840	NG_065504 (Sogonov et al. 2005)	NR_111128 (Schoch et al. 2014)	NG_069863 (Vu et al. 2019)
Pseudogymnoascus destructans	655981	NG_065563	NR_111838	KF017865 (Minnis and Lindner 2013)

Table 2 (continued)

Species name	ID[a]	18S	ITS	28S
		(Blehert et al. 2009)	(Schoch et al. 2014)	
Allantophomopsiella pseudotsugae	1564656	AF203467 (Gernandt et al. 2001)	MH857221 (Vu et al. 2019)	MH868759 (Vu et al. 2019)
Phacidium italicum	2716951	MT214979 (Li et al. 2020)	NR_171297 (Li et al. 2020)	MT183509 (Li et al. 2020)
Collophorina africana	744430	GQ154630 (Damm et al. 2010)	NR_119748 (Schoch et al. 2014)	MK314588 (Bien et al. 2020)
Pallidophorina paarla	744432	GQ154634 (Damm et al. 2010)	NR_119749 (Schoch et al. 2014)	MK314610 (Bien et al. 2020)
Ramoconidiophora euphorbiae	2315864	NG_067663 (Nasr et al. 2018)	NR_165868 (Nasr et al. 2018)	MK314602 (Bien et al. 2020)
Lanceolata brunnea	2544970	MK585028 (Ekanayaka et al. 2019a)	NR_163777 (Ekanayaka et al. 2019b)	MK591967 (Ekanayaka et al. 2019b)
Thelebolus globosus	319062	FJ176851 (Schoch et al. 2009)	NR_138367 (De Hoog et al. 2005)	FJ176905 (Schoch et al. 2009)
Beauveria caledonica	38006	AY245650 (Kuo et al., unpublished)	HQ880817 (Rehner et al. 2011)	AF339520 (Sung et al. 2001)
Trichoderma reesei	51453	MH047196 (Heeger et al. 2018)	MH047196 (Heeger et al. 2018)	MH047196 (Heeger et al. 2018)

[a] NCBI taxonomy ID

biomass or in alkaline solution was also analyzed (Bárcena et al. 2018). The chemical composition of pigments contained in the dry biomass was also analyzed by Fourier transform infrared (FTIR) spectroscopy (Bárcena et al. 2018). The experiment was performed in a completely randomized design with three replicate cultures.

Melanin synthesis inhibition

The PDA medium was supplemented, once at a time, with three inhibitors of melanin synthesis: (i) tricyclazole (5-methyl-1,2,4-triazolo[3,4-b] benzothiazole, Ultra Scientific Analytical Solutions, USA); (ii) sulcotrione (Riedel-de Haën™, Seelze, Germany) or; (iii) kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, Parafarm, Argentina) at a concentration of 1, 10, and 100 ppm. These inhibitors target each specific enzyme in the biosynthetic pathway of 1,8-dihydroxynaphthalene (DHN)-melanin, pyomelanin, or DOPA-melanin, respectively. The experiment was performed in a completely randomized design with three replicate cultures per concentration and type of inhibitor tested, including a control that consisted in unamended growth medium. Plates were inoculated with spores and mycelia suspensions of LPSC 1155 and were incubated in the dark at 25 ± 2 °C for 21 days. Pigmentation of the colonies was estimated according to Bárcena et al. (2015). Also, the oxidative enzyme activity was measured on aliquots of cell-free extracts obtained from colonies grown on PDA (control) and PDA supplemented with tricyclazole at 100 ppm (Saparrat et al. 2010).

Colony pigmentation and chlamydospore production

Colony pigmentation and chlamydospore production by the isolate LPSC 1155 were estimated on cultures grown on (2%, w v^{-1}) basal medium supplemented with 0.5% sodium-carboxy-methylcellulose (CMC, BDH Chemicals Ltd) (pH 5.5), as the main C source. Cultures were incubated in the dark for 10 days at three temperatures: 5 ± 1 °C, 15 ± 1 °C, and 25 ± 1 °C according to Scorsetti et al. (2012). The experimental design was completely randomized with three replicates per incubation temperature. Colony color was measured as described above. Chlamydospore production was measured on each plate, by making a suspension by melting the agar supplemented with 20 mL of water in a water bath. The spore suspension was homogenized with a Waring blender, and chlamydospores were counted in a Neubauer chamber, and values were expressed as chlamydospore number/plate. Also, the influence of light on colony pigmentation and chlamydospore production was analyzed on fungal cultures grown on PDA and CMC media, by exposing plates to a UV irradiation using a lamp Osram ST III 220–240 V, white light (provided by a T8 L18W/835 Osram 26Ø fluorescent tube with a wavelength range of 400–700 nm) and continuous darkness conditions. This experiment was performed in a completely

Table 3 Fungal species and GenBank accession numbers of the deduced amino acid sequences used in the phylogenetic reconstruction of the KS domain of the PKS

Species name	*pks* gene	GenBank accession numbers	KS region
Aspergillus flavus	*pksA*	AAS89999 (Ehrlich and Cotty 2002)	375–803
Aspergillus fumigatus	*alb1*	AAC39471 (Tsai et al. 1998)	378–806
Aspergillus nidulans	*wa*	CAA46695 (Mayorga and Timberlake 1992)	379–807
Aspergillus oryzae	*pksL1*	BAC45240 (Tominaga et al. 2006)	375–803
Aspergillus parasiticus	*wa*	CAB44699 (Bingle et al. 1999)	1–216
Aspergillus parasiticus	*pksL1*	AAC41675 (Feng and Leonard 1995)	375–803
Aspergillus parasiticus	*wa*	CAB44698 (Bingle et al. 1999)	1–212
Aspergillus terreus	*lovB*	Q9Y8A5 (Hendrickson et al. 1999)	1–449
Ascochyta fabae	*pks1*	ACS74441 (Hendrickson et al. 1999)	1–207
Ascochyta pinodes	*pks1*	ACS74443 (Akamatsu et al. 2010)	1–207
Bipolaris maydis	*pks18*	AAR90272 Kroken et al. 2003)	373–802
Bipolaris oryzae	*pks1*	BAD22832 Moriwaki et al. 2004)	373–802
Byssochlamys nivea	*6msas*	AAK48943 Puel et al. 2007)	35–457
Cladosporium phlei	*nr*[a]	AFP89392 So et al. 2012)	104–329
Cladosporium cladosporioides	*pks1*	AEV59802 (Llorente et al. 2012)	1–217
Colletotrichum lagenarium	*pks1*	BAA18956 (Takano et al. 1995)	385–813
Didymella rabiei	*pks1*	ACS74449 (Akamatsu et al. 2010)	213–642
Exophiala dermatitidis	*pks1*	AAD31436 (Ye et al. 1999)	369–798
Fungal endophyte sp. CR61	*pks1*	AAP68704 (Sauer et al. 2002)	1–229
Fungal endophyte sp. CR475	*pks1*	AAP68701 (Sauer et al. 2002)	1–229
Fusarium fujikuroi	*pks2*	CAD19100 (Linnemannstöns et al. 2002)	30–451
Fusarium fujikuroi	*pks1*	CAC44633 (Linnemannstöns and Tudzynski, unpublished)	7–435
H. cephalosporioides	*pks1*	AGH27151 (this study)	5–213
Penicillium griseofulvum	*wa*	CAB44712 (Bingle et al. 1999)	1–216
Penicillium griseofulvum	*msas*	CAA39295 (Beck et al. 1990)	35–457
Pertusaria hymenea	*nr*a	AAY00101 (Schmitt et al. 2005)	1–206
Pertusaria lecanina	*nr*	AAY00108 (Schmitt et al. 2005)	1–209
Phoma sp. *C2932*	*wa*	CAB44719 Bingle et al. 1999)	1–217
Phoma sp. *C2932*	*pks1*	AAO62426 Nicholson et al. 2001)	32–455

