Description of a new Coniochaeta species isolated from clinical sample

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Research

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Abstract

Coniochaeta is a teleomorphic genus belonging to the *Ascomycota* class and *Coniochaetaceae* family. Some of the *Coniochaeta* species are plant and animal pathogens, while others are known to be primarily involved in human disease. In the last decades, case reports of human infections with *Coniochaeta* have increased, mainly in immunocompromised hosts. We have described and characterised a new species in the *Coniochaeta* genus, here named *Coniochaeta massiliensis* (PMML0158) that was isolated from a clinical sample. Species identification and thorough description were based on apposite and reliable phylogenetic and phenotypic approaches. The phylogenetic methods included a multilocus nucleotide analysis of four genomic regions: ITS (rRNA internal transcribed spacers 1 and 2), TEF-1α (translation elongation factor-1alpha), TUB2 (β-tubulin2), and D1/D2 domains (28S LSU rRNA). The phenotypic characterisation first consisted of a physiological analysis using both EDX (Energy-Dispersive X-ray Spectroscopy) and Biolog™ advanced phenotypic technology for fixing the chemical mapping and carbon source oxidation/assimilation profiles. Afterwards, morphological characteristics were highlighted by optical microscopy and scanning electron microscopy. The in vitro antifungal susceptibility profile was characterised using the E-test™ exponential gradient method. The molecular analysis revealed the genetic distance between the novel species *Coniochaeta massiliensis* (PMML0158) and other known taxa, and the phenotypic analysis confirmed its unique chemical and physiological profile when compared to all other species of this genus.

Introduction

Coniochaeta spp. are pleomorphic ascomycetous fungi belonging to the *Coniochaetaceae* family (Weber 2002). Some of these yeasts are also classified within dematiaceous fungi due to the presence of melanin in cell walls, known for emitting dark pigments in culture, which is perceived as a virulence factor (Nosanchuk and Casadevall, 2006). *Coniochaeta* species are ubiquitously distributed in the environment. They have been isolated from several natural substrates, including soil (Guarro et al. 1997; Kamiya et al. 1995), wood (Yuan and Mohamed, 1997), plants (Mahoney and Lafavre, 1981), water (Crane and Shearer, 1995), and food (Perdomo et al., 2013). Following the recent “One fungus, one name” nomenclature change in January 2013, the previously described teleomorph name, *Coniochaeta*, has been retained for the anamorphic *Lecythophora* genus. Therefore, the six species of the *Lecythophora* genus (*L. lignicola*, *L decumbens*, *L. fasciculata*, *L. luteoviridis*, *L. mutabilis* and *L. hoffmannii*) were transferred to the *Coniochaeta* genus (Khan et al., 2013; Weber and Begerow, 2002). *Coniochaeta hoffmannii* and *C. mutabilis* are considered the most frequent species of the *Coniochaeta* genus found in clinical samples. These anamorphic yeasts are known to be human pathogens, causing invasive fungal infections, occasionally with fatal outcomes, particularly in immunocompromised patients (de Hoog et al., 2000). *C. hoffmannii* has been described as a plant pathogen (Gams and McGinnis, 1983). It has also been involved in emerging human fungal infections, including subcutaneous abscess (Gams and McGinnis, 1983), keratitis (Fintelmann, 2011), and sinusitis (Marriott, 1997), and in canine osteomyelitis in a dog (Sakaeyama, 2007). However, *C. mutabilis* (Gams and McGinnis, 1983) is known to be more frequently involved in severe infections. It has been involved in human peritonitis (Ahmad, 1985), endocarditis (Slifkin and Bowers, 1975; Drees et al., 2007), septic shock (Taniguchi et al., 2009), endophthalmitis (Scott et al., 2004; Marcus et al., 1999) and keratomycosis (Ho et al., 1991). The present study aimed to properly describe and characterise genetically and phenotypically a new species of *Coniochaeta* isolated from a human abscess.

Materials And Methods

Fungal strains

The phenotypic features of *Coniochaeta massiliensis* PMML0158 were compared to those of two type strains, *C. mutabilis* DSM 10716 and *C. hoffmannii* DSM 2693. The genotypic characteristics of these three strains were also compared with those of seven other type strains in the genus *Coniochaeta*, including: *C. fasciculate* CBS 205.38, *C. lignicola* CBS 267.33, *C. luteoviridis* CBS 206.38, *C. hoffmannii* CBS 245.38, *C. mutabilis* CBS 157.44, *C. lignaria* DWS9m2/SMH2569/95.605, and *C. cateniformis* UTHSC 01-1644. *Phialemonium obovatum* CBS 279.76 was used as the outgroup in the phylogenetic analysis.

Macroscopic characterisation

The temperature growth profiles and macroscopic features, including colony time of growth, aspect and surface/reverse colour for all strains was determined on five-day-old colonies cultivated on SDA GC plates. Colonies were inoculated on other new plates of SDA GC at: 4, 25, 30, 33, 37, 40, and 45°C.

