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Full Length Research Paper

Isolation and identification of swainsonine-producing fungi found in locoweeds and their rhizosphere soil

Hao LU, Ji Ping CHEN, Wei LU, Yao MA, BaoYu ZHAO* and Jin Yi WANG

College of Veterinary Medicine, Northwest A&F University, Shaanxi Yangling 712100, P. R. China.

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This study concerns isolation and identification of fungal on swainsonine-producing from locoweeds and rhizosphere soil in western China. Five locoweed populations from various sites were assessed for fungi in inner Mongolia Autonomous Region, Qinghai and Ningxia Province. Fungus were isolated from all parts and rhizosphere soil samples of five locoweeds *Astragalus variabilis*, *Oxytropis glabra DC*, *O. kansuensis*, *O. deflexa* and *O. ochrocephala.* Fungal cultures grew very slowly and sporadically produced subcylindrical hypha with very dark transverse septa. Eight strains fungi were isolated and obtained from above samples, but only FS-5 and EFG-7 produced the alkaloid swainsonine and SW yield is 0.773 and 0.11 mg/g, respectively. Morphological evidence and sequence analysis of the ITS region suggest that FS-5 is most closely related to *Schizophyllu*m sp, and EFG-7 is *Fusarium tricinctum*. However, with the paucity of *Schizophyllum* species represented in sequence databases, precise taxonomic placement will await further study.

Key words: Swainsonine, fungal endophyte, *Schizophyllum* sp*, Fusarium tricinctum*, locoweed.

INTRODUCTION

Locoweeds are those species of the genera *Astragalus* and *Oxytropis* (Family Leguminosae) that specifically contain the 1,2,8-trihydroxyindolizidine alkaloid, swainsonine (SW) (Dorling et al., 1980; Molyneux and James, 1982). The swainsonine is an inhibitor of α-mannosidase, which was first isolated and identified from *Swainsona canescens* in 1979 (Colegate et al., 1979) and then from locoweed *Astragalus lentiginosus* in 1982 (Molyneux and James, 1982) and others (Taylor and Strickland, 2002; Martyn et al., 2003; Smith et al., 1992; Medeiros et al., 2003; Cao et al., 1992; Zhao et al., 2003).

So far, it has been 30 years since swainsonine was discovered. At first, people only considered it as an origin of plant toxin and studied its toxicity, metabolic dynamics and poisoning mechanism and other details. Until 1985, Hino et al. (1985) first reported that swainsonine possessed the function of inhibiting tumor cells' growth and metastasis, then a number of scholars began to study their pharmacological activity and found that swainsonine is a strong α-mannosidase competitive inhibitor, which not only can inhibit glycoprotein expression on tumor cell surface and tumor cell growth and metastasis and induce apoptosis, but also stimulate the body's immune system and boost the proliferation of immunocytes and enhance the ability of killing tumor cells. The traditional anti-cancer drugs result in immune injury, but swainsonine can stimulate the proliferation of medullary cells and enhance the body's immune system (Klein et al., 1999; Rooprai et al., 2001; Sun et al., 2006; Liu et al., 2006; Jun et al., 2007). It has been screened as an antineoplastic drug at home and abroad because of its anti-tumor activity and immune-enhancing effects. However, the scarcity of swainsonine inhibits its research as an anticancer drug, commercialization and application. Recently, microbial fermentation as a method of swainsonine production, was frequently used due to the fact that it cannot only obtain swainsonine, but also has the advantages of low cost and easily industrial production. Braun et al. (2003) isolated *Embellisia* sp which can be used to produce swainsonine from the American locoweed (*Astragalus mollissimus, Oxytropis lambertii* and *O. sericea*), and proved that the content of

*Corresponding author. E-mail: zhaobaoyu12005@163.com. Tel: +86 29 87092429. Fax: +86 29 87091032.

swainsonine in locoweed is positively correlated with the level of endophyte infection. Subsequently, *Embellisia oxytropis* is isolated from *O.kansuensis* and *O. flabra* (Li and Nan, 2007; Yu et al., 2009a, 2009b; Lu et al., 2009).

Locoweeds possess wide geographical distribution from the Great Plains to the Rocky Mountains. They are multi-species and worldwide spread, such as in the United States (Taylor and Strickland, 2002), Australia (Martyn et al., 2003), Mexico (Smith et al., 1992), Brazil (Medeiros et al., 2003). In China, there are also very rich in locoweed resources (44 species according to incomplete statistics (Zhao et al., 2008) however, the area of endophytic fungi is poorly understood. Locoweed is widely spread in Qinghai, Inner Mongolia and Ningxia province; however, it is not clear whether locoweed was infected by swainsonsine-producing fungi in these areas. Therefore, fungi were isolate from five locoweeds and their rhizosphere soils, in order to provide a theoretical basis for screening strains of swainsonsine-producing and microbial diversity research of locoweeds.

MATERIALS AND METHODS

Test samples

Samples of *A. variabilis*, *O. flabra*, *O. kansuensis*, *O. deflexa*, *O. ochrocephala* and their rhizosphere soil were collected from Alashan league of Inner Mongolia, Qilian county of Qinghai and Haiyuan county of Ningxia province in July to August, 2008 (Table 1).

