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Curvularia shahidchamranensis sp. nov., a crude oil-tolerant fungus

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Abstract

Curvularia shahidchamranensis is newly described and illustrated from crude oil contaminated soils of Ahvaz in the Khuzestan province of Iran. This new species is supported with a phylogenetic analysis based on ITS and *gpd* regions in combination with morphology. In two-locus based tree, the isolates of *Curvularia shahidchamranensis* were distinguished from the other previously known species of *Curvularia*, except the *C. nicotiae.* Morphologically, this species easily distinguished from *C. nicotiae* in having less septate and narrower conidia with diamond-like cellular chambers and slightly flatted end in apex. In an *in vitro* test, both isolates showed 18.2%, 21.2% and 24.5% growth inhibition at the concentration of 30, 40 and 50 percent of the crude oil, respectively.

Key words – Ahvaz – Marun oil field – New fungal species

Introduction

Curvularia belongs to family *Pleosporaceae* of the order *Pleosporales* and is morphologically characterized by having dark-pigmented structures (including those of the mycelia, conidiophore, and conidia) (Webster & Weber 2007). Their conidiophores are percurrent, the conidiogenous cells are tretic and usually placed apically or intercalary in the conidiophore, and their conidia are sympodial and nearly elongated with transverse septa (Webster & Weber 2007). At the beginning, curvularia-like species that produce phragmoconidia were solely distinguished by their shape and size of the sexual and asexual structures. Homoplasy and variability in morphological characters have resulted in the fact that the taxonomic status of these fungi by the traditional methods never has been fully understood. Currently, the molecular phylogeny has made it possible to identify *Curvularia* species based on the nucleotide sequences of ITS (internal nuclear ribosomal transcribed spacer region), gpd (glyceraldehyde-3-phosphate dehydrogenase) and EF1α (translation elongation factor 1-a) (Manamgoda et al. 2012a, b, 2014, 2015, Madrid et al. 2014, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b, Heidari et al. 2018, Liang et al. 2018). According to the recent findings in the molecular phylogeny of the helminthosporium-like fungi (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b), some formerly known species of *Bipolaris* also transferred to *Curvularia* and the genus has been amended to accommodate them (Manamgoda et al. 2012a, b, 2014, 2015, Tan et al. 2014, Tomaso-Peterson et al. 2016).

The genus *Curvularia* is widely distributed in soil, water, human, animals and plants as well

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as anywhere in the ecological environment (Sivanesan 1987, Manamgoda et al. 2011, 2012 a, b, da Cunha et al. 2013, Rangaswamy et al. 2013, Verma et al. 2013, Hyde et al. 2014, Ariyawansa et al. 2015, Wijayawardene et al. 2017, 2018). *Curvularia* spp. are mainly saprobes (Manamgoda et al. 2011, 2012a, b, 2014, 2015, Scott & Carter 2014, Tan et al. 2014, 2018), however some of them also act as plant and human pathogen (Manamgoda et al. 2011, Madrid et al. 2014). Some species were also known as mutualistic endophytes (Tadych et al. 2012, Gautam et al. 2013, Jena & Tayung 2013).

In the present study, we report a new *Curvularia* species that was isolated from filed soils contaminated with crude oil in Iran. The morphological characteristics and phylogenetic analysis of the new species are provided. Furthermore, an *in vitro* assay carried out to compare the growth rates of this fungus on the PDA plates amended with different concentration of the crude oil.

Materials & Methods

Isolates and Purification

The isolates under study were obtained from filed soils contaminated with crude oil in Marun Oil Field (Marun 5) of Ahvaz, Iran. A serial dilution of the soil suspensions was prepared in sterile water containing 0.1% Tween 80 and plated on dishes containing potato dextrose agar (PDA, Difco, USA) supplemented with streptomycin (30 mg/L) using a L-shape spreader. The individual colonies were transferred to fresh media and purified by a single spore method. A diluted spore suspension (10^2 spores per ml) was made for each isolates and 100 µl of them was plated on water agar (WA). The plates were incubated in the dark at 28 °C for 24–48 h and a germinated spore was transferred to PDA medium.

The type specimens (dried cultures) were lodged with the Herbarium Ministerii Iranici Agriculturae, Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 16939F). Its ex-type living culture is deposited in the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 3133C). Furthermore, our isolates were maintained in Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran (SCUA-8-marun and SCUA-8.1-marun (Table 1).

Morphological study

The morphological characteristics were examined by growing each isolate on PDA at 28 °C in a 12-h fluorescent light-and-dark up to 14 days. Colony diameter was measured at eight days. Figures were mainly prepared from fungal structures grown on the slide culture (Beneke & Rogers 1996). After 8 days, morphometric and morphological characteristics (including 100 of the mycelia, conidiophore, and conidia) were determined with the 40x and 100x objective lens of a Leitz wetzlar (SM-LUX) Basic Biological Light Microscope (Riddle & Briggs 1950). The colour of the fungal structures was recorded at day eight as described in Methuen handbook of color (Kornerup & Wanscher 1967). The photographs were recorded with an OLYMPUS BX51 microscope fixed with an OLYMPUS DP12 digital camera. The isolates were morphologically compared with previously known species based on original and non-original description documented in literature review and MycoBank (Manamgoda et al. 2012a, b, 2014, 2015, Madrid et al. 2014, Tan et al. 2014, 2018, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b).

