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Full Length Research Paper

Endophytic fungi diversity of wild terrestrial plants in Kyrgyzstan

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A total of 255 species of wild medicinal plants were analyzed in Northern and Northeastern Kyrgyzstan; from 150 plant species, 278 endophytic fungal isolates were identified. Endophytic fungi were frequently recorded on members of large plant families: *Asteraceae*, *Ranunculaceae*, *Rutaceae*, *Fabaceae*, *Lamiaceae*, *Boraginaceae*, *Moraceae*, *Iridaceae*, *Euphorbiaceae* and *Brassicaceae*. In the foothill ecosystems, rich biodiversity and numerous species of endophytic fungi genera - *Fusarium*, *Acremonium*, *Cladosporium*, *Penicillium*, *Curvularia*, *Monilia*, *Rhizoctonia*, *Papularia*, *Botrytis* and *Stemphylium* - were registered. The comparison of the Shannon diversity index (HS) for the endophytic fungi community showed a higher diversity of light-colored species (HS = 3.47) in foothill ecosystems than in alpine systems (HS = 2.75), and a lower diversity in the middle mountains (HS = 2.46). Endophytes were identified from all organs of plants. The most favorite habitats for fungi in plant body were root tissues, followed by the stems and leaves, while the flower clusters and seeds were rarely colonized by endophytes.

Keywords: wild medicinal plants, biodiversity of endophytic fungi, distribution on vertical zonation, tissues colonized.

INTRODUCTION

Microscopic fungi are an essential component of terrestrial ecosystems. They are found in extreme environmental conditions, at high and low temperatures, in the Arctic ice, in the upper layers of the atmosphere, in the phyllosphere and in plant tissues. Among them, a special position is occupied by the endophytic hyphal fungi that live in the internal organs of land plants. G. C. Ainsworth (1971) defined the endophytes "as plants living inside another organism." Endophytic fungi are located in symbiotic associations with different plant species, without causing any external signs of damage and contamination, and they can be isolated from various parts of plants (Ainsworth 1971; Carroll 1988; Rajagopal et al., 2011; Arnold and Lutzoni 2007). All vascular plants feed the endophytic organisms (Wildman 2003). Between them, endophytic fungi and vascular plants have developed an evolutionarily mutualistic relationship, in which the plants provide the fungus with food and a stable habitat. Endophytes, for their part, produce different metabolites that positively affect the life and functioning of the plant.

Researchers note that the species diversity and frequency of endophyte species depend on the type of host plant tissue (Maheswari and Rajagopal 2013). The species derived from one plant body may not be derived from others. However, the dominant fungal species isolated from different plant organs can exhibit a sufficient degree

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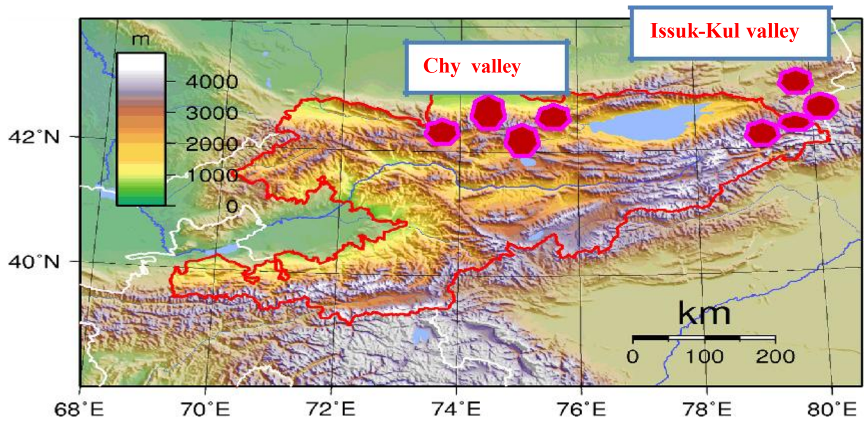

Figure 1. Map of Kyrgyz Republic, showing a location of studied areas in Chy and Issyk-Kul valleys

of repeatability (Kumar and Hyde 2004). Mechanisms through which an endophyte exists and responds to its environment must be better understood in order to suggest in what plants and at what times to look for them, thus facilitating the process of discovering a new producer.

There has been great interest in endophytic fungi as potential producers of novel, biologically active products (Wildman 2003; Schutz et al., 2002; Strobel and Daisy 2003; Tomita 2003; Urairuj et al., 2003; Manoharachary et al., 2013; Spiering et al., 2006). Unique species of endophytic fungi with a wide range of potential practical applications in plant protection as repellents, insecticides, antimicrobials, anthelmintic and vermicides have been found (Strobel et al., 2008; Vega et al., 2008). Furthermore, there is evidence of the use of endophytes for producing anticancer, antimicrobial and antioxidant compounds, and also in a biotransformation process (Pimentel et al., 2011).

Endophytic fungi from medicinal plants have been studied intensively over recent years, and fungal isolates with antimicrobial, anticancer and antimalarial activities were obtained (Wiyakrutta et al., 2004; Raviraja 2005; Gangadevi and Muthumary 2007 ; Mohanta et al., 2008).

Kyrgyzstan is a poorly studied region of the globe for microbial diversity, including mycological diversity. In Kyrgyzstan's natural areas, there are more than 22 different ecosystems, among them high alpine meadows, alpine steppes, alpine deserts, mountain lakes and rivers, juniper, tugai and broadleaf forests, etc. These ecosystems offer a habitat for various species of wild plants, and for their associated endophytic fungi, but the biodiversity, ecology and biology of endophytic fungi remain practically unexplored. Furthermore, the appropriateness of bioprospecting for endophytic fungi as sources of new active compounds is scientifically undetermined and almost unexplored, which means the relevance of these studies is unclear. The purpose of this study was to examine the species diversity and ecology of endophytic fungal symbionts of wild medicinal plants growing in different climatic conditions on the vertical zonation areas of Kyrgyzstan, in order to identify useful biological properties of their isolates.