Microscopic characterisation

Microscopic features were first analysed with optical microscopy. Microscopic slides were prepared using the cellophane adhesive tape method with lactophenol cotton blue (LCB). Photographs were taken with the DM 2500 (LEICA CAMERA SARL, Paris, France) camera. Further observation was performed using scanning electron microscopy. A fragment of fungal colonies was fixed on a microscope slide with 400 µL of glutaraldehyde 2.5% in 0.1M sodium cacodylate buffer and stored at 30°C for at least 30 minutes. Photographs were taken with the TM4000 Plus microscope (Hitachi High-Technologies, Tokyo, Japan) adjusted at 15KeV lens mode 4 with a back-scattered electron detector (BSE).

MALDI-TOF MS identification

The fungi were incubated on Sabouraud Dextrose Agar (SDA) plates supplemented with gentamycin and chloramphenicol (GC) at 30°C for five to eight days. Once grown, colonies were identified with Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), using the procedure described in Cassagne et al. (2016). The Microflex LT™ instrument and the MALDI Biotyper™ system (Bruker Daltonics GmbH, Bremen, Germany) were used, along with the manufacturer's and in-house reference spectra databases, as described in Normand et al. (2017). A dendrogram based on protein expression intensity was built with the MALDI-TOF Biotyper Compass Explorer (Bruker Daltonics).

Antifungal Susceptibility Testing (AFST)

The *in vitro* sensitivity of nine antifungal drugs was tested against the three *Coniochaeta* strains using the Sensititre YeastOne™ (Thermo Fisher Scientific, Dardilly, France) microdilution system, following the supplier's recommendations. Briefly, isolates were grown on SDA GC until maturity, the inoculum was suspended in 2 mL of saline and turbidity was adjusted to 0.5 McFarland, to obtain an inoculum of ~ 1.5 x 10^8 CFU/mL. Next, 20 µL of this solution was added to 10 mL of YeastBroth™ (ThermoFisher Scientific) before 100 µL of this final solution were transferred into each Sensititre YeastOne™ YO09 (Thermo Fisher Scientific) plate well. MICs were read after 48h incubation at 35°C.

Physiological analysis

1. Energy-Dispersive X-ray Spectroscopy (EDX) analysis

Fresh cultures of the three strains were fixed in 1 mL of 2.5% Glutaraldehyde in 0.1M Sodium Cacodylate buffer for at least 1h. The cytospin was done with 200 µL of the fixed fungal solution and centrifuged at 800 rpm for eight minutes. EDX was achieved with an INCA X-Stream-2 detector (Oxford Instruments) associated to the TM4000 Plus scanning electron microscopy and AztecOne software (Oxford instruments). The chemical mapping was performed blindly and took into consideration all chemical elements. Results of the weight and atomic percentages of chemical elements for each strain were subjected to a principal component analysis with the XLSTAT (Addinsoft, Paris, France) software.

2. Biolog™ phenotypic analysis

The Biolog's advanced phenotypic technology was used for the phenotypic analysis. YT MicroPlates™ (Gen III) (Biolog Catalog #1005) were used for oxidation tests and carbon source assimilation. The selected carbon sources can discriminate between the different profiles of fungal phenotypes (Bochner, 2001). The 96-well YT MicroPlate™ contains a patented Redox tetrazolium dye that changes colour when cellular respiration occurs, conferring a metabolic fingerprint. All strains were cultivated on Biolog Universal Yeast™ (BUY) Agar medium (Biolog Catalog #70005). Colonies must be fresh and well developed. Incubation time depends on the genus. For *Coniochaeta* spp., maximal growth was observed after four to six days of incubation. A fungal suspension was prepared by swabbing some conidia into the YT Inoculating Fluid™ (Biolog Catalog #72501) adjusted to a 47% transmittance level with the Biolog Turbidimeter™ (Biolog Catalog #3587) (29). Then, 100 µL of this suspension was pipetted into each YT MicroPlates™ well. The plates were incubated at 26°C for one week. The results are shown in the form of a heat map done with XLSTAT.

DNA extraction and sequencing

After five days of incubation on SDA GC at 30°C, DNA was extracted from the fungal colonies with the Qiagen™ Tissue kit after mechanical lysis using the FastPrep^{TM}-24 instrument in bead tubes with G2 lysis buffer (provided with the Qiagen™ Tissue kit). The extraction was performed with the EZ1 Advanced XL™ instrument, following the manufacturer's instructions.

Four genomic regions were amplified: the rRNA internal transcribed spacers 1 and 2 (ITS1-2), a fragment of the β-tubulin gene (TUB2), a fragment of the translation elongation factor 1-alpha gene (TEF-1-α), and the D1/D2 domains of the rRNA large-subunit (LSU) (Table 1). PCR mixes included 5 µL of DNA extract to 20 µL of Mix (12.5 µL ATG (Ampli Taq Gold™ 360 Master Mix, Applied Biosystems)/6 µL sterile water DNase/Rnase-free/0.75 µL forward/reverse primer) to a total volume of 25 µL/well. The PCR program for all gene amplifications was as follows: an initial denaturation step at 95°C for 15 minutes, followed by 39 cycles of: 1-minute denaturation at 95°C, 30-seconds annealing at 56°C, 1-minute extension at 72°C, and a final 5-minute extension at 72°C. The amplicons were visualized on 2% agarose gel with Sybr Safe DNA™ gel stain (Invitrogen) with the Safe Imager 2.0 Blue-Light Transilluminator™ (Invitrogen). Sequencing was performed on a 3500 Genetic Analyser™ (Applied Biosystems, Inc.). The sequences were assembled and corrected using Chromas Pro 2.0 and analysed using the BLASTn tool with the reference data available from the GenBank database of the National Center for Biotechnology Information (NCBI).