Reagents and apparatus

Swainsonine standard sample was provided by College of Veterinary Medicine of Northwest A&F University, purity ≥ 98%; N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and (trimethylchlorosilane) (TMCS) were purchased from SUPELCO Company in the United States; recycling kit of PCR products and cloning kit were purchased from Beijing Tian-gen Biochemical Technology Co., Ltd.; all of the other reagents were domestic products. Primer synthesis was finished by the Shanghai Jierui completion of Bio-Engineering Co., Ltd.; DNA sequence determination was finished by the Nanjing Jenkins Special Technology Co., Ltd. GC-14C gas chromatograph, Shimadzu, Weimar Long chromatography data workstations, AT.SE-54 capillary column (30 m × 0.25 mm × 0.25 μm), Chinese Academy of Sciences, Lanzhou Institute of Chemical Physics chromatography technology research and development center; PCR Instrument, Bio-Rad Inc.; eXplorist500 GPS positioning system, the U.S. Magellan navigation and positioning the company.

Fungi isolation

Isolation of fungi in plant samples

All parts of locoweed samples were washed with sterile distilled water and then disinfected the surface by 30 s-rinsing of 75% ethanol, 3 times washing with sterile distilled water and 90 s-rinsing of 0.1% mercuric chloride followed by the last 3 times washing. Sterile filter paper was used to remove the water of plant tissues which had gone through the surface sterilization and then cut tissue blocks into 0.5 × 0.5 cm and cultivated them in the potato dextrose agar (PDA) medium at 28°C. If there were appropriate size of the tissue growing around the tissue blocks, then transferred fungi into new PDA medium and purified them for 2 to 3 times, purified fungi were made numbers and cultivated in the potato carrot agar (PCA) culture medium and they were preserved in refrigerator at 4°C, rejuvenated once every 4 months. Meanwhile, The droplets in the last time washing of sample disinfection as a control were also seeded into the sterile PDA agar plate and kept at 28°C for 72 h for detecting whether there would be fungi on the surface of culture medium and verifying the effect of surface sterilization.

Isolation of fungi in soil samples

10 g soil samples were weighed with a sterile paper then made the soil suspension with concentration of 10^{-1} to 10^{-4}. 1 ml 10^{-2} and 10^{-4} suspension was imbibed and added 2 to 3 drops into PDA medium contained 3% lactic acid, in sterile conditions, inverted cultivation at 28°C, when colony of suitable size erupted in the culture medium, hypha was picked and transferred into new PDA medium, purified them for 2 to 3 times and obtained purified strains. Isolated fungi were given numbers then cultivated in the PCA culture medium and they were preserved in refrigerator at 4°C.

Screening of Swainsonsine producing fungi Isolated

Strain fermentation and extraction

Cultivate the above-mentioned strains on Czapek's liquid medium in 28°C for 14 days. Deal fermentation broth and mycelium with acid-base treatment, then extracted with n-butanol to get n-butanol extract, decompress and concentrate them in order to obtain liquid to be examined.

Qualitative detection of swainsonine

The TLC (Thin Layer Chromatography) technology was utilized here. After drop the liquid to be examined on the silicone plate-like, expand them with $V_{chloroform}$:$V_{methanol}$:$V_{ammonia}$:V_{water} = 70:26:2:2 as developing solvent, Ehrlich's reagent used as color indicator, observe displacement and spot color of the sample in the thin-layer plate and then record the color and calculate the R_f values. If the sample has the spots whose colors and displacement are the same with standard swainsonine, the corresponding fungi was initially confirmed to produce swainsonine.

Quantitative detection of swainsonine

First the pyridine reagent was used to make standard concentration gradient then swainsonine was examined by gas chromatography (GC). Chromatographic conditions were as follows: the temperature of column was 200°C, the temperature inlet was 300°C, the temperature of FID detector was 280°C, carrier gas was nitrogen, the pressure of carrier gas was 200 kPa, the flow rate was 2 ml/min, application of sample was 2 μl and the ratio of diffluent was 60:1. The standard curve of swainsonine was then produced with the concentration of swainsonine as abscissa and its peak area as vertical axis. After that, samples were examined by GC. If the samples presented the peak whose preserved time was the same with swainsonine, they would be confirmed to contain swainsonine. The content of swainsonine was calculated according to the standard curve equation.

Table 1. Locoweed, locations, period and habitat of collections.

Species	Locations	GPS	Altitude (m)	Growth period	Growth environment
O. glabra	Alashan in Inner Mongolia	N: 39.50.364 E: 104.37.368	1463	Flowering fruit bearing stage	Desert, drought, growth with harmel, malian
	Alashan in Inner Mongolia	N: 39.50.176 E: 104.37.674	1457	Fruit period	Desert, drought, growth with harmel, malian
	Alashan in Inner Mongolia	N: 40.13.480 E: 104.49.491	1305	Flowering fruit bearing stage	Desert, drought growth with harmel, malian
	Alashan in Inner Mongolia	N: 38.51.536 E: 105.39.707	1498	Fruit period	Forest, moisten, growth with *Sophora alopecuroides*, Lanceleaf Thermopsis Herb
	Yinchuan in Ningxia	N: 38.29.867 E: 108.08.289	1101	Flowering fruit bearing stage	Lakefront, moisten, growth with Lanceleaf Thermopsis Herb, *Agropyron cristatum*
A. variabilis	Alashan in Inner Mongolia	N: 40.12.036 E: 104.26.462	1479	Flowering fruit bearing stage	Gobi, drought, growth without other plant
O. kansuensis	Qilian in Qinghai	N: 38.16.048 E: 99.53.146	2991	Fruit period	Rangland, moisten, growth with forage grass, Fischer Euphorbia root
	Ebo in Qinghai	N: 37.53.745 E: 101.00.894	3463	Flowering fruit bearing stage	Rangland, moisten, growth with forage grass, Fischer Euphorbia root
	Ebo in Qinghai	N: 37.44.407 E: 101.15.078	3380	Flowering fruit bearing stage	Rangland, moisten, growth with forage grass
O. deflexa	Qilian in Qinghai	N: 38.15.973 E: 99.53.082	3005	Fruit period	Rangland, moisten, growth with forage grass, Fischer Euphorbia root
	Qilian in Qinghai	N: 38.18.844 E: 99.46.847	3113	Fruit period	Rangland, moisten, growth with forage grass
	Qilian in Qinghai	N: 38.33.299 E: 99.27.878	3460	Flowering fruit bearing stage	Rangland, drought, growth with forage grass, Fischer Euphorbia root

