Community composition and differential analysis of rhizosphere soil microorganism and endophytes in Schisandra sphenanthera Rehd. et Wils.

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Research Article

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Abstract

**Background and aims:** Endophyte and rhizosphere soil microorganism are vital microbial environments of the plant, namely plant microenvironments. A robust understanding of the structural composition of the microbiome present in below-ground and above-ground communities has remained elusive. *Schisandra sphenanthera* Rehd. et Wils. is a kind of traditional Chinese Medicine (TCM) of *Schisandra*, which can protect the kidney and liver.

**Methods:** In this study, high-throughput sequencing analysis is applied to unravel microbial communities in rhizosphere soil and different parts of wild *S. sphenanthera*, and the movement regularity of endophytes in plant tissues.

**Results:** There are differences in microbial composition and diversity between rhizosphere soil and four parts of *S. sphenanthera*. Proteobacteria, Cyanobacteria, and Acidobacteria are main bacteria, Ascomycota and Basidiomycota are main fungi at phylum level of microbe in *S. sphenanthera*. There are 12 common bacterial genera and 11 common fungal genera in rhizosphere soil and different parts of *S. sphenanthera*. In addition, each of the four parts and rhizosphere soil have its own dominant communities, such as *Achromobacter* (stem and leaf) and *Methyllobacterium* (leaf). OTUs clustering results indicate that the bacterial community of root is greatly influenced by rhizosphere soil, while the microbial community of stem and fruit are greatly affected by the microorganisms of leaf.

**Conclusions:** Understanding the microbial community structure and diversity in rhizosphere soil and different parts of *S. sphenanthera* can provide basis for further study of host-microbial interactions of *S. sphenanthera* in phytoremediation, sustainable utilization, and secondary metabolite production.

Introduction

The relationship between microorganisms and plants and animals are one of the most studied research areas in biology or microbiology in recent years. The massive interest in this area is reflected by numerous studies, including studies of human gut microfauna (Liu et al., 2021) and plant microbiome (Bai et al., 2015). In most cases, microorganisms maintain a close mutualistic relationship with animals and plants, through which they obtain nutrients (Hacquard et al., 2015), and the related microbial community can play an important role in the immune system of animals and plants (Kau et al., 2011; Lu et al., 2021). The plant microbiome comprises a variety of microorganisms, including bacteria and archaea, fungi, and viruses (Turner et al., 2013). Plant-microbe interactions are important, not only to better understand their role in plant growth and development, but also to discover their function in the formation of secondary metabolites (Hardoim et al., 2008; Nishad et al., 2020). Within plant microbiome research, most attention have been dedicated to endophyte and rhizosphere soil microorganisms (Wang et al., 2022; Tumer et al., 2013).

Endophytes, derived from the Greek "endon", are a class of fungi or bacteria that lives in various tissues and organs of healthy plants at one certain stage or at all stages of their life history, without causing substantial damage to the host plants (Kambach et al., 2021; Wani et al., 2015; Wijekoon and Quill, 2021). Endophytes include mutualistic symbiotic microorganisms, neutral symbiotic microorganisms, and pathogenic microorganisms lurking in the host (Jia et al., 2016; Rim et al., 2021). Several studies have shown that the diversity and community structures of endophytes are closely related to the species, growth stage, nutrition supply, living environment, and genotype of the host plants (Liu et al., 2019; Rim et al., 2021; Robinson et al., 2016). Endophytes play a positive role in plant growth and development, pest control, plant resistance, bioremediation, and other aspects (Durand et al., 2021; Jauri et al., 2020). For example, endophytes can not only relieve plant stress, but also significantly improve plant yield and height (Rojas-Tapias et al., 2012; Yaish et al., 2015). For the current findings, three major mechanisms by which endophytes affect plant metabolism, physiology and chemical composition occur at the molecular and cellular levels (Dong et al., 2003; Verhagen et al., 2010; Yedidia et al., 2003). Endophytes not only participate in synthesis or transformation of the plant secondary metabolites, but also form a large number of secondary metabolites that possessed biological active functions and great potential applications in the field of medicine, health and agriculture (Aghdam and Brown, 2021; Yuan et al., 2019). Furthermore, endophytes can produce antibiotics, enzymes, plant growth regulators, alkaloids, and a series of metabolites (Fuchs et al., 2017; Joo et al., 2021; Nisa et al., 2015).

The rhizosphere is a narrow band of soil directly influenced by plant roots (York et al., 2016). Rhizosphere systems are one of the most complex ecosystems on earth, containing numerous rhizosphere microorganisms, which are significantly more numerous and diverse than soils outside the rhizosphere (Lucas et al., 2020). Rhizosphere environment is an important place for plant growth, metabolism and absorption of soil nutrients, as well as the most direct interaction between roots and soil (Ma et al., 2021; McCully, 2007). Rhizosphere soil microorganisms can increase plant tolerance to biotic and abiotic stresses, improve soil nutrient absorption, and influence plant yield and quality (Choi et al., 2020; Vries et al., 2020). At the same time, rhizosphere soil microorganisms directly affect the biochemical activity of soil (Thiele-Bruhn et al., 2012).
The colonization and development of endophytes in plants are affected by many factors, such as temperature, humidity, soil microorganisms, and plant growth cycle. On the one hand, endophytes may enter the plant tissue cells by degrading the cellulose on the plant surface. On the other hand, endophytes may enter the plant through the stomata on the surface of the host plant, the rhizosphere, or by inducing the plant to form corresponding channels (Rangjaroen et al., 2017; Santi et al., 2013). Once the endophytes enter the plant, they can infect the adjacent plant tissues. However, the spatial mechanism of endophytic colonization in wild environment, and the relationship between below-ground (rhizosphere soil microorganisms) and above-ground (endophytes) communities has remained elusive.