Table 1
Sets of primers used for amplifying the ITS, TUB2, TEF1 and D1/D2 genetic regions.

Primers	Sequences	Targeted regions	References
ITS1	TCCGTAGGTGAACCTGCGG	18S-5.8S	White L. T. *et al*. (1990)
ITS2	GCTGCGTTCTTCATCGATGC	18S-5.8S	White L. T. *et al*. (1990)
ITS3	GCATCGATGAAGAACGCAGC	5.8S-28S	White L. T. *et al*. (1990)
ITS4	TCCTCCGCTTATTGATATGC	5.8S-28S	White L. T. *et al*. (1990)
ITS1	TCCGTAGGTGAACCTGCGG	18S-5.8S, 5.8S-28S	White L. T. *et al*. (1990)
ITS4	TCCTCCGCTTATTGATATGC	18S-5.8S, 5.8S-28S	White L. T. *et al*. (1990)
Bt-2a	GGTAACCAAATCGGTGCTGCTTTC	TUB2	Glass & Donaldson (1999)
Bt-2b	ACCCTCAGTGTAGTGACCCTTGGC	TUB2	Glass & Donaldson (1999)
EF1-728F	CATCGAGAAGTTCGAGAAGG	TEF1	Carbone & Kohn (1999)
EF1-986R	TACTTGAAGGAACCCTTACC	TEF1	Carbone & Kohn (1999)
D1	AACTTAAGCATATCAATAAGCGGAGGA	28S	De Hoog et al. (2005)
D2	GGT CCG TGT TTC AAG ACG G	28S	De Hoog et al. (2005)

Phylogenetic analysis

The phylogenetic tree was built after concatenating the ITS, TUB2 and D1/D2 nucleotide sequences of the three strains and other reference strains obtained from the GenBank database (accession numbers detailed in Table 2). All sequences were aligned with Muscle (tool available in MEGA 11 software). *Phialemonium obovatum* CBS 279.76 was used as the outgroup. The maximum parsimony multilocus phylogenetic tree was constructed with the default settings. The branch robustness estimation was tested using 1000 bootstrap replications with the molecular evolutionary genetics analysis (MEGA) software version 11.

Table 2
GenBank accession numbers of reference strains used for the phylogenetic analysis.

Species	Collection ID	GenBank accession numbers			
		ITS	TUB2	D1/D2	TEF1
Coniochaeta massiliensis	PMML0158	OM366153.1	ON000097.1	OM366268.1	OM640093.1
Coniochaeta mutabilis	DSM 10716	OM366154.1	ON000098.1	OM366269.1	OM640094.1
Coniochaeta hoffmannii	DSM 2693	OM366155.1	ON000099.1	OM366270.1	OM640095.1
Coniochaeta fasciculata	CBS 205.38	HE610336.1	HE610350.1	FR691988.1	
Coniochaeta lignicola	CBS 267.33	HE610335.1	HE610353.1	FR691986.1	
Coniochaeta luteoviridis	CBS 206.38	HE610333.1	HE610351.1	FR691987.1	
Coniochaeta hoffmannii	CBS 245.38	HE610332.1	HE610352.1	FR691982.1	
Coniochaeta mutabilis	CBS 157.44	HE610334.1	HE610349.1	AF353604.1	
Coniochaeta lignaria	DWS9m2/SMH2569/95.605	KJ188673.1	AY780113.1	AF353584.1	
Coniochaeta cateniformis	UTHSC 01-1644	HE610331.1	HE610347.1	HE610329.1	
Phialemonium obovatum	CBS 279.76	HE610365.1	HE610365.1	FR691997.1	

Results

Macroscopic characterisation

Macroscopic features confirmed the rapid growth time of *Coniochaeta* species on SDA GC medium at an optimal temperature of 25°C for all species (Fig. 2). However, none of the three yeasts were able to grow at 4 and 45°C. Colonies of the three isolates were initially white to beige, both on the surface and reverse. After four to five days of incubation, *Coniochaeta massiliensis* turned light orange to salmon. All colonies were flat and moist. *Coniochaeta hoffmannii* DSM 2693 and the newly isolated yeast (PMML0158) presented a glabrous aspect, while *Coniochaeta mutabilis* DSM 10716 was typified by an aerial mycelium growth.

Microscopic characterisation

The microscopic observation of the three strains was characterised by the presence of wide septate hyphae, numerous cylindrical adelophialides (short phialides without septum), discrete phialides with conical tips exhibiting ellipsoidal to cylindrical and rarely curved conidia, and nonseptate with thin and smooth conidial walls (2 to 3 by 6

to 10 µm). Several conidia were observed aggregating on the hyphae's side and most often in clusters. Collarettes were only found in both *Coniochaeta massiliensis* and *C. mutabilis*. No chlamydospore was observed (Fig. 3).

Antifungal susceptibility testing (AFST)

The minimum inhibitory concentration (MIC) values of the three species for all nine antifungal drugs are shown in (Table 3). The MIC endpoints for all strains were determined as the lowest concentration inhibiting the growth of 90% of the strains and were determined as described in Perdomo et al. (2011), since there are no validated AFST guidelines for this genus. The MICs of AMB, 5-FC, ITC, POS and VOR were low for the three isolates. The new species of *Coniochaeta* displayed low echinocandins (AND and CAS) MICs and a high FL MIC, while *C. hoffmannii* and *C. mutabilis* demonstrated the opposite for these four antifungal drugs.

