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Short communication

Diversity of fungal endophytes isolated from the invasive plant *Solanum rostratum*

Nigora Kuchkarova[1], Caixia Han[1], Zokir Toshmatov[1,4], Hongyang Chen[1], Hua Shao[1,2,3*]

[1] State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

[2] Research Center for Ecology and Environment of Central Asia, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

[3] University of Chinese Academy of Sciences, Beijing 100049, China

[4] Institute of Genetics and Plant Experimental Biology, Academy of Sciences of the Republic of Uzbekistan, Kibray, Tashkent region 111208, Uzbekistan

Abstract – A culture-dependent method was used to isolate fungal endophytes from the leaves, stems, and roots of the invasive plant *Solanum rostratum* Dunal. growing in Xinjiang Province, China. All isolates were identified according to ITS (internal transcribed spacer) region of ribosomal DNA sequences and analyzed by Nucleotide BLAST according to NCBI GenBank and Mycobank database. Altogether 176 endophytic fungal isolates corresponding to 44 OTUs were identified, which were classified into 12 genera, with *Penicillium* (59.66%) and *Aspergillus* (23.29%) being the highly dominant genera. Ten endophytic isolates (OTU1, OTU15, OTU16, OTU21, OTU23, OTU25, OTU26, OTU30, OTU37 and OTU44) were identified as potential new species.

Keywords: Culture-dependent endophyte isolation, endophytes, *Penicillium*, *Mucor circinelloides*

Introduction

Solanum rostratum Dunal. is an annual weed with a strong capacity for propagation and adaptation. A notorious invader, it also serves as the primary host of the potato leaf-roll virus and *Leptinotarsa decemlineata* (potato beetles), which pose substantial threats to biodiversity and the environment in China (Zhao et al. 2013, Liu et al. 2020). Additionally, *S. rostratum* contains abundant amounts of secondary metabolites, primarily flavonoids, alkaloids, steroids, and other compounds (Liu et al. 2020).

Endophytic fungi live in plants for all or part of their lives without harming the host (Ripa et al. 2019). They may affect a plant's ability to reproduce, grow, or resist abiotic stress or natural enemies (Rho et al. 2018). To the best of our knowledge, previous papers largely concentrated on the biological traits and phytochemical profile of *S. rostratum* and there is no report about the diversity of endophytic fungi of this invasive plant. The main goal of this study is to explore the community of the fungal endophytes of *S. rostratum*. Identification of the endophytes may help explain the invasive success of *S. rostratum* from the perspective of plant-microbe interaction; these endophytes are also potentially valuable resources of bioactive substances that have various biological activities.

Materials and methods

Forty-five mature *S. rostratum* plants at flowering stage were collected on June 28, 2018 from 3 different locations (15 plants from each location) in Urumqi and Changji city of Xinjiang province: location 1: 43° 55'60'' N, 87° 20'41'' E (Loc-1); location 2: 43° 56'0'' N, 87° 20'41'' E (Loc-2); location 3: 43° 46'14'' N, 87° 46'31'' E (Loc-3). Endophytes were isolated using a culture-dependent method within two days of collection of plants. Surface sterilization of plant parts (roots, stems, and leaves) and isolation of the endophytes were conducted following the protocol of Schulz et al. (1993). Colonization rate (CR) was counted by following Petrini et al. (1982).

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* Corresponding author e-mail: shaohua@ms.xjb.ac.cn

DNA of the endophytic isolates was extracted by using the DNA Extraction Kit for fungi (Solarbio Life Sciences, Beijing, China), according to the manufacturer's instructions (Abd-Elsalam et al. 2003). PCR amplification of the rDNA ITS (internal transcribe spacer) region was conducted with the use of ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') fungal primer pairs (White et al. 1990). The sequences of the fragments were identified using the basic local alignment search tool BLAST (http://www.ncbi.nlm.nih.gov/) of the NCBI and Mycobank (https://www.mycobank.org) databases. Fungal identities were generated by sequence alignment analysis with those previously submitted to GenBank.