Here, we evaluate the diversity and composition of endophytes associated with the rhizosphere soil, root, stem, leaf, and fruit of wild *Schisandra sphenanthera* Rehd. et Wils., using 16s rDNA and ITS high-throughput sequencing analysis. *S. sphenanthera*, a perennial deciduous woody vine of the genus *Schisandra* (Committee of Flora of China, 1996; Smith, 1947), is a high-value traditional Chinese Medicine (TCM). The dry and ripe fruit is often used as medicine, known as “Nanwuweizi”, to treat chronic cough, asthma, night sweats, and palpitations insomnia (Committee of National Pharmacopoeia, 2020; Li et al., 2020). The active ingredients in fruits include lignans, essential oils, polysaccharide, and so on (Gu et al., 2008; Lu et al., 2012; Wang et al., 2017). However, up until now, minimal studies are available that involve the *S. sphenanthera* endophytes, both domestically and abroad, even *Schisandra*. In the present study, we focussed on two main questions: (i) How variable are rhizosphere soil microorganisms and endophytes in different parts of *S. sphenanthera*? (ii) What are the formation mechanism of endophytes community structure, and the migration rule of endophytes in *S. sphenanthera* tissues? The purpose of this study is to provide reference for the development and utilization of microorganisms through the analysis of rhizosphere soil microorganisms and endophytes in different parts of *S. sphenanthera*.

**Materials And Methods**

**Sampling and sample processing**

The plant materials (*S. sphenanthera*) are collected from Zhashui County, Shaanxi province of China, south of Qinling Mountains, in August 2019. Five individuals are sampled for rhizosphere soil, root, stem, leaf, and fruit. The roots of similar thickness are collected at a depth of 10–20 cm below the ground, without damaging the taproot. The rhizosphere soil is soil particles adhered to the root system, and collected by shaking off the roots. Particles separated during the shaking of the roots are collected, and then be sifted through a 2 mm sieve. For stem and leaf, one complete branch is collected from each individual, and all leaves are collected from the sampled offshoot. And a few fruits are collected from each individual. The materials are transported to the laboratory with the sterile bag in ice boxes, and stored in a -40°C refrigerator as soon as possible. All voucher specimens of plant materials are kept in the College of Life Sciences, Shaanxi Normal University.

The samples are cleared and sterilized from epiphytic bacteria (surface sterilization) according to the following methods. 5 g of root, stem, leaves, and fruit are soaked by 75% alcohol for 1 min, washed with sterile water 3–5 times, respectively. Then, the samples are divided into small pieces with a sterile scalpel, transferred to a mortar, and ground to paste with few sterile water and quartz sand. Finally, the plant material (root, stem, leaf, and fruit) are stored at –80°C until DNA was extracted.

**DNA extraction**

The grated parts (root, stem, leaf, and fruit) are placed in a 20 mL centrifuge tube. 15 mL sterile water is added into the centrifuge tube, and 200×g centrifugation for 5 min to precipitate. 13 mL supernatant liquid are transferred to the centrifuge tube, added 0.23 g solid NaCl and 164 µL 10% SDS (w/v), inverted mix, placed at 4°C for 1 h, and the supernatant liquid are moved to a new centrifuge tube. The supernatant liquid is centrifuged at 4°C, 5000×g, 10 min. The supernatant liquid is discarded, the precipitate is thoroughly suspended with sterile water. Then add 40 mL sterile water to the precipitate solution, 0.36 g of solid NaCl and 252 µL of 10% SDS (w/v). After mixing, the mixture is stored overnight at 4°C, centrifuged at 4°C, 5000×g for 10 min, and the supernatant liquid is discarded, this time the precipitation was the endophyte. Then follow the Ezup column kit (Thermo Scientific) to get fungal, bacterial total DNA.

The DNA of rhizosphere soil microorganisms is extracted using Soil Kit (Thermo Scientific) following the manufacturer's instructions.

**16S rDNA and ITS PCR amplification and high-throughput sequencing**

Appropriate amount of microbial DNA is placed in the centrifuge tube, and diluted to 1 ng/µL with sterile water. In order to generate microbial libraries, using diluted genomic DNA as template, the V4 region of 16S rDNA gene is amplified by PCR with universal primers 515F and 806R, and ITS1 region of ITS gene is amplified with universal primers ITS5-1737F and ITS2-2043R (Table S1). The samples are mixed in equal amounts according to the concentration of PCR products. After full mixing, the PCR products are purified by 2% agarose gel electrophoresis (1×TAE). The products are recovered by GeneJET gel recovery kit (Thermo Scientific), and the target bands are cut and recycled.
The purified amplicon libraries are pooled in equal concentration, and sequenced with an Miseq (Illumina, USA) system following the manipulation instructions at SAGENE Guangzhou (China).