Table 3. Results of *in vitro* antifungal susceptibility testing.

MIC* (mg/L) read at 48h									
	AMB	AND	CAS	5-FC	FL	ITC	MIF	POS	VOR**
Coniochaeta massiliensis PMML0158	0,25	0,5	1	2	8	0,25	1	0,25	0,12
Coniochaeta hoffmannii DSM 2693	0,12	4	4	1	4	0,06	>32	0,12	0,12
Coniochaeta mutabilis DSM 10716	0,12	4	8	1	2	0,015	>32	0,03	0,03

*MIC, minimum inhibitory concentration

** AMB, amphotericin B; AND, anidulafungin; CAS, caspofungin; 5-FC, 5-fluorocytosine; FL, fluconazole; ITC, itraconazole; MIF, micafungin; POS, posaconazole; and VOR, voriconazole.

MALDI-TOF MS identification

The MALDI-TOF MS identification score (log score < 1.90) was below the limit required for obtaining a good identification. The isolate was identified at the genus level as *Coniochaeta sp.* The score value generated was MALDI-TOF MS spectra of the new isolate and the two other reference strains from the DSMZ collection, *Coniochaeta mutabilis* DSM 10716 and *Coniochaeta hoffmannii* DSM 2693, were collected and have been incremented in the MALDI-TOF MS database.

Physiological analysis

1. EDX (Energy-Dispersive X-ray Spectroscopy)

The weight and atomic percentages of chemical elements resulting from the chemical mapping performed on the three species of the *Coniochaeta* genus displayed three distinct profiles. In the principal component analysis (Fig. 4), each *Coniochaeta* species was very distant from the others, demonstrating highly heterogeneous chemical profiles.

2. Biolog™ system

The Biolog™ advanced phenotypic technology was very useful for the phenotypic characterisation. The oxidation and assimilation test results are illustrated using heat maps (Fig. 5, 6). The majority of the substrates were not oxidized/assimilated. Each heat map was quite heterogeneous, and both demonstrated similar findings: *Coniochaeta mutabilis* DSM 10716 appeared more divergent from *Coniochaeta massiliensis (*PMML0158) than from *Coniochaeta hoffmannii* DSM 2693. However, *Coniochaeta massiliensis (*PMML0158) appeared to be closely related to *Coniochaeta hoffmannii* DSM 2693.

Phylogenetic analysis

We built a phylogenetic tree using MEGA 11 software based on the assembled and concatenated sequences of ITS, Beta-tubulin2 and D1/D2 genetic regions for one clinical isolate, two reference strains and sequences of eight strains collected from GenBank (Table 2).

The multilocus analysis of the tree strains (Fig. 1) revealed the presence of 2 main clades; the first one is divided into five subclades. The second one includes the newly isolated strain of *Coniochaeta* PMML0158, which appears distinct from all other *Coniochaeta* species.

Taxonomy

Coniochaeta massiliensis Kabtani J. & Ranque S. **sp. nov.**

MycoBank: MB843839

(Fig. 3. A-L)

Etymology: Named in honour of Marseille (France), the city where it was isolated.

Diagnosis: Closely similar to the other two *Coniochaeta* species examined by displaying the same flat and moist colonies aspect as well as the absence of the dematiaceous appearance. However, relying on macroscopic features, it differs from *C. mutabilis* by lacking aerial growth. On the other hand, *C. massiliensis* presented the same microscopic structures as other species, such as the presence of several adelophialides, discrete phialides and cylindrical or curved conidia. At this point, *C. massiliensis* is closer to *C. mutabilis*, due to the decisive presence of the collarette.

Type: **France**: Marseille. Human body (abscess of the hand), 15 July 2020. (Holotype IHEM 28559). GenBank: OM366153 (ITS), ON000097 (Btub2), OM640093 (TEF-1a), OM366268 (D1/D2).

Description: the macroscopic features were characterised by a rapid growth time on SDA GC medium at an optimal temperature of 25°C. However, *Coniochaeta massiliensis* was not able to grow at 4 and 45°C. Colonies were first white to beige, both on the surface and reverse after four to five days of incubation, then turned light orange to salmon. Colonies were flat and moist, with a glabrous aspect. There was no aerial mycelium growth. The microscopic features were characterised by the presence of wide septate hyphae, numerous cylindrical adelophialides (short phialides without septum), discrete phialides with conical tips, exhibiting ellipsoidal to cylindrical and rarely curved conidia, nonseptate with thin and smooth conidial walls (2 to 3 by 6 to 10 µm). Several conidia were observed aggregating on the side of the hyphae and most often in clusters. Collarettes were present, but chlamydospores formation was not observed.

The Biolog™ carbon sources assimilation profile showed that *C. massiliensis* PMML0158 can assimilate different carbon substrates, such as 2 -keto-D-gluconic acid, D-gluconic acid, D-ribose, D-xylose, D-glucosamine, D-cellobiose, D-melibiose, Palatinose, Turanose, L-sorbose, and β-methyl-D-glucoside. Based on this phenotypic analysis, *C. massiliensis* PMMFL0158 appears similar to *C. hoffmannii* DSM 2693.

