Biodiversity and Antimicrobial Activity of Endophytic Fungi in *Angelica sinensis*

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Abstract: **Objective** To systematically investigate the biodiversity, ecological distribution, and antimicrobial activities of the endophytic fungi in *Angelica sinensis* growing in several natural habitats in China. **Methods** The isolation, culture, and identification of microorganism and mycelium growth inhibition test were adopted, and the relative data were analyzed by the statistical methods. **Results** A total of 206 isolates of endophytic fungi representing 22 species were collected at different time periods from *A. sinensis* in three locations. Melanconiaceae (45.1%) was the most prevalent followed by Dematiaceae (34.0%), mycelia sterilia (17.0%), Tuberculariaceae (2.9%), Moniliaceae (0.5%), and Leptostromataceae (0.5%). The main genera were *Gongromella* Ribaldi, *Coniosporieae* Lk. ex Fr., *Fusella* Sacc., *Myxormia* B. ex Br., *Ozonium* Lk. ex Fr., *Pestalotia* de Not., *Phacodium* Pers. ex Wallr., and *Sphaceloma* de Barry. Endophytes from the samples of Min county showed more diversity, percentage colonization, and species richness compared to other two locations. Endophytic colonization frequency was also greater in Min county (56.5%) than those in Heqing county (29.5%) and Baoxing county (17.0%). As to endophytic community in different plant tissues, the maximum endophytes species richness and diversity appeared in root tissues rather than in stem and leaf tissues from each location. Leaf samples were colonized by greater numbers of endophytes relative to the stem and root samples of the same location. Antimicrobial assay of the 206 endophytes showed that 20% was capable of inhibiting plant pathogenic fungi and eight strains displayed strong inhibition. **Conclusion** Endophytic fungi isolated from *A. sinensis* are specific in location, tissue, and season, and diverse in species. They are potential sources of antimicrobial agents which might improve the stress resistance and growth of this medicinal plant and play the important biological functions in ecosystem. Therefore, endophytic fungi will explore a new way to control plant diseases and develop healthful Chinese crude drugs.

Key words: *Angelica sinensis*; antimicrobial activity; biodiversity; ecological distribution; endophytic fungi

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Introduction

Endophytes are defined as organisms which inhibit plant tissues at some stages in their life cycle without causing apparent harm to their hosts (Rodriguez *et al*, 2009; Hanada *et al*, 2010). In recent years, many researches regarding the roles of the endophytic fungi in the host plants have been carried out. The stability or variability of the asymptomatic interaction between endophytic fungi and their host plants depends on numerous factors such as environmental stress, senescence of the hosts, virulence of the endophytes and the host defense response (Compant, Heijden, and Sessitsch, 2010). In general, the endophytic fungi might play an important role in the physiology of the plant by conferring the resistance to insects and herbivores, drought tolerance, protection against pathogens and enhancement of vegetative growth (Arnold *et al*, 2007; Higgins *et al*, 2007; Opelt *et al*, 2007; Senthilkumar, Govindasamy, and Annapurna, 2007; Brosi *et al*, 2011). In addition, the endophytes may not only produce special bioactive substances, but also induce or promote their host plants to synthesize or accumulate more

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secondary metabolites. And even on some occasions, the endophytic fungi may produce one or more of the same secondary metabolites which are known from the host plant. Such is the case of taxol, a potent anti-breast cancer drug, being produced by *Taxomyces andreanae* Strobel, Stierle & Hess, an endophyitc fungus isolated from *Taxus brevifolia* Nutt. (Stierle, Strobel, and Stierle, 1993).

Endophytic fungi have been screened from many plants ever investigated. In recent years, researches on the biodiversity of endophytic fungi from medicinal plants and their functions, such as antifungal activity, promotion of growth, and active compound accumulation of the host plants, are extensively explored (Li *et al*, 2001; Banerjee *et al*, 2009; Xu *et al*, 2009; Zhang *et al*, 2009). Endophytic fungi isolated from a Brazilian medicinal plant could produce guignardic acid (Rodrigues *et al*, 2001). Likewise, new bioactive metabolites were produced by *Colletotrichum* sp. Corda, an endophytic fungus in *Artemisia annua* L., a traditional Chinese medicinal herb (Lu *et al*, 2000).

