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PRIMARY INVESTIGATION OF THE DIVERSITY AND DISTRIBUTION CHARACTERISTICS OF *TRICHODERMA* SPP. IN THE SPECIFIC SOIL OF VOLCANIC FOREST PARK AND VOLCANO PLATFORM

YUAN CHANG[1O], RUI MIAO[1O], ABDUL MAJEED BALOCH[1], ZHIHONG YAO[1], ABDUL WAHID BALOCH[2], CHUANYING JIANG[1], ZHIHUA LIU[3] AND RONGSHU ZHANG[1*]

[1]*College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*
[2]*Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan*
[3]*School of Forestry, Northeast Forestry University, Harbin 150040, China*
**Corresponding author's email: zrs_nefu@163.com*
[O]*Authors contributed equally to this work*

Abstract

Trichoderma spp. are used in biological plant protection as biofungicides as well as in bioremediation. *Trichodrma* spp. are also utilized as bio-fertilizer to improve and amend soil. To obtain more optimized germplasm resources of *Trichodrma* spp., the diversity and distribution of *Trichoderma* species were investigated and explored in the Volcanic Forest Park and volcanic platform in Heilongjiang Province, China. Twenty-eight soil samples near the root, including the rhizosphere of different plants were collected. The *Trichoderma* strains were screened by dilution plate method. Species were identified through morphological characteristics, analyses of Internal Transcribed Spacer (ITS) sequences and genetic relationships based on phylogenetic tree. As a result, a total of 201 *Trichoderma* strains were isolated from the two sampling spots and they were identified to be members of 13 known species of *Trichoderma*, including *T. harzianum, T. hamatum, T. atroviride, T. citrinoviride, T. virens, T. koningii, T. koningiopsis, T. viride, T. tomentosum, T. petersenii, H. stellate, T. saturnisporum* and *T. asperellum*. Among all the identified species, *T. harzianum* was the dominant species, accounting for 43.28% of the total, followed by *T. viride*, which accounted for 14.93%. 12 species were obtained from the soil of the Volcanic Forest Park, while 8 species were acquired from the volcanic platform, which showed the difference of species diversity of *Trichoderma* between the two spots. In addition, there were differences in *Trichoderma* species and stains according to the differences of plants at each sampling spot. In short, our results would illustrate the elementary diversity and distribution characteristics of *Trichoderma* spp. in the two specific types of soil and provide novel germplasm resources for improved mixed *Trichoderma* bio-fertilizer.

Key words: *Trichoderma*, Diversity analysis, Volcanic Forest Park, Volcanic platform.

Introduction

At present, the agricultural development based on long term application of chemical fertilizers and pesticides has caused irreversible damage to the farmland ecosystem (Liu *et al*., 2015; Prashar & Shah, 2016). Nevertheless, certain microorganisms have significant effects on controlling plant diseases and improving the soil of farmland (Prashar & Shah, 2016; Mao *et al*., 2018). Studies have found that bio-fertilizers which are rich in *Trichoderma* spp. and Bacillus etc. have many usages such as recycling organic waste, providing organic material and increasing beneficial microbes (Xiong, 2017) for the soil. Therefore, the application of bio-fertilizers can provide a better way for sustainable development of agriculture.

Trichoderma spp. are known for their environment-friendly traits as biocontrol agents. They are generally abundant in nature and most of them are frequently found in the rhizophere of plants as well as on the trunk surface of living trees, leaves and decaying wood (Paz *et al*., 2010; Gal-Hemed *et al*., 2011). In recent years, studies have shown that *Trichoderma* has significant effects on improving plants' tolerance to salt (Jalali *et al*., 2017; Zhao *et al*., 2017), drought (Alwhibi *et al*., 2017) and high temperature (Hanson & Howell, 2004), and promoting plant growth (Azarmi *et al*., 2011; Bombiti *et al*., 2012), controling plant pathogenic fungi invasion (Rojo *et al*., 2007; Kowalska, 2014; Munir *et al*., 2019), and improving soil condition (Saravanakumar *et al*., 2013). In these processes, the compounds secreted by *Trichoderma* mycelium contributed and played crucial roles in promoting plant growth and resistance to biotic or abiotic stresses. (Martínez-Medina *et al*., 2011; Mclean *et al*., 2012; Tripathi *et al*., 2013; José *et al.*, 2015).