Table 3 (continued)

Species name	*pks* gene	GenBank accession numbers	KS region
Pseudocercospora fijiensis	*nr*	EME80693 Ohm et al. 2012)	2–423
Pseudocercospora griseola (S3b)	*pks1*	AGI04994 (Bárcena et al. 2015)	1–207
Pseudocercospora griseola (T4)	*pks1*	ALF44675 Bárcena et al. 2015)	1–235
Ramularia collo-cygni	*pks*	ADZ14597 (Kellner et al., unpublished)	1–213
Streptomyces coelicolor	*aveA4*	AGO88723 Zhang et al. 2013)	36–459
Streptomyces coelicolor	*aveA2*	AGO88719 Zhang et al. 2013)	2682–3106

[a] *nr*, gene name not reported

randomized design with three replicate cultures per each light condition and medium type.

Statistical analysis

Data upon colony pigmentation, chlamydospore counts, and size as well as laccase activity were analyzed by means of a one-way analysis of variance (ANOVA), and the means were compared by Tukey's test at a significance level of 0.05 using the Statistix 8.0 software for Windows.

Results

Morphological and molecular identification

The LPSC isolates studied presented similar morphological characteristics, and when they were grown on CMC medium for 21 days, they differentiated 2 types of unicellular spores (Fig. 1a–c). A hyaline one on the young peripheral mycelium that was ovoid to cylindrical (7–11 × 2–2.5 μm) that in some cases presented a distinctive curvature (Fig. 1b). The other

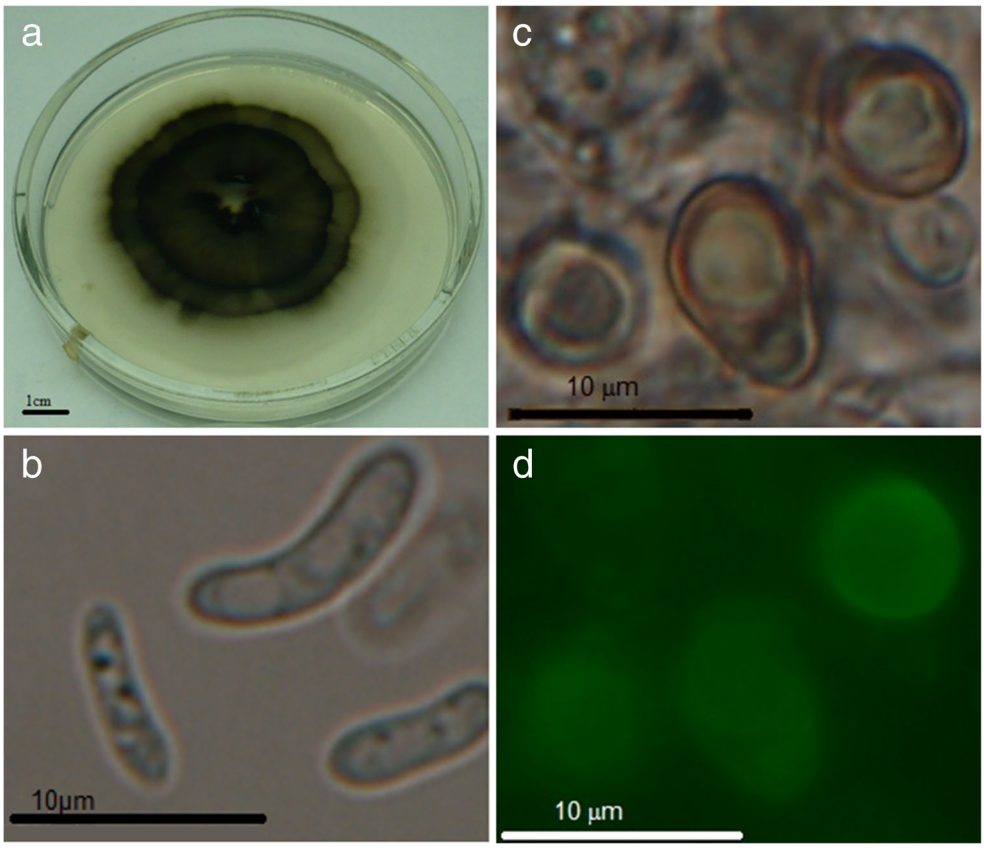

Fig. 1 *H. cephalosporioides* LPSC 1155 cultures and observations in the microscope: Colony on CMC medium (**a**); hyaline conidia (**b**) and chlamydospores (**c**) seen in the light microscope; chlamydospores (**d**) seen in the epifluorescence microscope. Melanization of chlamydospore walls was detected by pretreating slides for 1 h in 30 % hydrogen peroxide and light and then acetone to remove all other non-polymer cellular compounds and afterward seen

Table 4 Number of chlamydospores/plate and pigmentation (expressed as darkness "K" level) of *H. cephalosporioides* grown at 5, 15, and 25 °C for 21 days. Data with different letter are significantly different (Tukey test, $P < 0.05$)