METHODS

Study area

The areas visited during this study include the ***Northern Tien Shan*** **and the** ***Northeastern Tien Shan***, located between latitude 42° N and longitude 79° E (Figure. 1).

Northern Tien Shan includes almost latitudinal ranges: Kyrgyz Ala-Too, Terskey Ala-Too and Kungey Ala-Too. The basic herbarium material was collected in the Jong-Kurchak tract and Ala-Archa gorge, in Arashan and Tatyr villages. The rest of the material was collected in the vicinity of the Chui valley. The Jong-Kurchak tract is located in the subalpine zone of the central part of the northern macro-slope Kyrgyz ridge (watershed of Alamedin and Ala-Archa) at altitudes of 2300-3400 m. Ala-Archa Park is in Ala-Archa gorge, which is located on the northern macro-slope Kyrgyz ridge in the most sublime, its central part. The pool of Ala-Archa river within the National Park is strongly dissected by mountains with steep slopes and deeply incised canyons with altitudes of 1650-4875 m above sea level.

Northeastern Tien Shan includes the nonfreezing Lake Issyk-Kul surrounded by the ranges of Terskey Ala-Too and Kungey Ala-Too. Our research area was located mainly in the south-west of the eastern part of the Issyk-Kul basin, including the flat part of the shore of Lake Issyk-Kul and the mountain Terskey Ala-Too.

Plant samples

Systematic selection of plant samples was performed from 2004 to 2008; a total of 255 species of plants were analyzed in the surveyed habitats of Northern Tien Shan and Northeastern Tien Shan. Of these, 278 endophytic fungal isolates were isolated from 150 plant species. All plant parts - stems, roots, leaves, buds and seeds - have been used to isolate pure cultures of endophytic fungi.

Treatments of plant samples and detection of fungal isolates

Plant specimens were thoroughly washed in running tap water for 10 minutes. Disks of 4-6 mm diameter were cut from each leaf using a sterile cork borer. The stems and the roots were cut into 2 cm long segments. Flowers were treated as a separate entity, and 20 individual flowers were taken from each inflorescence. The surfaces of all samples were sterilized by immersion in 75 % ethanol for 1 minute, and then in 2.5 % solution of sodium hypochlorite (NaOCI) for 2 minutes, and finally 75 % ethanol for 30 seconds. The surface-sterilized samples were washed three times with sterile distilled water and blotted with sterile tissue paper (Schulz et al. 1993; Kumaresan and Suryanarayanan 2002). Plant segments were then transferred to potato dextrose agar (PDA, Oxoid) plates containing 1 % streptomycin to inhibit the growth of bacteria. Plates were labeled accordingly and incubated at 24 °C with a 12-hour cycle of dark and light (Lacap 2003**)**.

Cultivation of endophyte isolates

The growing edges of colonies from the plant segments were transferred to PDA plates by hyphal tipping (Strobel et al., 1996). Continuous transfer of fungi was carried out as new colonies continued to appear for up to two or three weeks. Plates were then incubated and periodically tested for purity by hyphal tipping. The fungal isolates were numbered and stored in sterile distilled water at 4 °C. To determine their physiological characteristics, the micromycetes were grown at different temperatures: 4 °C; 26-27 °C and 42 °C. To induce a sporulation, the isolates were cultured on potato-carrot agar. The cultures which failed to sporulate within 2-4 months of incubation were designated as '*mycelia sterilia*', and sorted into morphotypes according to cultural characteristics (Lacap 2003; Guo et al.,1998).

A set of carbohydrates (glucose, arabinose, maltose, D-mannitol, sucrose, D-galactose, mannose) and nitrogen sources were used to study the biochemical properties of fungal strains. To isolate primary cultures as well as to determine the physiological needs of the fungi for nutrients, the following media were used: potato dextrose agar (PDA), peptone-dextrose agar (PDA), starch-ammonium agar (SAA) and Czapek agar.

Identification

Pure cultures were examined periodically for sporulation and identified. Fungal identification methods were based on the morphology of the fungal culture and on the mechanism of spore production by following the standard mycological manuals (Ellis 1971; Sutton 1980; Bilai 1982; Geltser 1990; Nag 1993; Garibova and Lekomtseva 2005; Courtecuisse 1995; Cherepanova 2005; Petrini 1986; Singer 1986). Morphometric features of mycelium and the sporulation structure of fungi were examined using an advanced compound microscope, MEIJI model ML5500, and a MEIJI zoom stereo microscope, model EMZ-5TR-MA502-PBH (Japan). Micrographs were performed using a MOTIC 2.0 megapixel digital microscope camera with Images 2000 software, model MOTICAM 2000.

Statistical analysis

Species diversity of endophytic fungi was analyzed in terms of species richness and evenness. We have used Chao I and Chao II rarefaction methods, which allow us to compare the number of species found in two regions (Chy valley and Issyk-Kul valley) where the sampling efforts differed. When we have found the fungi species in the less-sampled region, this rarefaction takes hypothetical subsamples of organisms from the more-sampled region, and calculates the average number of species in such subsamples. Chao I method was used (Chao 1984) for nonparametric estimation of species richness to estimate the number of taxa when most of the information is concentrated in the low-order occupancy numbers:

$S_1 = S_{OBS} + (a / 2в), S_{OBS}$

S obs - the number of observed species; a - the number of species observed once; b - the number of species observed twice. When this formula is applied to a single collection, it is called the Chao-1 estimator. When applied to several collections, and "just once/twice" means "observed in just one/two collections", it is called the Chao-2 estimator (Chao 1987). In both cases, a standard deviation is computed using:

$SD = b [(a / (4b))^4 + (a/b)^3 + (a / (2b))^2$

The Shannon biodiversity index (HS) was used to show the extent of the species richness of endophytic fungal communities in the studied ecosystems.