The traditional identification methods for *Trichoderma* species mainly depended on morphological characteristics. Persoon (Błaszczyk *et al*., 2014), was the first person to discover *Trichoderma* in 1794 and divided it into four species by the color of conidia. According to the morphological variation, *Trichoderma* spp. were classified into four taxa: *Longibrachiatum*, *Trichoderma*, *Pachybasium* and *Hypocreanum*. However, based on molecular data (including protein markers, DNA barcode, phylogenetic markers) a new classification system has been introduced by Meincke *et al*., (Meincke *et al*., 2010). Most recently, using a DNA barcode within the Internal Transcribed Spacer (ITS) 1 and ITS2 sequences of the rDNA repeat has become an effective approach for quick identification of *Hypocrea/ Trichoderma* on the genus and species levels (Druzhinina *et al*., 2005). *Trich*OKey (www.isth.info) is a data base for *Trichoderma* species evolutionary relationships and morphological characteristics and can be used for *Trichoderma* identification. Meanwhile, *Tricho* BLAST (www.isth.info.) is a database that holds sequence identification and similarity search tool that can be applied for ITS identification. These tools can act as complements to traditional methods and avoid the subjective influence in morphological identification (Kopchinskiy *et al*., 2005).

The Volcanic Forest Park (E 128°53'-128°54', N 44°-44°19') is located in Ning'an City, Heilongjiang Province,

China and has typical climate of temperate monsoon zone. The temperature changes greatly within a year; the average temperature in summer is 22°C and -25°C in winter.

In addition, here the rainfall is abundant and humidity is high, making the area a virgin forest full of precious tree species including *Pinus koraiensis*, *Tilia amurensis*, *Phellodendron amurense*, *Fraxinus mandshurica*, *Larix olgensis*, *Picea jezoensis*, *Larix gmelini*, etc. (Xu and Yuan, 2010). The soil of the Volcanic Forest Park area is generally covered by plant debris and has sufficient moisture and abundant nutrients, making it a very rich and precious natural resource (Fig. 1A). On both sides of the road leading to the entrance of the Volcanic Forest Park, there are a large number of volcanic platforms where extremely precious natural resources are distributed in such soil of specific types (Huang *et al*., 2012) which evolved from ancient volcanic magma. Now some plants grow in its crevice; however, shrubs are relatively rare and woody plants are particularly rare (Fig. 1B). Above all, this place might contain abundant *Trichoderma* spp. resources. However, investigations on the distribution of *Trichoderma* spp. in these two spots have not been reported yet.

In this study, the diversity and distribution of *Hypocrea/ Trichoderma* in plant rhizosphere soil in the Volcanic Forest Park and volcanic platforms were investigated. Methods combining morphological characterization and rDNA ITS sequencing were comprehensively used to identify *Trichoderma* spp., after which the evolutionary relationships were analyzed based on the ITS sequences and constructing phylogenetic tree. The results of this study have great significance on identifying and investigating specific functional *Trichoderma* resources and this study laid a foundation for developing plant bio-control agents for local application.

Material and Method

Soil sample collection: Soil samples were collected seperately in the Volcanic Forest Park and the volcanic platform in early October 2015. Twenty soil samples were collected from the Volcanic Forest Park and eight soil samples were collected from the volcanic platform. Each soil sample was taken at a plant's rhizosphere at five random spots at 5-20 cm depths and combined, containing over 20 g of fresh (moistured) soil. The 20 soil samples taken at the Volcanic Forest Park included rhizosphere soil of five major coniferous trees, 13 major broad-leaved trees and two herbaceous plants. The eight soil samples collected from the volcanic platform included rhizosphere soil of four herbaceous plants, three major shrubs and one woody plant. Each soil sample was collected into sterile bags marked with the date and name according to the method of Oskiera *et al*., (2017) and stored at -20°C for *Trichoderma* spp. screening.

Isolation and purification of strains: For each soil sample, approximately 20.0 g of soil was weighed and mixed with 100 ml distilled water. The suspensions was then diluted for 10, 20 and 40 times and 200 µl of each diluted suspension was applied to basal rose bengal medium and then incubated at 28°C in dark to obtain isolates according to the method provided by Vargas-Gil *et al*., (2009) and Siddiqueea *et al*., (2017), and then purified on potato dextrose agar medium (PDA) according to Vizcaíno *et al*., (2005). The isolates were inoculated on PDA medium to culture in petri dishes and the culturing condition was as above. When colonies emerged, hyphae were repeatedly sub cultured on PDA medium in order to acquire non-contaminated single colony of *Trichoderma* strains. And these strains were named as NECC10001 to NECC10216.

20.00 g of each soil sample was weighed and thoroughly mixed with 100 ml of sterile water, then each sample was diluted for 10, 20 and 40 times and 200 µl of each diluted solution (suspension) was equally applied to solid basal rose bengal medium in petri dishes and incubated at 28°C in dark to obtain isolate colonies (Vargas-Gil *et al*., 2009; Siddiqueea *et al*., 2017); the isolates were then purified on potato dextrose agar medium (PDA) (Vizcaíno *et al*., 2005). The purified isolates were subsequently cultured on PDA medium and the culturing condition was consistent with the above. When colonies emerged, hyphae were repeatedly subcultured on PDA medium in order to acquire non-contaminated single colonies of individual *Trichoderma* strains. The isolated strains were respectively named as NECC10001 to NECC10216. Fifteen numbers in the NECC data were discontinuous, so the end final number is NECC10216.