Temperature (°C)	Chlamydospores/plate	Darkness (k)
5	2333 ± 516 (a)	75.17 ± 2.90 (a)
15	2083 ±736 (a)	80.08 ± 1.59 (ab)
25	2167 ± 683 (a)	81.33 ± 1.84 (b)

type of spores produced was chlamydospores. They were thick-walled ovoid, claviform, or irregular (7.5–11.5 × 5–7 μm) structures, either intercalary or terminally located, isolated or in chains that initially were hyaline that later turned to a dark, whose abundance gave colonies a dark appearance (Fig. 1c). The ITS and 18S rDNA amplicons generated by PCR for all the isolates were identical. A nucleotide blast analysis of the 28S rDNA and ITS sequences of isolate LPSC 1155 (GenBank accession numbers: MZ566634 and KY065162) was 100% and 99.95% homologous to the same loci of type strain *H. cephalosporioides* CBS 645.76 (NG_064108 and NG_160150) suggesting that isolates LPSC 1155, LPSC 1157, LPSC 1158, and LPSC 1159 are siblings of *H. cephalosporioides*.

Colony pigmentation, chlamydospore production, and environmental factors

We evaluated the effect of several environmental factors such as temperature, light, and nutrition on colony pigmentation and chlamydospore production in cultures of *H. cephalosporioides* LPSC 1155. Temperature did not affect chlamydospore production, but it did their pigmentation that was enhanced at 25 °C, compared to 5 °C (Table 4). Fungal colonies were of a clear brown color when grown under visible light, compared to those incubated in the darkness or under UV light, either on PDA or CMC medium. The fungus produced a higher number of spores on CMC than on PDA medium, though on the latter one only if incubated in the darkness or under UV light conditions (Tables 5, 6, 7).

Table 5 Number of chlamydospores/plate and pigmentation (expressed as darkness "K" level) of *H. cephalosporioides* on PDA or CMC for 21 days. Letter show significant differences for different culture medium for the same light condition. The asterisk (*) show on the same culture medium differences respect to dark (control)

Culture medium	Light conditions	Chlamidospores/plate	Darkness (k)
PDA	Dark	2917 ± 236 (a)	82.50 ± 1.09 (a)
PDA	Light	1208 ± 59 (a)*	78.42 ± 0.72 (b)*
PDA	UV	3500 ± 117 (a)	82.33 ± 2.36 (a)
CMC	Light	4777 ± 636 (b)	82.08 ± 0.63 (a)
CMC	Dark	3291 ± 412 (b)	78.50 ± 1.15 (b)*
CMC	UV	6083 ± 235 (b)	79.92 ± 0.72 (a)*

Table 6 ANOVA for chlamydospore production when *H. cephalosporioides* was inoculated on either CMC- or PDA medium and incubated under three light conditions: mean square significance

Source of variation	d.f.	MS	*F* value
Medium	1	2695069**	24.975
Light condition	2	841458*	7.797
Medium × light condition	2	83958ns	0.778
Error	9	107909	

d.f., degree of freedom; *MS*, means square; *$P < 0.05$; **$P < 0.001$; *ns*, not significant

Identification of *H. cephalosporioides* pigments

Twenty-one-day-old colonies of *H. cephalosporioides* LPSC 1155 treated with hydrogen peroxide and light developed chlamydospores that were brown under the light microscope, and green fluorescent in an epifluorescence microscope; therefore, we considered they were melanized (Fig. 1c, d). No fluorescence was observed if chlamydospores were not treated either with hydrogen peroxide or light. It is interesting to mention that hyaline chlamydospores differentiated earlier in cultures (data not shown).

Cultures of *H. cephalosporioides* LPSC 1155 contained pigmented chlamydospores whose dark pigments, which were pelleted, remained insoluble after rinsing them with water and organic solvents such as acetone, but were solubilized in a 1M NaOH solution at 121°C for 20 min and precipitated by adding 3N HCl. This alkaline solution containing pigments at a concentration of 0.1 mg ml^{-1} presented a broad wavelength absorbance spectrum with a peak at 230 nm (Fig. 2a) and the ESR analysis provided evidences of the presence of stable free radicals in pigments after their isolation as dry solid phase or when they were part of the starting fungal sample

Table 7 ANOVA for colony pigmentation when *H. cephalosporioides* was inoculated on either CMC- or PDA medium and incubated under three light conditions: mean square significance

Source of variation	d.f.	MS	*F* value
Medium	1	3.8ns	2.38
Light condition	2	23.2**	14.60
Medium x Light condition	2	2.6ns	1.65
Error	12	1.6	

d.f., degree of freedom; *MS*, means square; *$P < 0.05$; **$P < 0.001$; *ns*, not significant

(dry biomass, Fig. 2b, c), which additionally presented a conserved FTIR pattern of absorption (Fig. 2d) with peaks at 3440–3300 cm^{-1}, 2920–2850 cm^{-1}, 1656–1644 cm^{-1}, 1410–1370 cm^{-1}, 1280–1250 cm^{-1}, and 1100–1050 cm^{-1}. These peaks correspond to stretching vibrations of –OH and –NH groups, CH_2 and CH_3 stretching, conjugated double bonds (C=C and C=O) in the aromatic ring as well as C=O in secondary amines, CH groups adjacent to COOH and OH groups and C=O of quinones, CO groups of acid, ester and phenol groups, and C–O deformation vibrations of aliphatic alcohols, respectively. Among all these bands, the one around 1650 cm^{-1}, that is typical of a conjugated quinoid structure, is a key feature to define the pigment as melanin.

Melanin biosynthetic pathway in *H. cephalosporioides*

H. cephalosporioides LPSC 1155 was cultured in the presence of three inhibitors of melanin biosynthesis. While pigmentation of the colonies remained the same whether the fungus was cultured with kojic acid or sulcotrion, it changed in response to tricyclazole, whose concentrations generated different responses. While on 1 ppm of tricyclazole colonies were similar to those of control cultures, those developed in the presence of 10 and 100 ppm were of a brown to brownish light gray color (Fig. 3a). However, colony darkness "k" only was reduced in the presence of 100 ppm of tricyclazole (Fig. 3b), whose presence in the medium whether at low or high concentrations, turned it to a brown-orange color.