Table 1. Contd.

	Ebo in Qinghai	N: 37.53.745 E: 101.00.894	3463	Flowering fruit bearing stage	Rangland, moisten, growth with forage grass
O. ochrocephala	Huangzhong in Qinghai	N: 36.29.692 E: 101.34.988	2682	Flowering fruit bearing stage	Forest, moisten, growth with bush

Identification of swainsonsine-producing fungi

Morphological identification

Fungi were cultivated in the PDA medium, which used the direct picking method and insert method. The characteristics of fungi were observed such as colonies, hypha, conidial fructification, conidiophore, shape and color of spores. Primary identification was carried out according to Manual of Fungal Identification (Wei, 1979).

Identification of rDNA-ITS Sequences

The extraction of fungal DNA

CTAB extraction method was used to extract DNA of the samples. OD and the concentration of DNA were measured and the concentration was regulated from 100 to 300 ng/μl.

PCR proliferation of fungal DNA

Fungal universal primers designed by White were used (White et al., 1990), ITS1 (5'-TCCGTAGGTGAACCTGCGC-3') and ITS4 (5'-TCCTCCGCTTATTGAT ATGC-3'). 15 μl reaction systems was applied in PCR, procedures of PCR are as follows: predegeneration at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 90 s, this cycle should be repeated for 35 times, preservation at 72°C for 7 min and insulated at 4°C.

The detection and reclamation of PCR products

10 μL PCR products were detected by agar gel electrophoresis and reclaimed and purified by TIANgel Extraction Kit.

The clone of target fragments

The recovered fragments were connected to the pMD-18T and then transfected into *E. coli* DH5α, after that the *E. coli* DH5α were smeared to LB culture medium which has Amp, X-gal, IPTG, cultivated them at 37°C for 12 to 16 h. Some white positive colonies were selected and identified by PCR method; PCR system and procedure are the same with the above methods. Positive clones were cultivated in LB liquid culture medium and were sent to sequencing company.

The structure of phylogenetic trees

Phylogenetic analyses were performed using programs contained in PAUP Phylogenetic Software (version 4.08; Sinauer Associates, Sunderland, MA). For both sets of sequence comparisons, phylogenetic trees were produced by parsimony analysis, using closest step-wise addition and branch swapping by tree bisection-reconnection and distance analysis, using neighbor-joining with Kimura 2-parameter distances. For each analysis, 1000 bootstrap replicates were performed to assess statistical support for resulting phylogenetic tree topology.

RESULTS

Separation of fungi in locoweed and the soil samples

All parts and rhizosphere soils of five locoweeds were collected from Mongolia and Qinghai province. We removed the common root mould, leaf mould, *Aspergillus* and other bacteria, and obtained 137 fungi colonies. According to shape and cultural characteristics of colonies, they were merged into eight fungi, and named as EFB-1, FS-2, FS-3, EFX-4, FS-5, EFG-6, EFG-7 and EFH-8. After identification, the eight fungi were respectively *Hysterium* sp, *Asterodon* sp, *Oospora* sp, *Ascochyta* sp, *Schizophyllum* sp, *Sclerotinia sp*, *Fusarium* sp and *Colletotrichum* sp (Table 2).

The detection of swainsonsine-producting fungi

The detection by TLC

The contents of swainsonine were determined on fermentation broth and hypha of eight fungi by TLC, the results showed that purple spots whose color and R_f values are the same with swainsonine in EFG-7 and FS-5, they could be preliminarily made to produce swainsonine (Table 3).

The detection by GC

Swainsonine standard samples of different concentrations were detected by GC (2.2 mg/ml swainsonine was double diluted into 8 gradients).

Table 2. Results of isolation from locoweed and rhizosphere soil.

Plant sample		EFB-1	FS-2	FS-3	EFX-4	FS-5	EFG-6	EFG-7	EFH-8
A. variabilis	Seed	8	4	-	-	-	-	-	-
	Stem and leaf	2	6	-	-	-	-	-	-
	Root	6	4	-	-	-	-	-	-
	Soil	-	2	-	-	-	-	-	-
O. glabra	Seed	5	-	-	8	-	-	-	-
	Stem and leaf	6	2	-	7	-	-	-	-
	Root	-	5	1	8	-	-	-	-
	Soil	-	3	4	-	-	-	-	-
O. kansuensis	Seed	-	-	-	-	-	4	3	-
	Stem and leaf	-	-	-	-	-	5	2	-
	Root	-	-	-	-	-	6	1	-
	Soil	-	-	-	-	5	-	-	-
O. deflexa	Seed	-	-	-	-	-	2	3	-
	Stem and leaf	-	-	-	-	-	3	-	-
	Root	-	-	-	-	-	5	3	-
	Soil	-	-	-	-	2	-	-	-
O. ochrocephala	Flower	-	-	-	-	-	-	-	3
	Stem and leaf	-	-	-	-	-	-	-	4
	Root	-	-	-	-	-	-	-	4
	Soil	-	-	-	-	-	-	-	-

"-" means no fungi was isolated from the sample, the figure means the colony numbers separated from the sample.