Fig. 1. Partial scene of the two special plots. A the Volcanic Forest Park. B volcanic platform.

Morphological identification of *Trichoderma* strains: The purified strains were cultured on solid PDA medium in petri dishes for 5 days, then 5 mm-diameter-round-shaped inoculums were collected from the PDA culture medium and placed near the edge on a new PDA petri dish then incubated at 28°C in dark. The growth radius, shape and color of the colony were recorded after 24 hours, and the microscopic morphology was observed under an optical microscope (Leica DM2500, Germany) (Bissett, 1984, 1991a, 1991b, 1991c).

Molecular identification of *Trichoderma* spp.: The *Trichoderma* rDNA was extracted according to the instructions of DNA extraction kit (Omega, USA). Then rDNA was used as template, with ITS1-5-TCCGTAGGTGAACCTGCGG-3 and ITS4-5-TCCTCCGCTTATTGATATGC as the two primers for PCR amplification in optimal system referring to Takara Ex Taq® manufacturer's instructions (TaKaRa Biotechnology Co., Ltd, Dalian). The PCR cycles were set as: pre-denaturation at 94°C for 3 min, then 94°C 30 s, 55°C 30 s, 72°C 1 min for 40 cycles, then 72°C for 5 min at the end. The ITS PCR product was purified and recovered through gel extraction (Promega Cycle-Pure Kit) following agarose gel electrophoresis. Recovered ITS fragment was constructed into pMD-18T vector (Takara) then sequenced (Shanghai Sangon Co., China). The sequences were submitted to GenBank. Accession numbers are as in Table 1.

Statistical analysis

The ITS sequences of unidentified *Trichoderma* strains were searched against the ISTH database. Statistic analyses were performed using Microsoft Office Excel 2007 (Microsoft Co. USA). Phylogenetic trees of the identified *Trichoderma* strains isolated from the two sampling spots were constructed by Neighbor-Joining method using MEGA 6 software with bootstrap resampling (1000 trials).

Results

Morphological identification: As a result, 201 purified individual *Trichoderma* stains were obtained. For preliminary classification of the newly isolated *Trichoderma* spp., according to the instruction of Gams and Bissett's *Trichoderma* classification system, these 201 strains were roughly identified as the following six species: *T. harzianum*, *T. hamatum*, *T. atroviride*, *T. citrinoviride*, *T. virens* and *H. stellate*. Morphology conidiophore, phialomerist and conidium and characterization of these stains which grew on PDA for 5 days are shown in Fig. 2 and Table 2.

ITS sequence analysis: Through sequencing, the ITS sequences of the isolated 201 strains were obtained. Among them, 57 strains were found to have the highest consistency with previously existed data while searched against the ISTH database. The corresponding strains carrying these sequences were named and numbered by Genbank, respectively (Table 1). These 57 strains were identified as 13 species of *Trichoderma* separately; detailed species distribution within the 201 strains was diaplayed in Fig. 3. *T. harzianum* was the dominant species, which included 2.90 times as many strains as *T. virens*. On the contrary, *T. viride* consisted of the least strains, which was only about 0.2% of *T. harzianum*.

Phylogenetic tree of the *Trichoderma*: Phylogenetic trees were constructed based on the *Trichoderma* ITS sequences and the identified *Trichoderma* strains were divided into four major groups according to Zhang's classification system (Zhang *et al*., 2014). As shown in Fig. 4, Group I and Group III belonged to Pachybasium B according to the evolutionary relationship. In Group I and III, *T. harzianum*, *T. tomentosum* and *T. virens* could be evolutionarily classified as Lixii/catoptron or harzianum and Virens of the Pachybasium B. In Group III *H. stellate* was classified as Pachybasioides. Group II included *T. citrinoviride* and *T. saturnisporu*, belonging to Longibrachiatum. Group IV included *T. atroviride*, *T. koningiopsis*, *T. petersenii*, *T. koningii* and *T. asperellum*, which belonged to the group of *Trichoderma*.

Diversity analysis of *Trichoderma* in the two investigated spots: 163 *Trichoderma* strains collected in the Volcanic Forest Park were divided into 12 species, while 38 strains collected from the volcanic platform were divided into 8 species.