$H^{S} = \Sigma [(n_i/ N) \ln (n_i/ N)];$

H^1 - Shannon numerical value index, i = 1, changing and the sum of several members, S -number of species; N –

Table 1. Taxonomic structure of endophyte fungi isolated from wild medicinal plants of Northern and Northeastern Kyrgyzstan

The family of micromycetes	Genera of micromycetes	Number of isolates	
		Chu valley	Issyk-Kul valley
Moniliaceae	*Geotrichum*	5	-
	Monilia	-	3
	Trichoderma	22	-
	Aspergillus	4	2
	Gliocladium	3	-
	Penicillum	31	7
	Botrytis	8	1
	Verticillium	-	-
	Trichothecium	-	-
	Paecilomyces	2	1
	Blastotrihum	2	-
	Scopulariopsis	4	1
	Acremonium	21	2
Dematiaceae	*Fusicladium*	3	-
	Cladosporium	18	2
	Stemphylium	3	1
	Macrosporium	4	-
	Alternaria	12	2
	Curvularia	14	2
	Papularia	10	2
Tuberculariaceae	*Fusarium*	30	3
Mycelia sterilia	*Sclerotium*	1	-
	Rhizoctonia	2	-
Total		**255**	**23**

abundant taxon; n - number of samples, ln - natural logarithm.

RESULTS

Taxonomic analysis of endophytic fungi, isolated from wild medicinal plants

The systematic structure of endophytic fungi mycobiota identified in wild medicinal plants of Northern Kyrgyzstan and Northeastern Kyrgyzstan includes one class (*Fungi imperfecti*), one division (*Hyphomycetales*), four families and 23 genera.

Table 1 shows a taxonomic structure of endophyte fungi isolated from wild medicinal plants of Northern and Northeastern Kyrgyzstan. *Moniliaceae* family was the largest, represented by 13 genera. In this family, *Penicillium* genus was the richest in terms of number of isolates; we have identified 38 isolates belonging to this genus. Such species as *Penicillium notatum*, *P. chrysogenum* and *P. cyclopium* were frequently detected. *Trichoderma* and *Acremonium* genera also have a large number of representatives: 22 and 23 isolates were found, respectively. Other genera have a few identified isolates from medicinal plants growing in ecosystems of Northern and Northeastern Kyrgyzstan. Four endophyte fungal isolates were noted as a new species in this family. According to their phenotypic and morphometric characteristics, we have still not established the species affiliation of these isolates. The study to identify the taxonomic and systematic affiliation of these new isolates is continuing.

Dematiaceae family was in second position in terms of the richness of the identified genera and related isolates. In this family, *Cladosporium*, *Curvularia* and *Alternaria* genera were more significantly represented by identified isolates. *Cladosporium* genera included 20 isolates; 16 isolates were detected within *Curvularia* genus, and 14 isolates were identified within *Alternaria* genus. By contrast, *Fusicladium* and *Macrosporium* genera were insignificant; their representatives were isolated only from medicinal plants in Chui valley, so they can be considered rarely found symbionts.

Table 2. The dominant plant species in ecosystems of Chui valley that host endophyte fungi

№	Genera of endophytic fungi	Plant species	Family of feeding plants	Number of endophytic fungi isolates
1.	*Alternaria*, *Penicillium*	*Xeranthemum longipapposum*	*Asteraceae*	34
	Acremonium, *Fusarium*	*Lachnonphyllum gossypinum*		
	Alternaria,*Penicillium, Acremonium*, *Fusarium*	*Artemsia macrocephala*		
2.	*Trichoderma*, *Papularia.*	*Thalictrum simplex*	*Ranunculaceae*	8
	Cladosporium	*Ranunculus repens*		
3.	*Stemphylium*	*Polygonum convonvulus*	*Polygonaceae*	8
4.	*Penicillium*, *Acremonium*	*Astragalus schangınianus*	*Fabaceae*	14
5.	*Papularia*	*Haplophylum perforatum*	*Rutaceae*	4
6.	*Stemphylium*	*Humulus lupulus*	*Moraceae*	6
7.	*Fusarium*, *Penicillium, Acremonium*	*Neslia apiculata*	*Brassicaceae*	
8.	*Penicillium*, *Acremonium*	*Nonnea caspica*	*Boraginaceae*	14
	Alternaria	*Arnebia guttata*		
9.	*Penicillium*	*Euphorbia jaxartica*	*Euphorbiaceae*	6
10.	*Acremonium*	*Iris sogdiana*	*Iridaceae*	2
11.	*Papularia*, *Botrytis*	*Eremstachys speciosa*	*Lamiaceae*	9

Tuberculariaceae family was represented by only the *Fusarium* genus, which consisted of 33 isolates. We should note that the representatives of this genus have been confined to the plants growing in the foothills and middle mountain ecosystems of Chui valley.

We have obtained the endophyte fungal isolates belonging to the *Mycelia sterile* family. It was represented by only two genera, and the number of representatives was also low. They were found only in the medium and high mountain areas of Chui Valley; they were not found in the ecosystems of Issyk-Kul valley. A total of 150 strains of endophyte fungi were identified to genus level and 50 strains to species level, from wild medicinal plants of Northern and Northeastern Kyrgyzstan.

The distribution of endophytic fungi in the vegetation of Chui valley (Northern Kyrgyzstan)

Herbarium materials were collected on vertical zonation: at altitudes of 600-1600 m above sea level, in the foothill zone with sagebrush-steppe and ephemeral vegetation; then at 1200-3000 m above sea level, in the middle mountain zone and tall grassland and meadow steppes, bush, mountain tipchak steppes, juniper, deciduous and spruce forests; and at 3000-3500 m above sea level, in subalpine zone, mainly geranium meadows, forest-steppe, steppe and juniper creeping.