Table 3. Results of TLC detecting.

Strain number	Fermenting liquor	Hyphae	Spot color and R_f value
EFB-1	-	-	-
FS-2	-	-	-
FS-3	+	+	0.79 (purple), 0.44 (purple), 0.328 (purple)
EFX-4	+	+	0.58 (fuchsia)
FS-5	+	+	0.52 (fuchsia)
EFG-6	-	-	-
EFG-7	+	+	0.88 (fuchsia), 0.52 (fuchsia), 0.21 (fuchsia)
EFH-8	-	-	-

"+" means TLC coloration is violet, "-" means TLC no coloration or coloration not violet; SW TLC coloration is violet, R_f value is 0.52; Culture media without inoculate is no coloration; Developing system is chloroform: methanol: ammonia water: water (70:26:2:2).

The standard curve was drawn with concentration of swainsonine as abscissa and peak area as vertical axis and obtained standard curve equation Y = 80326X - 984.69 (R^2 = 0.9985). It means that when the concentration of swainsonine is 0.02 to 2.2 mg/ml, swainsonine concentration and its peak area will show good linear relationship (Figure 1). The retention time of swainsonine standard chromatographic peak is 6.07 min, chromatogram peaks appeared in FS-5 and EFG-7 during this period of time, indicating that the two fungal can produce swainsonine (Figures 2 and 3). Based on the standard curve equation and the peak area, the content of FS-5 fermentation broth is 1.233 mg/L, the content of the mycelia is 0.773 mg/g; the content of EFG-7 fermentation broth is 2.780 mg/L, the content of mycelia is 0.110 mg/g.

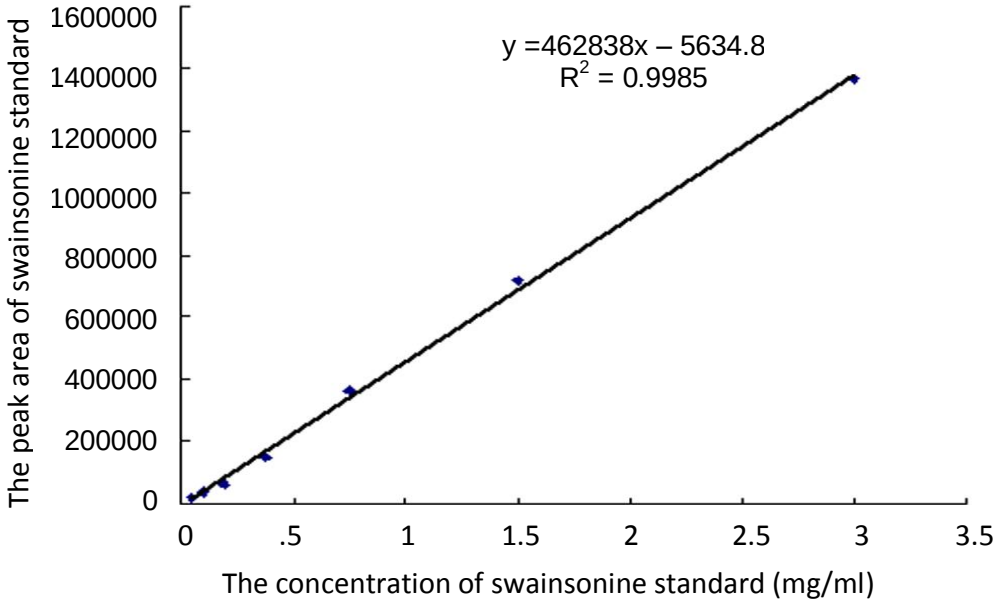

Figure 1. The standard curve of SW content and peak area.

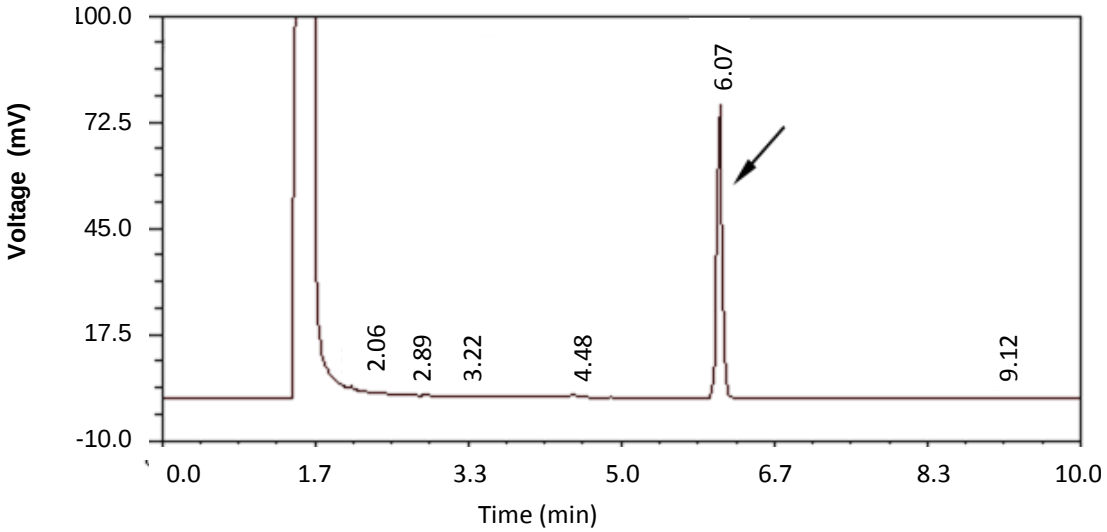

Figure 2. GC peak of swainsonine standard. The arrow show retention time of chromatographic peak on swainsonine standard is 6.07 min.