Sequencing data processing and analysis

The sequenced data has a certain proportion of dirty data. In order to make the analysis results more accurate and reliable, the original data is spliced and filtered to obtain clean data. Finally, high quality effective sequences are obtained by shearing and filtering reads for clustering and classification analysis. Then the high quality effective sequences are analyzed by clustering, and the sequences with sequence similarity ≥ 97% are named as OTUs (Operational taxonomic units). OTUs clustering and species classification analysis are performed based on available data. Because of the DNA conservation of the ribosome, one sequence obtained during sequencing represents one species.

Chao1 index, ACE index, Shannon, and Simpson index are used to analyze α diversity of rhizosphere soil and four parts. Principal component analysis (PCoA) and hierarchical cluster analysis are performed to assess the β diversity of rhizosphere soil and four parts, and the similarity and difference of community composition among rhizosphere soil and four parts. The obtained OTUs classification information is used to plot the structure and composition histogram of each sample and the visual heatmap with the R language (Version 2.17.0) software package.

Results

Analysis of OTUs data

By high-throughput sequencing, effective high quality sequences are obtained from rhizosphere soil and different parts of S. sphenanthera after the primers are removed, the quality control filtration, chimera removal, and non-specificity are performed, amplification and removal of mitochondrial chloroplasts (Table S2). The total OTUs of bacteria is significantly different from that of fungi, especially rhizosphere soil (total OTUs of bacteria are 17 times more than fungi). Among them, the OTUs content of bacterial is the highest in rhizosphere soil (17703), followed by the root (3462). In fungi, the OTUs content of leaf (1570) is the highest, the stem and soil are the second (exceed 1000), while the fruit and root are the least (between 600–800) (Table S2). OTUs cluster analysis show that the microorganisms in stem and fruit of S. sphenanthera are grouped into one group, and the endophyte in the root is different from the stem, leaf, and fruit (Fig. 1). Rhizosphere soil microorganisms and root endophytes are classified into one group (Fig. 1), and the classification of bacteria and endophytic fungi is consistent.

The Venn diagram results show that there are differences in composition and genetic relationships among rhizosphere soil and different parts of S. sphenanthera (Fig. 2). There are 10 common OTUs of bacteria in rhizosphere soil and four parts of S. sphenanthera (Fig. 2A), which belong to three phyla (Proteobacteria, Actinobacteria, and Cyanobacteria) respectively. Rhizosphere soil and root have the largest number of common OTUs (92), followed by stem and fruit (83), leaf and fruit (80). The number of OTUs in rhizosphere soil and above-ground parts (stem (27), leaf (19), and fruit (26)) is much lower than that in rhizosphere soil and root. At the same time, the difference between the number of common OTUs in root and above-ground parts (stem (46), leaf (36), and fruit (45)) is small, but it is higher than that in rhizosphere soil. In addition, the common OTUs number of stem and leaf is 68 (Fig. 2A). Among the fungi of S. sphenanthera, there are 6 common OTUs in rhizosphere soil and four parts (Fig. 2B) belonging to two phyla (Ascomycota and unclassified_Fungi). Consistent with the results of bacterial, there are more common OTUs in the above-ground parts (stem, leaf, and fruit), with the most common OTUs between leaf and fruit (88), followed by stem and leaf (81), and stem and fruit (61) (Fig. 2B). The number of common OTUs between roots and other three parts (stem, leaf, and fruit) (26, 27, and 25, respectively) is small and the difference is little, so is the rhizosphere soil (22, 34, and 25, respectively). Different from the results of bacterial (92), the number of common OTUs in rhizosphere soil and roots is not large (39) (Fig. 2B). The results show that stem, leaf, and fruit are closely related (Fig. 2). In addition, the number of specific OTUs to rhizosphere soil and leaf is large, so it can be inferred that the microbial community of rhizosphere soil and leaf may be greatly affected by external environment. For example, the influence of soil internal environment on rhizosphere soil microorganisms, and some microbial communities in leaf may come directly from the environment.

Microbial composition and community structure in rhizosphere soil and different parts of S. sphenanthera

By comparing the OTUs representative sequence of rhizosphere soil and four parts with SILVA database, the histogram abundance map can be drawn, which can analyze the composition and proportion of species between rhizosphere soil and different parts more intuitively, taking the level of phylum as an example (Fig. 3). The endophytic bacteria in the root, stem, leaf, and fruit are mainly composed of Cyanobacteria (53.70%, 48.73%, 54.89%, and 57.40%, respectively) and Proteobacteria (28.47%, 26.48%, 25.50%, and 29.96%, respectively). The bacteria of rhizosphere soil are mainly composed of Acidobacteria (35.57%) and Proteobacteria (30.80%) (Fig. 3A). The results show that there is little difference in the composition and abundance of endophytic bacteria in root, stem, leaf, and fruit at phylum level, but there are significant differences between four parts and rhizosphere soil bacteria. For example, the abundance of Acidobacteria in rhizosphere soil is nearly 10