Endophytic fungi were most often registered in the major representatives of wild plant families: *Asteraceae*, *Ranunculaceae*, *Rutaceae*, *Fabaceae*, *Lamiaceae*, *Boraginaceae*, *Moraceae*, *Iridaceae*, *Euphorbiaceae* and *Brassicaceae.*

From plants of the *Asteraceae* family, a rich diversity of endophytes was isolated: representatives of *Alternaria*, *Acremonium*, *Papularia*, *Botrytis*, *Penicillium*, *Fusarium* and *Curvularia* genera dominated in these plants. Meanwhile, *Penicillium* and *Acremonium* species dominated in the plants from the *Fabaceae* family. Endophyte fungi of the *Papularia* and *Botrytis* genera were often related to plants of the *Lamiaceae* family; endophytes from *Penicillium*, *Acremonium* and *Fusarium* genera were frequently associated with plants of the *Brassicaceae* family (Table. 2).

The analyzed wild medicinal plants differed in their content of endophytic fungi. The greatest number of endophytic fungi was isolated from the organs of dry flowers of long Hoholkova (*Xeranthemum longepapposum*) and large-flowered wormwood (*Artemisia macrocephala*). Numbers of endophyte species were relatively low in simple rue (*Thalictrum simplex*) and creeping buttercup (*Ranunculus repens*) from the *Ranunculaceae* family. Plants such as *Polygonum convolvulus*, *Astragalus schangınianus*, *Haplophyllum perforatum*, *Humulus lupulus*, *Nonea caspica*, *Arnebia guttata*, *Cotinus coggýgria*, *Eremostachys speciosa*, *Euphorbia jaxartica*, *Neslia apiculate* and *Iris sogdiana* contained in their bodies the same amount of endophytic fungi (Figure. 2; Figure. 3).

The distribution of light-colored, dark-colored and sterile mycelium of endophytic species was different in host plants

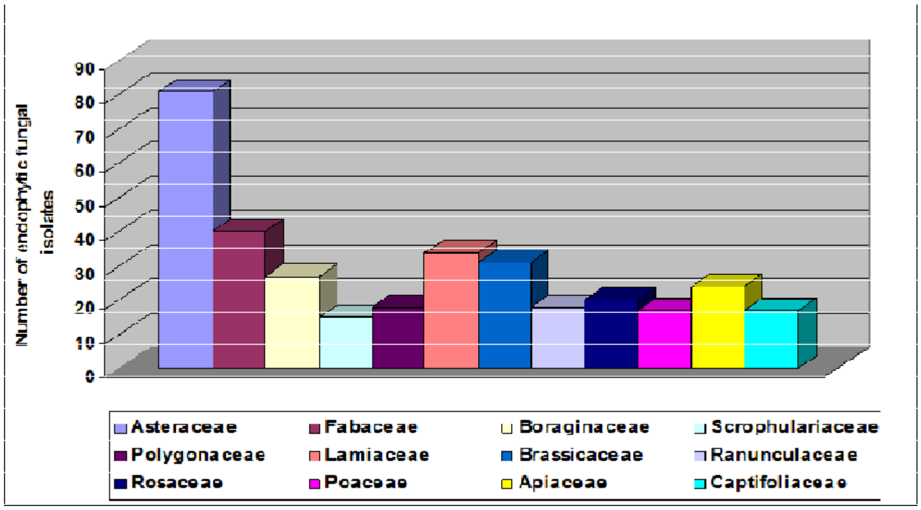

Figure 2. The largest families of plants with a high content of endophytic fungi

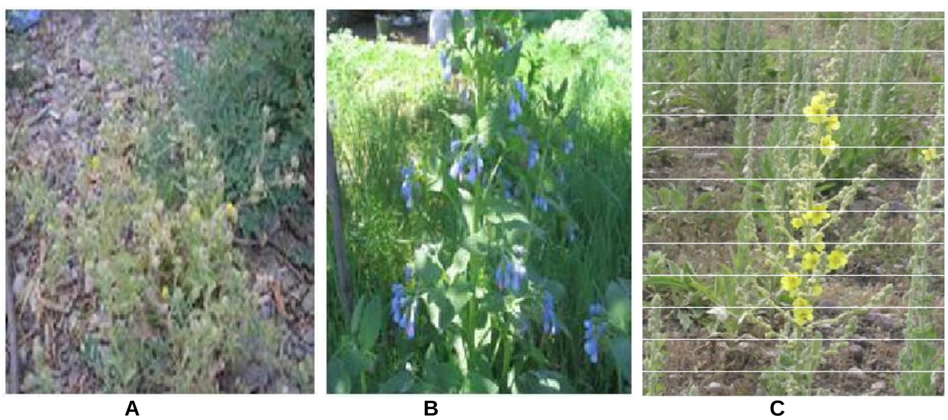

Figure3. Some host plant photos in nature: A-*L.gossypıum Bunge* **(***Asteraceae fam*.), B- *Caucasicum M.B. (Boraginaceae fam)*. C- *Songoricum Sch. (Scrophulariaceae fam)*

during the study period (four years). The frequency of fungal species in host plants fluctuated widely over the years. Table 3 shows the common species of endophytic fungi that dominated among other species.

Our analysis showed that the endophytic fungal isolates were unequally distributed in populations of plants on the vertical zonation. About 40 % of the isolates were found in plants grown at an altitude 1601-2085 m above sea level, 32 % at an altitude 636-814 m above sea level, 20 % at an altitude 1085-1540 m sea level, and only 8 % at an altitude 2530-3284 m above sea level (Figure. 4). In the foothill zone, a rich biodiversity of *Fusarium*, *Acremonium*, *Cladosporium*, *Penicillium*, *Curvularia*, *Monilia*, *Rhizoctonia*, *Papularia*, *Botrytis* and *Stemphylium* species, among others, was registered (Shannon index: 3.47). Among these species, *Penicillium*, *Alternaria*, *Cladosporium*, *Fusarium* and *Acremonium* genera dominated.