Fungal biomass preparation

A mono-conidial colony of each *Curvularia* isolates was grown into shaking flasks containing potato-dextrose-broth (PDB) medium. The growth condition was at 28 ºC for 10–15 days. The mycelial biomass was retrieved by passing through filter papers and washed using sterile distilled water. Collected mass were freeze-dried (Freeze-Dryer, Alpha 1–2LD Plus, Christ) and then powdered in the mortar containing liquid nitrogen. The tubes containing fungal powder was stored in the -20 ºC freezer until consumed.

Table 1 Strains representing most previously known species of *Curvularia* and new taxon used in phylogenetic analysis.

Species	Strain name*	Source	Origin	Accession numbers	
				ITS	*gpd*
B. drechsleri	MUS0028	*Microstegium vimineum*	USA	KF500532	KF500535
C. aeria	CBS 294.61	Air	Brazil	HE861850	HF565450
C. affinis	CBS 154.34	Unknown	Indonesia	KJ909780	KM230401
C. akaii	CBS 317.86	*Themada triandra*	Japan	KJ909782	KM230402
C. akaiiensis	BRIP 16080	–	–	KJ415539	KJ415407
C. alcornii	MFLUCC 100703	Zea	Thailand	JX256420	JX276433
C. americana	UTHSC 072649	Toe tissue	USA	HE861834	HF565486
C. americana	UTHSC 08-3414	*Homo sapiens*	USA	HE861833	HF565488
C. asianensis	MFLUCC 100711	*Panicum* sp.	Thailand	JX256424	JX276436
C. australiensis	BRIP 12044	*Oryza sativa*	–	KJ415540	KJ415406
C. australiensis	CBS 172.57	*O. sativa*	Vietnam	JN601026	JN601036
C. australis	BRIP 12247a	*Eragrostis cilianensis*	Australia	KC424609	KC747759
C. australis	BRIP 12521	*Sporobolus carolii*	–	KJ415541	KJ415405
C. bannonii	BRIP 16732	*Jacquemontia tamnifolia*	USA	KJ415542	KJ415404
C. beasleyi	BRIP 10972	*Chloris gayana*	Australia	MH414892	MH433638
C. beerburrumensis	BRIP 12942	*E. bahiensis*	Australia	MH414894	MH433634
C. boeremae	IMI 164633	*Portulaca oleracea*	India	MH414911	MH433641
C. borreriae	AR5176r	*Sorghum bicolor*	South Africa	KP400637	KP419986
C. borreriae	MFLUCC11–0422	Unknown grass	Thailand	KP400638	KP419987
C. bothriochloae	BRIP 12522	*Bothriochloa*	Australia	KJ415543	KJ415403
C. brachyspora	CBS 186.50	Soil	India	KJ922372	KM061784
C. brachyspora	ZW020185	–	–	HM053667	HM053655
C. buchloes	CBS 246.49	*Buchloe dactyloides*	USA	KJ909765	KM061789
C. caricapapayae	CBS 135941	*Carica papaya*	India	HG778984	HG779146
C. chlamydospora	UTHSC 072764	Toe nail	USA	HG779021	HG779151
C. clavata	BRIP:61680	*Oryza* sp.	Australia	KU552205	KU552167
C. coatesiae	BRIP 24261	*Litchi chinensis*	Australia	MH414897	MH433636
C. coicis	CBS 192.29	*Coix lacryma*	Japan	JN192373	JN600962
C. colbranii	BRIP 13066	*Crinum zeylanicum*	Australia	MH414898	MH433642
C. crustacea	BRIP 13524	*Sporobolus* sp.	Indonesia	KJ415544	KJ415402
C. cymbopogonis	CBS 419.78	*Yucca* sp.	Netherlands	HG778985	HG779129
C. dactyloctenicola	CPC 28810	*Dactyloctenium aegyptium*	Thailand	MF490815	MF490837
C. dactyloctenii	BRIP 12846	*D. radulans*	Australia	KJ415545	KJ415401
C. ellisii	CBS 193.62	Air	Pakistan	JN192375	JN600963
C. ellisii	IMI 75862	Air	Pakistan	KJ922379	KM061792
C. eragrosticola	BRIP 12538	*E. pilosa*	Australia	MH414899	MH433643
C. eragrostidis	CBS 189.48	–	–	HG778986	HG779154
C. geniculata	CBS 187.50	Unknown seed	Indonesia	KJ909781	KM083609
C. gladioli	CBS 210.79	–	–	HG778987	HG779123
C. gladioli	ICMP 6160	*Gladiolus* sp.	New Zealand	JX256426	JX276438
C. graminicola	BRIP 23186a	–	Australia	JN192376	JN600964
C. harveyi	BRIP 57412	*Triticum aestivum*	Australia	KJ415546	KJ415400
C. hawaiiensis	BRIP 11987	*O. sativa*	USA	KJ415547	KJ415399
C. heteropogonicola	BRIP 14579	*Heteropogon contortus*	India	KJ415548	KJ415398
C. heteropogonis	CBS 284.91	*Heteropogoncontortus*	Australia	JN192379	JN600969
C. hominis	CBS 136985	*Homo sapiens*	USA	HG779011	HG779106
C. homomorpha	CBS 156.60	–	–	JN192380	JN600970
C. inaequalis	CBS 102.42	*Sand dune soil*	France	KJ922375	KM061787
C. inaequalis	DAOM 20022	*Pisum sativum*	Canada	KJ922374	KM061786
C. intermedius	CBS 334.64	–	–	HG778991	HG779155