Results and discussion

In total, 176 endophytic fungal isolates corresponding to 44 OTUs (operational taxonomic units) were isolated and were classified into 12 genera (Tab. 1). Among the isolates, 55 (31.25%) were obtained from leaves, 55 from stems (31.25%), and 66 from roots (37.50%); 34 out of 44 OTUs had between 97.14% and 100.00% sequence similarity with relevant entries in Mycobank and GenBank databases, whereas OTU1, OTU15, OTU16, OTU21, OTU23, OTU25, OTU26, OTU30, OTU37 and OTU44 had between 70.13% and 95.87% sequence similarity with species belonging to the genera *Aspergillus*, *Penicillium*, *Microascus*, *Purpureocillium* and *Mucor* (Tab. 1), indicating they might be potential new species. *Penicillium* and *Aspergillus* were the dominant genera of the endophytic fungal community (Cheng et al. 2018). OTU23 (closest hit *Purpureocillium lilacinum* CBS 284.36) was chosen for further study on its secondary metabolites due to its high plant growth regulatory activity, which resulted in the isolation and identification of 3 bioactive compounds, i.e., adenosine, cerevisterol, and thymine, which were found to possess significant plant growth regulatory activity (Kuchkarova et al. 2020).

The percentage of endophytic isolates belonging to *Penicillium* (59.66%; 105/176) was much higher than those identified as *Aspergillus* (23.29%; 41/176), *Purpureocillium* (6.25%; 11/176), *Emericella* (3.41%; 6/176), *Fusarium* (2.27%; 4/176), *Paecilomyces* (1.14%; 2/176), *Geotrichum* (1.14%; 2/176) as well as *Altenaria*, *Microascus*, *Mucor*, *Pichia* and *Talaromyces,* which were detected only sporadically (< 1%). The CR of the roots of the plant was higher (43.33%) than that of the stems (30.00%) and leaves (26.67%) of the identical plants. Furthermore, the CR of fungal endophytes of plants acquired from Loc-1 was much higher than that from Loc-2 and Loc-3.

To the best of our knowledge, this is the first report on the diversity of the endophytic fungi isolated from the invasive plant *S. rostratum*. This study demonstrated the comparatively high multiplicity of the endophytic fungi of *S. rostratum* from three locations in Xinjiang. Our work revealed that the invasive plant *S. rostratum* harbours a variety of fungal endophytes in its leaves, stems, and roots. Given the fact that endophytes are able to produce biologically

Tab. 1. List of identified endophytic fungi isolated from *Solanum rostratum* plant parts. [a]BLASTN max score; [b]level of identification for pairwise alignments by calculating using the Martinez-Needleman-Wunsch algorithm; [c]level of similarity for pairwise alignments with the closest match, using the NCBI and Mycobank database; Accession number of the closest database match; [d]accession number of the closest database match. OUT - operational taxonomic unit.

OTU	Accession no.	Best Blast hit					Number of isolates						
		Closest taxa match	Score[a]	Query coverage (%)[b]	Ident (%)[c]	Accession no[d]	By tissue type			By location			Total observed
							Leaf	Stem	Root	1	2	3	
1	ON149677	*Aspergillus lentulus*	556	94	85.21	PWQ2395	0	0	1	1	0	0	1
2	ON149678	*Penicillium oxalicum*	843	95	100.00	FMR 14261	6	4	18	18	4	6	28
3	ON149679	*Pichia kudriavzevii*	711	96	99.36	CNRMA6.98	1	0	0	1	0	0	1
4	ON149680	*Aspergillus quadrilineatus*	786	92	99.40	IHEM 22705	1	1	0	1	0	1	2
5	ON149681	*Aspergillus rugulosus*	762	92	99.20	UOA/HCPF 10020	2	0	3	5	0	0	5
6	ON149682	*Emericella nidulans*	775	94	98.22	WM 06.100	4	2	0	6	0	0	6
7	ON149683	*Aspergillus creber*	637	94	97.42	FMR 14364	1	0	0	1	0	0	1
8	ON149684	*Fusarium verticillioides*	775	94	100.00	IHEM 9835	0	1	0	1	0	0	1
9	ON149708	*Penicillium citrinum*	795	96	99.60	NRRL 1841	0	0	2	1	1	0	2
10	ON149685	*Aspergillus niger*	857	94	100.00	WM 10.76	7	0	1	7	0	1	8
11	ON149686	*Aspergillus nidulans*	805	95	99.42	WM 11.60	0	0	2	1	1	0	2
12	ON149687	*Penicillium brasilianum*	843	94	100.00	FMR 14296	0	1	0	1	0	0	1

Tab. 1. Continued.