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times that of four parts, and the abundance of Cyanobacteria in four parts is nearly 50 times that of rhizosphere soil. However, there are differences in the dominant flora of endophytic bacteria at lower levels in root, stem, leaf and fruit, especially at order level (Fig. 4 and S1A-S3A, Table 1 and S3). At the order level, the dominant bacteria in rhizosphere soil mainly includes iii1-15 (20.158%), Rhizobiales (5.245%), Burkholderiales (4.820%), Sapropiriales (4.298%), and a small amount of Rhodospirillales (3.087%), and Xanthomonadales (3.038%) (Fig. S2A). These six orders belong to the five classes with higher concentrations in rhizosphere soils (Fig. S1A), and Rhizobiales also have high concentration in the remaining four parts (11.173% in root, 9.672% in leaf, 6.098% in stem, and 4.369% in fruit) (Fig. S2A). Streptophyta, belonging to the class Chloroplast, is the dominant bacterial community in fruit, leaf, root, and stem (57.323%, 54.777%, 53.672%, and 48.725%, respectively) (Fig. S2A). In addition to Streptophyta and Rhizobiales, Rickettsiales is dominant bacteria in fruit (11.260%) and root (5.754%), Burkholderiales and Actinomycetales are dominant in stem (10.174% and 7.128%) and leave (10.603% and 6.721%), and Bacillales is also dominant in stem (11.959%) (Fig. S2A). According to the above results, there are significant differences in the composition and diversity of microflora in rhizosphere soil and different parts.

There are 12 common bacterial genera belonging to rhizosphere soil and four parts of S. sphenanthera (Table 1). Achromobacter is the dominant genus of leaf and stem (7.130% and 7.010%), and Methylobacterium is the dominant genus of leaf (6.037%). In addition, Methylobacterium is found in fruit (2.960%) and stem (2.945%) (Table 1). Rhodoplanes is the dominant genus in root and rhizosphere soil (4.280% and 2.209%), and only existed in below-ground parts (Table S3). The heat map of species level clustering shows that rhizosphere soil and root are still clustered as one group (Fig. 4), and the clustering results of the three parts (stem, leaf, and fruit) are different from OTUs clustering results (Fig. 1A), indicating that microbes of rhizosphere soil and root are relatively close (Fig. 4).

The fungi in rhizosphere soil and different parts of S. sphenanthera are mainly composed of Ascomycota and Basidiomycota (Fig. 3B). Ascomycota have higher compositions in fruit, stem, leaf and root (49.74%, 45.31%, 26.47%, and 25.70%, especially), while Basidiomycota have higher concentrations in rhizosphere soil (45.74%) (Fig. 3B). The difference of rhizosphere soil and different parts fungal communities is more obvious at lower levels (Fig. 5 and S1B-S3B, Table 2 and S4). Agaricales belong to Agaricomycetes, which is the dominant fungal community at the order level of rhizosphere soil (41.595%) (Fig. S1B and S2B). At the same time, Agaricales is also the dominant fungal community in the root with high content (5.734%). Pleosporales has high concentration in the remaining four parts (14.739% in stem, 4.557% in fruit, 4.296% in leaf, and 2.577% in root). Capnodiales is the dominant fungal community in fruit (12.403%), leaf (11.281%), and stem (3.970%). In addition, Helotiales is the dominant fungal community in root (11.836%), and Dothideomycetidae_ord_Incertae_sedis is the dominant fungal community in stem (18.543%) (Fig. S2B).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Rhizosphere soil</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achromobacter</td>
<td>0.003</td>
<td>0.281</td>
<td>7.010</td>
<td>7.130</td>
<td>1.358</td>
</tr>
<tr>
<td>Actinomycetospora</td>
<td>0.002</td>
<td>0.008</td>
<td>0.199</td>
<td>1.262</td>
<td>0.248</td>
</tr>
<tr>
<td>Burkholderia</td>
<td>0.407</td>
<td>0.152</td>
<td>1.481</td>
<td>1.481</td>
<td>0.355</td>
</tr>
<tr>
<td>Hyphomicrobium</td>
<td>0.070</td>
<td>0.145</td>
<td>0.809</td>
<td>0.374</td>
<td>0.422</td>
</tr>
<tr>
<td>Methylobacterium</td>
<td>0.005</td>
<td>0.019</td>
<td>2.945</td>
<td>6.037</td>
<td>2.960</td>
</tr>
<tr>
<td>Nevskia</td>
<td>0.003</td>
<td>0.025</td>
<td>0.879</td>
<td>0.566</td>
<td>1.661</td>
</tr>
<tr>
<td>Nocardioides</td>
<td>0.002</td>
<td>0.054</td>
<td>0.144</td>
<td>0.212</td>
<td>0.085</td>
</tr>
<tr>
<td>Phenyllobacterium</td>
<td>0.830</td>
<td>0.216</td>
<td>0.081</td>
<td>0.005</td>
<td>0.083</td>
</tr>
<tr>
<td>Planctomyces</td>
<td>0.788</td>
<td>0.557</td>
<td>0.202</td>
<td>0.200</td>
<td>0.121</td>
</tr>
<tr>
<td>Plesiocystis</td>
<td>0.023</td>
<td>0.012</td>
<td>0.199</td>
<td>0.197</td>
<td>0.246</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>0.002</td>
<td>0.069</td>
<td>2.281</td>
<td>0.340</td>
<td>0.541</td>
</tr>
<tr>
<td>Sphingomonas</td>
<td>0.043</td>
<td>0.177</td>
<td>0.222</td>
<td>0.219</td>
<td>0.489</td>
</tr>
</tbody>
</table>
Table 2
The differences in abundance of common genus of fungal in rhizosphere soil and different parts of S. sphenanthera.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Rhizosphere soil</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>0.005</td>
<td>2.143</td>
<td>13.803</td>
<td>1.350</td>
<td>3.871</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>0.005</td>
<td>0.350</td>
<td>0.216</td>
<td>0.215</td>
<td>0.675</td>
</tr>
<tr>
<td>Cercospora</td>
<td>0.032</td>
<td>0.123</td>
<td>1.718</td>
<td>4.474</td>
<td>0.832</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>0.005</td>
<td>0.463</td>
<td>0.581</td>
<td>0.349</td>
<td>0.652</td>
</tr>
<tr>
<td>Penicillium</td>
<td>0.006</td>
<td>0.368</td>
<td>0.102</td>
<td>0.043</td>
<td>0.534</td>
</tr>
<tr>
<td>Peniophora</td>
<td>0.014</td>
<td>0.311</td>
<td>0.002</td>
<td>0.474</td>
<td>0.185</td>
</tr>
<tr>
<td>Schizophyllum</td>
<td>0.006</td>
<td>0.614</td>
<td>0.389</td>
<td>0.404</td>
<td>0.182</td>
</tr>
<tr>
<td>Stagonosporopsis</td>
<td>0.024</td>
<td>0.084</td>
<td>0.386</td>
<td>0.478</td>
<td>0.086</td>
</tr>
<tr>
<td>unclassified_Ascomycota</td>
<td>0.077</td>
<td>0.007</td>
<td>0.030</td>
<td>0.695</td>
<td>0.039</td>
</tr>
<tr>
<td>unclassified_Pleosporales</td>
<td>0.044</td>
<td>0.017</td>
<td>0.002</td>
<td>0.665</td>
<td>0.086</td>
</tr>
</tbody>
</table>

