DOI 10.12905/0380.sydowia74-2022-0287 Published online 21 January 2022

Endophytic *Cephalotrichum* spp. from *Solanum tuberosum* (potato) in Iran – a polyphasic analysis

Nasim Alijani Mamaghani[1], Hossein Saremi[1,*], Mohammad Javan-Nikkhah[1], Sophie De Respinis[2], Elisa Pianta[2], Mauro Tonolla[2]

[1] Laboratory of Mycology, Department of Plant Protection, Faculty of Agricultural Sciences Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj- Iran
[2] Laboratory of Applied Microbiology, Department of Environment Construction and Design, University of Applied Sciences and Arts of Southern Switzerland, Bellinzona, Switzerland

*e-mail: hsn.saremi@ut.ac.ir

Alijani Mamaghani N., Saremi H., Javan-Nikkhah M., de Respinis S., Pianta E. & Tonolla M. (2022) *Endophytic Cephalotrichum* spp. in *Solanum tuberosum* (potato) in Iran - a polyphasic analysis. – Sydowia 47: 287–301.

Thirty-three endophytic fungi isolated from *Solanum tuberosum* (potato) were identified as belonging to the genus *Cephalotrichum*. They were first grouped based on their morphological characters, and molecular identification by multi locus sequencing using ITS, *tub2*, *tef1* and LSU allowed assigning them *Cephalotrichum asperulum*, *C. gorgonifer*, and *C. tenuissimum*. Twenty isolates were also analysed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) and the spectra obtained were used to construct Superspectra for MALDI-TOF MS identification of these species. The remaining thirteen *Cephalotrichum* isolates were then used to validate the SuperSpectra. The concordance of the MALDI-TOF MS results with the phylogenetic analysis was 100 %. *C. asperulum*, *C. gorgonifer*, and *C. tenuissimum* have never been reported as endophytic fungi from any plant species. Regionally, they are also new to Iran, where they apparently have a broad distribution. This study has confirmed the usefulness of MALDI-TOF MS as a quick and comparatively cost-effective tool for the identification of fungi.

Keywords: Endophytes, MALDI-TOF, mass spectrometry, potato, taxonomy.

Solanum tuberosum L. (potato) is an important part of the diet of over 1.5 billion people (Ray et al. 2013). Worldwide, more than 800 million people, of which 50 000 are farmers in Iran, are involved in potato production (Imani et al. 2020). Iran is the fourth-largest potato producer in Asia. After wheat and maize, this crop is an important part of the Iranian diet, but its production cannot meet domestic demand (Dadrasi et al. 2020). *S. tuberosum* is cultivated mainly in the provinces of Ardebil, Hamedan, Esfahan, East Azarbaijan, and South Kerman, with the Ardebil province being the most important production center because of the favorable climate conditions and diverse production resources (Imani et al. 2020).

Endophytic fungi live inside healthy plant tissue without causing any apparent symptoms or damage to their host, and have now been reported from a large number of plants (Petrini 1991). They exhibit high species richness and phylogenetic diversity, and are known from all lineages of plants examined thus far (Oita et al. 2021). All nearly 300 000 known plant species are considered to host at least one endophytic taxon (Strobel & Daisy 2003). Conceptual aspects related to the nature of endophytic life are controversial (Hardoim et al. 2015). On one hand, endophytic associations may bring a vast range of beneficial effects to their host, as this kind of association may enhance immunological host plant defense (Clay 2014), limit pathogens damage (Arnold et al. 2003), modulate the seedling development of host plant by producing plant signal molecules and growth regulators, nutrient acquisition (White et al. 2019), as well as induce stress tolerance to both biotic and abiotic stresses (Lata et al. 2018). Some fungal endophytes may actually be detrimental to their host, causing disease symptoms to the host plant under stress conditions (Schulz & Boyle 2005), or being pathogenic to plant species other than their typical host (Schulz et al. 1998). Overall, the distinction between latent pathogen and mutualistic or neutral endophyte is blurred, because symptomless endophytes may become symptomatic when the host is stressed (Petrini 1991). Some pathogens may have evolved from endophyte ancestors (Carroll 1988) and, in turn, some endophytes could be

single-gene mutant of wild pathogen types (Redman et al. 1999). Thus, some of the fungi thought to be endophytes may actually be latent pathogens (Tadych & White 2019), and conversely potential plant mutualists can become pathogenic for their host. Pathogenicity or mutualism may depend on many factors, including plant and microbial genotype, microbial numbers, and environmental conditions (Hardoim et al. 2015).

The development of next generation sequencing and multiple omics techniques, such as metagenomics and metatranscriptomics approaches have increased our knowledge about the microbial community composition residing within the host plants and their associated functions (Knief 2014). For instance, soil types and plant genotypes have both been shown to play critical roles in the interactions between *Arabidopsis* plants and their root- associated endophytes (Bulgarelli et al. 2012). The culture- dependent techniques might have limitations to capture large amounts of microbes that cannot be cultured on media with known methods. However, it also has its advantages, since diverse microbial endophytes could be isolated, identified and further characterized for their function on the plant separately. Therefore, a lot of studies on investigation of endophytic fungi is being done, based on culture-dependent techniques (Xia et al. 2018, 2019, Pereira et al. 2019).

The endophytic mycobiota of potato are only poorly known. So far, only three studies have dealt with endophytic fungi of *S. tuberosum*. O'Callaghan et al. (2005) studied the microbial communities of *S. tuberosum* and magainin-producing transgenic lines in New Zealand. In Germany, Goetz et al. (2006) examined fungal endophytes from roots of field-grown transgenic T-4 lysozyme producing potatoes and the parental line by traditional isolation and cultivation- independent DNA-based methods. Marak & Kayang (2018) isolated and identified the endophytic fungi associated with *S. tuberosum* in South-West Garo Hills, Meghalaya, India.

So far, mainly only morphological and molecular characters have been used to characterise environmental fungal isolates, including endophytic fungi. Matrix-Assisted Laser Desorption/Ionization Time Of Flight (MALDI-TOF) mass spectrometry (MS) is widely used in diagnostic laboratories for the rapid identification of clinical pathogens (Welker et al. 2019, Tsuchida et al. 2020), including clinically important fungi (Sanguinetti & Posteraro 2017, Lau et al. 2019, Pinheiro et al. 2019, Wilkendorf et al. 2020, Lee et al. 2021), and more recently also food spoilage organisms (Quéro et al. 2019) and indoor air *Penicillium* species (Reboux et al. 2019) have been included in the MALDI-TOF MS databases. Environmental fungi, however, are still underrepresented in these databases (Rahi et al. 2016, Sanitá Lima et al. 2019). Clinical and environmental isolates of *Fusarium* (Triest et al. 2015) and *Trichoderma* (De Respinis et al. 2010), as well as some aquatic hyphomycetes (Cornut et al. 2019) are the notable exceptions and the identification by MALDI-TOF MS of environmental taxa is still problematic (Tsuchida et al. 2020). For instance, *Cephalotrichum* MALDI-TOF reference spectra are completely absent from the Bruker Biotyper Fungi (revision no 3) and VITEK® MS SARAMIS® RUO (version 4.11) libraries. The online available mass spectrometry identification platform (MSI, https://msi.happy-dev.fr; Normand et al. 2017) includes some *Cephalotrichum* strains, but only 24 references exist for *C. gorgonifer*, *C. microsporum*, *C. nanum* and *C. purpureofuscum*.

To our knowledge, *Cephalotricum* spp. have never been reported as endophytes of any plant species, in Iran or elsewhere. In addition, validated MALDI-TOF reference spectra for *Cephalotrichum* are not complete. We thus decided to use a polyphasic approach to characterise the endophytic *Cephalotrichum* isolates, using morphological and molecular data as well as MALDI-TOF MS, with the aim to build up and validate a MALDI-TOF MS library of *Cephalotrichum*.

Materials and methods

Sample collection

Eighty healthy, mature, symptomless potato plants were randomly collected in potato fields in the Ardebil (Northwestern Iran) and South Kerman (Southeastern Iran) provinces in August 2018 and February 2019. The material was stored in paper bags, kept at 4 °C during transportation, and immediately transferred to the laboratory. Surface sterilization and incubation of samples were carried out within 3 days.

Surface sterilization and isolation

Samples of leaf, stem, tuber, and root were cut into small pieces and washed in running tap water for at least 15 minutes. Surface sterilization was carried out according to Goetz et al. (2006). Briefly, samples were immersed in 70 % ethanol for 2 minutes, then in 5 % sodium hypochlorite for 5 minutes, washed in sterile distilled water 3 times for at least

5 minutes, dried between sterile tissues and kept in sterile conditions for at least 15 minutes.

Incubation, isolation, purification and preservation of strains was also based on the protocol by Goetz et al. (2006). Samples were cut into small 25 mm^2 pieces, placed onto potato dextrose agar (PDA, Merck, Germany), supplemented with a mixture of antibiotics (Penicillin G Na 60 mgl^{-1}, Streptomycin Sulphate 80 mgl^{-1}, Tetracycline HCL 50 mgl^{-1}) to suppress bacterial growth, and cultured at 20 °C in the dark for at least one month. Cultures were monitored during one month for fungal growth. Any emerging mycelium was transferred by hyphal tip or single spore isolation onto fresh PDA medium. The pure cultures were preserved on filter papers, and kept at –20 °C.