H. cephalosporioides differentiated chlamydospores on PDA and PDA supplemented with 100 ppm of tricyclazole, though in the latter one they were not pigmented (Fig. 4a, b). TEM analysis showed that the cell wall thickness of chlamydospores was of approximately 90 nm whether they developed in control or amended media ($P < 0.005$). However, tricyclazole at 100 ppm reduced darkness "k" of the electron-dense protoplast of chlamydospores, which was of 21.71 ± 9.90 and 9.80 ± 5.10 for cultures grown on PDA and PDA + tricyclazole respectively ($P < 0.005$; Fig. 5). Concomitantly, tricyclazole at a 100 ppm concentration also reduced laccase activity, which was of 34.81 ± 2.41 mU g^{-1} (fresh weight), quite low compared to the activity of control cultures on plain PDA (66.51 ± 5.04 mU g^{-1}).

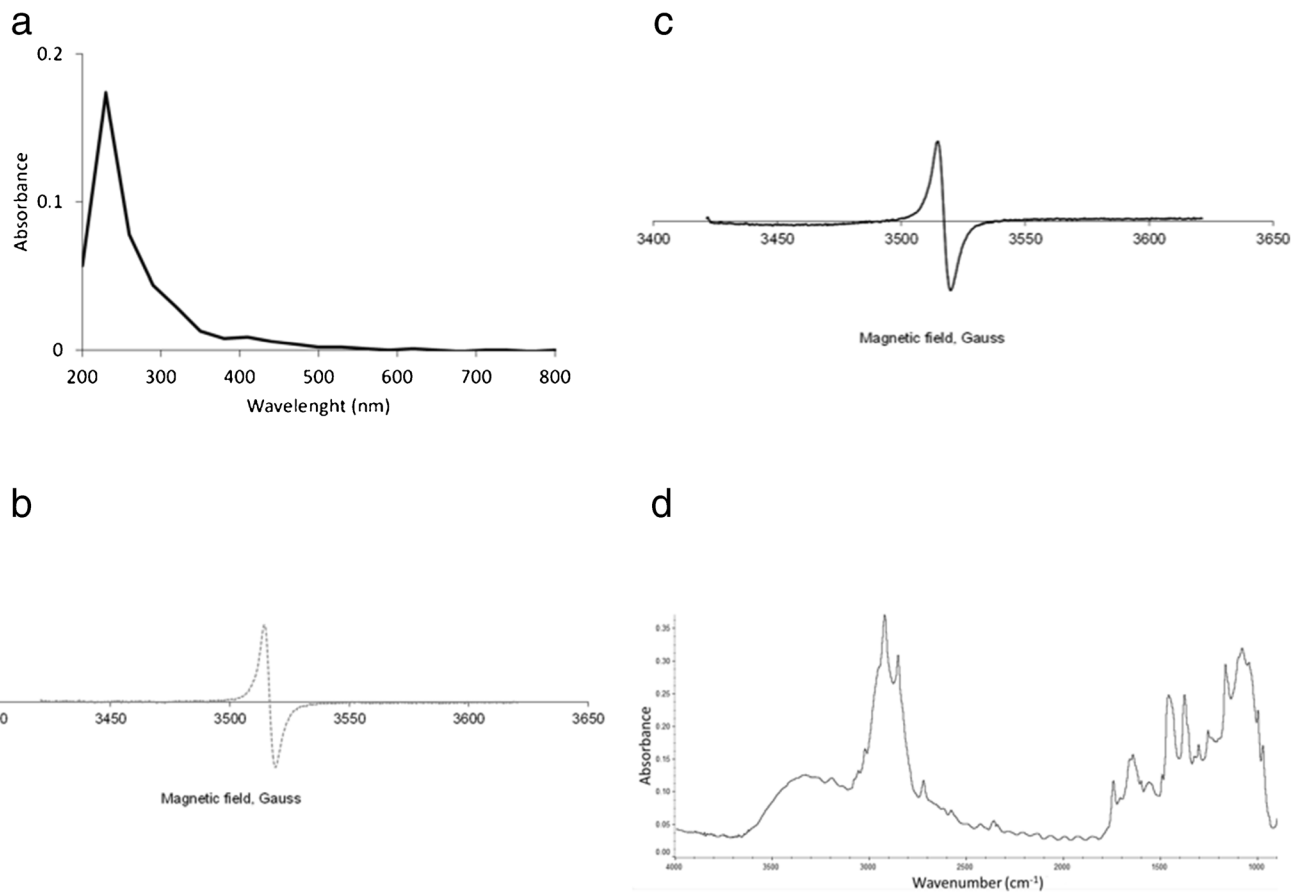

Fig. 2 Spectroscopical features of melanin of *H. cephalosporioides* LPSC 1155: Ultraviolet-visible spectrum (**a**), ESR spectrum of pigment isolated (**b**) and one available within biomass (**c**) as well as FTIR spectrum of pigment available within biomass (**d**)