Table 1 Continued.

Species	Strain name*	Source	Origin	Accession numbers	
				ITS	*gpd*
C. intermedius	UTHSC 09 3240	–	–	HE861855	HF565469
C. ischaemi	CBS 630.82	*Ischaemum indicum*	Fiji	JX256428	JX276440
C. ischaemi	ICMP 6172	*Ischaemum indicum*	New Zealand	JX256428	JX276440
C. kenpeggii	BRIP 14530	*Triticum aestivum*	Australia	MH414900	MH433644
C. kusanoi	CBS 137.29	*E. major*	Japon	NR152455	LT715862
C. lamingtonensis	BRIP 12259	*Microlaena stipoides*	Australia	MH414901	MH433645
C. lunata	CBS 730.96	Lung biopsy	USA	JX256429	JX276441
C. malina	CBS 131274	Zoysia grass	USA	JF812154	KP153179
C. malina	FLS–119	*Bermuda grass*	USA	KR493070	KR493083
C. mebaldsii	BRIP 12900 T	*Cynodon tranvaalensis*	Australia	MH414902	MH433647
C. mebaldsii	BRIP 13983	*C. dactylon x transvaalensis*	Australia	MH414903	MH433646
C. microspora	GUCC 6272	*Hippeastrum striatum*	China	MF139088	MF139106
C. microspora	GUCC 6273	*H. striatum*	China	MF139089	MF139107
C. miyakei	CBS 197.29	*E. pilosa*	Japan	KJ909770	KM083611
C. mosaddeghii	IRAN 3131C	*Syzygium cumini*	Iran	MG846737	MH392155
C. mosaddeghii	IRAN 3123C	*Vigna unguiculata*	Iran	MG971270	MG975597
C. muehlenbeckiae	CBS 144.63	*Sorghum* sp.	USA	KP400647	KP419996
C. neergaardii	BRIP 12919	*O. sativa*	Ghana	KJ415550	KJ415397
C. neoindica	IMI 129790	*Brassica nigra*	India	MH414910	MH433649
C. nicotiae	BRIP 11983	Soil	Algeria	KJ415551	KJ415396
C. nisikadoi	CBS 192.29	–	–	AF081447	AF081410
C. nodosa	CPC 28800	*Digitaria ciliaris*	Thailand	MF490816	MF490838
C. nodulosa	CBS 160.58	–	–	JN601033	JN600975
C. oryzae	CBS 169 53	*O. sativa*	Vietnam	KP400650	KP645344
C. ovariicola	CBS 286.91	–	–	HG778994	HG779145
C. ovariicola	CBS 470.90	*E. interrupta*	Australia	JN192384	JN600976
C. pallescens	CBS 156.35	Air	Java	KJ922380	KM083606
C. papendorfii	BRIP 57608	*Acacia karroo*	–	KJ415552	KJ415395
C. papendorfii	CBS 308.67	*A. karroo*	South Africa	KJ909774	KM083617
C. perotidis	CBS 350.90	*Perotis rara*	Cape York	JN192385	JN601021
C. petersonii	BRIP14642	*D. aegyptium*	Australia	MH414905	MH433650
C. pisi	CBS 190.48	*P. sativum*	Canada	KY905678	KY905690
C. platzii	BRIP 27703b	*Cenchrus clandestinum*	Australia	MH414906	MH433651
C. portulacae	BRIP 14541	*P. oleracea*	USA	KJ415553	KJ415393
C. portulacae	CBS 239.48	*P. oleracea*	USA	KJ909775	KM083616
C. prasadii	CBS 143.64	*Jasminum sambac*	India	KJ922373	KM061785
C. protuberata	5876	*Fragaria* sp.	–	KT012665	KT012626
C. protuberata	CBS 376.65	*Deschampsia flexuosa*	UK	KJ922376	KM083605
C. pseudobrachyspora	CPC 28808	*Eleusine indica*	Thailand	MF490819	MF490841
C. pseudolunata	UTHSC 092092	Nasal sinus	USA	HE861842	HF565459
C. pseudorobusta	UTHSC 083458	Nasal sinus	USA	HE861838	HF565476
C. ravenelii	BRIP 13165	*S. fertilis*	Australia	JN192386	JN600978
C. ravenelii	CBS 127709	–	–	HG778999	HG779109
C. reesii	BRIP 4358	Air	Australia	MH414907	MH433637
C. richardiae	BRIP 4371	*Richardia brasiliensis*	Australia	KJ415555	KJ415391
C. robusta	CBS 624 68	*Dichanthium annulatum*	USA	KJ909783	KM083613
C. ryleyi	BRIP 12554	*S. creber*	–	KJ415556	KJ415390
C. ryleyi	CBS 349.90	*S. creber*	Australia	KJ909766	KM083612
C. senegalensis	CBS 149.71	–	–	HG779001	HG779128

Table 1 Continued.