Identification of swainsonsine-producting fungi

Morphological features

The average growth rate of FS-5 was about 12 mm d^{-1} in the PDA medium, it covered the entire plate on the seventh day. The colonies were round, flat and velvet-like, the hypha was white, thin, dense and radial growth, the edges of colony were like snowflakes. The hypha in edges whose diameter was 2 to 5.5 μm are colorless, thin-walled and their aerial hypha were colorless, diameter was 1 to 3 μm, there are few branches. Hypha had diaphragm, granular salience which is long and black were growing on the surface and vegetative hypha grew from these black saliences. Conidia were not observed (Figure 4A and B).

The average growth rate of EFG-7 was about 7 mm d^{-1} in the PDA medium. At first the colonies were white, ring-shaped growth, after that the center of colonies turned pink, the outer layer turned white and finally the whole colonies turned into purple-red. In addition, ridge colonies were observed, strong aerial hypha, which were shaped like cotton, the opposite of matrix was purple, with purple materials infiltrating into medium. Hypha surface was

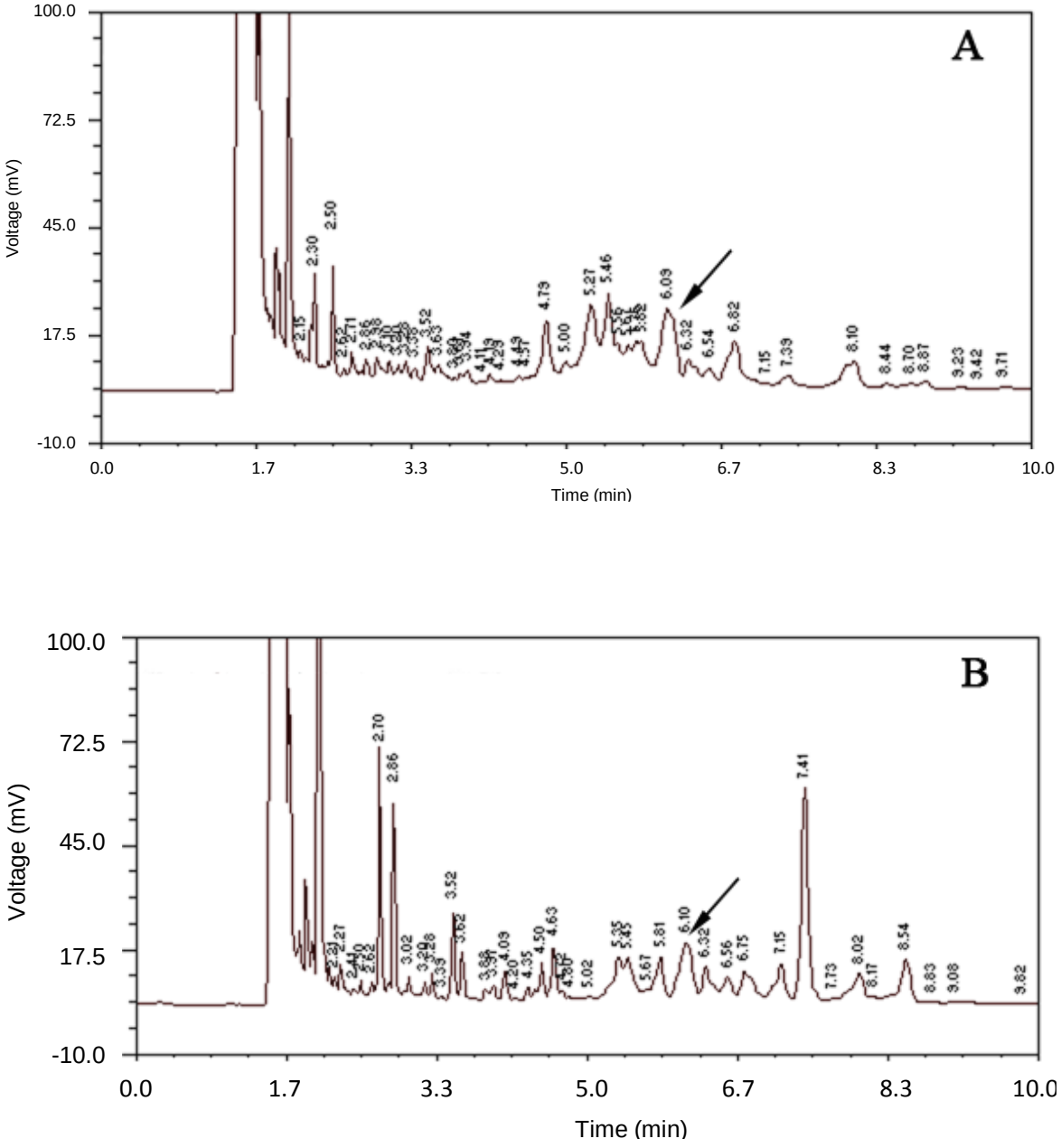

Figure 3. GC of extraction from FS-5 (A), EFG-7 (B). The arrows show retention time of chromatographic peak on FS-5 and EFG-7 fungus are essentially in accord with swainsonine standard, indicating that the two fungal can produce swainsonine.

smooth. Conidia were not observed (Figure 4C and D).