There are 11 common fungal genera belonging to rhizosphere soil and four parts of S. sphenanthera (Table 2). Among the common genera, except for unclassified_Fungi, Alternaria is found with high concentrations in root, stem, leaf, and fruit, especially in stem (13.803%). And Cercospora is found with high concentrations in stem and leaf (Table 2). In addition, Hebeloma (rhizosphere soil), unclassified_Helotiales (root), Stomiopeltis (stem), and Botrytis (fruit) ran to greater than 10% in their respective domains (Table S4). The clustering results of rhizosphere soil and four parts are consistent with OTUs clustering results (Fig. 1B). It is indicating that microbes of rhizosphere soil and root are relatively close, and the microbes of stem, leaf, and fruit are close (Fig. 5).

Fungal and bacterial species richness and diversity

Rarefaction curves are constructed for rhizosphere soil and different parts of S. sphenanthera to show the number of observed OTUs, and to evaluate the OTUs richness per sample generally approached saturation (Fig. 6). As expected, endophytic bacterial communities (Fig. 6A) are much less diverse than rhizospheric communities, while fungal communities are more diverse in leaf (Fig. 6B). Alpha diversity indices including Chao1, ACE, Shannon, and Simpson are applied for the estimating of endophytic community complexity, on the basis of the OTUs sequence, the diversity of the community is further reflected (Table 3). Chao1 and ACE are used to evaluate the microbial species richness, and the higher the index, the higher the microbial species richness. Shannon and Simpson are used to evaluate the species diversity of microflora. The higher the Simpson index is, the lower the community diversity is; the higher the Shannon index is, the higher the community diversity is.

Table 3
The total OTUs Alpha diversity index of bacteria and fungi.

<table>
<thead>
<tr>
<th>Alpha index</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhizosphere soil</td>
<td>Root</td>
</tr>
<tr>
<td>ACE</td>
<td>74802.77</td>
<td>7336.57</td>
</tr>
<tr>
<td>Chao1</td>
<td>70527.98</td>
<td>8624.06</td>
</tr>
<tr>
<td>Shannon</td>
<td>11.59</td>
<td>5.97</td>
</tr>
<tr>
<td>Simpson</td>
<td>1</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Among the endophytic and rhizosphere bacteria identified, the diversity of the below-ground parts is higher than the above-ground parts, and the soil Shannon index is nearly two times as high as that of the endophytes index. It indicated that the abundance of microbial species in the rhizosphere soil is high and distributed evenly. In the identified fungal microbial communities, the Simpson index is above 0.9, and the Shannon index in the stem and leaf are higher, and the microbial diversity of the fungi is lower than that of the bacteria (Table 3). This also explains the variability of fungi, bacteria in rhizosphere soil and different parts of the plant. And the diversity of endophytic microbial in different parts of the host and the specificity of the rhizosphere soil are fully reflected.
The Beta diversity of microbial community structure of rhizosphere soil and four parts of *S. sphenanthera* is evaluated according to the principal coordinate analysis diagram, which can intuitively show the microbial community difference of rhizosphere soil and different parts (Fig. 7). PCoA1 and PCoA2 explained 40.6% and 22.9% of the total variance of bacterial communities in rhizosphere soil and different parts of *S. sphenanthera*, respectively (Fig. 7A). Similarly, PCoA1 and PCoA2 explained 34.0% and 32.5% of the total variance of fungal communities, respectively (Fig. 7B). The distribution of the three points representing stem, leaf and fruit in the figure is not close to each other, and the separation distance is not very large, which illustrated that the endophyte community structures in the above-ground parts are different, but the differences are small. The part of the above-ground parts (stem, leaf, and fruit) is far apart from below-ground parts (root and rhizosphere soil), indicating that there are great differences in the composition of microbial between the above-ground parts and the below-ground parts (Fig. 7).