Species	Strain name*	Source	Origin	Accession numbers	
				ITS	*gpd*
C. shahidchamranensis	IRAN 3133C; SCUA-8-Marun	Crude oil contaminated soil	Iran	MH550084	MH550083
C. shahidchamranensis	SCUA-8.1-Marun	Crude oil contaminated soil	Iran	MH550087	MH550086
C. soli	CBS 222.96	Soil	Papua New Guinea	KY905679	KY905691
C. sorghina	BRIP 15900	*S. bicolor*	Australia	KJ415558	KJ415388
C. spicifera	CBS 274.52	Soil	Spain	JN192387	JN600979
C. sporobolicola	BRIP 23040b	*Sporobolus australasicus*	Australia	MH414908	MH433652
C. subpapendorfii	CBS 656.74	Desert soil	Egypt	KJ909777	KM061791
C. trifolii	CBS 173.55	*Trifolium repens*	USA	HG779023	HG779124
C. tripogonis	BRIP 12375	Unknown	Australia	JN192388	JN600980
C. tropicalis	BRIP 14834	*Coffea arabica*	India	KJ415559	KJ415387
C. tsudae	ATCC 44764	*C. gayana*	Japan	KC424596	KC747745
C. tsudae	MAFF 236750	*C. gayana*	Japan	KP400651	KM061790
C. tuberculata	CBS 14663	*Zea mays*	India	JX256433	JX276445
C. uncinata	CBS 221.52	*O. sativa*	Vietnam	HG779024	HG779134
C. variabilis	CPC 28813	*D. ciliaris*	Thailand	MF490820	MF490842
C. variabilis	CPC 28815	*C. barbata*	Thailand	NR154866	MF490844
C. verruciformis	CBS 537.75	*Vanellus miles*	New Zealand	HG779026	HG779133
C. verruculosa	CBS150 63	*Punica granatum*	India	KP400652	KP645346
C. verruculosa	MFLUCC 100690	*O. sativa*	Thailand	JX256437	JX276448
C. warraberensis	BRIP 14817	*D. aegyptium*	Australia	MH414909	MH433653
Curvularia sp.	AR5117	*Lolium perenne*	USA	KP400655	KP645349
Curvularia sp.	MFLUCC 100709	*O. sativa*	Thailand	JX256442	JX276453
Curvularia sp.	MFLUCC 100739	*O. sativa*	Thailand	JX256443	JX276454
Curvularia sp.	MFLUCC 120177	Unknown grass	Thailand	KP400654	KP645348
Curvularia sp.	UTHSC 08809	Human	USA	HE861826	HF565477

* BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, Kew, UK; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan, MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory, Department of Pathology at the University of Texas Health Science Center, San Antonio, Texas, USA; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; SCUA: the Collection of Fungal Cultures, Department of Plant Protection, Shahid Chamran University of Ahvaz, Iran.

DNA extraction and amplification

Fungal DNA was isolated from freeze-dried mycelia using an organic method as previously described by Raeder & Broda (1985), with some modification (Ahmadpour et al. 2017).

The partial amplification of nrDNA (internal transcribed spacer regions 1 & 2 and 5.8S) and *gpd* was performed using primers ITS1 and ITS4 (White et al. 1990) and gpd1 and gpd2 (Berbee et al. 1999), respectively. Each PCR reaction contained 0.4 mM deoxyribonucleotide triphosphates (mix), 0.4 µM of each primer, 0.06 U/µl Prime Taq DNA Polymerase, 1X prime Taq Reaction Buffer, 2.5 mM of MgCl2, and 5 ng/µL template DNA. The thermal cycling was carried out using a MJ Mini™ Gradient Thermal Cycler (BioRad, Hercules, CA, USA). An initial denaturation step at 94 °C for 3 min was followed by 35 amplification cycles of denaturation at 94 °C for 30 s,

annealing at 54 °C (ITS) or 56 °C (*gpd*) for 30 s and extension at 72°C for 60 s. The thermal cycling was followed by a final extension step at 72°C for 10 min.