Diversity of *Trichoderma* in the Volcanic Forest Park: According to our results, the Volcanic Forest Park held more *Trichoderma* species, which indicated more biodiversity, probably due to its integral natural environment. *T. harzianum* was the dominant species in the Volcanic Forest Park, accounting for 41.10% of the total, and it was followed by *T. virens*, which was about 17.79%. *T. atroviride*, *T. tomentosum* and *H. stellate* respectively accounted for 11.04%, 9.20% and 8.59% of total isolated strains, as displayed in Table 3. Nine species of *Trichoderma* were obtained in the rhizosphere soil of coniferous trees, 5 of which were from the rhizosphere soil of *Picea jezoensis*, and 4 were from *Picea koraiensis*, *Abies nephrolepis* and *Larix gmelini* respectively. On the other hand, nine species of *Trichoderma* were isolated from the rhizosphere soil of broad-leaf trees, among which six were from *Betula dahurica* and five were from *A. truncatum* and *Albizzia kalkora*.

Further analyses of *Trichoderma* spp. diversity in the rhizosphere of different plants in the Volcanic Forest Park revealed that a large number of *T. harzianum*, *T. virens* and *T. atroviride* strains existed in the rhizophere of coniferous trees, broad-leaf trees and herbaceous plants. But there were some differences in *Trichoderma* diversity about the 3 plant types. *T. petersenii*, *T. koningii* were obtained in the rhizosphere of coniferous trees but were not found in the rhizophere of broad-leaved trees and herbaceous plants. *T. hamatum*, *T. tomentosum* and *T. saturnisporum* were obtained in the rhizosphere of broad-leaf trees, however, they were not found in the rhizosphere of coniferous trees and herbaceous plants.

Table 1. Information of isolated *Trichoderma* strains.

Strains *No.* [a]	Quantity [b]	Accession *No.*	ITS strains	ISTH strains	Identities (%)
NECC10017	1	MF351642	*T. harzianum*	CPK839	100
NECC10018	7	MF351643	*T. harzianum*	DAOM231412	99
NECC10021	3	MF351644	*T. harzianum*	DAOM231412	100
NECC10023	8	MF351645	*T. harzianum*	DAOM231412	100
NECC10029	1	MF351646	*T. harzianum*	DAOM229907	99
NECC10030	8	MF351647	*T. hamatum*	DAOM167057	99
NECC10034	1	MF351648	*T. atroviride*	CBS142.95	100
NECC10035	2	MF351649	*T. harzianum*	DAOM229907	100
NECC10039	1	MF351650	*T.harzianum*	NR5555	99
NECC10040	1	MF351651	*T. harzianum*	DAOM231412	99
NECC10044	2	MF351652	*T. virens*	CBS249.59	98
NECC10046	1	MF351653	*T. harzianum*	DAOM231412	99
NECC10047	2	MF351654	*T. koningiopsis*	GJS93-20	100
NECC10048	6	MF351655	*T. harzianum*	NR5555	100
NECC10050	3	MF351656	*T. harzianum*	DAOM229907	100
NECC10055	2	MF351657	*T. viride*	GJS91-62	100
NECC10056	1	MF351658	*T. harzianum*	DAOM231412	99
NECC10057	1	MF351659	*T. petersenii*	GJS04-355	100
NECC10059	3	MF351660	*T. harzianum*	DAOM231412	99
NECC10060	1	MF351661	*T. harzianum*	DAOM229907	100
NECC10063	4	MF351662	*T. harzianum*	DAOM229907	100
NECC10064	2	MF351663	*T. atroviride*	CBS142.95	100
NECC10066	1	MF351664	*T. harzianum*	DAOM231412	99
NECC10067	2	MF351665	*T. citrinoviride*	CBS258.85	100
NECC10071	6	MF351666	*T. harzianum*	DAOM231412	99
NECC10072	1	MF351667	*T. virens*	CBS249.59	98
NECC10073	3	MF351668	*T. harzianum*	DAOM231412	99
NECC10075	3	MF351669	*T. virens*	CBS249.59	99
NECC10077	14	MF351670	*H. stellate*	GJS99-222	99
NECC10080	23	MF351671	*T. virens*	CBS249.59	98
NECC10081	11	MF351672	*T. harzianum*	DAOM229907	99
NECC10084	2	MF351673	*T. atroviride*	CBS142.95	99
NECC10091	1	MF351674	*T. virens*	CBS249.59	98
NECC10094	9	MF351675	*T. tomentosum*	DAOM178713	99
NECC10100	2	MF351676	*T. harzianum*	DAOM231643	99
NECC10104	6	MF351677	*T. tomentosum*	DAOM178713	99
NECC10112	1	MF351678	*T. harzianum*	DAOM231412	99
NECC10144	1	MF351679	*T. harzianum*	DAOM231617	99
NECC10161	3	MF351680	*T. koningii*	CBS459.96	99
NECC10180	5	MF351681	*T. harzianum*	DAOM231408	97
NECC10187	16	MF351682	*T. atroviride*	CBS142.95	100
NECC10188	2	MF351683	*T. saturnisporum*	ATCC18903	99
NECC10192	3	MF351684	*T. harzianum*	NR5555	99
NECC10196	2	MF351685	*T. harzianum*	DAOM231412	100
NECC10197	2	MF351686	*T. hamatum*	DAOM167057	99
NECC10198	2	MF351687	*T. harzianum*	DAOM231412	99
NECC10199	2	MF351688	*T. harzianum*	DAOM231412	99
NECC10200	4	MF351689	*T. citrinoviride*	CBS258.85	100
NECC10201	1	MF351690	*T. saturnisporum*	ATCC18903	100
NECC10202	2	MF351691	*T. harzianum*	DAOM231412	99
NECC10203	3	MF351692	*T. hamatum*	DAOM167057	99
NECC10206	1	MF351693	*T. harzianum*	DAOM231412	99
NECC10209	1	MF351694	*T. saturnisporum*	ATCC18903	100
NECC10210	1	MF351695	*T. asperellum*	CBS433.97	99
NECC10213	1	MF351696	*T. koningiopsis*	GJS93-20	99
NECC10214	2	MF351697	*T. saturnisporum*	ATCC18903	100
NECC10216	1	MF351698	*T. harzianum*	DAOM222149	100