**Discussion**

**Microbial diversity analysis**

Endophytes exist in specific tissues of plants and interact with plants through the exchange of nutrients, enzymes (catalase, oxidase, etc.), functional factors (biosurfactants, etc.), and signal transmission (Lu et al., 2021). They colonize in plant tissues for a long time and do not produce negative effects similar to pathogens, such as the destruction of photosynthesis, nutrient transfer, etc. On the contrary, the presence of these endophytes in host plants has beneficial effects on their health and growth. In recent years, high-throughput sequencing technology has been widely used to study the diversity of environmental, food, animal, plant, and microbial communities (Caravaca et al., 2020).

Endophytes determination by high-throughput sequencing technology, can obtain detailed information about the microbial structure in plants, analysis of plant internal micro level of ecological and environmental relation. At the same time, the study of endophytes of medicinal plants provide the possibility of screening high-quality strains and fermentation for the production of drug active ingredients, and establish a new mode of genuine identification of medicinal materials, which has gradually become a research focus of microbial resources of medicinal plants (Adeleke and Babalola, 2021).

In this study, the diversity of microorganisms is associated with rhizosphere soil and the different parts of *S. sphenanthera*. In the case of bacteria, Chao1 indices indicate that the rhizosphere soil samples had the highest diversity among all of the samples from the different parts. Chao1 also revealed that the diversity of root samples is higher than that in the leaf samples (Table 3). On the contrary, fungi (Chao 1) showed an increasing trend of species richness from root to stem and leaf samples. Additionally, the diversity of bacterial microorganisms showed richness than fungal microorganisms (Table 3). PCoA showed that below-ground parts are distinguishable from the above-ground parts (Fig. 7). This result is consistent with the principal component analysis (PCA) results of different parts of *S. chamaejasme* L, the leaf and stem are distributed in clusters, which are different from the root plots (Jin et al., 2014).

In this study, the results show that Proteobacteria, Cyanobacteria, and Acidobacteria are main bacteria, Ascomycota and Basidiomycota are the main fungi at phylum level (Fig. 3). According to research found that most microbial community have little difference at phylum level, which is basically consistent with the results of this study, which fully demonstrated the similarity of microbes in larger taxonomic units (Rim et al., 2021; Sun et al., 2021). At present, endophytes can be isolated from various parts and organs of studied plants, such as root, stem, leaf, fruit, and seed, and the structure composition and abundance of endophytes will change with different plant varieties, parts, and development periods (Dong et al., 2018; Lv et al., 2021). This study reveals that there are significant differences between the microbial communities screened from rhizosphere soil and endophytic communities screened from different parts of *S. sphenanthera* (Table S3 and S4). There are also significant differences in the community structure and composition of endophyte in different parts of *S. sphenanthera*, which may be related to the different physiological structures of different tissues and the diversity of endophyte sources. The microbial communities of rhizosphere soil and root are more similar than those of endophytes from stem, leaf, and fruit, suggesting that endophytes of root invaded from rhizosphere soil and reached root through transport tissue. However, the endophytic communities of stem and fruit are similar to leaf, suggesting that the microorganisms of stem and fruit may enter through the phyllosphere (Figs. 1 and 7).

**Bacterial function analysis (generic level)**

Most of the bacterial microorganisms identified in this study are involved in microbial degradation, producing a variety of metabolites that affect plant growth and development. They promote plant growth through a variety of direct and indirect mechanisms. Directly mechanisms include dissolution of phosphate, nitrogen fixation, degradation of environmental pollutants, and production of hormones. And indirect mechanisms protect host plants by inhibiting plant-pathogenic bacteria by producing amino acids, polysaccharide, and other metabolites (antibiotics or lyases) (Bhattacharyya et al., 2015). In this study, *Achromobacter, Methylobacterium*, and *Propionibacterium* with high abundance have been detected in rhizosphere soil and four parts of *S. sphenanthera*, among which there are more in above-ground parts (stem, leaf, and fruit) (Table 1). *Achromobacter* can inoculate into vetiver grass (*Chrysopogon zizanioides*) which utilizes aromatic compounds as a sole carbon source (Ho et al., 2013). *Methylobacterium* protects soybean (*Glycine max*) against pathogens by inducing
endophytic community changes (Christian et al., 2021). \textit{Propionibacterium} is a kind of bacterial microorganism in stem that can synthesize propionic acid using special carboxylic enzymes, and the study shows that \textit{Propionibacterium} is widely used in the production of vitamin B12, four pyrrole compounds and propionic acid, as well as probiotics and cheese industry (Ames et al., 2012; Guyomar et al., 2020).