Purification and sequencing

PCR products were separated by gel electrophoresis in 1% agarose (Hispan Agar, Spain) stained with commercial safe stain (SinaClon, Iran), and visualized under UV light. Amplicons of the expected size were excised and purified with GF-1 AmbiClean Kit (Vivantis, Malaysia). Sequencing was performed in both directions using original PCR primers by Macrogen Company (Humanizing Genomics, Macrogen, South Korea). Raw ABI chromatograms were edited and aligned using the BioEdit Sequence Alignment Editor Version 7.0.9.0 (Hall 1999). Multiple sequences of the same amplicons were assembled using DNA Baser Sequence Assembler v4 programs (2013, Heracle BioSoft, www.DnaBaser.com). The assembled sequences are deposited at GenBank (http://www.ncbi.nlm.nih.gov/genbank/; Table 1).

Phylogenetic analyses

Phylogenetic relationships among the isolates under survey and reference strains were inferred from the combined two-locus data set using maximum likelihood algorithm (ML) in MEGA version 6 (Tamura et al. 2013). Reference sequences belonging to the currently described species of *Curvularia* included in the phylogenetic analysis (as row data in Table 1, Fig. 1) were mostly from Manamgoda et al. (2012a, b, 2014, 2015), Madrid et al. (2014), Tan et al. (2014, 2018), Tomaso-Peterson et al. (2016), Marin-Felix et al. (2017a, b), Heidari et al. (2018), Liang et al. (2018). The species of *Bipolaris drechsleri* was used as outgroup taxon. ITS and *gpd* sequences were individually aligned using Clustal W in BioEdit program, Version 7.0.9.0 (Hall 1999). Alignments were checked visually and ambiguous sections were excluded before constructing two-locus data set. Composite alignments were generated with Clustal W in MEGA version 6 (Tamura et al. 2013) using optimum parameters. MEGA program identified the Tamura-Nei model with a proportion of invariant sites and gamma-distributed rate heterogeneity (TN93 + G + I) as the best-fit model of nucleotide substitution for ML analysis of the combined data set. Phylogenetic trees were generated with Subtree-Pruning-Regrafting (SPR) algorithm and following options: Gaps (insertion/deletions) were included in analysis, the bootstrap method (BP) was applied with 1000 replications. Two-locus matrix used for phylogenetic analysis was deposited in the Treebase database (http://purl.org/ phylo/ treebase/ phylows/study/TB2: S23053).

Fungus tolerance to crude oil

The isolates under study were tested for their ability to tolerate crude oil by visual assessment of the fungal growth on PDA medium. Desalted crude oil from Marun oil field was added into sterile Erlenmeyer flasks containing PDA supplemented with streptomycin and additional agar at the rate of 30, 40 and 50 percent (v/v). Two polar and nonpolar phases were mixed on heater shaker using 3-4% (v/v) Tween 80 and an orbital magnetic stirrer at 50–55 °C and then poured into Petri dishes. After medium solidifying, mycelial disks from the outer edge of an actively growing colony of each isolate were placed on the center of medium plates and were incubated at 28 ± 0.5 ºC. Control plates made of PDA without the addition of crude oil. Three plates were set for each concentration. The growth rates of the isolates were recorded daily by measuring the diameter of the colonies. Percentage of growth inhibition was calculated by Abbott formula as follow: percentage of growth inhibition = C – T/C × 100, where C and T mean diameter of the fungal colony in control and treatment, respectively.

Results

Phylogenetic analysis

DNA sequence analysis showed that both the ITS and *gpd* loci of the new species are identical with the previously described species *Curvularia nicotiae*. In phylogenetic analysis, two-

locus data set totaled 968 bp of aligned DNA sequence data, including 626 conserved sites, 39 parsimony uninformative nucleotide positions and 295 parsimony-informative nucleotide sites. The analysis of individual sequences indicated that the *gpd* locus contained more parsimony-informative nucleotide sites than the ITS locus. In two-locus based tree (Fig. 1), the isolates of *Curvularia shahidchamranensis* cluster in a well-supported clade (99 % bootstrap support) with type strain of the *Curvularia nicotiae*, but morphologically highly different.

Taxonomy

Curvularia shahidchamranensis M. Mehrabi-Koushki, sp. nov., Fig. 2

MycoBank: MB826923

Etymology – Name refers to the Shahid Chamran University of Ahvaz, a scientific center that has trained a large number of specialists in various disciplines for Iran.

Morphology on PDA – *Hyphae* sub-hyaline to brown, branched, septate, thin and smooth-walled, 2.6–7.8 µm diam, ($\overline{x}$ = 5.1, SD ± 1.4 µm, n = 100). *Conidiophores* arising singly, septate, wider in upper parts, straight or flexuous, geniculate in apex in medium and long type, un-branched, cells walls thicker than those of the vegetative hyphae, smooth-walled at the base and slightly verruculose towards apex, pale brown to brown, (26–)31.2–119(–260) × (2.6–)3.4–5.2 µm, 95% confidence limits = 56.1–82 × 4.5–4.9 µm, ($\overline{x}$ = 69 × 4.7, SD = 45.5 × 0.7 µm, n = 100).