Morphological identification

Pure cultures were incubated on malt extract agar (MEA, Merck, Germany), PDA, and oatmeal agar (OA, Merck, Germany) at 25 °C in the dark and their growth rates and colony characters were recorded after two weeks (Jiang et al. 2017, Woudenberg et al. 2017a). Morphological descriptions of microscopic characters were done according to current literature (Sandoval-Denis et al. 2016, Jiang et al. 2017, Kiyuna et al. 2017, Woudenberg et al. 2017a, Woudenberg et al. 2017b, Das et al. 2020), and were based on the colonies grown on SNA (Crous et al. 2009) at 25 °C in the dark during 14 days. The microscopic slides were mounted on 85 % lactic acid, and 30 measurements for each structure (×1000 magnification) were carried out. Color chart was used for color descriptions. Identification was carried out according to current literature (Rayner et al. 1970, Woudenberg et al. 2017b); Descriptive statistics [mean, standard deviation, 95 % confidence intervals of the means (95 % CI)] were computed; Measurements are presented as (minimum) lower 95 % CI – mean – upper 95 % CI (maximum). Pure cultures are deposited in the Mycology Laboratory of the University College of Agriculture and Natural Resources, University of Tehran, and The Agricultural Biotechnology Research Institute of Iran (ABRI), Karaj, Iran.

DNA extraction and sequencing

Genomic DNA was extracted from colonies grown on PDA at 25 °C for 7–10 days according to Zhong & Steffenson (2001). Internal transcribed spacer regions 1 and 2 including 5.8 S rDNA (ITS), β-tubulin (*tub2*), translation elongation factor 1α (*tef1*), and Large Subunit of rDNA (LSU) genes were partially amplified using the pair primers ITS1/ITS4, Bt2a/Bt2b, ef1-983F/ef1-2218R, and LROR/LR05 respectively. PCR was performed in the thermocycler (Corbett Research, Australia) in a total volume of 25 µl. The PCR mixtures contained 10–15 ng of genomic DNA, 0.2 µl of each primer, 1 × Taq PCR buffer, 20 µm dNTP, 2 mM $MgCl_2$, and 0.75 µl DMSO and 0.25 U Smart Tag DNA Polymerase (CinnaGen, Iran). The PCR conditions for all genomic regions consisted of an initial denaturation step of 94 °C for 5 min followed by 35 cycles for ITS and LSU, and 40 cycles for *tub2* and *tef1* at 94 °C for 30 s, 57 °C for 30 s (ITS and LSU), or 58 °C for 45 s (*tub2* and *tef1*), and 72 °C for 50 s, and a final elongation step of 10 min at 72 °C was used (Woudenberg et al. 2017b).

PCR products were purified by centrifugation through columns containing Sephadex G-100. Sequencing was performed with primers ITS4, Bt2a

Tab 1. Frequency and distribution of *Cephalotrichum* strains among sampling areas and different parts of potato plants. Percentages reflect the colonisation rate observed over the whole sample (922 isolates).

	C. asperulum N (%)	*C. gorgonifer* N (%)	*C. tenuissimum* N (%)	Frequency compared to total isolates
Ardebil	0	8 (0.8 %)	0	8/248 (3.2 %)
Leaf	0	1 (0.1 %)	0	1/57 (1.75 %)
Stem	0	0	0	0/70 (0 %)
Root	0	0	0	0/81 (0 %)
Tuber	0	7 (0.7 %)	0	7/40 (17.5 %)
South Kerman	3 (0.3 %)	12 (1.2 %)	10 (1 %)	25/674 (3.7)
Leaf	0	0	0	0/150 (0 %)
Stem	0	0	2 (0.2 %)	2/141 (1.4 %)
Root	1 (0.1 %)	2 (0.2 %)	4 (0.4 %)	7/223 (3.1 %)
Tuber	2 (0.2 %)	10 (1 %)	4 (0.4 %)	16/160 (10 %)

Tab 2. Isolates used in this study, and their origin and accession numbers in GenBank, as well as sequences downloaded from GenBank and used for the phylogenetic analysis.

Species	Strain number	source	Country	GeneBank accession no.				Reference
				ITS	*tub2*	*tef1*	LSU	
C. asperulum	CBS 127.22	Seed	Netherlands	LN850959	LN851113	LN851060	LN851006	Jiang et al. 2017
	CBS 215.49	Unknown	Indonesia	KY249250	KY249291	KY249329		Woudenberg et al. 2017a
	CBS 582.71T	Soil	Argentina	LN850960	LN851114	LN851061	KX924027	Woudenberg et al. 2017a
	UT33CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW717893**	**MW732188**	**MW732208**	**MW721022**	**This study**
	UT34CE	***Solanum tuberosum* Root**	**Iran, Kerman**	**MW717894**	**MW732189**	**MW732209**	**MW721023**	**This study**
C. brevistipitatum	CBS 157.57T	Solanum tuberosum	Netherlands	LN850984	LN851138	LN851084	LN851031	Jiang et al. 2017
C. domesticum	CBS 395.67	Indoor, plaster	Netherlands	KY249279	KY249317	KY249359		Woudenberg et al. 2017b
	CBS 142035T	Indoor air, house	Netherlands	KY249280	KY249318	KY249360		Woudenberg et al. 2017b
	CBS 255.50	Mushroom compost	Netherlands	KY249278	KY249316	KY249358		Woudenberg et al. 2017b
	CBS 139.42	Manure	Netherlands	KY249277	KY249315	KY249357		Woudenberg et al. 2017b
C. gorgonifer	CBS 131.08	Unknown	USA	LN850974	LN851128	KY249333	LN851021	Woudenberg et al. 2017b, Jiang et al. 2017
	CBS 877.68	Wheat field soil	Germany	KY249256	KY249297	KY249336		Woudenberg et al. 2017b
	CBS 496.62	Compost ground domestic waste	Italy	KY249255	KY249296	KY249335		Woudenberg et al. 2017b
	CBS 635.78ET	Human hair	Netherlands	LN850977	LN851131	LN851077		Woudenberg et al. 2017b
	UT9CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718110**	**MW732190**	**MW732210**	**MW721024**	**This study**
	UT29CE	***Solanum tuberosum* Tuber**	**Iran, Ardebil**	**MW718111**	**MW732191**	**MW732211**	**MW721025**	**This study**
	UT31CE	***Solanum tuberosum* Leaf**	**Iran, Ardebil**	**MW718112**	**MW732192**	**MW732212**	**MW721026**	**This study**
	UT38CE	***Solanum tuberosum* Root**	**Iran, Kerman**	**MW718113**	**MW732193**	**MW732213**	**MW721027**	**This study**
	UT39CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718114**	**MW732194**	**MW732214**	**MW721028**	**This study**
	UT44CE	***Solanum tuberosum* Tuber**	**Iran, Ardebil**	**MW718115**	**MW732195**	**MW732215**	**MW721029**	**This study**
	UT45CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718116**	**MW732196**	**MW732216**	**MW721030**	**This study**
	UT46CE	***Solanum tuberosum* Root**	**Iran, Kerman**	**MW718117**	**MW732197**	**MW732217**	**MW721031**	**This study**
	UT47CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718118**	**MW732198**	**MW732218**	**MW721032**	**This study**
	UT48CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718119**	**MW732199**	**–**	**MW721033**	**This study**
	UT49CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**–**	**MW732200**	**MW732219**	**MW721034**	**This study**
C. telluricum	CBS 336.32T	Soil	Cyprus	NR154845	KY249325	KY249367	MH866802	Das et al. 2020
	CBS 568.50	Soil	Canada	KY249288	KY249326	KY249368		Woudenberg et al. 2017b
C. tenuissimum	CBS 127792T	Soil	USA	KY249286	KY249324	KY249366	MH876141	Das et al. 2020, Woudenberg et al. 2017b
	UT6CE	***Solanum tuberosum* Root**	**Iran, Kerman**	**MW718242**	**MW732201**	**MW732220**	**MW721036**	**This study**
	UT8CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718243**	**MW732202**	**MW732221**	**MW721037**	**This study**
	UT35CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718244**	**MW732203**	**MW732222**	**MW721038**	**This study**
	UT36CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718245**	**MW732204**	**MW732223**	**MW721039**	**This study**
	UT37CE	***Solanum tuberosum* Tuber**	**Iran, Kerman**	**MW718246**	**MW732205**	**MW732224**	**MW721040**	**This study**
	UT40CE	***Solanum tuberosum* Root**	**Iran, Kerman**	**MW718247**	**MW732206**	**MW732225**	**MW721041**	**This study**
	UT50CE	***Solanum tuberosum* Stem**	**Iran, Kerman**	**–**	**MW732207**	**MW732226**	**MW721042**	**This study**
Wardomyces inflatus	CBS 216.61T	Wood, Acer sp.	Canada: Québec	LM652496	LN851152	LN851098	LN851047	Jiang et al. 2017
	CBS 367.62T			NR146270	LN851153	LN851099	MH869775	Das et al. 2020

and ef1-983F for ITS, *tub2* and *tef1* regions respectively, as well as both LROR and LR05 primers for LSU gene. The mixture for sequencing reaction was performed in a total volume of 10 µl, it contained 2 µl of primer (1 µM), 1 µl of BigDye® Terminator v3.1 Ready Reaction Mix, 1.5 µl of Sequencing buffer 5X and 5 to 20 ng of PCR product. Amplification was performed in a Thermocycler (Veriti 9902, AB Prism) with the following conditions: initial denaturation step of 96 °C for 1 min followed by 25 cycles at 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. After a purification step by centrifugation through columns containing Sephadex G-50, samples were submitted to sequencing in an ABI3500 sequencer (Applied Biosystems, USA).