Angelica sinensis (Oliv.) Diels is a small, perennial, slender, and erect bushy herb that grows at the cool and moist mountain with an elevation of 1700 to 3000 m. *Angelicae Sinensis Radix*, called *Danggui* in China, has been used as a traditional Chinese medicinal material (TCMM) for thousands of years to treat menstrual disorders, amenorrhea, dysmenorrheal, and so on (Zhang, 2001; Liu, Mei, and Cheng, 2005). Previous phytochemical studies on this herb had resulted in isolation and identification of different constituents, including phthalides, organic acids, polysaccharides, polyacetylenes, amino acids, and trace elements (Lü *et al*, 2009a; 2009b). In China, this medicinal plant is mainly found in some regions of Gansu, Yunnan, Sichuan, Shanxi, and Hubei provinces. However, the qualities of *A. sinensis* from various areas are quite different. *A. sinensis* with the best quality mainly grows in regions of Min county (Gansu, China) (Chen and Yang, 2004). At present, a systematic research (phytochemical constituents, ecological factors like soil, climate, and water, etc) on the quality formation of this TCMM has been carried out in our laboratory. We had isolated a new phthalide dimmer and ceramides from *A. sinensis*, which was reported for the first time (Lü *et al*, 2009a; 2009b). After analyzing the dynamic accumulations of volatile constituents in the roots of *A. sinensis* from various regions and harvest times, we proposed that the late autumn (October) is the appropriate harvest time (Ge *et al*, 2009). Currently, the endophytic community of this herb has not been investigated. Thus, it would first be critical to do a systematic study on the biology and distribution of endophytes with the important effects on the growth of this plant. This report shows the relative importance of location, tissue, and season preference of endophytes, their biodiversity and antimicrobial activities. It represents an important initial step in beginning to understand the role of endophytes to this herb. In addition, our research is also a part of an ongoing project on the conservation of medicinal plants and their associated microorganisms in China.

Materials and methods

Sample collection

Plant samples were selected from three places, Min county, Gansu province; Heqing county, Yunnan province; and Baoxing county, Sichuan province, China (Table 1). Two hundred samples of the leaves, stems, and roots from *A. sinensis* were collected on four separated occasions (August, September, October, and November, 2011) from twenty individual plants at each location, respectively. All samples were immediately brought to the laboratory in an icebox, and the tissues were screened for endophytic fungi.

Screening, identification, and preservation of endophytes

All the samples were washed properly in running tap water followed by double distilled water before surface sterilization. Surface sterilization was performed by the following immersion sequence: 75% ethanol for 1 min, NaOCl (6% available chlorine) for 3 min, and 75% ethanol for 0.5 min. The samples were then rinsed with sterile distilled water for three times and dried under aseptic conditions. The samples were cut into small pieces. Leaves were cut into small discs; stems and roots were cut into 1.0 cm × 1.0 cm pieces using a sterile pinch cutter. Segments of each sample were placed on potato dextrose agar (PDA) supplemented with streptomycin (100 mg/L) and incubated at 25 ℃ for up to 21 d. The plant segments were observed once a day for the growth of endophytic fungi. Hyphal tips growing from the plant segments were then subcultured.

Table 1 Ecological distribution of *A. sinensis* in China

No.	Locations	Ecological conditions	Brief description of radix
1	Min county	34°16′—34°28′ N and 103°49′—104°03′ E, in the northwest of China, an average annual rainfall of 570—650 mm, an annual mean temperature of 4.5—5.7 ℃, cool and moist climate, mountainous regions, brown, fertile and loose soil	Main producing area, high yields (over 80% total yields of China), large individual, compact texture, high concentration of volatile constituents
2	Heqing county	25°57′—26°42′ N and 100°01′—100°29′ E, in the southwest of China, an average annual rainfall of 959 mm, an annual mean temperature of 13.5 ℃, cool climate, many landforms (mountains, hills basins, and river valley), brown soil	About 10% total yields of China, compact texture, high concentration of volatile constituents
3	Baoxing county	30°24′—30°25′ N and 102°40′621—102°40′951 E, in the middle of China, an average annual rainfall of 993 mm, an annual mean temperature of 15 ℃, mild climate, mountainous region, brown soil	About 3% total yields of China, compact texture, high concentration of volatile constituents

The endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology. Standard taxonomic manuals were used to identify the fungal genera (Wei, 1979). All isolated endophytic fungi were maintained in cryovials on PDA layered with 20% glycerol and also in a lyophilized form at −20 ℃ in a deep freezer.

Preparation of fungal crude extract

Endophytic fungal isolates were grown on PDA at 25 ℃ for 5 d. Three pieces (0.5 cm × 0.5 cm) of mycelia agar plugs were inoculated into 500 mL Erlenmeyer flasks containing 200 mL potato dextrose broth, followed by shaking in an incubator at 120 r/min at 25 ℃ for three weeks. The broth culture was filtered to separate the culture broth and mycelia. Filtrate was extracted for three times with ethyl acetate, freeze-dried, and then dissolved in sterilized water.

Antimicrobial assay

The fungal extracts were screened for anti-microbial activity using the agar diffusion method (Rios, Recio, and Villar, 1988) against potentially plant pathogenic fungi [*Fusarium graminearum* Schwabe, *Alternaria solani* (Ell. et Mart.) Jones et Grout, *Botrytis cinerea* Pers. ex Fr., and *Rhizoctonia solani* Kühn]. The antimicrobial activity was assessed by the size (diameter in mm) of the inhibition zones. Sterilized water was used as the control. Each inhibition experiment was repeated for five times.

Statistical analysis

A total of 200 segments in different tissues of *A. sinensis* were isolated and statistically analyzed. The relative colonization frequency (CF) of endophytic species was calculated using the formula outlined by Hata and Futai (1995).

$$CF = (N_{col} / N_t) \times 100$$

where N_{col} stands for the number of segments colonized by each endophyte, and N_t stands for the total number of segments

The dominant endophytes were calculated as the CF percentage of a given endophyte divided by the sum of the CF percentage of all endophytes × 100. Based on the data of CF percentage in roots, stems, and leaves of different locations, diversity, evenness, and dominant indexes were calculated using the formulae (Guo *et al*, 2004).

$$\text{diversity index } (H) = -\sum \text{pi} \ln \text{pi}$$

$$\text{evenness index } (J) = -\sum \text{pi} \ln \text{pi} / \ln Sn$$

$$\text{dominant index } (D) = \sum (\text{pi})^2$$

where pi stands for CF proportion of the *i*th species in a sample, *Sn* stands for the total number of species present in a sample

Results

Endophytic communities of *A. sinensis* at different locations

Endophytic fungi from roots, stems, and leaves of *A. sinensis* at three locations were isolated and evaluated with the procedures described. A total of 113 isolates of endophytic fungi representing six genera were obtained from Min county, Gansu province, 59 isolates of endophytic fungi representing four genera from Heqing county, Yunnan province, and 34 isolates of endophytic fungi representing four genera from