Conidiogenous cells frequently integrated, mostly with verruculose nodes, terminal or intercalary, with sympodial proliferation, with circular thickened scars, brown, subcylindrical to slightly swollen. *Conidia* smooth-walled, ellipsoidal to sub-cylindrical or even barrel-shaped, highest diameter at the third cell from base, straight or sometimes very slightly curved, (1–)3(–4)-distoseptate, with diamond-like cellular chambers, rounded or hilate at the basal end, slightly flatted in apex, (23–)25–36.7(–39) × (10–)11–18.2 µm, 95% confidence limits = 29.1–30.3 × 14.6–15.4 µm, ($\overline{x}$ = 29.7 × 15, SD = 3.6 × 2.1 µm, n = 100). *Hilum* no protruding, darkened, thickened. *Chlamydospore* and sexual morph not observed.

Cultural characters on PDA – *Colonies on PDA* growing 80–85 mm diam after 8 d incubation at 28 °C, 4–5 cm diam. at 35°C and 0.4 cm diam at 40 °C, circular with fimbriate margin, greenish black, aerial mycelium abundant, floccose; reverse black.

Habitats – Soil

Distribution – Iran (Ahvaz).

Material Examined – IRAN, KHUZESTAN PROVINCE: Ahvaz. From crude oil contaminated soils in Marun oil field (Marun 5), Sep-2016, *Farzaneh Dehdari and Mehdi Mehrabi-Koushki* (holotype: IRAN 16939F, ex-type cultures: IRAN 3133C = SCUA-8-marun).

Notes – In phylogenetic tree based on ITS-gpd dataset (Fig. 1), the closest related species to *Curvularia shahidchamranensis* is *C. nicotiae* (Mouch.) Y.P. Tan & R.G. Shivas (Manamgoda et al. 2015), with 99% bootstrap support. However, *C. shahidchamranensis* can be easily distinguished from *C. nicotiae* by the production of less septate and narrower conidia [(1–)3(–4) – distoseptate and 29.7 × 15 µm average size in *C. shahidchamranensis* vs. 3–5 (–6)-distoseptate and 31 × 19 µm average size in *C. nicotiae*]. In addition, in *C. shahidchamranensis* the conidia have diamond-like cellular chambers with slightly flatted end in apex, while in *C. nicotiae* the conidia have elliptical to rectangular cellular chambers with rounded end at apex.

Fungus tolerance to crude oil

The results showed that this new species was able to grow on PDA amended with different concentration of crude oil (Fig. 3). The highest concentration of crude oil used (50%) inhibited mycelial growth by an average of 24.5% based on the diameter of fungal growth. In addition, both isolates showed 18.2% and 21.2% growth inhibition at the concentration of 30% and 40% of the crude oil, respectively.