Molecular identification and phylogenetic analysis

The sequences were uploaded in BLASTn (National Center for Biotechnology Information, NCBI: https://blast.ncbi.nlm.nih.gov/) and compared with already available sequences. Fourteen related and recently published sequences of *Cephalotrichum* species, belonging to the CBS culture collection (Jiang et al. 2017, Woudenberg et al. 2017b, Das et al. 2020) were downloaded from the NCBI database and used as reference sequences in phylogenetic analysis. Overall, the sequences of thirty-four strains (14 CBS strains and 20 endophytic isolates) were included in the phylogenetic analysis. Sequences of two *Wardomyces inflatus* strains were used as outgroup (Tab. 2).

Alignments of the individual and combined genes were done with MEGA 7 (Tamura et al. 2013) using the Clustal W function. A maximum likelihood (ML) phylogenetic tree was constructed using the Tamura-Nei method, and with bootstrap values from 1000 replicates. Nearest-Neighbor-Interchange (NNI) was used as heuristic method for the ML tree, and patterns among lineages were treated as same (homogeneous). Gaps or missing data were treated as complete deletions. Branches with more than 70 % bootstrap values were considered meaningful. All sequences of the endophytic isolates are deposited in GenBank (Tab. 2).

MALDI-TOF MS analysis

Samples were grown at 25 °C for 3 days on Sabouraud, Gentamicin-Chloramphenicol (SGC2, bioMérieux, France) agar plates, supplemented with a semi-permeable cellulose membrane. Inactivation and protein extraction of the samples were carried out as in De Respinis et al. (2014).

Each sample was prepared in quadruplicate. Spectrum acquisition was performed with a VITEK MS RUO mass spectrometer (AXIMA Confidence; bioMérieux, France) equipped with a 50 Hz nitrogen laser (pulse of 3 ns). Mass spectra were collected in positive linear mode in the range of 3,000–20,000 mass-to-charge ratio (m/z) with delayed, positive ion extraction (delay time of 104 ns with a scale factor of 800) and an acceleration voltage of 20 kV. For each analysis, 100 averaged profile spectra were collected and those fulfilling the quality criteria (peak intensity between 20 and 100 mV, cumulative intensity of all 100 spectra >3000 mV, main peaks resolution strictly higher than 600) were processed using the MALDI MS Launchpad 2.9.3 software (bioMérieux, France) with baseline correction, peak filtering, and smoothing procedures.

Peak lists (m/z 3,000 to 20,000) were imported into the VITEK® MS SARAMIS® RUO software (Spectral Archiving and Microbial Identification, Version. 4.1.0.9, bioMérieux, France) for taxonomic analysis, using a proprietary single linkage agglomerative cluster analysis (0.08 % error). SuperSpectra™ for *C. asperulum, C. gorgonifer*, and *C. tenuissimum* were computed and integrated in the SARAMIS® RUO database for future rapid identification of new samples (Erhard et al. 2008). SuperSpectra™ are used for automatic microorganism identification and contain characteristic genus, species, and strain biomarkers that are representative for the respective group of microorganisms. The new *Cephalotrichum* SuperSpectra™ contain between 29 and 43 biomarkers that are present in at least 90 %, 80 % and 70–90 % of the individual mass spectra from, respectively, *C. asperulum, C. gorgonifer*, and *C. tenuissimum*.

Concordance of the MALDI-TOF MS classification of 20 isolates with the identification by molecular methods was checked using classic nonmetric multidimensional scaling (nMDS), after binary transformation of the matrix obtained from the VITEK® MS SARAMIS® RUO software (mass range 3,000–20,000 *m/z*, 0.08 % error), using the Dice similarity coefficient and the stress criterion normalized by distances. For clarity, only the centroids of each taxon are presented in the graphical display of the first 2 dimensions. All calculations and graphics have been carried out using Stata SE v. 16 (StataCorp, College Station, Texas, USA).

Fifteen additional endophytic isolates were used to validate the SuperSpectra™ created with the spectra obtained from the 20 *Cephalotrichum* isolates used in the first MALDI-TOF MS run.

Tab 3. Measurements of microscopic features (Metulae, conidiogenous cell, conidia) on SNA in µm.

Species	synnemata	Metulae	Conidiogenous cell	Conidia	Reference
C. asperulum	300–750	(2)3–3.2–3.4(4.5) × (5)7–7.4–7.8(11)	(2)2.8–2.9–3(3.5) × (6.5)7.5–7.9–8.2(10)	(3)3.7–3.9– 4(5) × (5.5)6.3–6.5–6.7(7)	Sandoval-Denis et al. 2016
C. gorgonifer	510–930	(3.5)4.2–4.4–4.7(7) × (5.5)7–7.5–8(10)	(2)2.9–3–3.1(4) × (5)6.5–6.8–7.1(10)	(3)3.4–3.6–3.7(4) × (5)5.3–5.5– 5.6(6)	Sandoval-Denis et al. 2016
C. tenuissimum	650–1100	(2.5)4–4.4–4.6(5.5) × (6)7.4–7.8–8.2(11)	(2.5)3.2–3.4–3.5(4) × (5)7.5–8.1–8.7(11)	(2.5)3.4–3.6–3.8(5) × (4.5)5.8–6.0–6.4(8.50)	Woudenberg, et al. 2017b

Results

Phylogeny based on multilocus sequencing

During this large-scale investigation on endophytic fungi of potato plants in Iran, of 922 endophytic fungal isolates obtained from different parts of the potato plants, 33 of them (3.57 %) could be identified as belonging to the genus *Cephalotrichum* Link (= *Doratomyces* Corda). Of these, eight originated from Ardebil and 25 from the south Kerman province; most of them were isolated from the tubers (Tab. 1). Morphologically, they could be identified as *C. asperulum*, *C. gorgonifer* and *C. tenuissimum*. *C. gorgonifer* was the most frequent species and was recovered from both sampling areas, whereas *C. asperulum* and *C. gorgonifer* were reported only from the Ardebil province.

The combined sequences alignment consists of 2453 characters including gaps (529 characters belong to ITS, 463 to *tub2*, 670 to LSU, and 791 to *tef1*). Maximum Likelihood (ML) and Neighbor Joining trees were similar to each other in topology and only the ML tree is presented here (Fig. 1). The sequences obtained from NCBI (CBS strains) clustered in 6 well- supported monophyletic clades, which corresponded to the six species *C. gorgonifer*, *C. telluricum*, *C. asperulum*, *C. brevistipitatum*, *C. domesticum*, and *C. tenuissimum*. The twenty endophytic strains of *Cephalotrichum* obtained from potato clustered in three different monophyletic clades with more than 98 % bootstrap supports.

Concordance between sequencing and MALDI-TOF MS analysis

The nMDS plot obtained with the MALDI-TOF MS data showed 100 % concordance (Fig. 2). In both nMDS and molecular analysis, isolate UT37 did not cluster closely with the other strains of *C. tenuissimum*, possibly indicating some sort of intraspecific variability.

Description of the Iranian endophytic isolates

Cephalotrichum asperulum (J.E. Wright S. Marchand) Sand-Den., Guarro Gené 2016. – (Fig. 3)

D e s c r i p t i o n . – Colonies on MEA, PDA, and OA 43, 31, 40 mm diameters. On SNA, synnemata pale brown and clavate; setae in the head absent. *Echinobotryum*-like synanamorph absent. The size of synnemata of the isolates was similar to that of the Type (120- 1000 µm), but conidia were somewhat larger (3–4 × 5–8.5 µm). Conidiogenous cells ampulliform, conidia oval to ellipsoidal, slightly verrucous and some of them smooth; they were apically pointed with a truncate base but their surface was finely and not coarsely roughened as in the Type description. Sexual morph not observed. Microscopic measurements are presented in Tab. 3.

In the phylogenetic analysis, BRIICC 10324 (UT33) and BRIICC 10325 (UT34) clustered in a monophyletic clade with other CBS isolates of *C. asperulum* with 99 % bootstrap value and are entirely distinct from the *C. telluricum* and *C. brevistipitatum* CBS isolates (Fig. 1). MALDI-TOF MS results also show a similar clustering of the isolates (Fig. 2).