Host: Human

Additional specimen examined: *C. hoffmannii*: Type: **Country of origin unknown**. Human body, before 08 July 1983. (Holotype DSM 2693 - ATCC 34158 – SP33–4). GenBank: OM366155 (ITS), ON000099 (Btub2), OM640095 (TEF-1a), OM366270 (D1/D2).

Additional specimen examined: *C. mutabilis*: Type: **Sweden**. Human body, before 14 June 1996. (Holotype DSM 10716 - EMPA 573, S24 E). GenBank: OM366154 (ITS), ON000098 (Btub2), OM640094 (TEF-1a), OM366269 (D1/D2).

Discussion

Coniochaeta mutabilis and *Coniochaeta hoffmannii* are the most familiar human pathogens of the *Coniochaeta* genus, and the most widespread and commonly encountered in human samples and severe infections. MALDI-TOF MS identification for these two species was relevant, with a log score > 2.0. However, this tool was not able to perform a species identification for the new isolate, which led to a molecular analysis targeting four relevant genes: the internal transcribed spacer (ITS1/ITS2) in the RNA ribosomal small-subunit (SSU), a fragment of the translation elongation factor 1-alpha gene (TEF-1-α), a fragment of β-tubulin gene (TUB2), and D1/D2 domains of the ribosomal DNA large-subunit (LSU).

The phylogenetic tree (Fig. 1) constructed with the concatenated sequences of these three genes (ITS/TUB2/D1D2) showed that *Coniochaeta mutabilis* DSM 10716 and *Coniochaeta hoffmannii* DSM 2693 clustered within distinct clades, and both were distant from *Coniochaeta massiliensis*. Based on phylogeny, the new isolate seems divergent from the group's other known taxa of *Coniochaeta*. de Hoog et al. (2000) described *C. lignaria* as the teleomorph of *C. hoffmannii*. However, in our phylogenetic tree, these yeasts clustered into two different clades, indicating a large distance between the teleomorphic and anamorphic species. These findings are in line with those of both Perdomo et al. (2011) and Weber and Begerow (2002), who reported a significant difference between these species.

Antifungal susceptibility testing showed relatively low MICs for AMB, 5-FC, ITC, POS and VOR in all strains. These results are in line with those of Perdomo et al. (2011), but contrast with those of other authors (Marriott et al., 1997; Scott et al., 2004; Khan et al., 2013) who reported relatively high MICs for AMB and ITC against *C. hoffmannii* and *C. mutabilis.*

We macroscopically observed a fast-growing phenotype in *Coniochaeta* species. This finding contrasts with those of Ahmad et al. (1985), who reported slow colony growth. All species displayed the same flat and moist colonies aspect. Only *C. mutabilis* additionally displayed low aerial growth, as reported by Drees et al. (2007). The primary colony colour remained unchanged (white/beige to orange/salmon). None of the colonies turned brown/dark in culture even after one month of incubation. This aspect has been well described in *C. hoffmanii*, a species that lacks the dematiaceous aspect, whereas *C. mutabilis* is classified within dematiaceous fungi, which are particularly typified by the presence of melanin in hyphae and conidia cell walls responsible for dark pigment emission. Occasionally, certain *C. mutabilis* species lack the melanin property, as mentioned in Khan et al. (2013), Dress *et al.*

(2007), and Perdomo et al. (2011). This was noted for the *C. mutabilis* DSM 10716 strain. Furthermore, in this study, the three species lacked the dematiaceous aspect.

The most remarkable morphological characteristics of the *Coniochaeta* species that have been described were the presence of several adelophialides, discrete phialides and cylindrical or curved conidia, in addition to the presence or absence of collarettes and chlamydospores, depending on the species (Sakaeyama et al., 2007; Drees et al., 2007; Perdomo et al., 2011; Khan et al. 2013). Most of these specific characteristics were observed in the newly described species (PMML0158). Based on this microscopic analysis, we infer that *Coniochaeta massiliensis* is closer to *C. mutabilis*, due to the decisive presence of a collarette.

Morphological characterisation helped for species differentiation, and physiological analysis was more convincing in distinguishing the three species, with the aim of describing the new one as thoroughly as possible. EDX (Energy-Dispersive X-ray Spectroscopy) and Biolog™ phenotypic technology revealed divergent chemical mapping and carbon source oxidation/assimilation profiles between the new isolate and the two main species of *Coniochaeta* genus. Moreover, the Biolog™ system findings were more relevant, as they revealed that the physiological profile of *Coniochaeta massiliensis* was closer to *C. hoffmannii* DSM 2693 than to *C. mutabilis* DSM 10716.

In conclusion, the clinical yeast isolate PMML0158 is herein described as *Coniochaeta massiliensis*, a new species that can be easily discriminated from the other species in the *Coniochaeta* genus owing to distinct genomic sequences and chemical and physiological profiles.