ITS sequence analysis

The ITS sequence length of FS-5 and EFG-7 were between 500 to 750 bp (Figure 5), the fragment contained the entire sequence of ITS1, 5.8S rDNA and ITS2, as well as the partial sequence of 18S rDNA and 28S rDNA. Gene sequencing results showed that the ITS sequence length of FS-5 was 639 bp, which has the similarity of 98% with the *Schizophyllum* sequence (Genebank No. FJ196608). The ITS sequence length of EFG-7 was 563 bp, which has the similarity of 99% with the *Tricinctum* sequence (Genebank No. FJ233196). The FS-5 and EFG-7 sequences were treated by splice and adverse complementation; phylogenetic trees were constructed (Figures 6 and 7). Phylogenetic tree consisted of three groups in Figure 6 (expressed A, B, C), group A consisted of *Schizophyllum*, group B consisted of *Lentinus*,

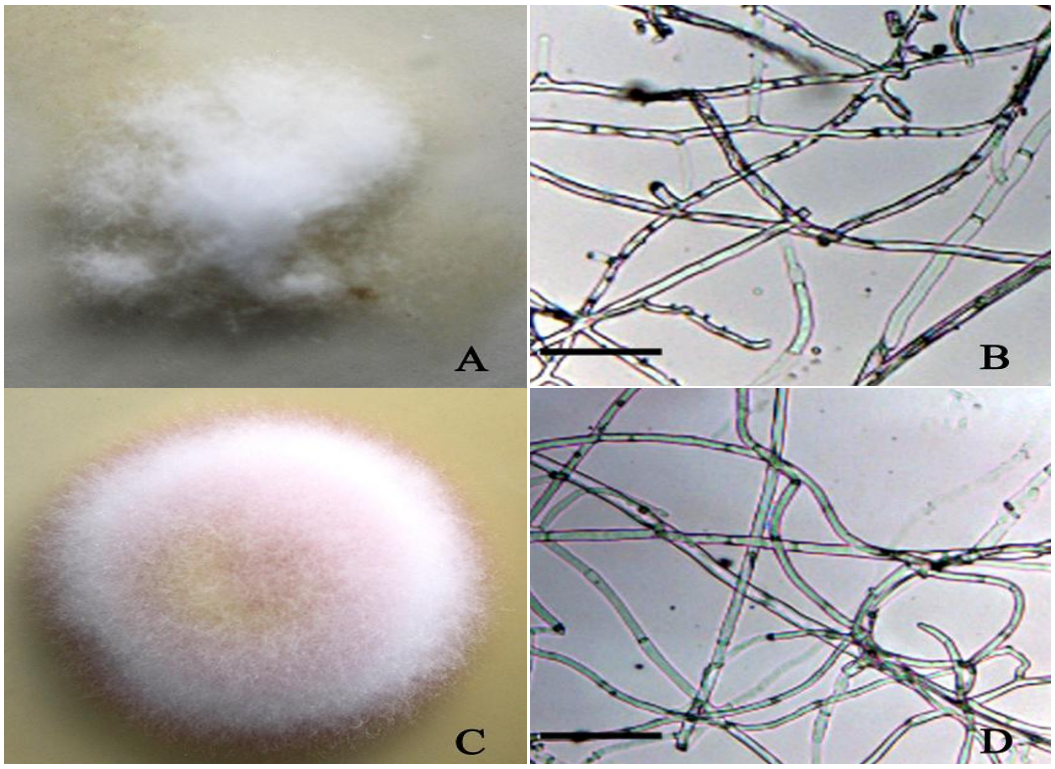

Figure 4. The colony shape and hyphae characteristic of FS-5 and EFG-7; A: The colony of FS-5 was round, and flat, the edges of colony was like snowflakes (×400); B: The hyphae of FS-5 had diaphragm, granular salience which is long and black were growing on the surface, vegetative hypha growed from these black saliences (bars = 50 μm); C: The colony of EFG-7 was white, ring-shaped growth, the center of colonies was pink and the outer layer was white (×400); D: The surface of hyphae of EFG-7 was smooth, and it had diaphragm (bars = 50 μm).