Cephalotrichum gorgonifer **(**Bainier) Sand.-Den., Gené Guarro 2016 (Fig. 4)

D e s c r i p t i o n . – Colonies on MEA, PDA, and OA 49, 51, 64 mm diameter respectively. On SNA, synnemata with flexuous, spirally coiled and unbranched 3–5 µm wide setae. *Echinobotryum*-like synanamorph present. Conidiogeous cells ampulli-

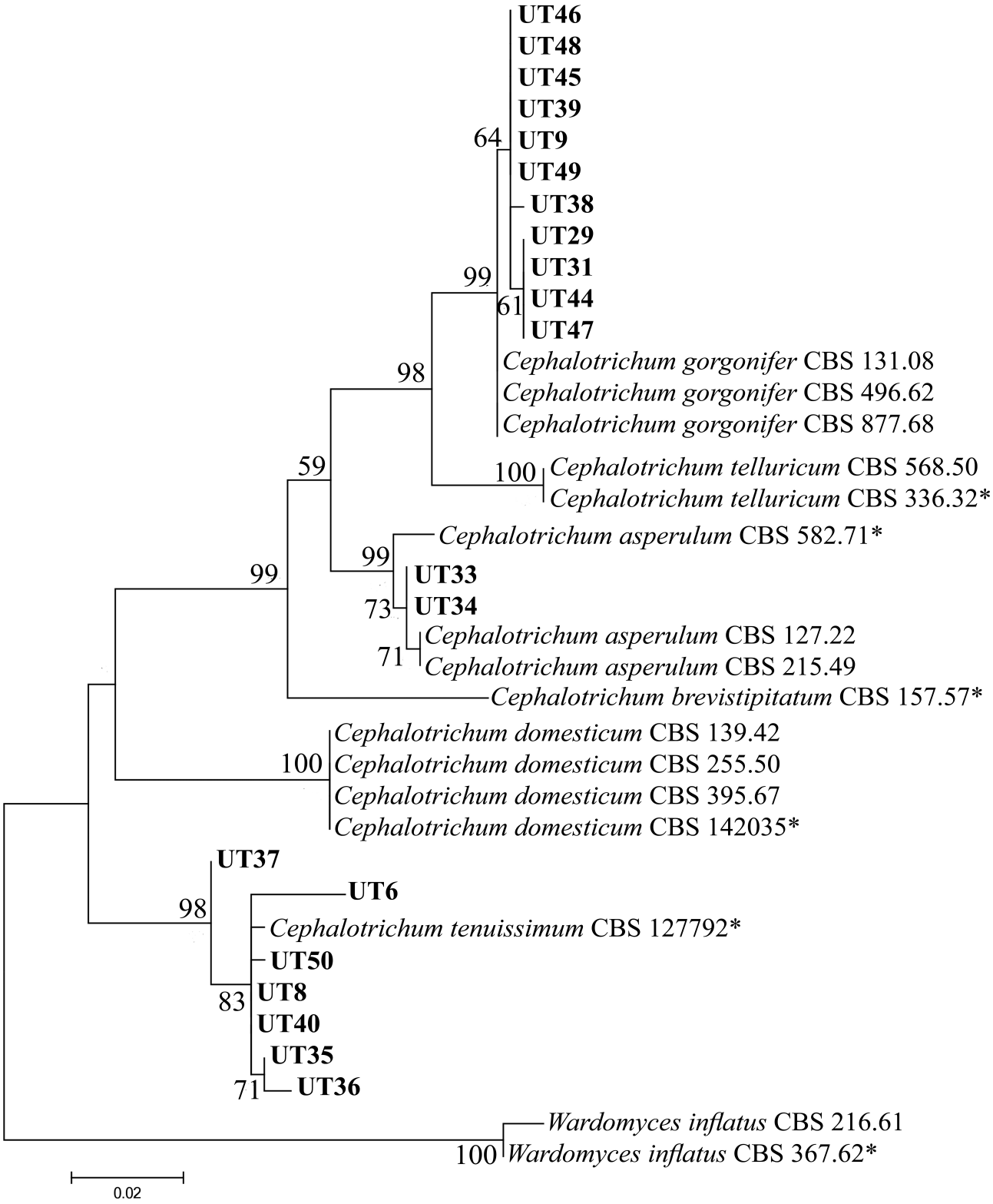

Fig. 1. Phylogenetic tree inferred from the Maximum likelihood analysis based on ITS, LSU, *EF-1α* and TUB2 combined sequences. Ex-type strains are marked with an asterisk. *Wardomyces inflatus* strains CBS 216.61T and CBS 367.62T were used as outgroups. Bootstrap values >50 % are shown above the branches obtained from 1000 replicates. The strains originating from Iran (this study) are indicated in bold.

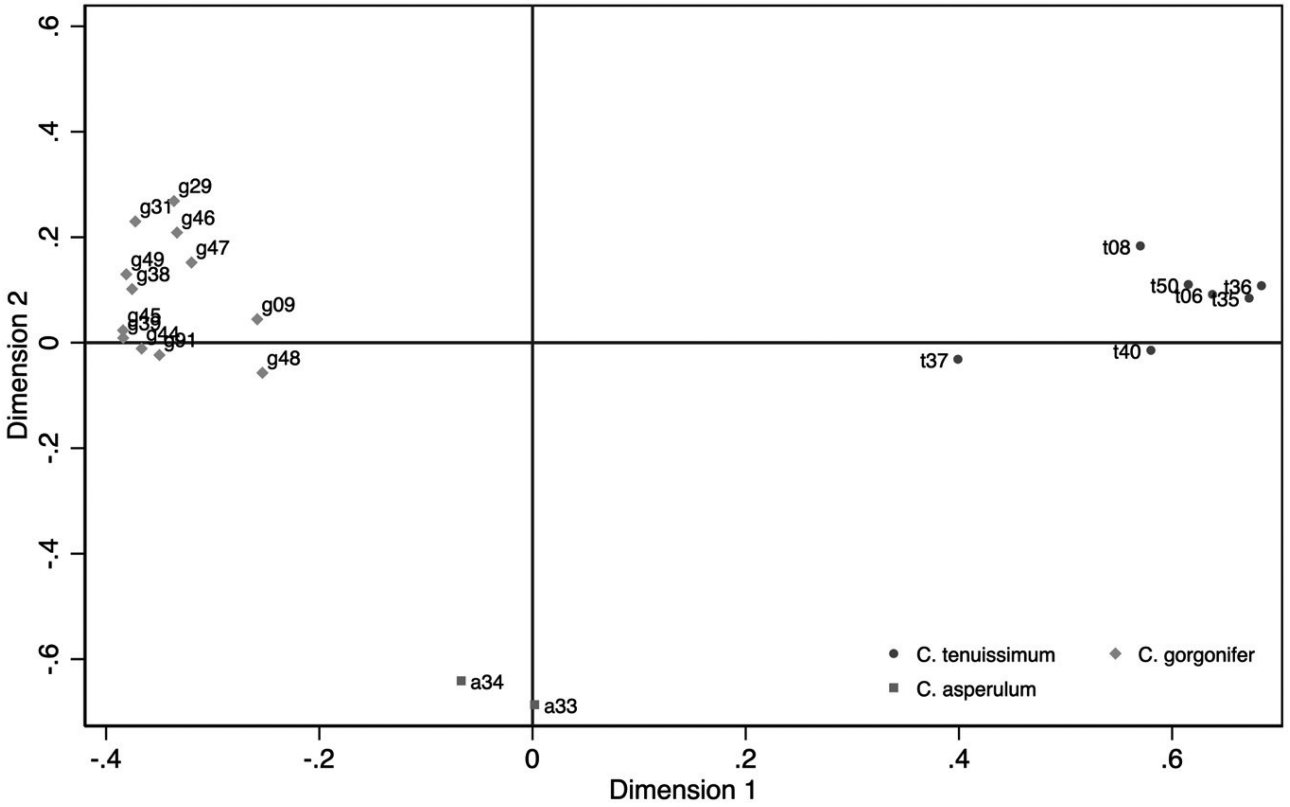

Fig. 2. Results of the nonmetric Multidimensional Scaling of the MALDI-TOF MS analysis carried out on the 20 isolates used for the creation of SuperSpectra™. Kruskal loss: 0.30.

form. Conidia oval to ellipsoidal, smooth, with truncate base and rounded apex. Synnemata and conidia sizes were exactly the same as for the Ex-epitype (500–1000, and 2.5–4 × 4–8 µm), but conidia were slightly shorter. Sexual morph not observed. Microscopic measurements are presented in Tab. 3.

In the phylogenetic analysis, strains UT 9, 29, 31, 38, 39, 44, 45, 46, 47, 48 and 49 clustered in a monophyletic clade with other CBS isolates of *C. gorgonifer* with 99 % bootstrap value and are entirely distinct from the *C. telluricum* CBS isolates (Fig 1). Strains UT 29, 31, 44 were isolated form the leaf and tuber of potato plants in Ardebil province (north part of Iran). These three strains, together with UT 47, originated from tuber in south Kerman, formed a monophyletic group in the *C. gorgonifer* clade. The same clustering was observed in the MALDI-TOF MS analysis (Fig. 2).