Baoxing county, Sichuan province. Collectively, among the total endophytic isolates recovered from *A. sinensis* in this study, there were 45.1% Melanconiaceae, 34.0% Dematiaceae, 17.0% mycelia sterilia, 2.9% Tuberculariaceae, 0.5% Moniliaceae, and 0.5% Leptostromataceae. Among all the isolates, the dominant fungi were *Coniosporium arundinis* (Corda) Sacc., *Fusella* sp. Sacc., *Sphaceloma batatas* Saw., *Myxormia* sp. B. ex Br., and *Ozonium* sp. Lk. ex Fr. in Min county, *Myxormia* sp. B. ex Br., *Phacodium* sp. Pers. ex Wallr. and *Ozonium* sp. Lk. ex Fr. in Heqing county and *Myxormia* sp. B. ex Br. in Baoxing county. The endophytes only appeared in Min county were *Gongromeriza* sp. Preuss., *Coccosporella* sp. Karst., *Fusella* sp. Sacc., and *Hainesia* sp. Ellis. et Sacc.; and *Alternaria* sp. Nees ex Wallr., *C. cladosporioides*, *Naemospora* sp. Pers. ex Fr. only occurred in Heqing county. All the data above indicated that the endophyte communities in *A. sinensis* had obviously regional distribution characteristics (Table 2). As mathematically determined, the samples from Min county showed more diversity, percentage colonization, and species richness of endophytes than those in the other two locations. The maximum of 56.5% endophytes of the total in this study was recovered from Min county, while the minimum, at 17.0%, was recorded from the No. 3 location (Table 3).

Distribution of endophytic fungi in different parts of *A. sinensis*

The predominant endophytic fungus, *Myxormia* sp. B. ex Br., observed from the three places, distributed in the stems and leaves of *A. sinensis*. However, *Phacodium* sp. Pers. ex Wallr. mainly distributed in the roots and stems, *Ozonium* sp. Lk. ex Fr. in the stems and leaves. It is especially to be found, the dominance in Min county, *Fusella* sp. Sacc., distributed in the stems and leaves, did not appear in Heqing county and Baoxing county (Table 2). On the other hand, *Hainesia*

Table 2 Composition of endophytic fungi from root, stem, and leaf segments of *A. sinensis* in three different locations

Endophytic fungi	No. 1			No. 2			No. 3			Total isolates	CF / %	Dominance of fungi
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf			
Dematiaceae												
Alternaria sp.	0	0	0	0	0	2	0	0	0	2	1.0	0.97
C. cladosporioides	0	0	0	0	0	2	0	0	0	2	1.0	0.97
C. arundinis	0	3	9	0	2	0	0	1	1	16	8.0	7.77
Fusella sp.	1	12	27	0	0	0	0	0	0	40	20.0	19.42
Gongromeriza sp.	1	2	2	0	0	0	0	0	0	5	2.5	2.43
Nigrospora oryzae	1	0	1	0	1	0	0	0	0	3	1.5	1.46
Torula rhododendri	0	0	1	0	1	0	0	0	0	2	1.0	0.97
Leptostromataceae												
Leptothyrium cameliae	0	0	1	0	0	0	0	0	0	1	0.5	0.49
Melanconiaceae												
Colletotrichum gloeosporioides	2	0	0	1	0	1	1	0	3	8	4.0	3.88
Cylindrosporium humuli	3	0	0	1	0	0	1	0	0	5	2.5	2.43
Hainesia sp.	1	0	0	0	0	0	0	0	0	1	0.5	0.49
Hypodermium sp.	1	0	0	1	0	0	1	0	0	3	1.5	1.46
Melanconium oblongum	1	0	0	1	1	0	3	1	0	7	3.5	3.40
Myxormia sp.	1	5	7	2	6	14	1	2	5	43	21.5	20.87
Myxosporium corticola	0	0	0	1	0	0	2	0	0	3	1.5	1.46
Naemospora sp.	0	0	0	2	0	0	0	0	0	2	1.0	0.97
Pestalotia macrotricha	0	0	3	1	0	1	1	1	3	10	5.0	4.85
S. batatas	1	3	3	0	1	1	0	1	1	11	5.5	5.34
Moniliaceae												
Coccosporella sp.	0	1	0	0	0	0	0	0	0	1	0.5	0.49
mycelia sterilia												
Phacodium sp.	3	2	0	5	2	0	2	0	0	14	7.0	6.80
Ozonium sp.	7	3	2	0	0	7	0	2	0	21	10.5	10.19
Tuberculariaceae												
Fusarium chlaydosporum	0	0	3	0	0	2	0	1	0	6	3.0	2.91
Total isolates	23	31	59	15	14	30	12	9	13			

Table 3 Species richness, CF percentage, and dominant endophytic fungi in *A. sinensis* at each location