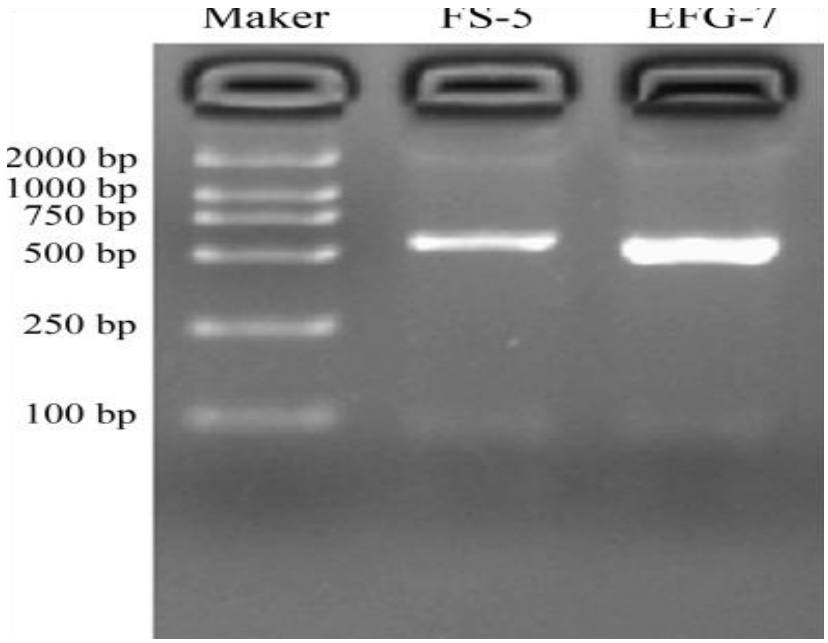

Figure 5. Agion electrophoresis of amplified 5.8S rDNA ITS region from strain FS-5, EFG-7 by PCR method. The ITS sequence length of FS-5 and EFG-7 were between 500 to 750 bp.

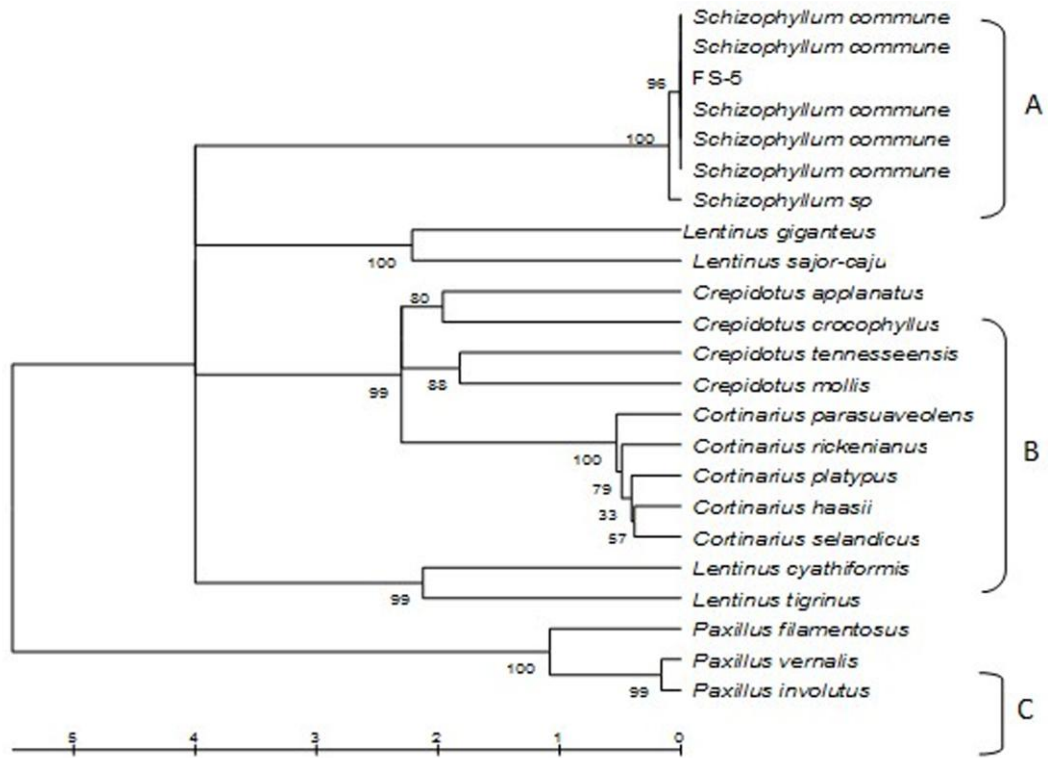

Figure 6. Phylogenetic tree of FS-5 obtained from the program N J (Neighbor joining). The phylogenetic tree consisted of three groups, group A consisted of *Schizophyllum*, group B consisted of *Lentinus* and *Cortinarius* and *Crepidotus*, group C consisted of *Paxillus*, group A and B constituted a complex group.