Cephalotrichum tenuissimum Woudenb. Seifert 2017 (Fig. 5)

Description. – Colonies on MEA, PDA, and OA 30, 22, and 37 mm diameter. On SNA, synnemata, Setae in the head absent. Metulae normally cylindrical, conidiogenous cells ampulliform. Conidia smooth, oval to ellipsoidal, thick-walled with truncate base and rounded apex. Synnemata, conidiogenous cells, and conidia were somewhat larger than in the Type (495–900, 2.5–3.5 × 5–8.5, and 3–4 × 4.5–6.5 µm respectively) and some of the conidia were oval, and never pale green unlike in the Type description. *Echinobotryum*-like synanamorph and sexual morph not observed. Microscopic measurements are presented in Tab. 3.

In the phylogenetic analysis, strains UT 6, 8, 35, 36, 37, 40, and 50 clustered in a monophyletic clade with the CBS type strain of *C. tenuissimum* with 98 % bootstrap value and are entirely distinct from the *C. domesticum* CBS isolates. (Fig. 1). The *C. tenuissimum* isolates were also grouped after MALDI-TOF MS analysis (Fig. 2).

Validation of the MALDI-TOF MS identification

To validate the created SARAMIS SuperSpectra™ and to allow future *Cephalotrichum* identifications using MALDI-TOF MS, fifteen unknown endo-

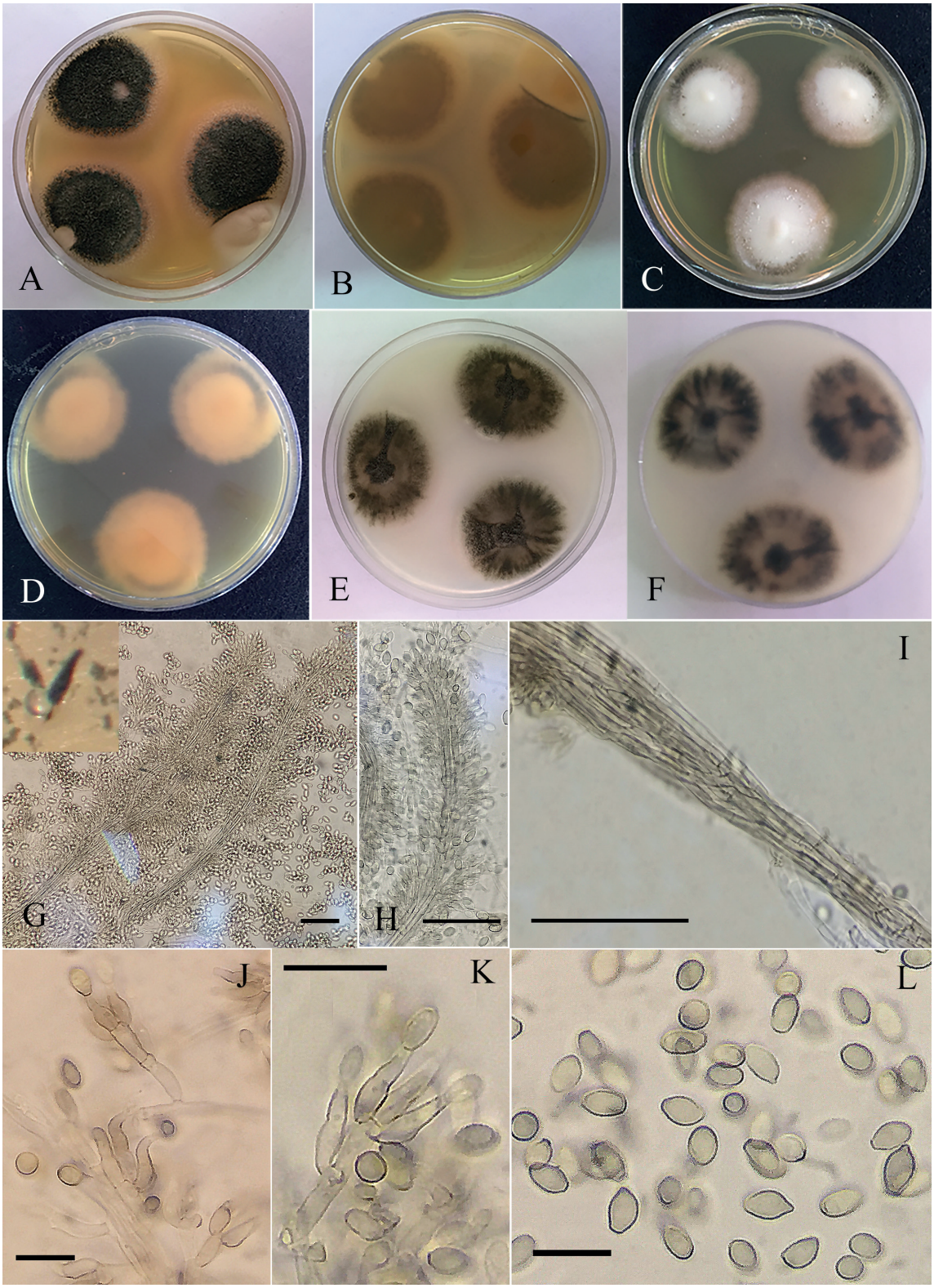

Fig. 3. *Cephalotrichum asperulum*. **A–F**. Colony on MEA, PDA and OA incubated at 25 °C for 14 days respectively (front and reverse). **G**. Synnemata. **H.** Head. **I.** Stipe. **J** and **K.** Conidiophores, metula, and conidiogenous cells. **L.** Conidia. Scale bars G–I: 50 µm, and J–L: 10 µm.

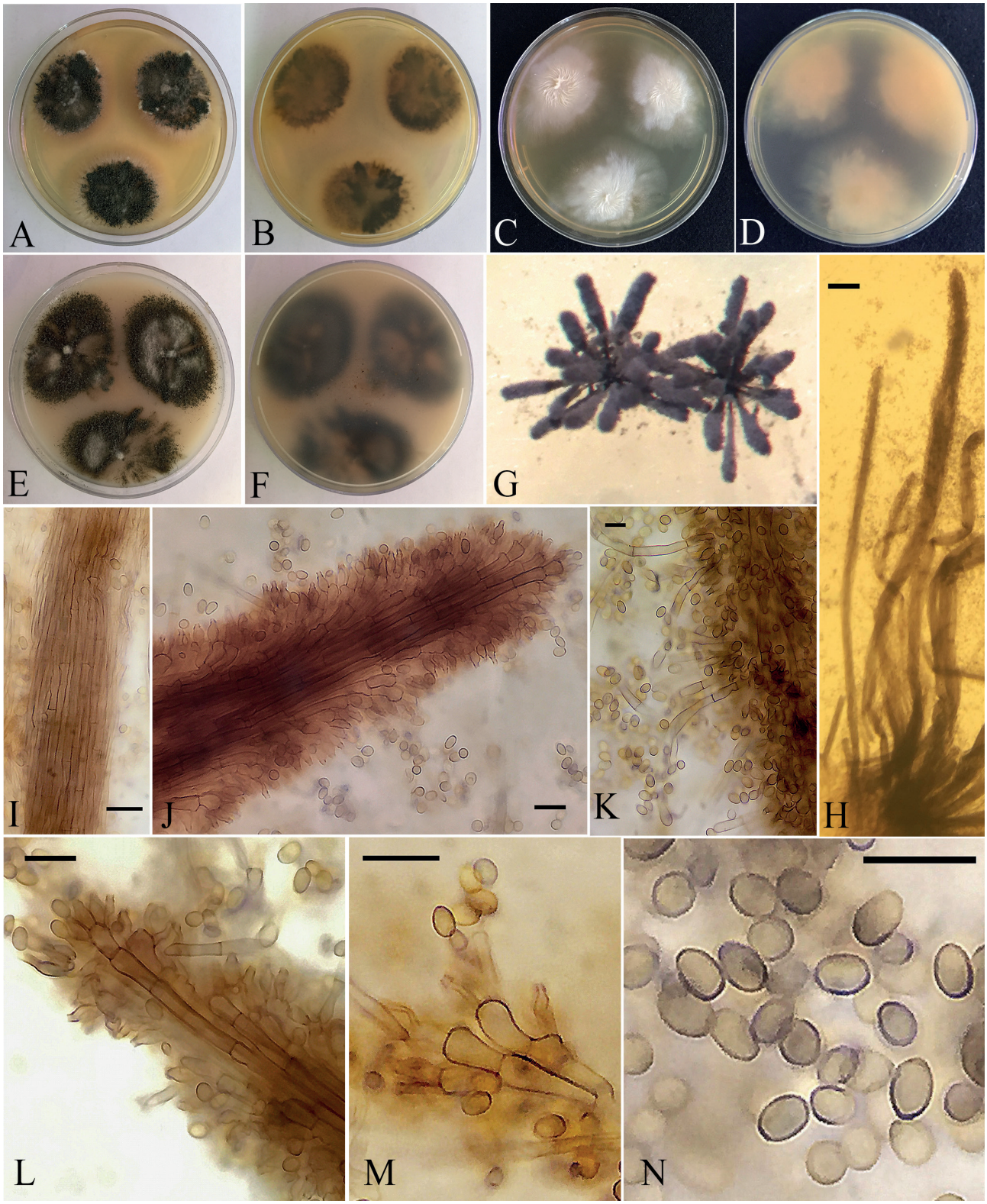

Fig. 4. *Cephalotrichum gorgonifer* . **A–F**.Colony on MEA, PDA and OA incubated at 25 °C for 14 days respectively (front and reverse). **G** and **H**. Synnemata. **I.** Stipe. **J.** Head. **K.** Sterile coiled hyphae. **L** and **M.** Conidiophores, metula, and conidiogenous cells. **N.** Conidia. Scale bars H: 50 µm, I–N: 10 µm.