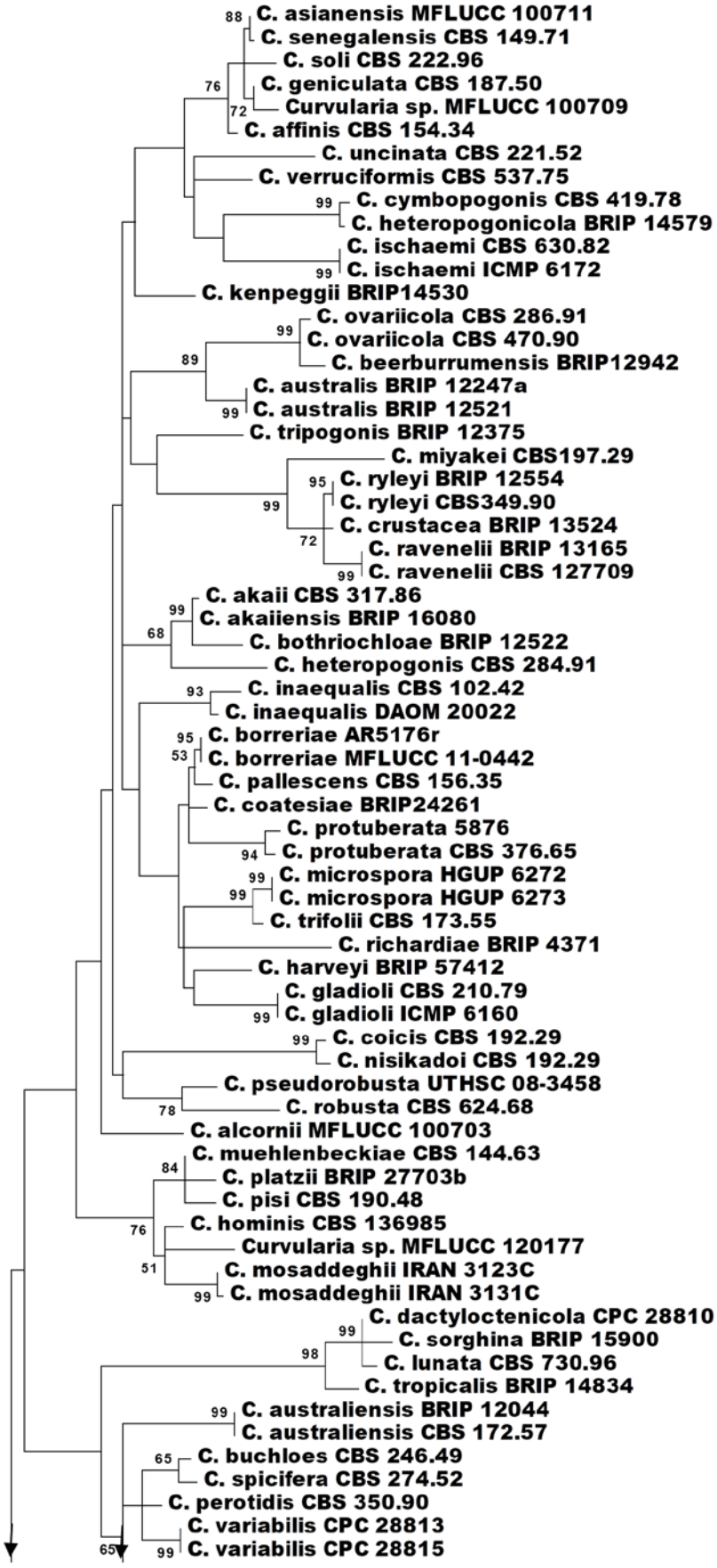

Fig. 1 – Phylogram generated from a ML analysis based on a concatenated alignment of ITS and *gpd* sequences of 123 *Curvullaria* strains representing most previously known species and new taxon. Bootstrap values greater than 50% are shown at the nodes.

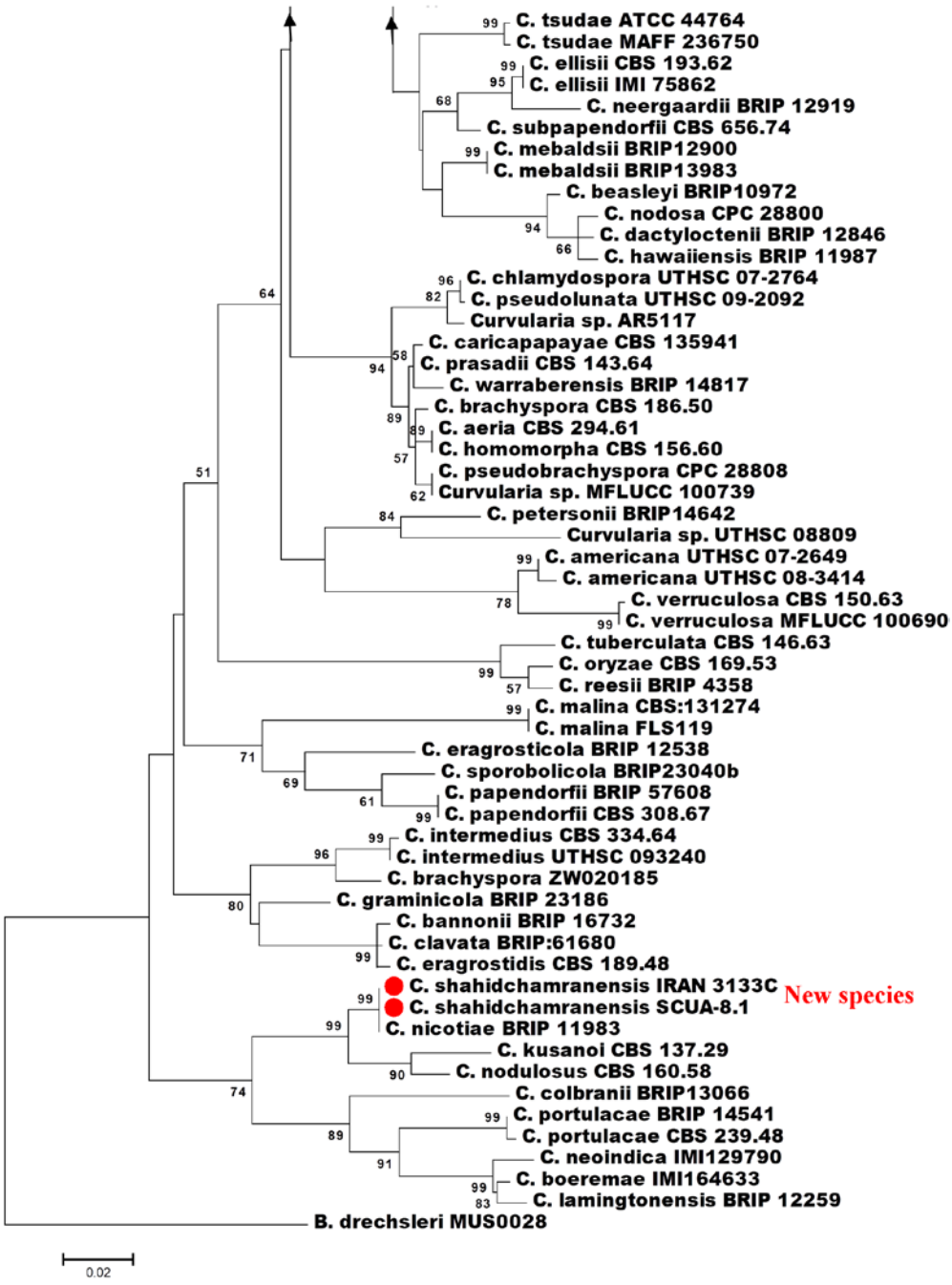

Fig. 1 – Continued.