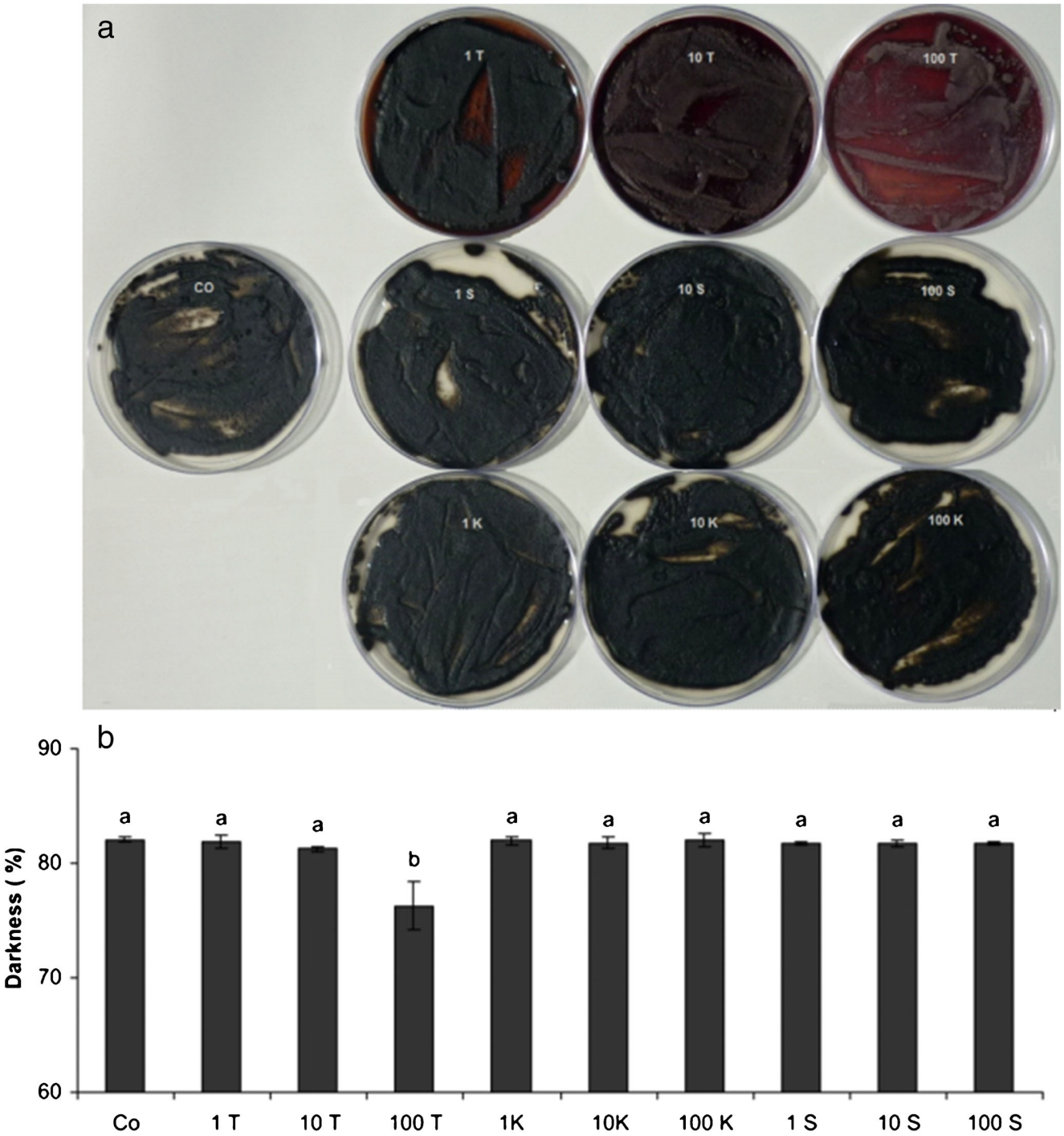

Fig. 3 *H. cephalosporioides* LPSC 1155 grown on PDA (control, Co) and PDA supplemented with 1, 10 and 100 ppm of kojic acid (K), sulcotrione (S) and tricyclazole (T) for 21 days in the dark at 25 ± 2°C: Colonies (**a**) and pigmentation (**b**). Values are means of four replicates; error bars correspond to standard deviation. Bars with the same capital letter are not significantly different ($P < 0.05$). Pigmentation is expressed as darkness "K" level

pks identification

By means of LC1-LC2 primers we obtained a 721 bp amplicon (Fig. 6), whose sequence was 75% homologous to a putative *pks* domain belonging to a fungal endophyte (JQ717039.1). The phylogenetic analysis of the amino acid sequence deduced from the KS domain of the PKS of *H. cephalosporioides* LPSC 1155 and that of other fungal PKSs as well as that of *Streptomyces avermitilis*, generated a tree where the *H. cephalosporioides pks* sequence was clustered with PKSs sequences involved in pigments synthesis, including DHN-melanin (Fig. 7).

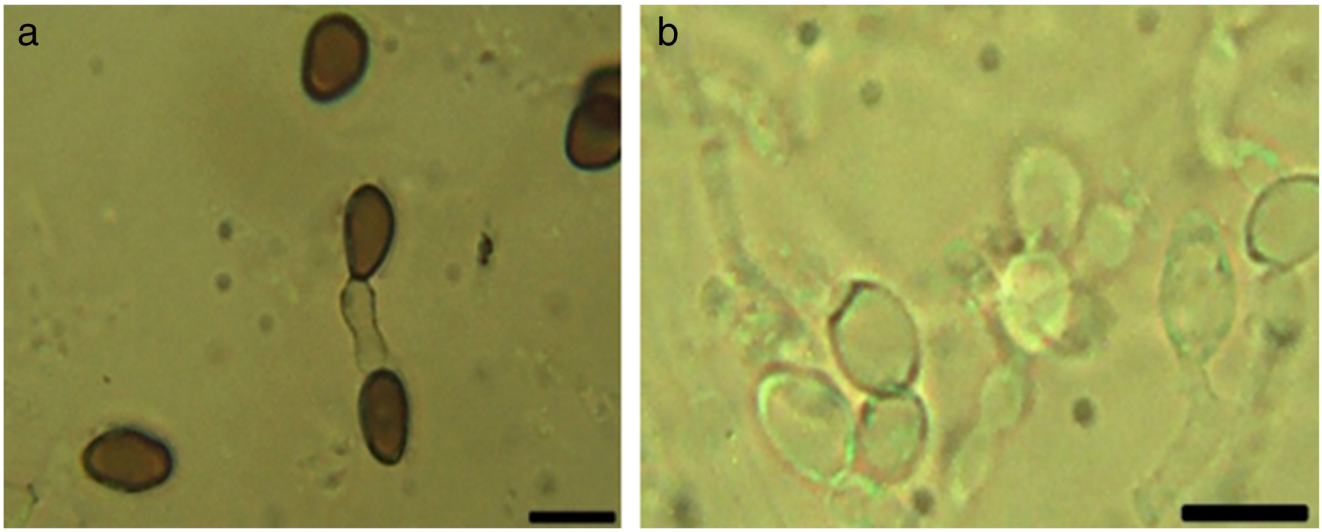

Fig. 4 Light microscope analysis of chlamydospores and hyphae differentiated by *H. cephalosporoides* grown in PDA (**a**) and in PDA supplemented with 100 ppm tricyclazole (**b**). Scale 10 μm

Discussion

H. cephalosporioides was considered for a long time as incertae sedis in the phylum *Ascomycota*, and recently, it was placed by Ekanayaka et al. (2019a) in the class *Leotiomycetes*, specifically in *Helicogoniaceae*, in an undescribed new order (*Helicogoniales*). However, this classification was based on a single isolate (CBS 645.76, BAFC 71, IMI 180354) collected from the soil of a *Nothofagus antarctica* forest (https://www.uamh.ca/details.php?id=5077) that turn out to be the type specimen, whose phylogenetic relationships were poorly resolved by LSU and ITS markers. Johnston et al. (2019) made a multigene phylogeny analysis for the classification of *Leotiomycetes*, but *H. cephalosporioides* was not included in the study. In addition to this, the 28 S rDNA partial sequence of *H. cephalosporioides* did not provide substantial information for a phylogenetic analysis by the ML method with sequences of isolate LPSC 1155 and *Leotiomycetes* type strains (Fig. 8). This generates an unresolved clade for the taxonomical position of this fungus, since a low bootstrap (415/1000) value related it to several representatives of the *Phacidiales*, an order that includes the family *Helicogoniaceae* (Johnston et al. 2019). Future studies aimed at defining the taxonomical position of *H. cephalosporioides* should include additional conserved sequences for contributing to its delimitation in class *Leotiomycetes*.