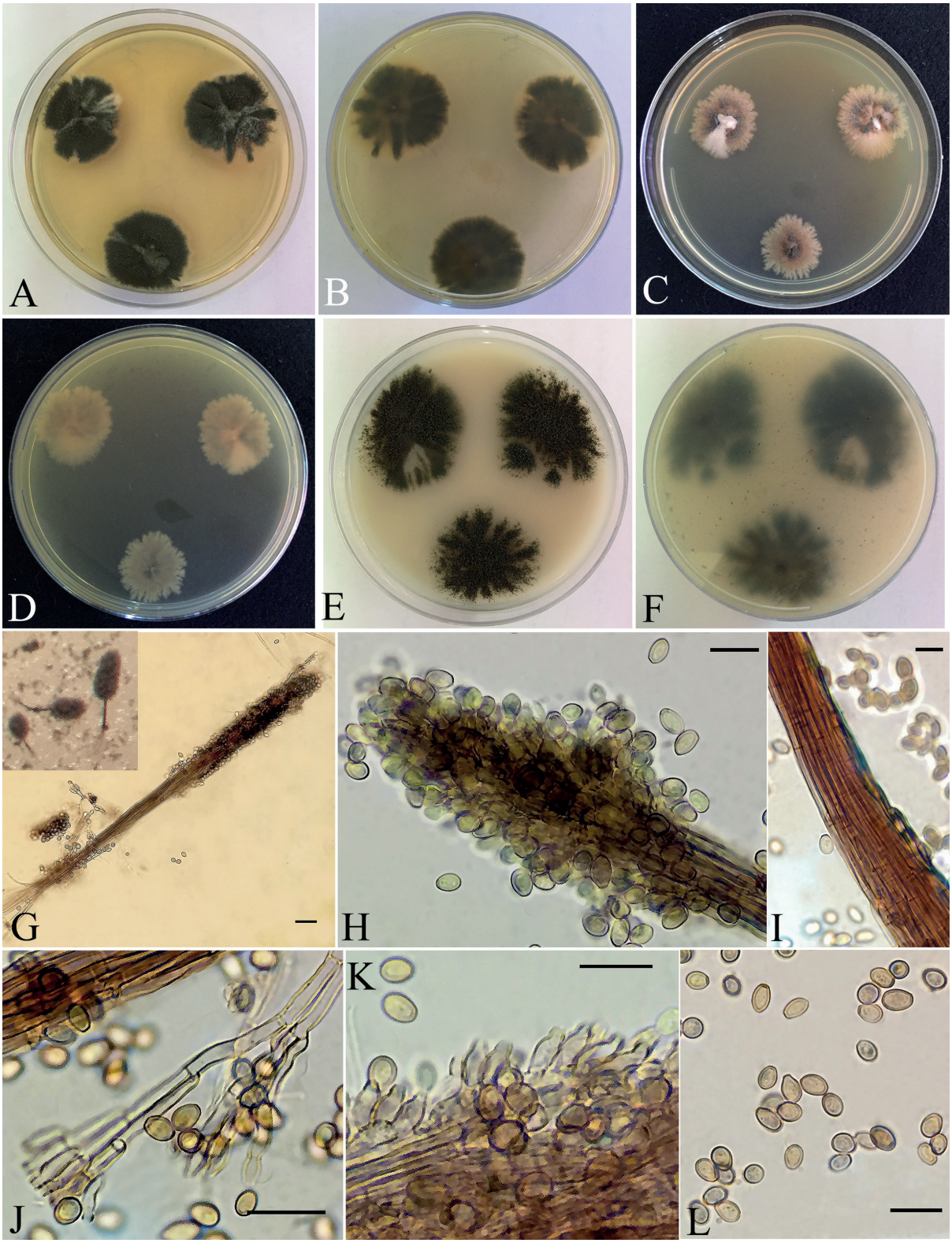

Fig. 5. *Cephalotrichum tenuissimum*. **A-F.** Colony on MEA, PDA and OA incubated at 25 °C for 14 days respectively (front and reverse). **G.** Synnemata. **H.** Stipe. **I.** Head. **J** and **K.** Conidiophores, metulae, and conidiogenous cells. **L.** Conidia. Scale bars G: 50 µm, H–L: 10 µm.

phytic fungi were submitted to a blind MALDI-TOF MS identification. One was identified as *C. asperulum* (90 % similarity with the corresponding SuperSpectrum™), 9 belonged the *C. gorgonifer* species (all of them with 99.9 % similarity), and 3 to *C. tenuissimum* (99.9 % similarity). Two isolates could not be identified as belonging to any of the three species, and after submission to the online MSI database, they were identified as *Petriella sordida*. The MALDI-TOF MS identification of the thirteen *Cephalotrichum* isolates was subsequently confirmed by ITS sequencing, with 100 % concordance between MALDI-TOF MS analysis and sequencing.

Discussion

Cephalotrichum spp. as endophytes

In this study, twenty strains belonging to *Cephalotrichum* were identified, based on their morphology and multilocus sequencing using ITS, *tub2*, *tef1* and LSU gene segments. *C. gorgonifer* was the most important species recorded from both sites and more frequently from the south Kerman province. *C. asperulum* and *C. tenuissimum* ultimately originated from potato plants in south Kerman province, thus they are apparently most common in dry and hot climates, whereas *C. gorgonifer* is observed in different climate conditions.

This is the first report of *C. asperulum*, *C. gorgonifer* and *C. tenuissimum* as endophytic fungi in general and from potato plants in particular, and at the same time they are new records for Iran mycobiota. Most *Cephalotrichum* species occur on decaying plant material, straw, dung, wood, soil, and are infrequently reported from the indoor or built environment (Woudenberg et al. 2017b). Some of them have been isolated from agricultural soil, cones and decayed wood of white spruce, or soil under elm wood (Das et al. 2020), but they have never been reported from living plants as endophytic fungi. There is just one report of *Doratomyces* sp. as fungal pathogen in potato causing "speck rot" disease (Farr et al. 1989), but for instance, O'Callaghan et al. (2005) did not report any *Cephalotrichum* from their investigations. Likewise, Goetz et al. (2006) isolated 63 fungal taxa, with *Verticillium dahliae*, *Cylindrocarpon destructans*, *Colletotrichum coccodes,* and *Plectosporium tabacinum* as the most frequent endophytes, but they did not report any species of *Cephalotrichum*. Similarly, Marak & Kayang (2018) reported also important potato phytopathogens such as *Alternaria solani*, *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii*, but no species of *Cephalotrichum*.

Cephalotrichum species are not known to produce mycotoxins, but they are involved in cellulose degradation, xylan decomposition (Domsch et al. 2008), and in general in the wood decay process (Nilsson 1973). Thus, *Cephalotrichum* species may behave like endophytic species of *Colletotrichum*, *Corynespora*, *Curvularia*, *Nodulisporium*, *Robillarda* and xylariaceous endophytes, which produce alkaline protease; many of these are also positive for acidic proteases (Petrini 1995, Whalley 1996, Suryanarayanan et al. 2011, Osono et al. 2013, U'Ren et al. 2019), and appear to inhabit both living and non-living plant tissues, with potentially important roles as saprotrophs. Endophytic *Colletotrichum*, *Corynespora*, *Curvularia*, *Nodulisporium*, *Robillarda* and xylariaceous endophytes persist in leaf litter, reflecting their ability to decompose lignocellulose and the capacity of some species to colonize decaying leaves (U'Ren et al. 2019). Therefore, endophytic *Cephalotrichum,* like these fungi, may be dormant and wait for their host to senesce to eventually start decomposition of its materials (Davis et al. 2003), to ultimately become active saprobes as soon as the tissues they have colonized as endophytes become senescent (Rajulu et al. 2013). As lignin is a major structural component often limiting decomposition, these ligninolytic endophytes are of particular importance in terms of their role in carbon turnover and nutrient cycling in ecosystems and deserve further studies on their ecology and functioning (Koide et al. 2005, Osono 2011, Osono & Hirose 2011).

Validation of the MALDI-TOF MS database for *Cephalotrichum*

The *Cephalotrichum* species isolated could be added to the existing MALDI-TOF MS database. The *C. asperulum*, *C. gorgonifer* and C. *tenuissimum* MALDI-TOF mass spectra were clearly separated according to their taxonomic position (Fig. 2) and could thus be used to construct MALDI-TOF SuperSpectra™ for further identification of isolates belonging to these three taxa. For the validation of the *Cephalotrichum* SuperSpectra™, thirteen isolates were blindly analysed by MALDI-TOF MS, and their identification was checked by ITS sequencing. The results gave a 100 % concordance between sequencing and MALDI-TOF MS identification. In addition, the percentages of similarity between SuperSpectra™ and the MALDI-TOF MS mass spectra of the unknown isolates were above 90 % for all thirteen isolates.