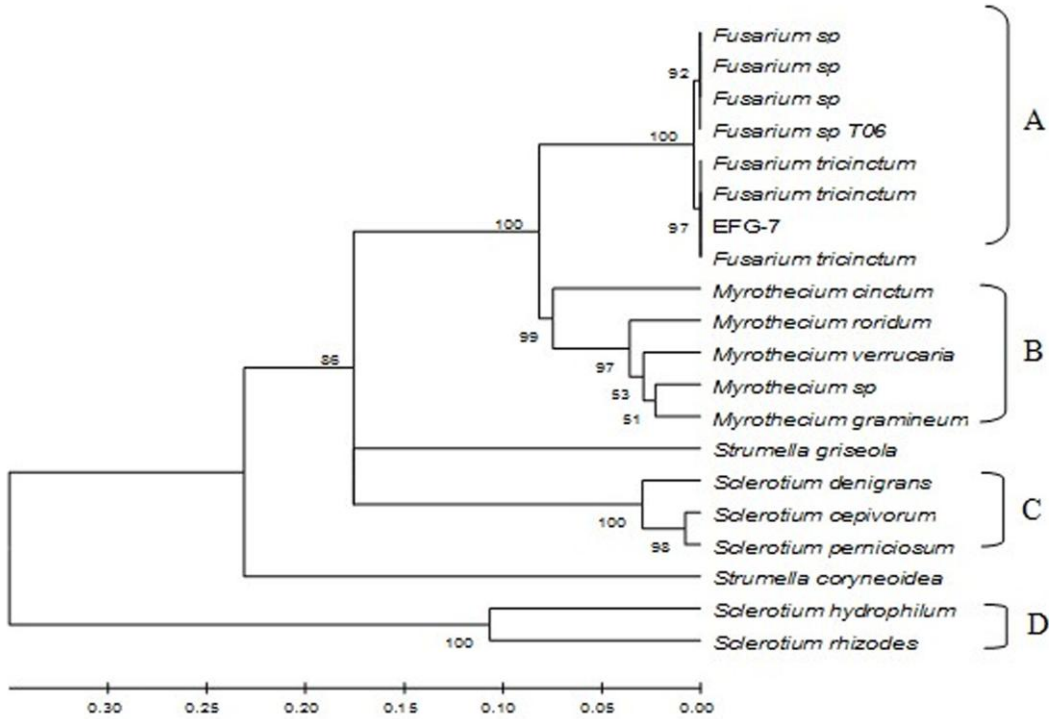

Figure 7. Phylogenetic tree of EFG-7 obtained from the program N J (Neighbor joining). The phylogenetic tree can be divided into A, B, C, D groups, group A was *Schizophyllum*, group B consisted of *Myrothecium*, group C consisted of *Strumella* genus and two species of the *Sclerotium*, group D consisted of three species of *Sclerotium*, group A, B, C together constituted a large complex group.

Cortinarius, *Crepidotus*, group C consisted of *Paxillus*. phylogenetic tree was composed of four groups in Figure 7 (expressed A, B, C, D); group A was *Schizophyllum*, group B consisted of *Myrothecium*, group C consisted of *Strumella* genus and two species of the *Sclerotium*, group D consisted of three species of *Sclerotium*.

DISCUSSION

Fungi were isolated and cultivated from five locoweeds and their rhizosphere soils which came from different geographical distribution, fungal secondary metabolites were detected and two strains fungi of swainsonine-producing were found from above samples. Morphological characteristics were observed on two strains fungi (FS-5 and EFG-7). FS-5 hyphae had diaphragm and its surface had black grainy processes, and its morphology is the same as *Schizophyllum* according to the Manual of fungi identification, but there was no fruiting body on the medium. EFG-7 produced a large number of aerial hyphae, which was woolly cloud-like on the medium and the mycelium produced purple pigment in the medium; however, the conidiospore is not found. Therefore, it is difficult to classify them according to the mycelium morphology.

As seen from the phylogenetic tree, the genetic relationship between FS-5 and *Schizophyllum* commune is relatively close and the similarity reached to 96%, meanwhile, there was a distant genetic relationship between FS-5 and any other genus. The genetic distance between EFG-7 and *F. tricinctum* is nearest (97% similarity). There is a certain distance with the other four species in this genus and a much farther distance with the remaining groups, suggesting that EFG-7 and *F. tricincum* were the same species of fungi. Fungal ribosomal rDNA gene is considered to be the ideal sequence in the ongoing molecular systematic studies and useful to understand the phylogenetic relationship of fungi and evolutionary history (Viaud et al., 2000; Martin and Rygiewicz, 2005; Li, 2005). However, due to influenced on comparison of genebank, the sequence analysis of rDNA-ITS cannot identify all species of fungi. For example, FS-5 should belong to *Schizophyllum*, but since it lack the sequence of any other related species of this genus in the genebank database and it could not be defined whether it belonged to the *S. commune*. However, there had been a very small difference between FS-5 and another unidentified fungus *Schizophyllum* sp (99% similarity); it still need further study for its classification of species.

The biosynthesis of swainsonine evolved from pipecolinic acid in fungus and it was highly relevant with the content of swainsonine in the host plants. This suggests that endophytic fungi at least partly control the toxicity of plants or swainsonine is the same secondary metabolites in endophytic fungi and the host, endophytic fungi may reinforce or complement its yield and enhance the toxicity of the host plant to herbivorous animals (Lu et al., 2009). On the other hand, genetic characteristics and geographical distribution of the host plants influenced colonization of endophytic fungi. The isolated fungi in this study possessed the properties of geographical distribution. The fungi isolated from Qinghai and Inner Mongolia samples are not the same and swainsonine producing fungi were isolated only from the samples in Qinghai, but failed to separate the type of fungi in Inner Mongolia samples. This may be related to flora of samples and local climate. *Schizophyllum* was a kind of precious medicinal fungus, whose polysaccharide could enhance immune function and had anti-tumor activity and its biological activity were very similar to swainsonine, but it was not clear that S*chizophyllum* could produce swainsonine. The nonsexual generations of *F. tricinctum* belonged to *Fusarium*, and sexual generations belonged to *Gibberella* sp, and whether they could produce swainsonine was not reported either (Cui et al., 2007). The research on the endophytic fungi of Taxol showed that certain genes of endophytic fungi may come from the transformation of host-related gene (Li et al., 2006). In our study, two fungi isolated can both produce swainsonine, so it could speculate that swainsonine-related genes of fungi may also come from the host plant-related gene transformation, but this remains to be confirmed by further studies.

In recent years, domestic and foreign scholars have made broad and intensive studies about the relationship between locoweed toxin-swainsonine and endophytic fungi (Yu et al., 2009; Lu et al., 2011; Cook et al., 2011, 2012). For now, more and more people will focus on the systematic classification of locoweed and the position of swainsonine-related genes of fungi and technological processes of microbial fermentation. With more fungi of swainsonine- producing found, Swainsonine as new anti-cancer drug is bound to play a greater role in the biological, medical and other areas.

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