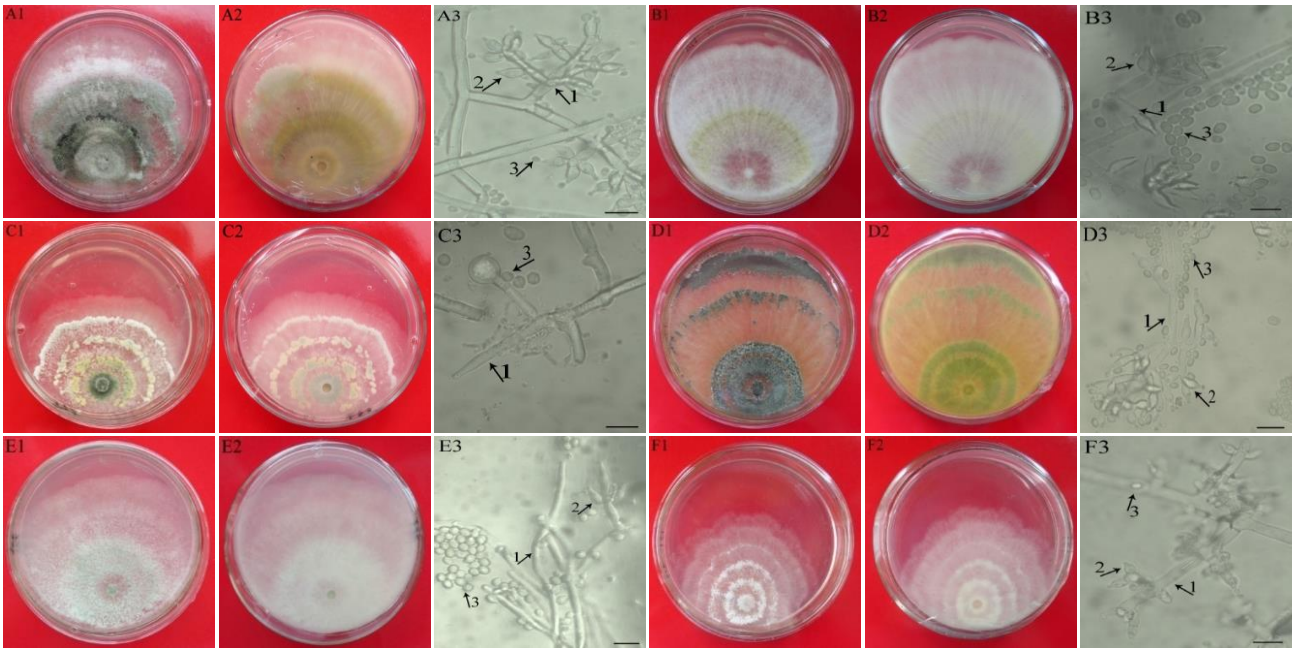

Fig. 2. The colony and microscopic morphology of *Trichoderma* on PDA after cultured 5 days. A. *T. harzianum*; B. *T. hamatum*; C. *T. atroviride*; D. *T. citrinoviride*; E. *T. virens*; F. *H. stellat*. A1-F1 The front of colony growth state; A2-F2. The back of colony growth state; A3-F3. Microscopic morphology. 1 Conidiophore; 2 Phialomeristem; 3 Conidium. Scale bars: 10 μm.