Abbreivations

5-FC: 5-fluorocytosine

AMB: amphotericin B

AND: anidulafungin

CAS: caspofungin

DNA: deoxyribonucleic acid

EDX: Energy-Dispersive X-ray Spectroscopy

FL: Fluconazole

GC: Gentamycin and chloramphenicol

IHU Méditerranée Infection: Institut Hospitalo-Universitaire Méditerranée Infection

ITC: Itraconazole

ITS: internal transcribed spacers of the rRNA

SDA: Sabouraud Dextrose Agar

LSU: large subunit of rRNA

MALDI-TOF MS: Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

MICs: Minimum Inhibitory Concentrations

MIF: Micafungin

POS: posaconazole

rRNA: ribosomal ribonucleic acid

SSU: small subunit of rRNA

TEF-1α: partial translation elongation factor 1-alpha gene

TUB2: partial β-tubulin 2 gene

VOR: voriconazole

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The *Coniochaeta massiliensis* holotype is available at the IHU MI (No PMML0158) and IHEM (No 28559) strain collections. The nucleotide sequences are available on GenBank (Accession Numbers: OM366153, OM366268, OM640093, and ON000097). The datasets analysed during the current study are available from the corresponding author on reasonable request

Competing interests: The authors have no conflict of interest to declare regarding this study.

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Data Availability Statement: The *Coniochaeta massiliensis* holotype is available at the IHU MI (No PMML0158) and IHEM (No 28559) strain collections. The nucleotide sequences are available on GenBank (Accession No:

OM366153, ON000097, OM640093, and OM366268). The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Figures

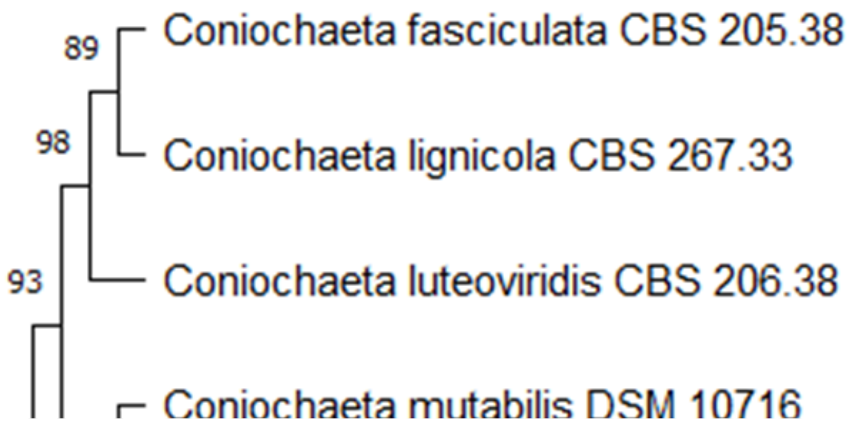

Figure 1

Phylogenetic tree of newly isolated species *Coniochaeta massiliensis* (PMML0158) and 10 reference strains, based on the concatenated ITS, BTUB2 and D1/D2 sequences. *Phialemonium obovatum* CBS 279.76 was used as an outgroup. The maximum parsimony method tree was used using the MEGA 11 software, with 1000 replications bootstrap values.

Figure 2

Culture growth on Sabouraud Dextrose Agar + gentamicin and chloramphenicol after five days of incubation at 25°C. The colour of both recto and verso of the colonies was white/beige to salmon. **A:** *Coniochaeta massiliensis* PMML0158*,* **B:** *Coniochaeta hoffmannii* DSM 2693, **C:** *Coniochaeta mutabilis* DSM 10716.

Figure 3

Morphology of *Coniochaeta massiliensis* (PMML0158). **A-B:** Formation of conidiogenous cells (adelophialides) on hyphae and presence of collarette (arrow), **C-F:** Conidia aggregating in clusters, **G:** Conidia aggregating along the side of the hyphae, **H:** Phialoconidia assembled at the phialide tip, **I-J:** Cylindrical conidia with thin and smooth walls, **K-L:** Wide hyphae. Optical microscopy (Magnification x1000). Scale bars: 50 µm. Scanning Electron Microscopy (15KeV lens mode 4). Scale bars: **J** = 10 µm, **B, F, H** = 30 µm, **D** = 40 µm, **L** = 50 µm.