H. cephalosporioides is a fungal taxon so far only isolated from soils of *N. antarctica* and *N. pumilio* forests in South American, specifically in Patagonia, Argentina (Marchand et al. 1976; Elíades et al. 2019; Mestre and Fontenla 2021). Several other species of *Nothofagus* are native to the Southern South America and other places such as Australia, New Caledonia, New Guinea, New Zealand, and Tasmania, which all together are part of the paleo supercontinent Gondwana. It will be interesting to see if bulk-soil of all these areas host this

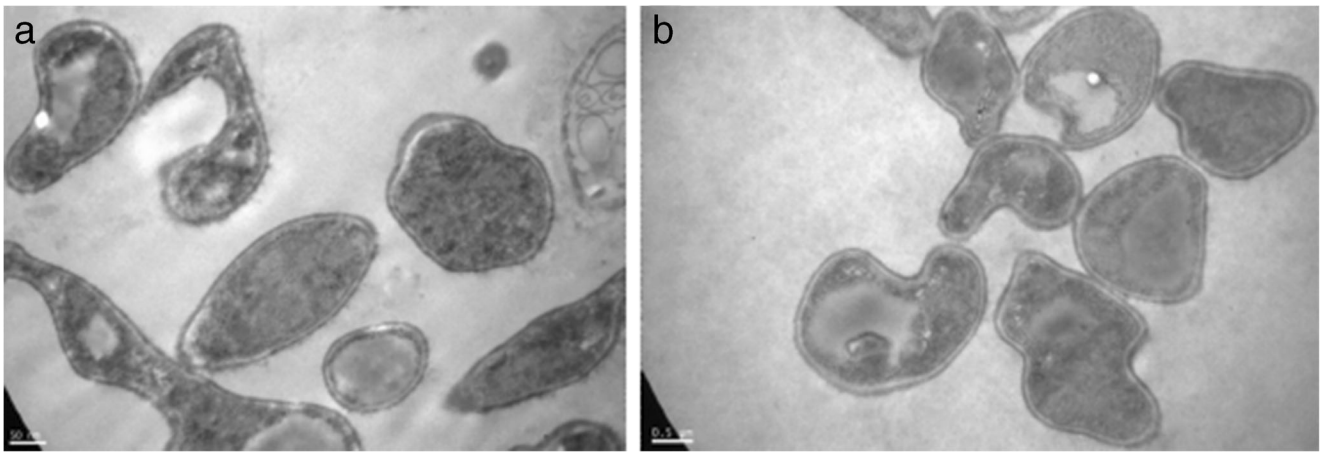

Fig. 5 Transmission electron microscopy of *H. cephalosporioides* chlamydospores differentiated when the fungus was grown on PDA in absence (**a**) and in the presence of 100 ppm of tricyclazole (**b**)

fungus or if it is specifically associated to this plant genus as a whole, or to a group of species or if is a member of the microbiome associated with *Nothofagus* forests and their ectomycorrhizae as suggested by Gutiérrez et al. (1991) and Mestre and Fontenla (2021).

In volcanic and shallow soils of South of Patagonia, microorganisms are exposed to several stresses such as low temperature and layers of frozen water, which might provoke anoxia in soils, exposition to daily freeze-thaw events as well as high UV radiation (Ruisi et al. 2007). Fungi living within these environments might develop survival capacities such as mechanisms leading to fast sporulation, shorter life cycles and/or synthesis of pigments (Robinson 2001). In *H. cephalosporioides*, conidiation, fragmentation of hyphae, and conversion of hyphal elements into chlamydospores are common ways of reproduction, like other taxa of *Helicogoniaceae* unable to form ascospores (Ekanayaka et al. 2019a). *H. cephalosporioides* differentiates two spore types, sympodial conidia and dark chlamydospores, an ability shared with *S. dimorphospora*, formerly classified as *H. dimorphospora* that produce blastic chlamydospores (Madrid et al. 2010). *H. cephalosporioides* chlamydospores are ontogenetically different and are dark enlarged structures that look like thick-walled vegetative cells of diverse forms that are differentiated within hyphae or at hyphal tips. Chlamydospore development is a complex process encoded by a specific set of genes that probably are related with regulatory pathways under the influence of the prevailing environmental conditions (Lin and Heitman 2005; Palige et al. 2013). Such processes remain to be studied in detail.

In many fungi, pigmentation is associated with cell differentiation, particularly the development of melanized chlamydospores as reported by Cooper and Gadd (1984) for *Microdochium bolleyi* (*Xylariales*). Chlamydospores of *H. cephalosporioides* contain dark pigments that were characterized by a peak within the ESR spectrum that is typical of melanins as suggested by Kumar et al. (2011), Llorente et al. (2012), and Pal et al. (2014). Specifically, the ESR signals indicate that the *H. cephalosporioides* melanin has o-semiquinone free radicals (Zdybel et al. 2013), and a structure with carbon-centered organic free radicals conjugated with functional groups containing oxygen (Atherton and Willder 1993), which is in accordance with the quinoid structure, inferred by FT-IR analysis. All these findings share typical signals of melanins like those described in *Amorphotheca resinae* Parbery (syn. *Cladosporium resinae* Vries) and *Cladosporium cladosporioides* (Llorente et al. 2012), which is strongly supporting that the extracted pigment from *H. cephalosporioides* is a DHN-melanin.