Discussion

Curvularia species were mainly found in living or dead tissues of plants and human (Manamgoda et al. 2012a, b, 2014, 2015, da Cunha et al. 2013, Tan et al. 2014, 2018, Madrid et al. 2014, Tomaso-Peterson et al. 2016, Marin-Felix et al. 2017a, b). As new species described above, some *Curvularia* species were also recovered from the soil, i.e. *C. brachyspora* (Boedijn 1933), *C. inequalis* (Shear 1907), *C. nicotiae* (Mouchacca 1973), *C. soli* (Marin-Felix et al. 2017a), *C. spicifera* (Bainier 1908) and *C. subpapendorfii* (Mouchacca 1973). A number of *Curvularia* species have been known as pathogens causing seed rot and seedling blight, root rot, leaf spot and blight, and grain discoloration and deformation (Benoit & Mathur 1970); some of them were also reported known as causal agents of leaf spots on grasses (Agrios 2005). In this study, the new species was isolated from the filed soils which had been affected and contaminated with crude oil. The presence of *Curvularia* in this stressful soil feasibly could be due to the vegetation around contaminated areas such as wheat, barley and wild grasses.

Fig. 2 – *Curvularia shahidchamranensis* (IRAN 3133C). a Colony on PDA. b–d Conidiophores and young and mature conidia.

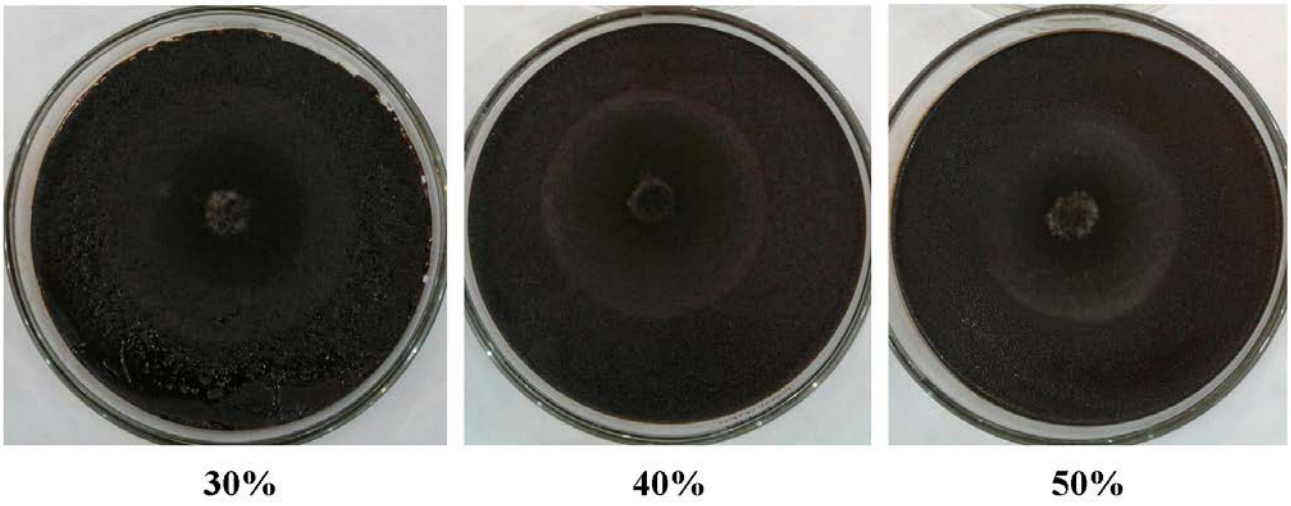

Fig. 3 – *Curvularia shahidchamranensis* (IRAN 3133C). Colony growth on PDA amended with different concentrations of crude oil.

In this study, both isolates of the new species were known as a crude oil-tolerate fungus while they showed low growth inhibition on medium amended up to 50% (v/v) crude oil used. Based on literature review (Norton 2012, Anastasi et al. 2013), some filamentous fungi are capable to

degrade oil compounds, once they grow quickly on the contaminated substrata, digesting them through the secretion of extracellular enzymes. Of those fungi, *Alternaria*, *Aspergillus*, *Candida*, *Cephalosporium*, *Cladosporium*, *Cochliobolus*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Lentinus*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pleurotus*, *Phanerochaete*, *Polyporus*, *Rhizopus*, *Rhodotolura*, *Saccharomyces*, *Talaromyces*, *Torulopsis*, *Trametes* and *Trichoderma* are the most common members reported from marine and terrestrial environments (Odu et al. 1978, Andrea et al. 2001, Saraswathy & Hallberg 2002, Adekunle & Adebambo 2007, Gesinde et al. 2008, Obire & Anyanwu 2009, Hadibarata & Tachibana 2010, Al-Nasrawi et al. 2012).

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