In addition, \textit{Rhodoplanes}, \textit{Candidatus Solibacter} and \textit{Gemmata} are also the dominant flora in the below-ground parts (rhizosphere soil and root). \textit{Rhodoplanes} and \textit{Candidatus Solibacter} are the exclusive bacteria of the below-ground parts (Table S3). \textit{Rhodoplanes} are facultative photoorganics and potential nitrate fixation bacteria (Hiraishi and Ueda, 1994). \textit{Candidatus Solibacter} is associated with food spoilage or foodborne diseases, and \textit{Gemmata} is pathogenic (the epidemiology of \textit{Gemmata} bacteremia) (Christen et al., 2018; Muriuki et al., 2021). At the same time, the content of dominant bacteria unique to the above-ground parts is less, and the total content is lower than 4% (3.147% in stem, 2.898% in leaf, and 1.512% in fruit) (Table S3). However, there are still some bacteria (not unique) with the higher contents in the above-ground parts, such as \textit{Anoxybacillus}, \textit{Methyloversatilis}, \textit{Staphylococcus}, \textit{Hymenobacter}, etc. (Table S3). \textit{Bacillus} and \textit{Anoxybacillus} can be used to promote plant growth, they compete to plant roots competitively, and can be used as biological fertilizers and identified root pathogens, such as bacteria, fungi, and nematodes (biological pesticides) (Shaikh and Sayyed, 2015). \textit{Methyloversatilis} is capable of reducing NO$_3^-$ or NO$_2^-$, and capable of C$_1$-assimilation via the serine cycle and Calvin cycle (Anthony, 1982; Lu et al., 2014). Most of \textit{Staphylococcus} is non-pathogenic parasitic bacteria, and some can cause infectious diseases in poultry (Othman et al., 2021). \textit{Hymenobacter} is a radiation-resistant bacterium commonly found in rhizosphere soil and phyllosphere (Park et al., 2022; Stevens et al., 2021). In this experiment, \textit{Hymenobacter} is only present in leaf (2.210%) (Table S3).

Among the dominant bacteria (genus level) mentioned above, \textit{Candidatus Solibacter}, \textit{Gemmmata} and \textit{Staphylococcus} are pathogenic. The remaining bacteria are involved in plant protection, growth promotion, and functional secondary metabolites. The results also confirm that endophytic bacteria and rhizosphere soil microorganisms have a low risk to plants.

**Fungal function analysis (generic level)**

The total abundance of fungi (genus level) detected in rhizosphere soil and different parts of \textit{S. sphenanthera} is higher than that of bacteria, especially the fungi in rhizosphere soil is more than four times as abundant as bacteria (Table 4). There are 6 genera of fungi unique to rhizosphere soil (Table S4), and no genus unique to bacteria (Table S3). Among six genera, \textit{Hebeloma} and \textit{Clavulinopsis} have higher abundances (Table S4). \textit{Hebeloma} affects the flow of phosphorus in litter, the transformation of soil organic matter itself, and the retardation of humification by participating in fungal interactions (Mrnka et al., 2020). \textit{Clavulinopsis}, a member of the Clavariaceae family, assimilates and transfers $^{15}$N-depleted N form to the leaves of host plants, and builds fungal cell walls by absorbing $^{15}$N-enriched N (Birkebak et al., 2013; Mayor et al., 2009). Moreover, \textit{Cortinarius} and \textit{unclassified_Xylariales} are dominant genera in rhizosphere soil, and almost absent from four parts of \textit{S. sphenanthera} (Table S4). \textit{Clavulinopsis} and \textit{Cortinarius} both belong to Agaricales, but most of \textit{Cortinarius} are edible (Li et al., 2018). On the other hand, \textit{Cortinarius} can form ectomycorrhiza with some trees and shrubs, and play an important role in plant growth and forest ecosystem (Bödeker et al., 2014). \textit{Unclassified_Xylariales}, which is within Xylariales, is pathogenicity to plants. And Xylariales has a wide range of metabolites, which can be used as biocontrol agents and biofuel producers (Helaly et al., 2018).

<table>
<thead>
<tr>
<th>Abundance</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizosphere soil</td>
<td>9.378</td>
<td>39.006</td>
</tr>
<tr>
<td>Root</td>
<td>11.789</td>
<td>26.080</td>
</tr>
<tr>
<td>Stem</td>
<td>37.004</td>
<td>45.683</td>
</tr>
<tr>
<td>Leaf</td>
<td>30.749</td>
<td>23.212</td>
</tr>
<tr>
<td>Friut</td>
<td>18.008</td>
<td>45.999</td>
</tr>
</tbody>
</table>

In addition to the four dominant fungi mentioned above, \textit{unclassified_Helotiales}, \textit{unclassified_Clavariaceae}, \textit{unclassified_Thelephoraceae}, \textit{unclassified_Agaricales} and \textit{Tuber} are also dominant fungi in rhizosphere soil. These five dominant fungi are also dominant in the root, especially \textit{unclassified_Helotiales} with high abundance in root, which is belong to Helotiales (Table S4). Helotiales has a high level of species diversity and ecological diversity, which may be reflected in their metabolic diversity (Hosoya, 2020). \textit{Unclassified_Clavariaceae} belongs to the Clavariaceae family (Agaricales), which is of great importance in studying fungus system. Many of Clavariaceae are ectomycorrhizal fungi, which play an important role in the protection and reconstruction of forest ecosystem, as well as maintaining the material cycle and energy
flow of forest ecosystem (Birkebak et al., 2013). And some species of Clavariaceae, such as Ramaria botrytis (Pers.) Ricken and Ramaria bottrooides (Peck) Comer, are not only edible, but also used as medicinal materials in TCM (Dai and Yang, 2008). Unclassified_Thelephoraceae belongs to Thelephoraceae, and unclassified_Agaricales belong to Agaricales. Some of Thelephoraceae can form ectomycorrhizas with a broad range of hosts (Miguel et al., 2016). Some of Tuber has high commercial and gastronomic values (Schneider-Maunoury et al., 2020).