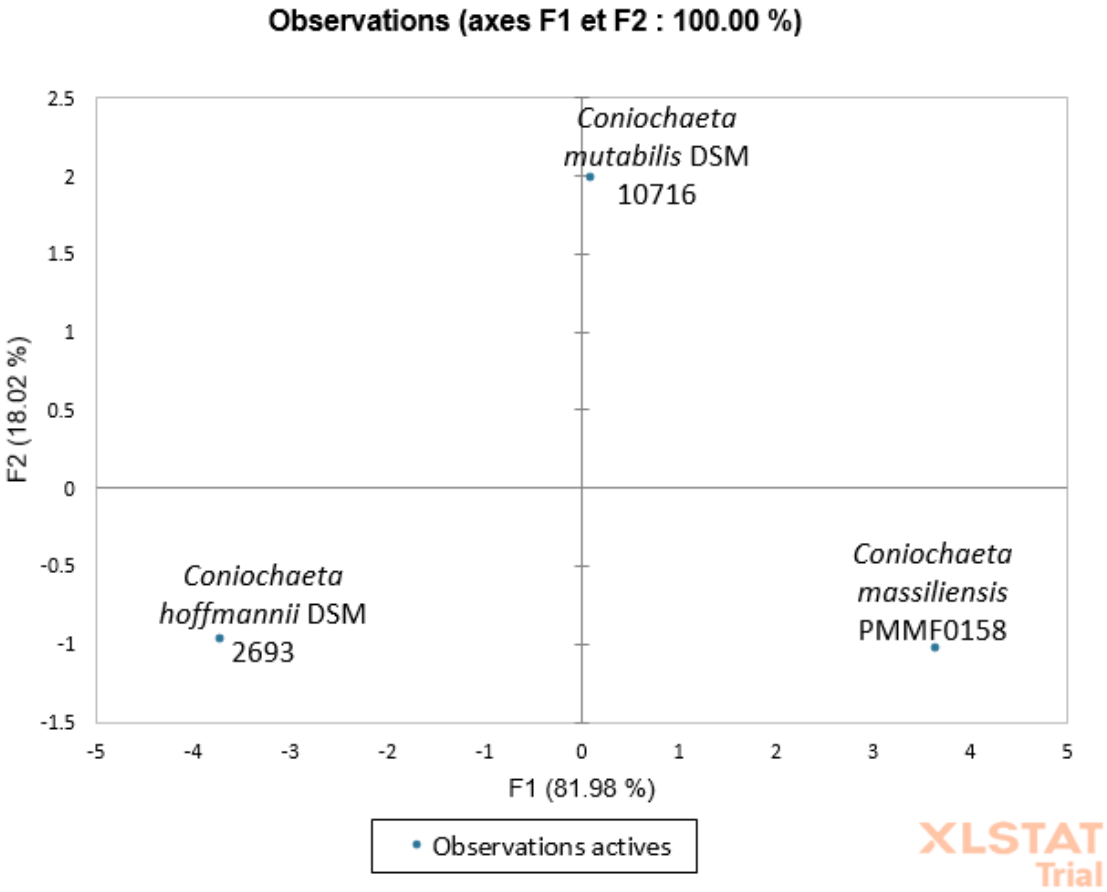

Figure 4

Principal component analysis (PCA) processed with the XLSTAT software of Energy-Dispersive X-ray Spectroscopy chemical mapping profile, performed for the novel species *Coniochaeta massiliensis* (PMML0158) and two reference strains in the genus. The principal components F1 and F2 explained 100% of the chemical mapping profile variance.