Inclusion of additional *Cephalotrichum* species in the MALDI-TOF MS database is now needed to complete the database and the available SuperSpectra™ must be validated against additional isolates. Overall, however, MALDI-TOF MS has been confirmed to be a reliable, time-saving and cost-effective method that can be used also for the identification of environmental fungal taxa.

Acknowledgments

This research was supported financially by the Iran National Science Foundation (INSF) with the project number: 98015699. We thank the University of Tehran and the University of Applied Sciences and Arts of Southern Switzerland for providing facilities for us to conduct this research.

References

Arnold A.E., Mejía L.C., Kyllo D., Rojas E.I., Maynard Z., Robbins N., Herre E.A. (2003) Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* 100(26): 15649–15654. https://doi.org/10.1073/pnas.2533483100.

Bulgarelli D., Rott M., Schlaeppi K., Ver Loren van Themaat E., Ahmadinejad N., Assenza F., Rauf P., Huettel B., Reinhardt R., Schmelzer E., Peplies J., Gloeckner F.O., Amann R., Eickhorst T., Schulze-Lefert P. (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488(7409): 91–95. https://doi.org/10.1038/nature11336.

Carroll G. (1988) Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. *Ecology* 69(1): 2–9. https://doi.org/10.2307/1943154.

Clay K. (2014) Defensive symbiosis: A microbial perspective. *Functional Ecology* 28(2): 293–298. https://doi.org/10.1111/1365-2435.12258.

Cornut J., De Respinis S., Tonolla M., Petrini O., Bärlocher F., Chauvet E., Bruder A. (2019) Rapid characterization of aquatic hyphomycetes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Mycologia* 111(1): 177–189. https://doi.org/10.1080/00275514.2018.1528129.

Crous P.W., Cock A.W.A.M. de, CBS-KNAW Fungal Biodiversity Centre (2009) *Fungal biodiversity*. CBS-KNAW Fungal Biodiversity Centre. Utrecht.

Dadrasi A., Torabi B., Rahimi A., Soltani A., Zeinali E. (2020) Parameterization and evaluation of a simple simulation model (ssm-icrop2) for potato (*solanum tuberosum* L.) growth and yield in Iran. *Potato Research* 63(4): 545–563. https://doi.org/10.1007/s11540-020-09456-y.

Das K., You Y.-H., Lee S.-Y., Jung H.-Y. (2020) A new species of *Thelonectria* and a new record of *Cephalotrichum hinnuleum* from Gunwi and Ulleungdo in Korea. *Mycobiology* 48(5): 341–350. https://doi.org/10.1080/12298093.2020.1807454.

Davis E.C., Franklin J.B., Shaw A.J., Vilgalys R. (2003) Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: Phylogenetics, distribution, and symbiosis. *American Journal of Botany* 90(11): 1661–1667. https://doi.org/10.3732/ajb.90.11.1661.

De Respinis S., Monnin V., Girard V., Welker M., Arsac M., Celliere B., Durand G., Bosshard P.P., Farina C., Passera M., Van Belkum A., Petrini O., Tonolla M. (2014) Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry using the vitek ms system for rapid and accurate identification of Dermatophytes on solid cultures. *Journal of Clinical Microbiology* 52(12): 4286–4292. https://doi.org/10.1128/JCM.02199-14.

De Respinis S., Vogel G., Benagli C., Tonolla M., Petrini O., Samuels G.J. (2010) MALDI-TOF MS of *Trichoderma*: A model system for the identification of microfungi. *Mycological Progress* 9(1): 79–100. https://doi.org/10.1007/s11557-009-0621-5.

Domsch K.H., Gams W., Anderson T.-H., Seifert K.A. (2008) Compendium of soil fungi. *European Journal of Soil Science* 59(5): 1007–1007. https://doi.org/10.1111/j.1365-2389.2008.01052_1.x.

Erhard M., Hipler U.-C., Burmester A., Brakhage A.A., Wöstemeyer J. (2008) Identification of dermatophyte species causing onychomycosis and tinea pedis by MALDI-TOF mass spectrometry. *Experimental Dermatology* 17(4): 356–361. https://doi.org/10.1111/j.1600-0625.2007.00649.x.

Farr D.F., Bills G.F., Chamuris G.P., Rossman A.Y. (1989) *Fungi on Plants and Plant Products in the United States*. APS, St Paul.

Goetz M., Nirenberg H., Krause S., Wolters H., Draeger S., Buchner A., Lottmann J., Berg G., Smalla K. (2006) Fungal endophytes in potato roots studied by traditional isolation and cultivation-independent DNA-based methods: Fungal endophytes in potato roots. *FEMS Microbiology Ecology* 58(3): 404–413. https://doi.org/10.1111/j.1574-6941.2006.00169.x.

Hardoim P.R., van Overbeek L.S., Berg G., Pirttilä A.M., Compant S., Campisano A., Döring M., Sessitsch A. (2015) The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* 79(3): 293–320. https://doi.org/10.1128/MMBR.00050-14.

Imani B., Allahyari M.S., Bondori A., Emami N., El Bilali H. (2020) Adoption of organic potato production in Ardabil plain, Iran: An application of the extended theory of planned behaviour. *Potato Research* 64(2): 177–195. https://doi.org/10.1007/s11540-020-09471-z.

Jiang J.R., Cai L., Liu F. (2017) Oligotrophic fungi from a carbonate cave, with three new species of *Cephalotrichum*. *Mycology* 8(3): 164–177. https://doi.org/10.1080/21501203.2017.1366370.

Xu J., Wu Y.M., Zhang Y.L., Liu H.M., Pan H.Q., Zhang T.Y. (2011) Studies on *Cephalotrichum* from soils in China—Twelve new species and two new combinations. *Mycotaxon* 117(1): 207–225. https://doi.org/10.5248/117.207.

Kiyuna T., An K.-D., Kigawa R., Sano C., Sugiyama J. (2017) Noteworthy anamorphic fungi, *Cephalotrichum verrucisporum*, *Sagenomella striatispora*, and *Sagenomella griseoviridis*, isolated from biodeteriorated samples in the Takamatsuzuka and Kitora Tumuli, Nara, Japan. *Mycoscience* 58(5): 320–327. https://doi.org/10.1016/j.myc.2017.02.003.

Knief C. (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. *Frontiers in Plant Science* 5: 216. https://doi.org/10.3389/fpls.2014.00216.

Koide K., Osono T., Takeda H. (2005) Colonization and lignin decomposition of *Camellia japonica* leaf litter by endophytic fungi. *Mycoscience* 46: 280–286. https://doi.org/10.1007/s10267-005-0247-7.

Lata R., Chowdhury S., Gond S.K., White J.F. (2018) Induction of abiotic stress tolerance in plants by endophytic microbes. *Letters in Applied Microbiology* 66(4): 268–276. https://doi.org/10.1111/lam.12855.

Lau A. F., Walchak R. C., Miller H.B., Slechta E.S., Kamboj K., Riebe K., Robertson A.E., Gilbreath J.J., Mitchell K.F., Wallace M.A., Bryson A.L., Balada-Llasat J.-M., Bulman A., Buchan B.W., Burnham C.-A.D., Butler-Wu S., Desai U., Doern C.D., Hanson K.E., Frank K.M. (2019) Multicenter study demonstrates standardization requirements for mold identification by MALDI-TOF MS. *Frontiers in Microbiology* 10: 2098. https://doi.org/10.3389/fmicb.2019.02098.

Lee H., Oh J., Sung G.-H., Koo J., Lee M.-H., Lee H.J., Cho S.-I., Choi J. S., Park Y.-J., Shin J.H., Lee H.K., Kim S.-Y., Lee C.H., Kim Y.R., Sohn Y.-H., Kim W.J., Ryu S.W., Lee N.Y., Huh H.J., Kim J. (2021) Multilaboratory evaluation of the MALDI-TOF mass spectrometry system, microidsys elite, for the identification of medically important filamentous fungi. *Mycopathologia* 186(1): 15–26. https://doi.org/10.1007/s11046-020-00507-z.

Marak M.C.N., Kayang H. (2018) Isolation and identification of endophytic fungi associated with *Solanum tuberosum* l. Of south-west Garo Hills, Meghalaya. *International Journal of Advances in Agricultural Science and Technology (IJAAST)* 5(1): 58–65.

Nilsson T. (1973) *Studies on wood degradation and cellulolytic activity of microfungi*. Report 104(104): 40–40. https://pub.epsilon.slu.se/5711/.

Normand A.C., Becker P., Gabriel F., Cassagne C., Accoceberry I., Gari-Toussaint M., Hasseine L., De Geyter D., Pierard D., Surmont I., Djenad F., Donnadieu J.L., Piarroux M., Ranque S., Hendrickx M., Piarroux R. (2017) Validation of a new web application for identification of fungi by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology* 55(9): 2661–2670. https://doi.org/10.1128/JCM.00263-17.

O'Callaghan M., Gerard E.M., Waipara N.W., Young S.D., Glare T.R., Barrell P.J., Conner A.J. (2005) Microbial communities of *Solanum tuberosum* and magainin-producing transgenic lines. *Plant and Soil* 266(1–2): 47–56. https://doi.org/10.1007/s11104-005-3714-1.