Penicillium, *Fusarium*, *Trichoderma* and *Acremonium* species were more characteristic of midland ecosystems (Shannon index: 2.46). A low number and poor diversity of endophytes was discovered in highlands with subalpine, alpine and nival vegetation. In total, only 8 % of endophytic fungi were isolated from this zone's vegetation, and *Penicillium* and *Cladosporium* species dominated (Shannon index: 1.6). However, some endophytic fungal species were found in all vegetation zones, while certain species preferred only one vegetation zone.

Our results have also confirmed the findings of other authors that the lowest number of endophytic fungi is to be found in the alpine zone, where evidently the high intensity of sunlight, low night temperatures and constant wind have

Table 3. Detection frequency of endophytic fungi in plants of Northern Kyrgyzstan (Chui valley), in %

List of endophyte species	2006-2007						2008-2009					
	1	2	3	4	5	6	1	2	3	4	5	6
	Deuteromycota, Hyphomycetes Light-colored species						Deuteromycota Hyphomycetes Light-colored species					
Acremonium charticola (Lindau) W. Gams	14	8	18	4	0.3	0.7	5	0.3	0.3	1.2	0	0.7
A. sclerotigenum (Moreau & R. Moreau ex Valenta)	3	0	12	4	1.2	0.7	0.7	0.7	0.3	2	0.3	0.3
Trichoderma viride Pers.	14	23	5	4	0.3	0.7	1.5	0.7	2	0.3	0.3	0.7
Penicillium chrysogenum Thom.	14	8	12	11	0.3	0.7	2	0.3	2	0.3	0.7	0
P. chatkalicum	10	0	5	4	0.7	0.7	2	1.2	0.3	0.7	0.7	0.3
Fusarium merismoides Corda	14	12	12	4	0.3	1.2	1.5	0.3	0.3	0.7	1.2	0.3
F.sporotrichioides Sherb.	7	8	5	17	0.7	0	1.5	0.3	0.7	0.3	0.3	0.3
	Deuteromycota Hyphomycetes Dark-colored species						Deuteromycota Hyphomycetes Dark-colored species					
Cladosporium herbarium (Pers.) Link	3	12	5	4	0.7	0.7	1.2	0.7	0.3	0.7	0.7	0
Papularia sphaerosperma (Pers.) Höhn	10	8	5	0	0	0.3	1.2	0.3	1.2	0.7	0.7	0.3
Stemphylium sp.	3	0	5	2	0.3	0.3	0.7	1.5	0.7	0.3	0.7	0.7
Alternaria alternata (Fr.) Keissl.	10	8	12	11	0.3	0	0.7	0.7	0.7	0.3	5	0.3
Alternaria tenuis Link	3	8	0	4	1.2	0.7	1.2	1.5	0.3	0.7	0.3	0.3
	Mycelia sterilia											
	0.7			1.2			0.7	0.3			0.3	0.3
The number of species	28	13	17	16	18	19	36	24	19	20	22	13

Note: The families of host plants: 1- *Asteraceae*; 2- *Ranunculaceae*; 3- *Fabaceae*; 4- *Brassicaceae*; 5- *Boraginaceae*; 6- *Lamiaceae*

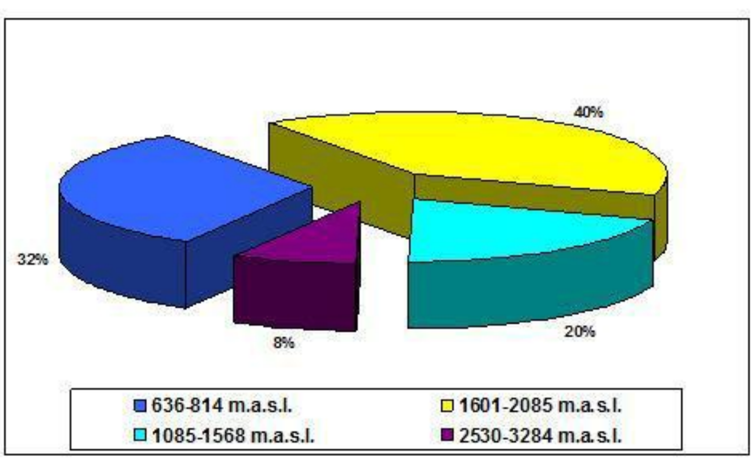

Figure 4. The distribution of endophytic fungi in the vertical zonation

Table 4. Detection frequency of endophytic fungi depending on the vertical zonation, in %

The list of endophyte species	2006		2007		2008		2009	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**
	Deuteromycota Hyphomycetes Light-colored species							
Acremonium charticola	10	7.5	6	16	5	13	4	15
A. sclerotigenum	10	10	9	21	13	10	0	3
Trichoderma viride Pers.	13	7.5	0	21	11	13	17	9
Penicillium chrysogenum Thom.	8	7.5	15	0	13	13	9	1
P.chatkalicum	10	10	9	21	8	10	17	9
Fusarium merismoides Corda	8	10	9	10	8	0	13	12
F.sporotrichioides Sherb.	5	7.5	12	0	8	6	0	9
	Deuteromycota Hyphomycetes Dark-colored species							
Cladosporium herbarum	5	7.5	9	0	11	6	13	0
Papularia sphaerosperma	10	7.5	9	10	0	0	4	9
Stemphylium sp.	5	2.5	0	0	8	10	9	3
Alternaria alternata	8	7.5	12	0	5	10	0	12
A. tenuis	5	5	9	0	5	10	13	9

Note: Plant associations: 1- foothills with sagebrush-steppe vegetation, ephemeral; 2- zone of tall grassland and meadow steppes, bush, mountain tipchak steppes, juniper and leafy forests, and spruce forests

an adverse effect on the formation of symbiotic flora. Representatives of *Alternaria* genus with dark-colored conidia, as well as species of *Penicillium* and *Fusarium* genera were characteristic of this zone.

