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Diversity and Antimicrobial Activity of Endophytic Fungi from Traditional Medicinal Plant *Curcuma wenyujin*

Jvfen Yan, Ningbo Qi, Suping Wang and Shulin Yang[1]

School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing - 210094, China.

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Endophytic fungi are ubiquitous in plants and reside their hosts for a part of their life cycle. Their various diversity associated with the potential bioactivity has played an important role in human health, especially that from Chinese traditional medicinal herbs. Here, we isolated endophytic fungi from plant *Curcuma wenyujin* and evaluated their antimicrobial activity. Sixty-six endophytic fungi were recovered from the fresh and healthy root, leaf and stem tissues of *C. wenyujin*. Of these isolates, the common species *Penicillium*, *Fusarium* and *Aspergillus* were the dominant species. 65% of endophytic fungi had the potential antibacterial activity against one or more tested microorganism of *E. coli*, *P. vulgaris*, *B. subtilis*, *S. aureus*, *Rhizopus*, *Microzyme*, *A. niger* and *Mucor*, and 4.5% strains could inhibit eight tested microbes, exhibiting a broad antimicrobial spectrum. These findings provide a strong platform for the isolation and purification of natural antimicrobial agents from endophytic fungi of *C. wenyujin.*

Key words: bioactivity, isolation, distribution, Chinese traditional herb.

The endophytic fungi are ubiquitous in the plant tissues by transmission via air or water-splash from surrounding saprobic colonies [1,2]. Its common definition is living at least part of their lifecycle inside plant tissues without causing any visible manifestation of disease to the host plant [3]. Although endophytic fungi are common in the herb plants, the composition of endophytic communities varies from the ecological and physiological factors in plants [4-7], such as geographical location, age and characteristics of host plant, seasonal changes (temperature, humidity, water content in the soil, etc.). These endophytic fungal interactions with their hosts range from mutualistic to antagonistic [8-11], providing resistance to hosts against different biotic and abiotic stresses and playing an important role in plant community health [12,13]. In turn, the host plants make the fungal communities increase the absorption of soil nutrients and also change in nutrient cycle occurs [14].

Furthermore, endophytic fungi are the origins of active secondary metabolites [15] and have been recognized as a repository of novel metabolites of pharmaceutical importance because of their unusual environments [16,17], which may directly or indirectly be used as drugs against many diseases [18-22]. Occasionally, fungi associated with medicinal plant are able to produce the same bioactive compounds as the host plant itself or valued metabolites [23-26]. Therefore, exploiting endophytic fungi for the production of bioactive secondary metabolites is a challenging and rewarding work.

On the basis of these successful experiments and the theory of "co-evolution" and "gene horizontal transfer" [27], the traditional Chinese medicinal plant *Curcuma wenyujin* was taken as target for the study of its endophytic fungi. *C. wenyujin* is famous for the volatile components including curcumol, β-elemene,

* To whom all correspondence should be addressed.
Tel: +86-25-84315945;
E-mail: bioshuliny@yahoo.com.cn

curzerene, curzerenone, curcumenol, etc. that is anticancer activity and used for therapeutical drugs[28].

The foregoing contents suggest fungi associated with plant especially the medicinal herb plant are important both from an ecological perspective and from a biochemical and molecular standpoint. So, the aim of this paper is to isolate the endophytic fungi from the herb *C. wenyujin*, study the characteristics of fungal communities and evaluate their potential antimicrobial ability.

MATERIALS AND METHODS

Endophytic fungi isolation and identification

The fresh healthy plant *C. wenyujin* (*C. wenyujin* Y.H.Chen & C.Ling) collected from Zhejiang Province, China was carefully washed under running water to discard the surface debris. Then following the procedure of surface-sterilization method [29], the samples were steeped successively in 75% ethanol (v/v) for 1 min, 0.1% mercuric chloride (v/v) for 1 min to roots (5 mm^3), 30 s for stems (5 mm) and leaves (5×3 mm), finally washed with sterile distilled water for 1 min several times. These sterilized segments were placed in Petri dishes containing potato dextrose agar (PDA) medium with 50 ìg/ml streptomycin, incubated at 28 ! with darkness. Meanwhile, both the last washing water and the print of sterilized tissue segments were tested on the respective PDA as negative control to check the effective of sterilization. After several days, the colonies appeared around the segments were then subcultured in fresh PDA medium on the premise that no microbe grew on the control medium. The pure endophytic fungal strains were then preserved in the laboratory of Institute of Biological Engineering, Nanjing University of Science and Technology and identified according to their morphology based on the manual [30].

Antimicrobial activity assay

These isolated endophytic fungi were evaluated for their antibacterial activity against eight tested strains including bacterial of *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus* and fungi of *Rhizopus*, *Microzyme*, *Aspergillus niger* and *Mucor* using the agar diffusion method [31]. The endophytes were inoculated into a 250 ml conical flask containing 100 ml of potato dextrose broth (PDB) and cultured at 28 !, 180 rpm for 10 days. Acquired fermentation liquid was centrifuged to remove mycelium. The supernatant liquid was then condensed for antimicrobial assay. The inhibition zones were observed, measured and recorded.