Oita S., Carey J., Kline I., Ibáñez A., Yang N., Hom E., Carbone I., U'Ren J., Arnold A. (2021) Methodological approaches frame insights into endophyte richness and community composition. *Microbial Ecology* 82: 21–34. https://doi.org/10.1007/s00248-020-01654-y.

Osono T. (2011) Phyllosphere fungi on leaf litter of *Fagus crenata*: Occurrence, colonization, and succession. *Canadian Journal of Botany 80*: 460–469. https://doi.org/10.1139/b02-028.

Osono T., Hirose D. (2011) Colonization and lignin decomposition of pine needle litter by *Lophodermium pinastri*. *Forest Pathology* 41(2): 156–162. https://doi.org/10.1111/j.1439-0329.2010.00648.x.

Osono T., Tateno O., Masuya H. (2013) Diversity and ubiquity of xylariaceous endophytes in live and dead leaves of temperate forest trees. *Mycoscience 54*: 54–61. https://doi.org/10.1016/j.myc.2012.08.003.

Patel R. (2019) A moldy application of maldi: MALDI-TOF mass spectrometry for fungal identification. *Journal of Fungi (Basel, Switzerland) 5*(1): E4. https://doi.org/10.3390/jof5010004.

Pereira E., Vázquez de Aldana B.R., San Emeterio L., Zabalgogeazcoa I. (2019) A survey of culturable fungal endophytes from *Festuca rubra* subsp. *pruinosa*, a grass from marine cliffs, reveals a core microbiome. *Frontiers in Microbiology* 9: 3321. https://doi.org/10.3389/fmicb.2018.03321.

Petrini O. (1995) Xylariaceous endophytes: An exercise in biodiversity. *Fitopatologia Brasileira* 20: 531–539.

Petrini O. (1991) Fungal endophytes of tree leaves. In Andrews J. H., Hirano S. S. (Eds.), *Microbial Ecology of Leaves*: 179–197. Springer. https://doi.org/10.1007/978-1-4612-3168-4_9

Pinheiro D., Monteiro C., Faria M.A., Pinto E. (2019) Vitek® MS v3.0 System in the Identification of Filamentous Fungi. *Mycopathologia* 184(5): 645–651. https://doi.org/10.1007/s11046-019-00377-0.

Quéro L., Girard V., Pawtowski A., Tréguer S., Weill A., Arend S., Cellière B., Polsinelli S., Monnin V., van Belkum A., Vasseur V., Nodet P., Mounier J. (2019) Development and application of MALDI-TOF MS for identification of food spoilage fungi. *Food Microbiology* 81: 76–88. https://doi.org/10.1016/j.fm.2018.05.001.

Rahi P., Prakash O., Shouche Y.S. (2016) Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) based microbial identifications: Challenges and scopes for microbial ecologists. *Frontiers in Microbiology* 7: 1359. https://doi.org/10.3389/fmicb.2016.01359.

Rajulu M.B.G., Thirunavukkarasu N., Babu A.G., Aggarwal A., Suryanarayanan T.S., Reddy M. S. (2013) Endophytic Xylariaceae from the forests of Western Ghats, southern India: Distribution and biological activities. *Mycology* 4(1): 29–37. https://doi.org/10.1080/21501203.2013.776648.

Ray D., Mueller N., West P., Foley J. (2013) Yield trends are insufficient to double global crop production by 2050. *PloS One* 8(6): e66428. https://doi.org/10.1371/journal.pone.0066428.

Rayner R.W., Commonwealth Mycological Institute (Great Britain), British Mycological Society. (1970) *A mycological colour chart*. Commonwealth Mycological Institute; British Mycological Society.

Reboux G., Rocchi S., Vacheyrou M., Millon L. (2019) Identifying indoor air *Penicillium* species: A challenge for allergic patients. *Journal of Medical Microbiology* 68(5): 812–821. https://doi.org/10.1099/jmm.0.000960.

Redman R., Freeman S., Clifton D., Morrel J., Brown G., Rodriguez R. (1999) Biochemical analysis of plant protection afforded by a nonpathogenic endophytic mutant of *Colletotrichum magna*. *Plant Physiology* 119(2): 795–804. https://doi.org/10.1104/pp.119.2.795.

Sandoval-Denis M., Guarro J., Cano-Lira J.F., Sutton D.A., Wiederhold N.P., de Hoog G S., Abbott S.P., Decock C., Sigler L., Gené J. (2016) Phylogeny and taxonomic revision of Microascaceae with emphasis on synnematous fungi. *Studies in Mycology* 83: 193–233. https://doi.org/10.1016/j.simyco.2016.07.002.

Sanguinetti M., Posteraro B. (2017) Identification of molds by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Journal of Clinical Microbiology* 55(2): 369–379. https://doi.org/10.1128/JCM.01640-16.

Sanitá Lima M., Coutinho de Lucas R., Lima N., Polizeli M.de L.T., Santos C. (2019) Fungal community ecology using MALDI-TOF MS demands curated mass spectral databases. *Frontiers in Microbiology* 10: 315. https://doi.org/10.3389/fmicb.2019.00315.

Schulz B., Boyle C. (2005) The endophytic continuum. *Mycological Research* 109(6): 661–686. https://doi.org/10.1017/S095375620500273X.

Schulz B., Guske S., Dammann U., Boyle C. (1998) Endophyte-host interactions. II. Defining symbiosis of the endophyte-host interaction. *Symbiosis* 25: 213–227.

Strobel G., Daisy B. (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67(4): 491. https://doi.org/10.1128/MMBR.67.4.491-502.2003.

Suryanarayanan T.S., Murali T.S., Thirunavukkarasu N., Rajulu M.G., Venkatesan G., Sukumar R. (2011) Endophytic fungal communities in woody perennials of three tropical forest types of the Western Ghats, southern India. *Biodiversity and Conservation* 20(5): 913–928.

Tadych M., White J. (2019) Endophytic Microbes. In *Reference Module in Life Sciences* :123–136. https://doi.org/10.1016/B978-0-12-809633-8.13036-5.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197.

Triest D., Stubbe D., De Cremer K., Piérard D., Normand A.-C., Piarroux R., Detandt M., Hendrickx M. (2015) Use of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of molds of the *Fusarium* genus. *Journal of Clinical Microbiology* 53(2): 465–476. https://doi.org/10.1128/JCM.02213-14.

Tsuchida S., Umemura H., Nakayama T. (2020) Current status of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology. *Molecules (Basel, Switzerland)* 25(20): E4775. https://doi.org/10.3390/molecules25204775.

U'Ren J. M., Lutzoni F., Miadlikowska J., Zimmerman N. B., Carbone I., May G., Arnold A.E. (2019) Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology Evolution* 3(10): 1430–1437. https://doi.org/10.1038/s41559-019-0975-2.

Welker M., Van Belkum A., Girard V., Charrier J.-P., Pincus D. (2019) An update on the routine application of MALDI-TOF MS in clinical microbiology. *Expert Review of Proteomics* 16(8): 695–710. https://doi.org/10.1080/14789450.2019.1645603.

Whalley A.J.S. (1996) The xylariaceous way of life. *Mycological Research* 100(8): 897–922. https://doi.org/10.1016/S0953-7562(96)80042-6.

White J.F., Kingsley K.L., Zhang Q., Verma R., Obi N., Dvinskikh S., Elmore M.T., Verma S.K., Gond S.K., Kowalski K.P. (2019) Review: Endophytic microbes and their potential applications in crop management. *Pest Management Science* 75(10): 2558–2565. https://doi.org/10.1002/ps.5527.

Wilkendorf L.S., Bowles E., Buil J.B., van der Lee H.A.L., Posteraro B., Sanguinetti M., Verweij P.E. (2020) Update on matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of filamentous fungi. *Journal of Clinical Microbiology* 58(12): e01263-20. https://doi.org/10.1128/JCM.01263-20.

Woudenberg J.H.C., Meijer Houbraken J., Samson R.A. (2017a) *Scopulariopsis* and scopulariopsis-like species from indoor environments. *Studies in Mycology* 88: 1–35. https://doi.org/10.1016/j.simyco.2017.03.001.

Woudenberg J.H.C., Sandoval-Denis M., Houbraken J., Seifert K.A., Samson R.A. (2017b) *Cephalotrichum* and related synnematous fungi with notes on species from the built environment. *Studies in Mycology* 88: 137–159. https://doi.org/10.1016/j.simyco.2017.09.001.

Xia Y., Amna A., Opiyo S.O. (2018) The culturable endophytic fungal communities of switch grass grown on a coal-mining site and their effects on plant growth. *PLOS ONE* 13(6): e0198994. https://doi.org/10.1371/journal.pone.0198994.

Xia Y., Sahib M.R., Amna A., Opiyo S.O., Zhao Z., Gao Y.G. (2019) Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. *Scientific Reports* 9(1): 1669. https://doi.org/10.1038/s41598-018-38230-x.

Zhong S., Steffenson B.J. (2001) Virulence and molecular diversity in *Cochliobolus sativus*. *Phytopathology®* 91(5): 469–476. https://doi.org/10.1094/PHYTO.2001.91.5.469.

(Manuscript accepted 20 October 2021; Corresponding Editor: I. Krisai-Greilhuber)