It follows that the species of endophytic fungi are distributed in all plant zones, as well as hyphal fungi in the phyllosphere. Endophytes were colonized in more significant numbers in the foothill vegetation than in the alpine zone. Foothill vegetation in all investigated regions of Kyrgyzstan was a favorable host for the symbiotic existence of endophytic fungi (Table 4).

The distribution of endophytic fungi in the vegetation of Issyk-Kul valley (Northeastern Kyrgyzstan)

I. V Vikhodtsev (1956) made a great contribution to the study of the vegetation of Issyk-Kul valley. He differentiated the zonation of vegetation on the slopes of the Kungey and Terskey Ala-Too mountains as follows:

1. Warm foothills zone;
2. Middle mountain zone (this includes grass-forb meadows and meadow steppes, bush land and spruce forests);
3. Subalpine zone with fine sod cereal associations and tall meadows;
4. Alpine zone (includes *Picea schrenkiana*, *Juniperus*, *Kobresia*, *Carex*, etc.).

Our research area was located mainly in the south-west of the eastern part of the Issyk-Kul basin, including the flat part of the shore of Lake Issyk-Kul and the mountain of Terskey Ala-Too. Vegetation cover of Issyk-Kul and Terskey Ala-Too is very varied in terms of plant species and also plant associations. In total, 23 isolates of endophytic fungi were found in this area, and the most isolates were detected in the ragwort groves (*Senecio nemorensis*) and eyebright bayangolskoy (*Euphrasia bajankolica*). Fungi species of *Stemphylium* and *Acremonium* genera dominated. The same representatives of microflora were found in both the eastern and western parts of this region. These species tend to evolve in plants related to mesophilic forbs growing along the banks of rivers and irrigation ditches. A favorable combination of environmental factors and a wide variety of wild plant flora in the middle mountain zone has led to the development of endophyte microdiversity of the *Fusarium*, *Trichoderma*, *Cladosporium*, *Stemphylium* and *Papularia* (Figure. 5).

Quantitative and generic composition of endophytic fungi in the subalpine zone (where the vegetation is small turf grass associations and tall grasslands) decreased sharply compared to other zones. In total, five isolates of

Table 5. Species diversity in terms of richness and evenness of endophytic assemblages in different ecosystems

Indexes of evaluation	In ecosystems of foothills		In middle mountain ecosystems		In alpine ecosystems	
Chao I and Chao II	light-colored	dark-colored	light-colored	dark-colored	light-colored	dark-colored
	25.0	12.0	20.0	11.0	5.0	3.0
Shannon index	3.47± 0.41	2.86± 0.37	2.46± 0.85	2.73± 0.92	2.75± 0.27	1.66± 0.19

A B C D

Figure 5. Photomicrographs of conidia of endophytic fungi. (A): *Varicosporium elodeae.* (B): *Papularia sphaerosperma;* (C): *Acremonium charticola;* (D) : *Fusicladium sp. X 100*

endophytic fungi were isolated in this zone. Our studies have showed a periodicity of occurrence of certain endophyte groups during the growing season in this zone; apparently some species have an evolutionary adaptation to a particular habitat, to a certain body of plants and to the distribution to local nature zones.

An abundant species diversity of fungi was discovered in the first and second zones of vertical zoning in studied ecosystems. The first zone was characterized by the presence of representatives of *Penicillium*, *Acremonium* and *Alternaria* genera. In the second zone, the representatives of *Cladosporium*, *Stemphylium*, *Papularia* and *Acremonium* genera dominated. In the third zone, the isolates of *Penicillium* genus dominated. The representatives of *Alternaria* and *Cladosporium* were typical for the alpine zone. Our studies have revealed that endophytic fungi most often entered into association with the following families of land plants: *Asteraceae*, *Fabaceae*, *Lamiaceae* and *Brassicaceae*.

The species richness of endophytic fungi in ecosystems of Northern and Northeastern Tien Shan

We estimated the species richness of endophytic fungi in all studied ecosystems of Northern and Northeastern Tien Shan by using Chao I and II methods and the Shannon index. These index parameters have revealed a significant difference in the distribution of fungi species on the vertical zonation and on the quantitative ratio of pigment-forming species (Table 5). Chao I and Chao II methods let us estimate and compare the number of dark- and light-colored species in the ecosystems of two regions (Chy valley and Issyk-Kul valley) on vertical zonation. In all zones, the light-colored fungi species were more numerous than the dark-colored. The highest number of light-colored endophytes were found in ecosystems of foothills, and the next-highest in middle mountains, while the lowest number were detected in alpine vegetation.

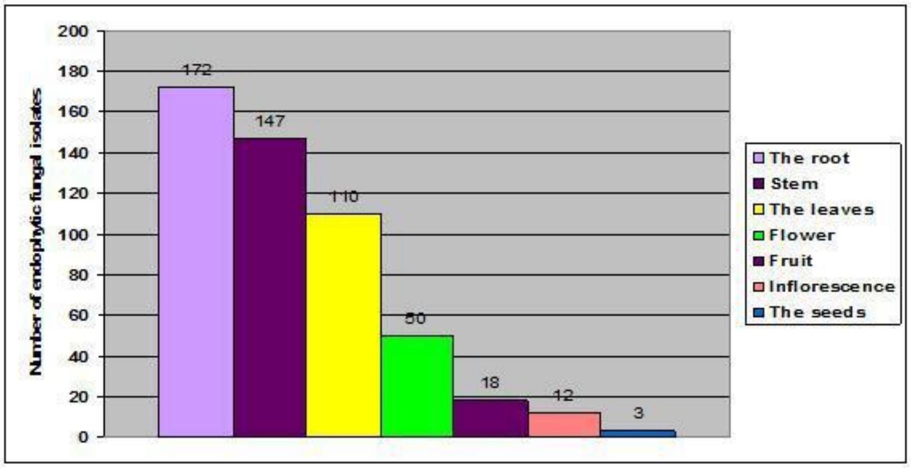

Figure 6. The distribution of endophytic fungi on different plant organs.