No.	Total isolates	Species richness	CF / %	Dominance of fungi
1	113	18	56.5	0.549
2	59	17	29.5	0.286
3	34	12	17.0	0.165

sp. Ellis. et Sacc., *Hypodermium* sp. Lk. ex Cda., and *M. oblongum* were mainly isolated from the roots and *F. chlaydosporum* mainly from the leaves. Some rare and incidental species, such as *Alternaria* sp. Nees ex Wallr. and *C. cladosporioides*, were recovered only from the leaves in Heqing county, *M. corticola* and *Naemospora* sp. Pers. ex Fr. only in the roots, and *Coccosporella* sp. Karst. only in the stems.

Four separate indices, the diversity, richness, evenness, and domination indices, were used to determine the species diversity of endophytic fungi colonizing *A. sinensis*. Generally, the root samples from each location showed more diversity of endophytic fungi than the leaf and stem samples. However, the leaf samples from each location were colonized by greater numbers of endophytes as compared to the stem and root samples from the same location. Endophytes from the stem samples in Baoxing county had the maximum evenness index, and the minimum endophytic evenness index was from the leaf samples in Min county (0.746). On the other hand, the dominant index ranged between 0 and 1, whereby, the larger index value proportionally reflects the greater species diversity. For example, the leaf samples in Heqing county had the maximum domination index, i.e., a greater diversity for the leaf samples from Heqing county (Table 4).

Table 4 *H*, richness index (*S*), *J*, and *D* of endophytic fungi from different tissues of *A. sinensis* at three different locations

No.	Parts	Total isolates	*H*	*S*	*J*	*D*
1	root	23	2.196	12	0.884	0.149
	stem	31	1.804	8	0.868	0.282
	leaf	59	1.789	11	0.746	0.258
2	root	15	1.987	9	0.904	0.173
	stem	14	1.673	7	0.805	0.245
	leaf	30	1.577	8	0.758	0.289
3	root	12	1.979	8	0.952	0.153
	stem	9	1.889	7	0.971	0.160
	leaf	13	1.439	5	0.894	0.266

Occurrence of endophytic fungi from *A. sinensis* in different months

In Table 5, the number of endophytic fungi found in the different locations and seasons is shown. Several endophytic fungi were shown to be exclusive of the seasons and more of them were found in September and October in each location. Further analysis examined how endophytic populations varied according to the sampling period. Results showed that 22 species of endophytic fungi varied in the four different months. Two endophytic fungi were found mainly (*S. batatas* and *Ozonium* sp. Lk. ex Fr.) or exclusively (*Alternaria* sp. Nees ex Wallr. and *C. cladosporioides*) in September and October, three (*T. rhododendri*, *L. cameliae*, and *Hainesia* sp. Ellis. et Sacc.) exclusively in September, one (*C. humuli*) mainly in November and one (*M. corticola*) did not vary significantly among the four seasons at each location. In addition, *Fusella* sp. Sacc. and *Gongromeriza* sp. Preuss. distributed during all the seasons studied exclusively in No. 1 sample.

The endophytic diversity and richness indices in October were relatively higher than the other months. However, endophytes from November/Min County had the maximum diversity index (2.398), and the maximum richness index (13) was from October/ Heqing County. On the other hand, the larger dominant index (0.344) of endophytes was from August/Heqing county, and the greater evenness index (1.000) was from August and November/Baoxing county (Table 6).

Antifungal activity

All the isolates were fermented and the crude extract of each fermentation broth was evaluated *in vitro* for antimicrobial activity. Among the 206 isolates, the inhibitory rates of endophytic fungi against *F. graminearum* and *A. solani* were 24.3% and 28.6%, respectively (Fig. 1). There were eight strains with strong antimicrobial activity against the test pathogenic fungi, two strains (*Coccosporella* sp. Karst. and *C. arundinis*) only against *A. solani*, two strains (*Fusella* sp. 3 and *Myxormia* sp. 3) only against *R. solani*, and *Hypodermium* sp. 1 only against *F. graminearum*. *Fusella* sp. 2 and *Myxormia* sp. 2 displayed strong inhibition against *B. cinerea*. Moreover, *Phacodium* sp. Pers. ex Wallr. showed a relatively broader spectrum of antimicrobial activity (Table 7).