Table 2. Morphological identity of *Trichoderma*.

***Trichoderma* species**	**Colony morphology on PDA**	**Microscopic morphology**
T. harzianum	Colony growed fastlywith mycelium curly and sometimes present yellow zone, the surface was granular or powder with sporulated clusters which fromed the pale green to dark green. (Fig. 2 A1, A2)	The conidiophore was complicated, and the primary branch angle was about 90°, the secondary branch arranged in a spiral which was also complicated. Conidium morphology was subglobose or obovate with round dome and slender bottom. (Fig. 2 A3)
T. hamatum	Fast growth with no concentric rings, almost no conidia formation, mycelium was slender and regular with a little yellow zone. (Fig. 2 B1, B2)	The upper part of the conidial spindle was often curved, serrated or hooked and the branch was irregular; phialomeristem was subglobose with constricted bottom and the top narrowed abruptly. Conidium was white and elliptical, the size was about 3 μm width with 4-5 μm length. (Fig. 2 B3)
T. atroviride	Rapid growth with clear concentric rings and irregular margin; mycelium was white and denser at fast then conidia was formed which color became dark green latterly. (Fig. 2 C1, C2)	Didymous conidiophore was common, but the typical branch of conidiophore was unilateral branch. Conidium was globose and slick, the diameter was about 3 μm. (Fig. 2 C3)
T. citrinoviride	Grow quickly with concentric rings, mycelium was regular with a small amount of yellow, conidiophores were dark green. (Fig. 2 D1, D2)	The conidiophore has a longer spindle with fewer branches, the phialomeristem was cylindrical with middle slightly thicker. In most cases, conidium produced at the edge of the colony, shape was oval, size was about 2-3 μm width with 3-4 μm length. (Fig. 2 D3)
T. virens	Grow faster, mycelium was curly and the color was from white to gray. (Fig. 2 E1, E2)	The conidiophore was thicker and more complex with 2 to 5 phialomeristem branches arranged in a colyliform; the bottom of phialomeristem was constricted and enlarged in the middle then gradually attenuated to the top. Conidium was globose about 2-4 μm width with 3-6 μm length. (Fig. 2 E3)

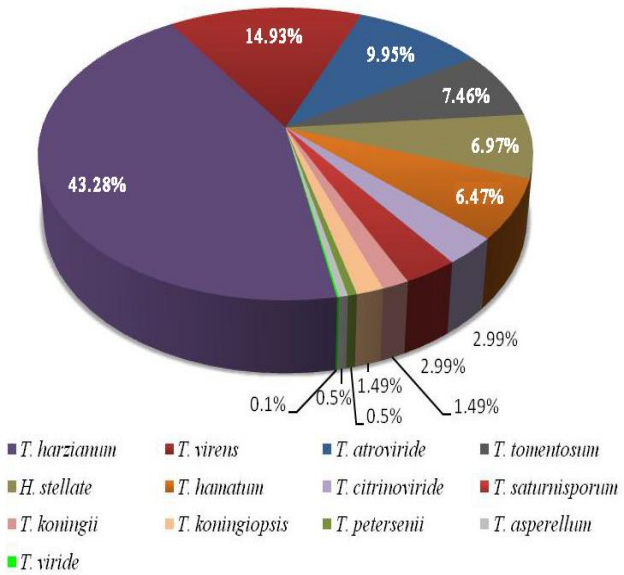

Fig. 3. The species and distribution of the total 201 isolated *Trichoderma* strains.

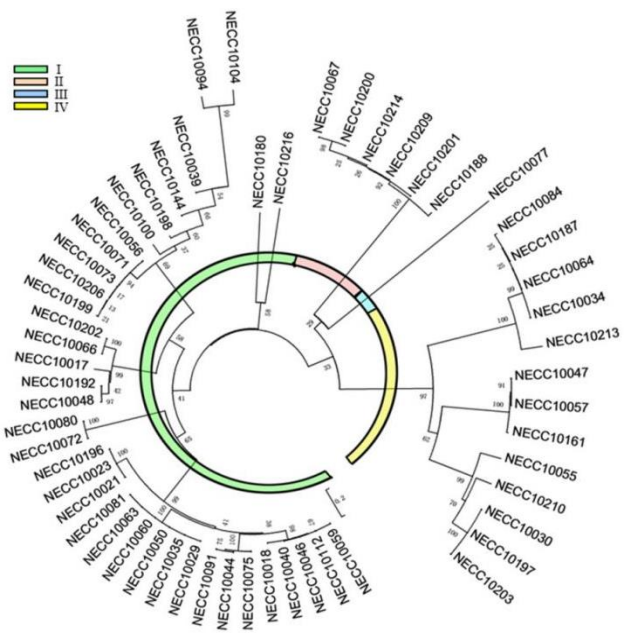

Fig. 4. Phylogenetic analysis of the 57 *Trichoderma* strains. The curves of green, pink, blue and yellow represent groups I, II, III and IV respectively. Evolutionary tree was constructed by NJ method with 1000 bootstrap resampling.