RESULTS AND DISCUSSION

During the process of isolation, there was no microbe occurred on the control medium plates, excluding the possibility of surface fungi and suggesting the isolated fungi was endophytic. On this premise, sixty-six isolates endophytic fungi

Table 1. The endophytic fungi isolated from *C. wenyujin*

Family	Species	Number	Root	Stem	Leaf
Moniliaceae	*Penicillium* sp.	14	10	3	1
	Aspergillus sp.	8	3	3	2
	Ceohalosporium sp.	2	1	1	
	Staphlosporonites sp.	1	1		
	Mycogone sp.	3	2	1	
Dematiaceae	*Cladosporium* sp.	4	2	1	1
	Alternaria sp.	2	2		
	Monotospora sp.	3	2	1	
Tuberculariaceae	*Fusarium* sp.	13	9	3	1
Saccharomycetaceae	*Saccharomyces* sp.	1	1		
Mucoraceae	*Mucor* sp.	4	2	1	1
Sphaeropsidaceae	*Hendersonula* sp.	2			2
Agonomycetaceae	*Rhizoctonia* sp.	5	3		2
	Mycelia sterilia	4	2		2
		66	40	14	12

were recovered from the healthy, symptomless leaves, stems and roots of *C. wenyujin* followed by the surface sterilization method. Among, forty-three strains isolated from the roots belonging to thirteen genera, fourteen strains from stems under eight ranks and twelve from leaves (Table 1). Obviously, *Penicillium* (21.2%), *Fusarium* (19.7%) and *Aspergillus* (12.1%) were the dominant species. This result was similar to that of Xuan and Zhang [32] and Wang *et al*. [33] who also isolate endophytic fungi from *Cucurma*. They are common a genus of ascomycetous fungi of major importance in the natural environment, but can also occur as endophytes. The common species *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp. and *Mucor* sp. were found in all the tissues (Table 1), but *Staphlosporonites*, *Alternaria* and *Saccharomyces* were occurred in the roots and *Hendersonula* only from leaves, which illustrated that on the one hand, a flora of diversity endophytic fungi resided in the medicinal plant *C. wenyujin* and on the other hand there were some differences in the quantity and composition of endophytic fungi associated with different tissues.

Endophytic fungi have been described as mutualists that protect host plants against insect herbivory through producing biologically active secondary metabolites [34]. Huang *et al.* [35] isolated 172 endophytic fungi from three medicinal plants and tested their fermentation broths for cytotoxicity, showing that the percentage of the broths at a dilution at 1:50 displayed a cytotoxic activity of 50% growth inhibition. Moreover, Phongpaichit *et al.*. [36] obtained 65 crude extracts from 51 endophytic fungi isolated from *Garcinia* plants and assessed these extracts for various bioactivities. In this research, 66 endophytic fungi isolated from *C. wenyujin* were tested for antibacterial activity by Oxford cup diffusion assay against eight isolates. Of these endophytes, 65% of endophytic fungi showed antibacterial activity against one or more than one microorganism, and 4.5% strains could inhibit eight tested microbes, exhibiting a broad antimicrobial spectrum (Fig 1). These active isolates mainly recovered from the root of *C. wenyujin* and distributed in the species of *Penicillium* (18.2%), *Fusarium* (12.1%) and *Aspergillus* (9.1%) (Fig 2). These findings provide

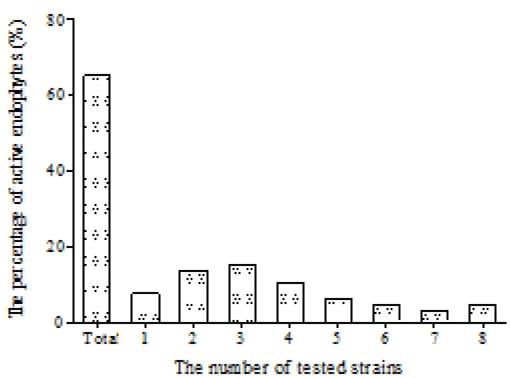

Fig 1. The summary of active endophytic fungi from *C. wenyujin*. X axis stands for the number of tested microbes which can be inhibited

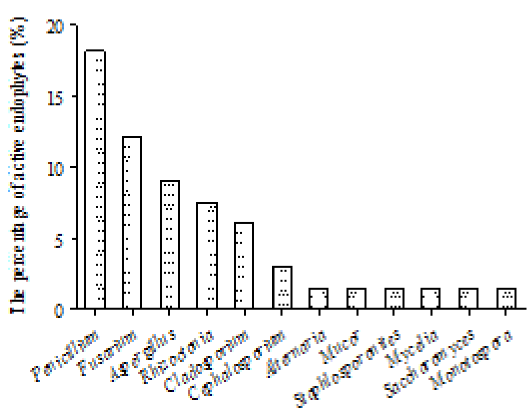

Fig. 2. The distribution of active entophytic fungi in *C. wenyujin*

a strong platform for the isolation and purification of natural antimicrobial agents from endophytic fungi of *Curcuma*.

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