The comparison of the Shannon diversity index (HS) for endophytic fungi communities showed a high diversity of light-colored species (HS = 3.47) in foothill ecosystems; the next-highest was in alpine ecosystems (HS = 2.75) and a lower diversity was found in the middle mountain zone (HS = 2.46).

Whereas a significant diversity of dark-colored fungi was also noted in foothill ecosystems (HS = 2.86), and to a slightly lower degree in the middle mountain zone (HS = 2.73), the lowest biodiversity of dark-colored fungi was noted in the alpine zone (HS = 1.66).

Colonization of the tissues of medicinal plants by endophytic fungi

Endophytic fungi were identified from all organs and tissues of plant samples. As Figure 6 shows, the root tissues of higher plants were a more favored habitat for endophytes in their choice of hosts. The most preferred bodies were stems and leaves, while endophytic fungi colonized the tissues of plants' inflorescences and seeds less frequently.

Put simply, the representatives of *Trichoderma*, *Acremonium*, *Cladosporium*, *Alternaria*, *Penicillium* and *Papularia* genera preferred the tissue of the root system of their host plants, whereas species of *Geotrichum*, *Verticillium*, *Acremonium*, *Cladosporium*, *Fusarium* and *Aspergillus* genera could also colonize the root system and leaf tissue (Table 6).

Growth characteristics at different temperatures

Under natural conditions, endophytic fungi, using various organs of higher plants for their symbiotic existence, indirectly enter into relations with environmental factors. Environmental temperature, together with moisture, is a major factor in the development and life support of wild medical plants and their microflora. Our research results have created a collection of endophytic fungi isolated from plants of different vertical zones and different climatic conditions..

In order to understand physiological requirements in respect of the ambient temperature, the endophytic fungal cultures were grown at four temperature regimes: 4 °C; 12 °C; 27 °C and 40 °C. As the results show, most endophytes were mesophiles adapted to existence at moderate temperature regimes (25-27 °C). This indicates that these species are mainly associated with host plants growing in foothills and middle zones, and their symbiotic existence in nature is determined by the moderate temperature regime of habitats.

Among the endophytes, obligate psychrophiles (*Penicillium chrysogenum* Thom. *Curvularia geniculata*, *Fusarium moniliforme*, *Alternaria tenuis*, etc.) have been discovered. They can develop and form mycelium and reproductive organs at 4 °C. These species colonize the plants growing in the high alpine and subalpine areas. Apparently, their symbiotic existence occurs at a low temperature range. The plants and endophytic fungi could provide for each other by supplying the required properties, protecting each other from the harsh climatic conditions of high altitude habitats. In addition, we have identified the psychrotrophs, which are better adapted to varying temperatures. These strains were slowly developed at 12 °C. It seems that their host plants can be distributed in different zones of vegetation, in both middle and high zones. Among the endophytes, the facultative thermophiles were also found. They were capable of developing at 40 °C (*Fusarium oxysporum*, *Alternatia alternata*, *Neurospora sitophila*, *Aspergillus fumigatus*, etc.). Apparently, these

Table 6. Colonization rate of differentplant organs by endophyticfungi

Family	Genera	Chu Valley								Issyk-Kul valley							
		Number ofisolates															
		the root	stem	the leaves	the flower	Theinflorescen ce	the fruits	the seeds	Total number ofspecies	the root	stem	the leaves	the flower	The inflorescence	the fruits	the seeds	Total number ofspecies
Moniliaceae	Geotrichum	2		2	1				5								
	Monilia	3	1		3				7	1					1		2
	Trichoderma	9	2	1			1		16	3		1					4
	Aspergillus	4	2	4	1	3			11				1				1
	Gliocladium		1		2				3								
	Penicillium	12	5	2	1		1		21	1							1
	Botrytis	4	2	1	2		1		10								
	Verticillıum	1		2				1	3								
	Trichothecium		1			3			4								
	Paecilomyces	1	2		1		1		5								
	Blastotrihum	1	1						2								
	Scopulariopsis	2		1	2				5								
	Acremonium	9	7	6		3	1		26	2		1					6
Dematiaceae	Fusicladium		1		2				3		3						3
	Cladosporium	7	5	11	5				27	2	1						3
	Stemphylium	4	6		2	1			13			1					1
	Macrosporium	2		1	1		1		5		1						
	Alternaria	11	5	7	--3	1	1		28	2		1					3
	Curvularia	4	2	1	1				8								
	Papularia	9	4	6		1			20								
Tuberculariaceae	Fusarium	4	6	5	2	3	1		21								
Mycelia Sterilia	Sclerotium	2		1		1			5								
	Rhizoctonia	4		1	1		1		7								
Total		95	53	52	30	16	12		**255**	**12**	**5**	**4**	**1**		**1**		**23**

species could enter into coexistence with host plants, developing in the steppes and foothill areas in hot climatic conditions.

Of obtained endophytic isolates, 82 % were unable to grow at 4 °C; however, during incubation at 27 °C, the mycelium growth was renewed. A total of 19 isolates were able to grow at 4 °C. All tested strains grew very slowly at 12 °C compared to other temperature ranges. The highest growth rate of most strains was 8-10 mm per day at 27 °C.