Table 5 Occurrence of endophytic fungi from *A. sinensis* at different months

Endophytic fungi	No. 1				No. 2				No. 3			
	Aug.	Sep.	Oct.	Nov.	Aug.	Sep.	Oct.	Nov.	Aug.	Sep.	Oct.	Nov.
Dematiaceae												
Alternaria sp.	0	0	0	0	0	1	1	0	0	0	0	0
C. cladosporioides	0	0	0	0	0	1	1	0	0	0	0	0
C. arundinis	3	5	2	2	0	0	1	1	0	0	1	1
Fusella sp.	6	21	8	5	0	0	0	0	0	0	0	0
Gongromeriza sp	1	2	1	1	0	0	0	0	0	0	0	0
N. oryzae	0	0	0	2	1	0	0	0	0	0	0	0
T. rhododendri	0	1	0	0	0	1	0	0	0	0	0	0
Leptostromataceae												
L. cameliae	0	1	0	0	0	0	0	0	0	0	0	0
Melanconiaceae												
C. gloeosporioides	0	1	0	1	0	1	1	0	1	1	1	1
C. humuli	0	0	1	2	0	0	1	0	0	0	1	0
Hainesia sp.	0	1	0	0	0	0	0	0	0	0	0	0
Hypodermium sp.	0	1	0	0	0	0	0	1	0	0	1	0
M. oblongum	1	0	0	0	0	0	1	1	0	2	1	1
Myxormia sp.	2	7	3	1	4	8	7	3	1	5	1	1
M. corticola	0	0	0	0	0	0	1	0	0	0	1	1
Naemospora sp.	0	0	0	0	0	0	2	0	0	0	0	0
P. macrotricha	1	1	1	0	0	1	1	0	1	1	2	1
S. batatas	1	2	2	2	0	1	1	0	0	1	1	0
Moniliaceae												
Coccosporella sp.	0	0	1	0	0	0	0	0	0	0	0	0
mycelia sterilia												
Phacodium sp.	1	1	1	2	2	3	1	1	0	0	1	1
Ozonium sp.	2	2	4	4	1	3	2	1	0	1	1	0
Tuberculariaceae												
F. chlaydosporum	0	0	2	1	0	0	0	2	0	1	0	0

Table 6 *H, S, J,* and *D* of endophytic fungi from *A. sinensis* in different months

Sampling month	Sampling locations	Total isolates	*H*	*S*	*J*	*D*
Aug.	Min countiy	18	1.956	9	0.890	0.179
	Heqing county	8	1.213	4	0.875	0.344
	Baoxing county	3	1.099	3	1.000	0.333
Sep.	Min countiy	46	1.877	13	0.732	0.252
	Heqing county	20	1.834	9	0.835	0.220
	Baoxing county	12	1.699	7	0.873	0.236
Oct.	Min countiy	26	2.118	11	0.883	0.157
	Heqing county	21	2.264	13	0.883	0.152
	Baoxing county	12	2.369	11	0.988	0.097
Nov.	Min countiy	23	2.398	11	0.935	0.123
	Heqing county	10	1.834	7	0.943	0.180
	Baoxing county	7	1.946	7	1.000	0.143

Discussion

Recently, various works have been done to study the relationships between environmental conditions and good quality formation of Chinese medicinal herbs, such factors as climate (illumination, temperature, humidity, and air), soil, and water quality, which are the main indexes to evaluate the suitableness of these herbs. However, these studies just limited to the contribution of external environmental factors, but not the internal environment of plants. The communities and distribution of endophytes are the important internal cause that could affect the growth and development of plants, which are correlated with the hosts and biogeocoenosis (region, climate, etc).