DHN-melanin synthesis is initiated by the activity of type I PKS enzymes, which have up to eight types of functional domains like acyl transferase, acyl carrier protein, ketosynthase (KS), ketoreductase, enoyl reductase, dehydratase, thioesterase, and methyltransferase. In line with this, we found that the genome of *H. cephalosporioides* has a sequence for the KS domain of a non-reducing type I putative PKS, which was highly homologous to other fungal PKSs catalyzing the synthesis of DHN-melanin. Griffiths et al. (2018), Klau et al. (2022), Medina et al. (2018), and Minami et al. (2020) have also performed similar phylogenetic analyses for the preliminary investigation of novel *pks* genes in other fungi. Based on the finding that tricyclazole reduces pigmentation by inhibiting enzymes other than PKSs such as reductases, coupled with the fact that the release of diffusible metabolites that gave a brown-orange color to the medium derived from the spontaneous oxidation of 1,3,6,8-tetrahydroxynaphthalene to flaviolin (Bárcena et al. 2015), we concluded that chlamydospores synthesize DHN-melanin. It appears that this is the main and probably only chromophoric polymer in chlamydospores of *H. cephalosporioides* as described for other spores and spore-structures differentiated by fungi (Nitiu et al. 2020). This is one of the first evidence of the melanin synthesis in fungi belonging to class *Leotiomycetes*. Future studies, such as the disruption of the *pks* gene of *H. cephalosporioides*, will confirm whether other genes that codify the reductases inhibited by tricyclazole vary along chlamydospore development.

Dark pigments in chlamydospore's walls and/or the generation of new chromophore groups as well as their oxidative polymerization driven by oxygen or metals might be activated by rises in temperature, which might impact on other fungal

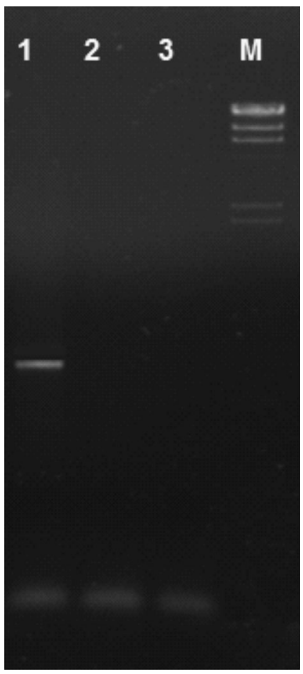

Fig. 6 Amplification of the DNA sequence coding for the KS domain using the primers LC1-F/LC2-R. Lane 1, PCR amplification product using genomic DNA of *H. cephalosporioides*. Lane 2, PCR control (without DNA). Lane 3, negative control using genomic DNA of *Grammothele subargentea* LPSC 436. Lane M, molecular weight marker 100–1000 bp (Highway)

structures containing DHN-melanins and heterogeneous ones (Nitiu et al. 2020). Chlamydospore development in *H. cephalosporioides* was unaffected by temperature, but white light–UV-driven photochemical reactions affected its melanin synthesis. Bárcena et al. (2018) reported that walls of *Pseudocercospora griseola* containing melanins or the isolated pigments differed in absorption due to differences in molecular weight, structural units, and/or chemical groups, as well as their interaction with light (scattering). Therefore, pure melanins from *H. cephalosporioides* and their relative amount in spores, differentiated along treatments, might contribute to differences in surface pigmentation. Such process should be studied further, though this will require a larger amount of melanin to make an accurate estimation. Since the amount of chlamydospores also varied according to the media composition and/or light conditions used to incubate the fungus, probably spore development in *H. cephalosporioides* is modulated by specific nutritional and/or environmental factors, as reported for *Cryptococcus*

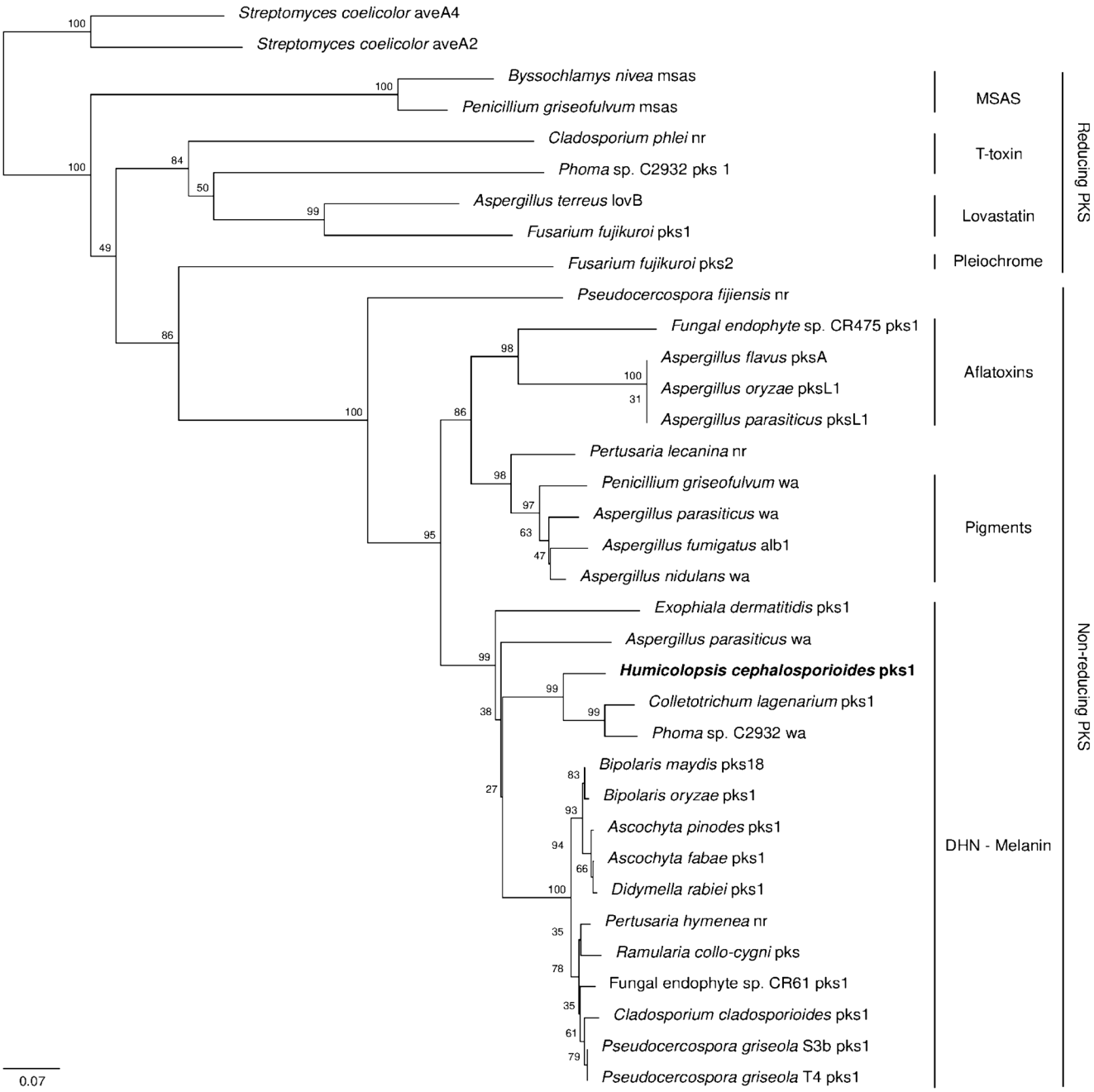

Fig. 7 Phylogenetic relationships of the amino acid sequence deduced from the KS domain of the PKS of *H. cephalosporioides* with other fungal PKSs, including those involved in DHN-melanin synthesis. The other types of metabolites synthesized by the enzymes are also indicated. Numbers at nodes indicate bootstrap support. The scale bar indicates substitutions per amino acid site