Diversity of *Trichoderma* in the volcanic platform: Plants in volcanic platform were mostly herbs. With land close to bare state, the growth environment of plants was poor. However, such growth conditions might suggest that the *Trichoderma* isolates may have high adaptability. Our results showed that *T. harzianum* was still the dominant specie in this sampling spot, accounting for 52.63% of all isolated strains, while *T. hamatum* and *T. saturnisporum* accounted for 15.79% and 13.16%, respectively. As shown in Table 4, the numbers of *T. harzianum*, *T. hamatum* and *T. saturnisporum* strains were much higher in this sampling spot. *T. virens*, *T. citrinoviride* and *T. koningiopsis* were only found in the rhizophere of shrubs. *T. harzianum*, *T. citrinoviride* and *T. saturnisporum* were found in the rhizophere of fern. Compared to the rhizosphere of herbs, there were much more *Trichoderma* in the rhizophere of shrubs. In the rhizophere of *Quercus mongolica*, *Populus cathayana* and even *Pteridophyta* existed the largest amount of *Trichoderma* species.

Discussion

The collection and collation work of *Trichoderma* has been carried out for years and greatly enriched the resources of *Trichoderma*. There are 256 species of *Trichoderma* that have been identified currently, including 117 kinds of new species in China, up to 2016 (Zhu & Li, 2016; Zhang *et al*., 2016; Qin & Zhuang, 2016). However, *Trichoderma* has unstable morphology, which makes the identification results susceptible to subjective and objective factors. This fact leads to the conclusion that it is necessary to describe precisely by many kinds of identification method for clear classification (Bissett, 1991). Therefore, the identification methods combined with morphology and molecular biology were used in our investigation on the distribution of *Trichoderma* to effectively avoid the inaccuracy of mere morphological identification.

Trichoderma is widely distributed in the soil. The results of previous studies showed that factors such as the soil type, fertility status, temperature and moisture have great effects on the distribution of *Trichoderma*, and these studies also indicated that the good edatope is beneficial to the colonization and conidial germination of *Trichoderma* (Yeakub *et al*., 2017). At present, the researches on *Trichoderma* resources are mainly concentrated in the south of China (Xia, 2009; Sun, 2013), the Shandong Peninsula (Zhang *et al*., 2011) and other areas, while studies on the distribution of *Trichoderma* in the northeastern cold region were less, especially in the original forest environment and volcanic platform. The soil in the Volcanic Forest Park and volcanic platform in Ning'an city, Heilongjiang Province is less investigated soil, which developed on volcanic lava and nurtured different plant communities. The characteristics of microbial communities are related to the vegetation types in the process of primary succession (Huang *et al*., 2012). Due to the differences of both terrain and soil weathering degree, the soil type has also changed greatly. For example, the soil of the Volcanic Forest Park was good in integrity, and has more plant residues, which was conducive to the discovery new species of *Trichoderma*. Soil of the volcanic platform was rich in mineral content and maintained the most primitive environment after volcanic eruption. In addition, we collected soil samples in October, and the average temperature of the northeast cold area of China was 5^{o}C at this time. Under this condition, the isolated *Trichoderma* spp. were supposed to be more adaptable to cold environment. So we selected the Volcanic Forest Park and the volcanic platform as the two special sampling spots.

Table 3. Distribution of *Trichoderma* spp. in rhizosphere of plants in Volcanic Forest Park.

Trichoderma strains plant types		*T. harzianum*	*T. hamatum*	*T. virens*	*T. koningiopsis*	*T. petersenii*	*T. viride*	*T. atroviride*	*T. citrinoviride*	*T. tomentosum*	*T. koningii*	*H. stellate*	*T. saturnisporum*
Coniferous species	*Pinus koraiensis*	4											
	Abies nephrolepis	2		1				2			3		
	Larix gmelini	2		2				2	2				
	Picea jezoensis	13		2	1	1	1						
	Picea koraiensis	2		1				3				3	
Broad-leaf species	*Betula platyphylla*	6	4	2									
	B. costata			2				1	2				
	B. dahurica	3	1	1				1		1		4	
	Tilia mandshurica	3					1	1					
	T. amurensis	2		3						1			
	Phellodendron amurense		1	2						3		3	
	Populus davidiana	3		2						4			
	P. cathayana			1				1					
	Quercus mongolica	4		2									
	Ulmus pumila	11											
	Acer mono	2						2		2			
	A. truncatum	2		2				1		3			1
	Albizzia kalkora	2	1	4				2		1			
Groud covers	*Bryophyte*	3		1								1	
	Pteridophyta	3		1	1			2				3	
Total		**67**	**7**	**29**	**2**	**1**	**2**	**18**	**4**	**15**	**3**	**14**	**1**

Table 4. Distribution of *Trichoderma* spp. in rhizosphere of plants in volcanic platform.