Thus, an optimum temperature for growth of most studied strains was 27 °C; for others, it was 12 °C. Ten strains were able to grow at 40 °C. Therefore, we can suggest that an optimal temperature condition for the best growth and expression of symbiotic life and biological activity of the studied endophyte cultures was 25-27 °C. This fact indicates that the rich biodiversity and quantitative dominance of endophyte species in the foothills and middle zones are directly connected to temperature factors, which limit their distribution in habitats.

DISCUSSION

The “endophyte-plant” association represents an excellent model for the study of fundamental problems of symbiosis. In this context, the study of the “endophyte-plant” in poorly studied regions of the world is of particular scientific and practical interest for the understanding of the symbiotic relationship between the micro- and macroorganisms in different climatic environmental conditions. Furthermore, the identification of new species of endophytic fungi will make a significant contribution to the study of the biological diversity of microscopic fungi, as endophytes are now considered an important component of biodiversity.

Thus, for the first time in Kyrgyzstan, research was carried out to identify the species diversity of endophyte fungi - symbionts of wild medicinal plants growing in different climatic conditions on the vertical zonation areas. The results show that endophyte fungi are unequally distributed in the studied regions of Kyrgyzstan; this is primarily related to the composition of flora, grass density, climatic conditions, and the physical and geographical location of the region.

We have found endophytic fungi in wild medicinal plants growing in all areas of vertical zoning from 600 m to 3000-3300 m above sea level. However, there was a certain restriction of fungal species to certain types of plants growing in a particular zone of verticality. For example, *Fusarium* genus representatives (33 isolates) were confined to the plants growing in the foothills and middle mountain ecosystems of Chui valley.

There is a definite pattern of fungi distribution in altitude zones. A high frequency of fungal isolates was recorded at an altitude between 1601-2085 m above sea level, and a low frequency at an altitude of 2530-3284 m above sea level. Some species were dominant in one zone, while others were dominant in other zones. Thus, the species of *Penicillium*, *Alternaria*, *Cladosporium*, *Fusarium* and *Acremonium* genera were dominant in the foothill zone (600-1600 m), while *Penicillium*, *Fusarium*, *Trichoderma* and *Acremonium* species were more characteristic of the midland ecosystem (1200-3000 m). *Penicillium* and *Cladosporium* species dominated in highlands with subalpine, alpine and nival vegetation (3000-3300 m).

Apparently, the climatic conditions and temperature of the foothill zone are optimal for the coexistence of endophytic fungi and their host plants. By contrast, colonization of the host plants by endophytic fungi was much lower in vegetation of high mountain areas. Many researchers have noted that the poorest areas for micromycetes are highlands, and the richest for these organisms are foothill and middle mountain areas, due to favorable environmental conditions and a variety of host plants (Taslakhchyan and Nanogyulyan 1996). Consequently, the highland climate and vegetation can have a direct impact on the diversity of fungi.

The results showed that not all medicinal plants can be a favorite host for endophytic fungi, and a symbiosis of plant-symbionts has developed in a long evolutionary process, which has determined which plant may be the best host. In our studies, the plants from the *Asteraceae* family were preferred hosts for most obtained endophyte species. Among these plants, a rich diversity of *Alternaria*, *Papularia*, *Botrytis*, *Penicillium*, *Fusarium* and *Curvularia* genera was detected.

As our data have shown, endophytes colonize the tissue of host plants selectively. For many species, it is very convenient to colonize the root tissues of plants, and for others, to colonize stem tissues. A few species colonized the leaves, flowers and fruits. More specifically, a colonization of the root system by endophytes apparently ensures a survival in water-deficient arid regions, such as in the mountain steppes and deserts of Northern Kyrgyzstan. As is well known, endophytes enhance the resistance of plants to drought by increasing the surface area of absorption. Endophytes cause elongation of roots and a simultaneous decrease in their diameter. During a drought, when there is little water in the soil, plants which host endophyte fungi can reduce water loss through transpiration. Moreover, symbiotic fungi can improve plant nutrition; endophytes change the reactivity of the roots so they can more effectively extract nutrients from the soil. Thus, these symbiotic associations offer various strategies for plant behavior in drought conditions.

By studying the growth rate of endophytes in different temperature regimes, we have managed to identity the mesophiles, psychrophiles, psychrotrophs and thermophiles among them. Of these, the majority were mesophiles, developing actively at 26-27 °C temperate. Psychrophiles and thermophiles grow at below 4 and above 40 °C, respectively; both were negligible. These data allowed us to make the assumption that the rich biodiversity and quantitative dominance of endophyte species in the foothills and middle zones are directly connected to temperature factors, which limits their distribution in habitats.

CONCLUSIONS

- We identified 278 symbiotic endophytic fungal isolates on 150 plant species growing in different ecosystems of Northern and Northeastern Kyrgyzstan. Morphological, phenotypical and chemotaxonomic characteristics of 50 strains were studied and identified to species level.
- The systematic structure of endophytic fungi mycobiota identified in wild medicinal plants of Northern Kyrgyzstan and Northeastern Kyrgyzstan includes one class (*Fungi imperfecti*), one division (*Hyphomycetales*), four families and 23 genera. *Moniliaceae* family was the largest, which was represented by 13 genera.
- The vegetation of Northern Kyrgyzstan, compared with other regions, is characterized by a rich biodiversity of endophytic fungi.
- An unequal distribution of endophytic fungi on the vertical zonation of the studied area was found. The most favorable habitats for the symbiotic existence of endophytic fungi in land plants were the foothills and midlands areas; the least favorable area was the high mountain zone.
- A rich diversity of endophyte species was found in plants of the *Asteraceae* family.
- The most favorable habitat for endophytic fungi within the body of land plants was the root tissue, followed by the tissue of stems and leaves, while the tissue of inflorescences and seeds was infrequently colonized by endophytic fungi.

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