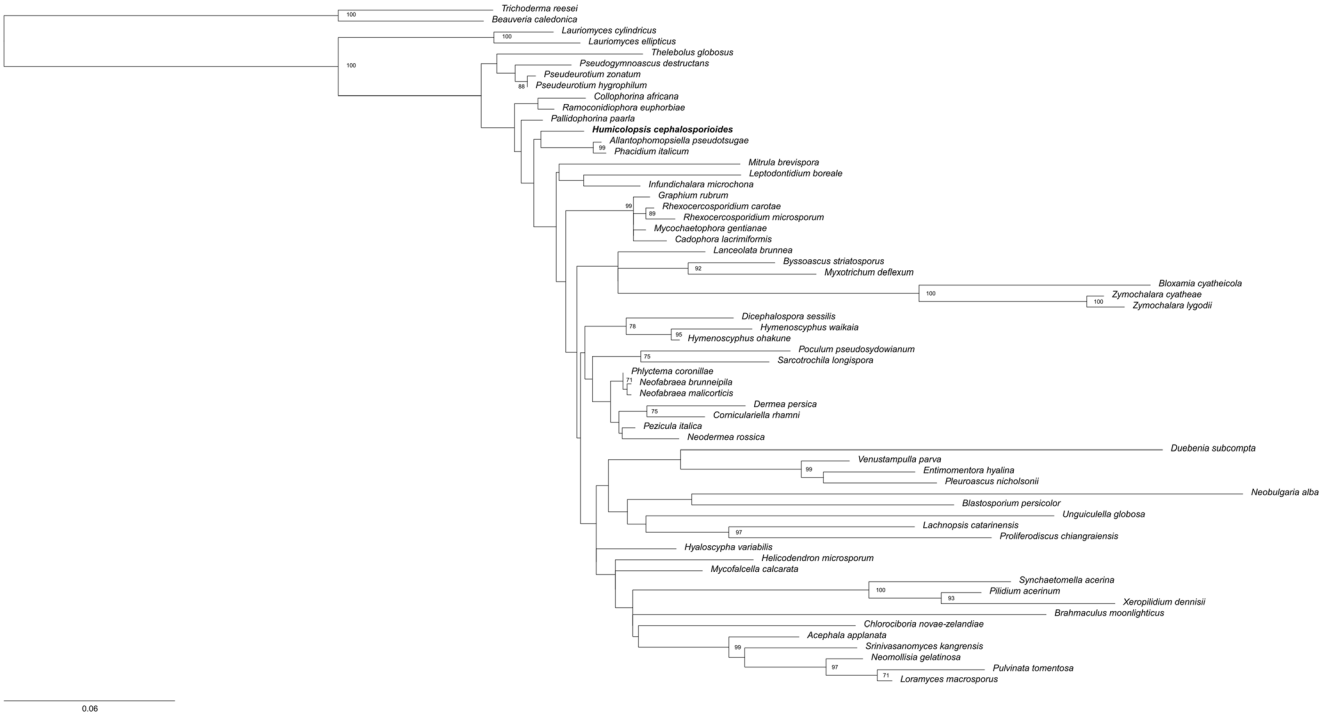

Fig. 8 Maximum-likelihood tree of *Leotiomycetes* type strains inferred from the concatenated 5.8S-18S-28S dataset. The *Sordariomycetes Beauveria caledonica* ARSEF 2567 and *Trichoderma reesei* DSM 768 were included as outgroups. Numbers at the nodes represent bootstrap support values as a percentage of 1000 replicates, being only shown when values were higher than 70%. The scale bar shows the average number of nucleotide substitutions per site

neoformans by Lin and Heitman (2005). Therefore, melanization of *H. cephalosporioides*, like in other fungi, might play a key role in chlamydospore morphogenesis and its survival in a stressful environment such as the one in *Nothofagus* forests in sub-Antarctica region.

Conclusions

H. cephalosporioides is a fungus that survives under extreme environmental conditions, such as those in *Nothofagus* forests in Patagonia. This is probably due to the development of chlamydospores, whose survival ability relies on the synthesis of DHN-melanin. The ITS, 18S rDNA, and 28S rDNA sequences of *H. cephalosporioides* did not provide enough information to delineate the phylogenetic relationships of the fungus within the class *Leotiomycetes*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01853-6.

Acknowledgements We thank Ana M. Gennaro from the INTEC (CONICET-UNL, Santa Fe, Argentina) for performing the ESR analysis. AB, RM, LAE, and MCNS are members of the Carrera del Investigador Científico CONICET, Argentina. MNC and PAB are researchers of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina.

Availability of data and material Not applicable for that section.

Author contribution AB designed and performed experiments on biomass production, extraction, and characterization of pigments and melanin synthesis inhibition. RM analyzed data, provided funding acquisition, and co-wrote the paper. MEEF made DNA extraction, polymerase chain reactions, sequence alignments, and phylogenetic analyses. LAE helped performing different tasks in laboratory. MNC provided fungal strains and participated actively in species identification using morphological features. CPT contributed on the revision and conceptualization of this manuscript. PAB was involved in the analysis and discussion of the data obtained, provided funding acquisition and assisted with the writing of the paper. MCNS supervised the research, provided funding acquisition and co-wrote the paper.

Funding This research was partially financed by the National Agency for Scientific and Technological Promotion (ANPCyT) of the Ministry of Science, Technology and Productive Innovation through the PICT 2018-3144 project (RM), PICT 2019-00207 project (MCNS), PICT 2020-02736 project (PAB), CONICET (PUE INFIVE and PIP 11220200100527CO) and UNLP, Argentina, and by the Secretary of Science and Technique of the National University of La Plata, through the R&D Projects A344 (MCNS).

Declarations

Ethics approval and consent to participate Not applicable for that section.

Competing interests The authors declare no competing interests.

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