In this study, Botrytis (20.768% in fruit), Stomiopeltis (18.506% in stem), Zygophiala (5.657% in fruit), and Ramularia (3.544% in leaf and 2.815% in fruit) are extremely abundant in the above-ground parts (Table S4). Most of the fungal microorganisms in this study are pathogenic. Although they temporarily lose their pathogenicity, they may recover their pathogenicity under environmental selection, such as Botrytis (Backman and Sikora, 2008; Kan et al., 2014), Stomiopeltis (Ajitomi et al., 2017), and Zygophiala (Batzer et al., 2008). At the same time, pathogens can be used as inducer to enhance the host's resistance to disease, activate the host defense system, and improve the host's defense ability against pathogens (Backman and Sikora, 2008). In addition, some endophytic fungi and their metabolites in above-ground parts can promote the growth and development of host plants, such as Trametes and Eremothecium (Table S4). The secondary metabolites of Eremothecium and Trametes have medicinal value. Eremothecium can synthesize essential oil, which has a composition similar to that of rose oil (Semenova et al., 2022). Trametes versicolor, the most important medicinal mushrooms of Trametes, can produce polysaccharide krestin (PSK) and polysaccharide peptide (PSP), which can reduce cancer metastases (Zmitrovich et al., 2012). Moreover, the abundances of Cercospora and Alternaria (especially in stems) in common genus of fungal in four parts are very high (Table 2). The extracts of Alternaria can be used as biological attractants to increase the content of secondary metabolites in Catharanthus roseusus (Birat et al., 2021). The secondary metabolites of Cercospora can inhibit the growth of Candida albicans (Bashir et al., 2022).

The abundance (genus level) of fungi detected in this experiment is higher than that of bacteria (except leaf) (Table 4), and the dominant fungi are far more than that of bacteria. Endophytic fungi have multiple functions. Unclassified_Xylariales, Botrytis, Stomiopeltis, and Zygophiala are pathogenic, but can be used as inducers to enhance the host's resistance to disease. There are several kinds of fungi belonging to Agaricales, mainly distributed in the below-ground part, which can be eaten and some can also be used as TCM. In addition, some fungi belong to growth-promoting fungi, whose metabolites can promote the growth and development of plants. And these growth-promoting fungi mainly distributed in four parts of S. sphenanthera (Table 2 and S4).

In view of the roles played by endophytes in plant growth, our findings contribute to the expansion of endophyte use in the production of S. sphenanthera and its important metabolites. The information on the differences of endophytes in the above-ground and below-ground parts can serve as basis for the selection of functional microorganisms.

Conclusion

A variety of microorganisms in different tissues and rhizosphere soil of S. sphenanthera are identified in this study. The results show that the species of microorganisms are rich and the distribution is uniform. The microorganisms in S. sphenanthera has a certain distribution rule: (1) the relationship between below-ground parts (rhizosphere soil and roots) is relatively close, and that between above-ground parts (stem, leaf, and fruit) is relatively close; (2) rhizosphere soil and four parts of S. sphenanthera have the common genus and own specific strains; (3) the diversity index of microorganisms in rhizosphere soil and four parts of S. sphenanthera is different. The results show that endophytes in different tissues have certain connections and certain specificity with microorganisms in rhizosphere soil of S. sphenanthera. The diversity and richness of microorganism structure of S. sphenanthera are affected by rhizosphere soil and different parts, and the migration of the microbe existed in different parts. Later, localization technology can be used to assist the further explore the movement mechanism of endophytes in S. sphenanthera. Most of the species in this study are unnamed endophytes at the species level, and secondary metabolite resources have not been fully developed and reported, which lays a foundation for our subsequent research on endophytic secondary metabolite analysis and product development.

Declarations

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xiao-Lu Qin, Han Pu, and Xi-Lin Fang. The first draft of the manuscript was written by Xiao-Rui Wang and all authors commented on previous versions of
Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:

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Figures
Figure 1

The cluster dendrogram of identified bacteria (A) and fungi (B) on the basis of the < 0.1% OTUs.

Figure 2

Venn diagram analysis on OTUs. The sequence with OTUs greater than 100 is selected to draw the Venn diagram. A: Bacteria. B: Fungal.
Figure 3

Stack diagram at the level of phylum of sample. A: Bacteria. B: Fungal
Figure 4

Cluster heat map of relative abundance at the level of bacterial species of sample
Figure 5

Cluster heat map of relative abundance at the level of fungal species of sample
Figure 6

Rarefaction curves of rhizosphere soil and different parts of *S. sphenanthera*. A: Bacteria. B: Fungal. R. soil: Rhizosphere soil
Figure 7

The principal coordinate analysis of rhizosphere soil and different parts of *S. sphenanthera*. A: Bacteria. B: Fungal. a-e and A-E: Rhizosphere soil, Root, Stem, Leaf, and Fruit

Supplementary Files

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