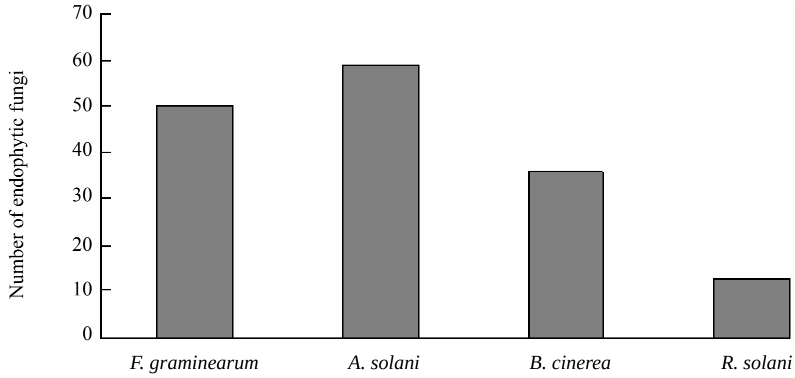

Fig. 1 Composition of endophytes with inhibition on plant pathogenic fungi

Table 7 Antimicrobial activity of metabolites from some endophytic fungi

Endophytic fungi	Inhibitory rate / %			
	F. graminearum	*A. solani*	*B. cinerea*	*R. solani*
Coccosporella sp.	0	57.1 ± 1.2	0	0
C. arundinis	0	62.9 ± 2.9	0	0
Fusella sp. 2	42.5 ± 1.6	0	76.6 ± 1.9	0
Fusella sp. 3	0	0	0	60.7 ± 2.7
Hypodermium sp. 1	71.6 ± 2.1	0	0	0
Myxormia sp. 2	0	0	68.3 ± 1.8	0
Myxormia sp. 3	0	0	0	40.6 ± 1.2
Phacodium sp.	60.8 ± 1.6	39.2 ± 1.8	16.3 ± 0.5	0

Accordingly, it is important to investigate the biodiversity and ecological distribution of endophytes and to learn the relationships between the endophytes and their hosts. This systematic study on the population structure, ecological distributions and dynamic fluctuation of endophytes in *A. sinensis* contributes to the further research on the influence on the quality formation of TCMM.

The results set a nice example for the other studies on endophytic microbes given the fact that multiple sites, multiple plant tissues, different months and mathematical analyses have helped define how maximum biodiversity and ecological distribution may be found in a given medicinal plant species. Because of the difference among the geographic setting, temperature, humidity, and illumination in different locations, the infection rate, CF percenteage, and growth are changed, and consequently, parts of the endophytes have the obvious region and tissue specificity, and appear the dynamic change along with the different growth and development periods of *A. sinensis*. Similar findings were obtained in case of *Neotyphodium* Pers. ex Fr., whose colonization in the *Bromus setifolius* L. was studied by Novas, Collantes, and Cabral (2007). The tissue specificity of endophytes in the different tissues of *A. sinensis* may be caused by the microenvironments among the tissues. Similar results on the ecological distribution of endophytes in roots, stems, and barks of *Azadirachta indica* A. Juss by Verma *et al* (2007) showed that the maximum species richness and CF percenteage of endophytes appeared in the leaf segments rather than the stem and bark tissues, whereas the bark samples showed the maximum diversity. Endophytic fungi living in the healthy tissues of plants are relatively unstudied and potential sources of novel natural products for the exploitation in medicine, agriculture, and industry (Strobel and Daisy, 2003). Upon exposure to virulent pathogens, endophyte colonized plants activate host defenses more quickly than non-symbiotic plants (Kari *et al*, 2004; Higgins *et al*, 2007). In this study, more than 20% strains had antimicrobial activity. Indeed, endophytic fungi inside the host plants are a versatile reservoir of the various bioactive metabolites and could be of potential use to modern medicine, industry and agriculture. Our results clearly confirmed that endophytic fungi from *A. sinensis* were sources of novel and antimicrobial compounds which might protect this herb against pathogens and be helpful to the growth. Further studies of characterizing the bioactive metabolites of the potent fungal strains from *A. sinensis* are in progress.

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