OTU	Accession no.	Best Blast hit					Number of isolates						
		Closest taxa match	Score[a]	Query coverage (%)[b]	Ident (%)[c]	Accession no[d]	By tissue type			By location			Total observed
							Leaf	Stem	Root	1	2	3	
13	ON149688	*Aspergillus oryzae*	852	95	99.81	WM 10.120	0	0	1	1	0	0	1
14	ON149689	*Aspergillus tubingensis*	848	94	100.00	IHEM 17440	3	3	2	4	0	4	8
15	ON149690	*Penicillium rolfsii*	695	95	95.10	FMR 14307	0	0	1	1	0	0	1
16	OM698374	*Microascus cirrosus*	166	62	70.13	FMR 12256	0	1	0	1	0	0	1
17	ON149691	*Penicillium chrysogenum*	835	94	100.00	FMR 14008	14	19	15	5	15	28	48
18	ON149692	*Fusarium pseudonygami*	735	89	100.00	U34563	0	0	1	1	0	0	1
19	ON149693	*Fusarium oxysporum*	732	93	98.77	UOA/HCPF AB82	0	0	1	1	0	0	1
20	ON149694	*Aspergillus terreus*	848	92	100.00	WM 03.218	0	0	2	2	0	0	2
21	ON149695	*Aspergillus calidoustus*	667	95	95.87	UOA/HCPF 9236	1	0	0	1	0	0	1
22	ON149696	*Aspergillus fumigatus*	863	95	100.00	ATCC 1022	1	0	0	1	0	0	1
23	ON149697	*Purpureocillium lilacinum*	624	76	95.59	CBS 284.36	4	5	2	4	3	4	11
24	ON149698	*Penicillium coprophilum*	817	99	99.24	FMR 13998	1	1	0	1	1	0	2
25	ON149699	*Penicillium glabrum*	516	75	94.12	FMR 14292	0	2	0	2	0	0	2
26	ON149700	*Penicillium frequentans*	513	75	94.09	FMR 14318	0	1	0	1	0	0	1
27	ON149701	*Talaromyces pinophilus*	816	94	99.42	FMR 14017	0	0	1	1	0	0	1
28	ON149702	*Aspergillus aculeatus*	795	92	100.00	CBS 172.66	0	0	4	1	0	3	4
29	ON149703	*Paecilomyces lilacinus*	791	83	98.85	WM 04.457	0	1	1	0	2	0	2
30	ON149704	*Aspergillus brasiliensis*	732	95	95.48	ATCC MY-A4553	1	0	0	0	1	0	1
31	ON149705	*Aspergillus flavus*	836	93	100.00	PWQ 2335	4	0	0	0	4	0	4
32	ON149706	*Penicillium echinulatum*	770	94	98.48	FMR 13945	0	0	1	0	1	0	1
33	ON149707	*Penicillium rubens*	827	93	99.81	FMR 13874	0	0	1	0	1	0	1
34	ON149709	*Penicillium crustosum*	726	85	99.16	FMR 1430	0	6	1	0	1	6	7
35	ON149710	*Penicillium allii*	808	93	99.04	FMR 14251	0	0	1	0	1	0	1
36	ON149711	*Penicillium commune*	764	93	97.14	CBS 311.48	0	0	1	0	0	1	1
37	ON149712	*Mucor circinelloides f. circinelloides*	754	91	92.19	IHEM 24129	1	0	0	0	0	1	1
38	ON149713	*Geotrichum candidum*	482	90	99.03	WM 07.304	1	0	0	0	0	1	1
39	ON149714	*Geotrichum bryndzae*	436	82	98.93	PMM09-440L	1	0	0	0	0	1	1
40	ON149715	*Fusarium keratoplasticum*	798	93	100.00	FRC S-2465	0	1	0	0	0	1	1
41	ON149716	*Penicillium brevicompactum*	738	93	97.67	WM 06.340	0	5	2	0	0	7	7
42	ON149717	*Alternaria alternata*	817	95	100.00	WM 04.486	0	1	0	0	0	1	1
43	ON149718	*Penicillium griseofulvum*	813	94	99.44	CBS 185.27	0	0	1	0	0	1	1
44	OM698376	*Penicillium palitans*	507	72	91.09	FMR 14268	1	0	0	0	0	1	1
Total							55	55	66	72	36	68	176

active secondary metabolites that affect the growth of their hosts, we speculate that the endophytic fungi might contribute to the invasive success of *S. rostratum*.

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