Trichoderma strains plant types		*T. harzianum*	*T. hamatum*	*T. virens*	*T. atroviride*	*T. citrinoviride*	*T. koningiopsis*	*T. saturnisporum*	*T. asperellum*
Trees and Shrubs	*U. pumila*	5							
	Q. mongolica	6	1		1	1			
	Malus baccata	1		1				2	
	P. cathayana	2	2				1	1	
Herbage	*Eragrostis pilosa*	1							
	Plantago depressa	1	1		1				
	Setaria viridis	1	2					1	
	Pteridophyta	3				1		1	1
Total		**20**	**6**	**1**	**2**	**2**	**1**	**5**	**1**

In this study, the ITS sequences of the *Trichoderma* strains were compared in the *Trich*OKEY and *Tricho*BLAST databases. It was found that 41 strains, accounting for 71.93% of total, had consistent ITS sequences when searched in either of the two databases; while 16 strains did not have inconsistent sequence data, 5 strains of which were unidentified species of *Trichoderma* in the *Trich*OKEY database. There were 21 strains who had ITS sequences of 100% identity compared with the existing ITS sequences in *Tricho*BLAST database; 31 strains with the 99% identity, 4 strains and 1 strain with 98% and 97% identity, respectively. As the identification process of *Trichoderma* was complex, the identification results in this study were comprehensively based on the results of morphological observation and molecular identification of ITS sequences. As for the identification work of suspected new species of *Trichoderma*, it will be carried out in the future.

From the analysis of the diversity of *Trichoderma*, the dominant specie of both sampling spots is *T. harzianum*. This result was consistent with Sun's findings of the diversity of *Trichoderma* in Liaoning Province (Sun *et al*., 2006). Studies have found that *T. harzianum* not only was dominant specie in northeastern China, but also in other parts of China (Wen *et al*., 1993; Zhang & Xu, 2005; Cheng, 2006; Xia, 2009). At some extent, all of these studies have proved that *T. harzianum* is highly adaptable to the environment.

Based on the results of this study, the differences of plant species in the sampling spots had a great influence on the space and quantiy distribution of *Trichoderma*. Soil samples were collected from 13 kinds of plants' rhizosphere soil in the Volcanic Forest Park and the species of *Trichoderma* were the most abundant in the rhizosphere soil of *Picea*, followed by the *Betula*, *Acer* and *Albizia*. Differences in the species and quantity distribution of *Trichoderma* in different plants' rhizosphere may be affected by root exudates, the physical and chemical properties of soil, and plant allelopathy. On the other hand, the distribution of *Trichoderma* species found in volcanic platform was different from that of Volcanic Forest Park, which was likely to be caused by edatope. *Trichoderma* could still be isolated from the soil in volcanic platform, where the surface was exposed in great day-night temperature difference; it suggested that in this region temperature-adaptable *Trichoderma* strains may exist. In addition, the reason why bryophytes still grow well on volcanic platform though they were sensitive to drought might be that *Trichoderma* could enhanced soil moisture (Chen *et al*., 2016).

China is a country of great biodiversity, but there are relatively few studies on the *Trichoderma* diversity, and it is difficult to accurately elucidate the distribution of *Trichoderma* species in a certain region of China. With the increasing application value of *Trichoderma* in industry, agriculture and environment protection and other fields, the investigation and function analysis of *Trichoderma* have become more and more important. It is of great significance to research the resources of *Trichoderma* and finding special functional strains for the development of *Trichoderma*–based soil amendment agents and developing bio-fertilizers in cold areas.

Conclusion

The results of this study showed that the dominant specie of the two sampling investigated sampling spots were both *T. harzianum*, but there were some differences in *Trichoderma* species variety and their distribution, possibly because of the different environments. The number of *Trichoderma* species obtained in the rhizosphere of *Q. mongolica* and *P. cathayana* from both sampling spots suggested that there are chances that more *Trichoderma* species exist in the volcanic platform. It might be because that the soil of volcanic platform contains high variety and large quantity of minerals, which might benefit the growth of various *Trichooderma* species. Additionally, secretions of the plant root in volcanic platforms might be more conducive to the survival of *Trichoderma*. After all, plants need the help of *Trichoderma* in order to better survive, and this beneficial symbiosis could promote the growth of both *Trichoderma* and plants.

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