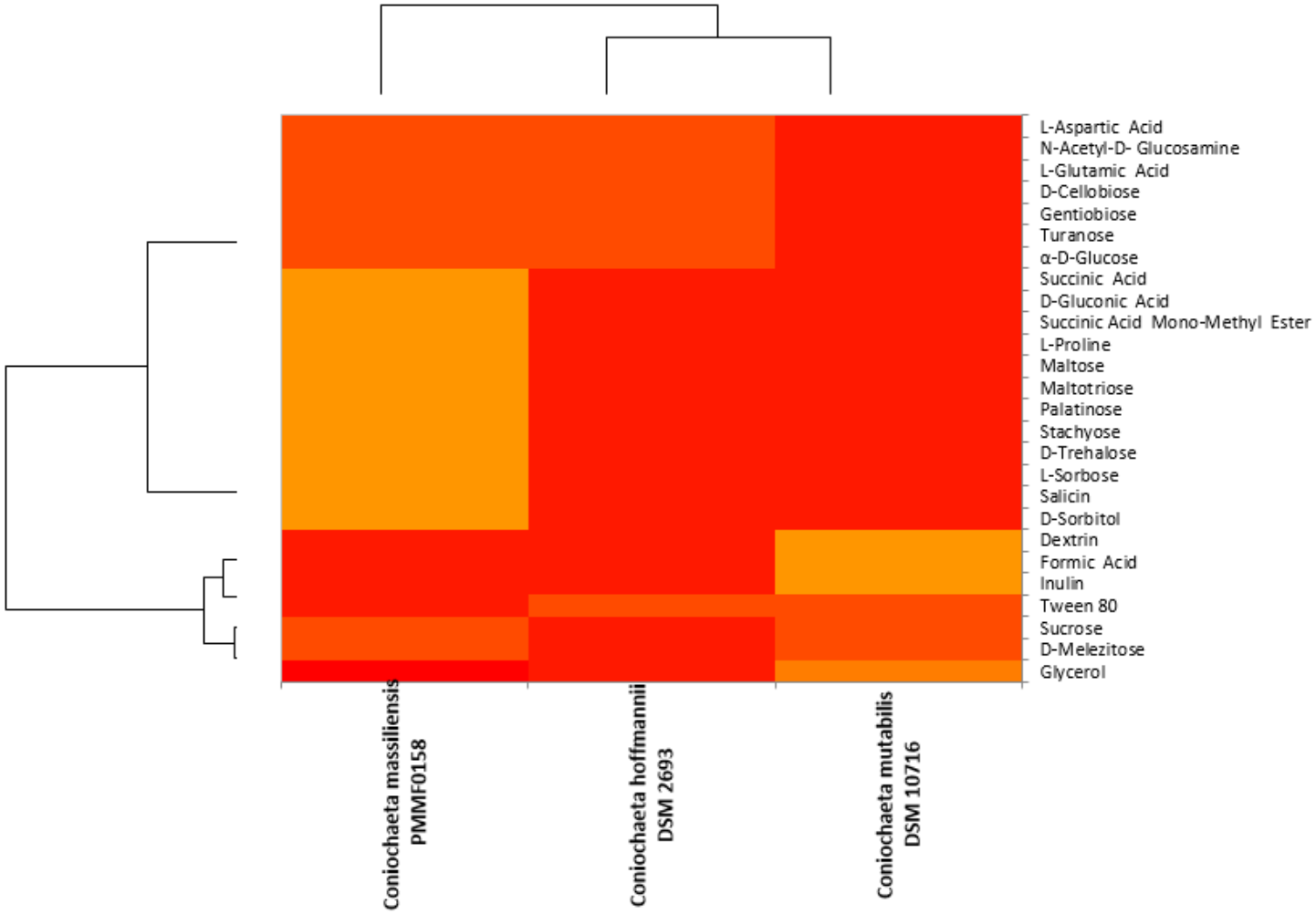

Figure 5

Principal component analysis (PCA) processed with the XLSTAT software of Energy-Dispersive X-ray Spectroscopy chemical mapping profile, performed for the novel species *Coniochaeta massiliensis* (PMML0158) and two reference strains in the genus. The principal components F1 and F2 explained 100% of the chemical mapping profile variance.

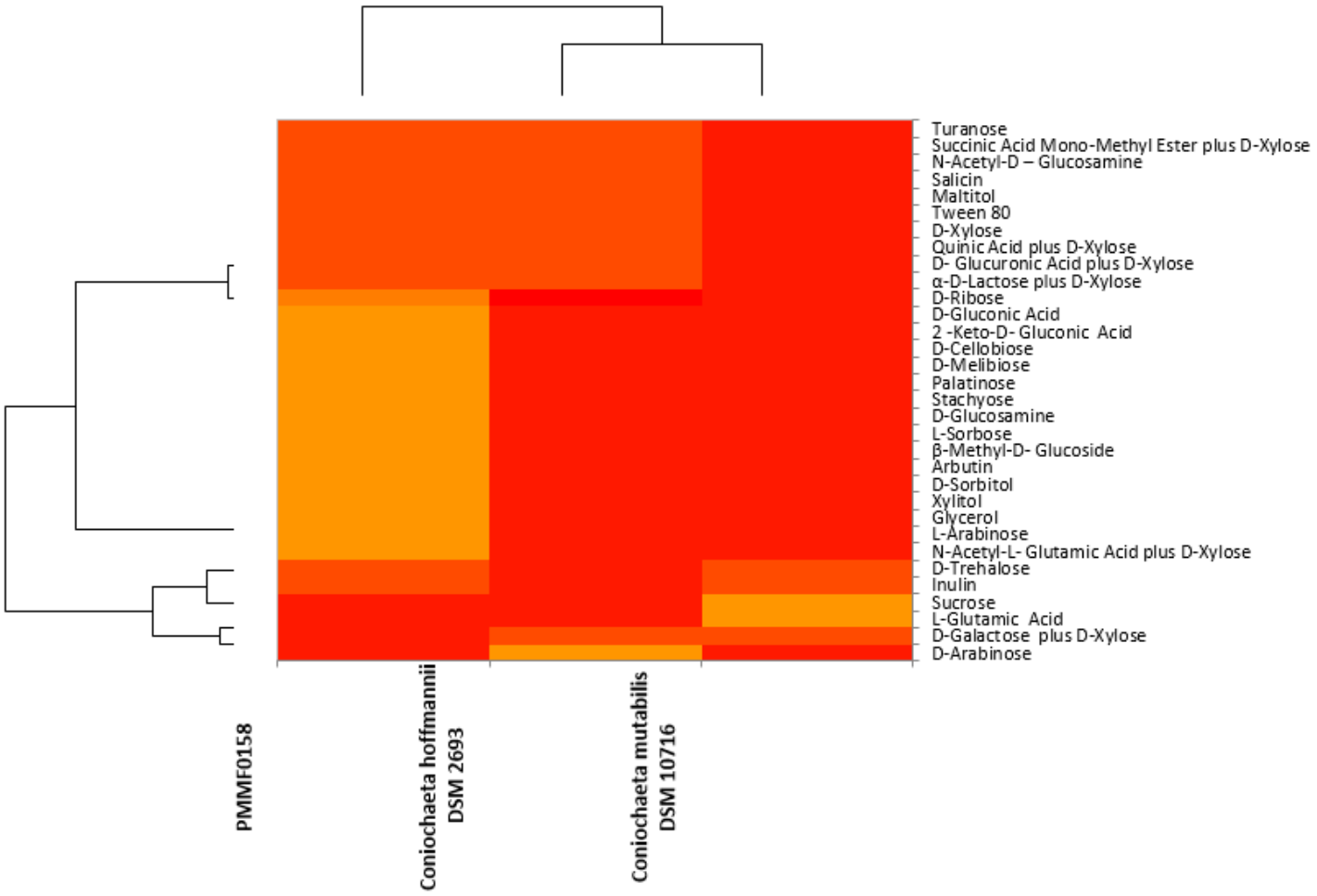

Figure 6

Heat map computed with the XLSTAT software for carbon sources assimilation by Biolog^{TM} system for the novel species *Coniochaeta massiliensis* (PMML0158) and two reference strains. Colour gradient interpretation: the most assimilated substrates are in light orange, and the least assimilated substrate in red.