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Diversity and functional analysis of epiphytic and endophytic bacteria in three different parts of Brasenia schreberi

Wen Luo
Nanchang Normal University
Pei Liu
Nanchang Normal University
Yue Qiu
Nanchang Normal University
Mingxing Li
Nanchang Normal University
Yizhong Huang
hyzjxlab@163.com

Nanchang Normal University

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Abstract

Brasenia schreberi J.F. Gmel (*B. schreberi*)is a traditional medicinal and edible plant in China, with its mucilage exhibiting antibacterial, anti-inflammatory, and antioxidant properties. This study aimed to investigate the community structure and functional roles of epiphytic and endophytic microorganisms in mature leaves, young leaves, and petioles of *B. schreberi*, as well as their influence on mucilage biosynthesis. A total of 560 epiphytic and 118 endophytic bacterial ASVs were obtained from *B. schreberi* samples. There were significant differences in OTU counts, ACE, Chao1, Simpson, and Shannon indices between mature leaves/petioles and young leaves of *B. schreberi* ($P < 0.05$), with the values of mature leaves and petioles being significantly higher than those of young leaves. *Proteobacteria* and *Bacteroidota*dominated the epiphytic bacterial communities across all tissues, while *Proteobacteria*and *Firmicutes* were dominant in the endophytic bacteria. The KEGG functional classification of epiphytic bacteria in mature and young leaves of *B. schreberi* showed significant differences in metabolic pathways, including amino acid metabolism, metabolism of terpenoids and polyketides, and energy metabolism. Our results elucidate the composition and diversity of bacterial communities on three parts of *B. schreberi* and preliminarily demonstrate the critical role of epiphytic bacteria in the biosynthesis of its mucilage.

1. Introduction

Brasenia schreberi J.F. Gmel (*B. schreberi*), also known as water shield, water mallow, lake vegetable, etc., is a perennial aquatic herb belonging to the family *Cabombaceae* [1, 2]. It prefers warm and humid aquatic environments and mainly grows in ponds, marshes and lakes, especially in areas with water depths of 20 to 60 cm. The species has been cultivated as an aquatic vegetable in East Asia for over 3,000 years, but its natural populations have declined significantly in recent decades and have become endangered in several regional countries [3, 4]. In September 2021, the new edition of the List of National Key Protected Wild Plants was published, and *B. schreberi* was listed as a Grade II nationally protected wild plant [5]. *B. schreberi* is a traditional medicinal and edible plant in China, and its young leaves and petioles are covered with a layer of transparent mucilage [6]. This mucilage has been found to possess anti-algal and antibacterial properties, functioning as a herbivore defense mechanism, protecting tender buds from abrasion, and acting as an excellent bio-lubricant [7]. Additionally, the amount of mucilage not only serves as an indicator for assessing the growth status of *B. schreberi* plants but also significantly influences their growth [4, 8]. The main component of *B. schreberi* mucilage is polysaccharides, which also contain many bioactive constituents such as polyphenols, proteins, terpenes and polyketones [9, 10]. *B. schreberi* polysaccharides have been shown to reduce plasma cholesterol levels [11], and exhibit bioactive properties such as antidiabetic [12, 13], anti-inflammatory [14, 15], and antioxidant properties [16, 17], indicating significant therapeutic potential. It is worth mentioning that *B. schreberi* plays an important role in the field of antioxidant activity [16]. Its polysaccharide and conjugate have been used in the food and pharmaceutical industries, and have recently gained considerable attention due to their biological properties.

Due to its high economic value and health benefits, *B. schreberi* has attracted wide attention from researchers in recent years. Previous studies have shown that the rubber production efficiency of *B. schreberi* is greatly influenced by its own development stage and environmental factors. For example, Shi et al. found that the growth and mucilage accumulation of *B. schreberi* depended on clean water quality and nutrient-rich sediment [18]. Furthermore, key factors in aquatic environments, such as the permanganate index, total nitrogen, conductivity, dissolved oxygen in water bodies, as well as sediment organic carbon and total nitrogen also significantly impact mucilage accumulation in *B. schreberi* plants [4, 19]. Regarding the origin of *B. schreberi* mucilage, two mainstream theories are widely accepted: host plant production and microbial sources. Substantial research has confirmed that the plant's structures and metabolism significantly influence mucilage secretion. Lü et al. observed glandular cells with secretory functions on the plant surface, noting that their distribution positively correlates with mucilage abundance. Chemical analysis confirmed that the mucilage produced by these glandular cells shared primary components with *B. schreberi* mucilage, leading to the conclusion that mucilage was an endogenous metabolic product of the plant [20]. Moreover, multi-omics analysis, including genomics, transcriptomics, proteomics and metabolomics, has been used to study the mechanisms of mucilage production or disappearance [1, 7, 8, 10]. However, research on the diversity of phyllosphere microbiota associated with *B. schreberi* and their specific impact on mucilage production capacity remains inconclusive. Many scholars contend that these co-colonizing microorganisms exert direct or indirect influences on mucilage biosynthesis. Studies indicate that epiphytic microorganisms may exhibit synergistic effects in mucilage production [21, 22]. Current investigations into microbe-mucilage interactions are still in the preliminary exploration phase, necessitating deeper research to elucidate detailed underlying mechanisms.

Jiangxi Province has established a wild germplasm conservation zone at the native habitat of *B. schreberi*, implementing artificial population restoration, restricting transplantation and harvesting, while promoting sustainable cultivation and expansion [23]. As the edible and medicinal values of *B. schreberi* gain increasing recognition, its industrialized cultivation has progressively matured through refined agronomic techniques. However, current research remains fragmented across domestic and international studies, primarily focusing on cultivation systems, mucilage purification, morphological anatomy, and culinary applications, with no integrated framework established. Crucially, investigations into the diversity of epiphytic and endophytic microbiota remain notably scarce [22]. Therefore, this study employed *B. schreberi* from Longhu Mountain (Jiangxi) as experimental material. Utilizing Illumina MiSeq high-throughput sequencing, we analyze the V3–V4 hypervariable regions of bacterial 16S rRNA genes to: (1) Decipher taxonomic composition and distribution patterns of epiphytic bacteria; (2) Elucidate structural characteristics of the microbial community; (3) Investigate potential correlations between epiphytic microbiota and biosynthesis of key functional metabolites. The findings will provide theoretical groundwork for applied research on *B. schreberi* polysaccharides and related bioproduct development.

2. Materials and Methods

2.1 Materials

Plant samples: *B. schreberi* samples from Jiangxi Chunyuan Agricultural Co., Ltd in Longhu Mountain in YingTan City, Jiangxi Province, were selected based on the following criteria: moderately-sized leaves fully coated with translucent mucilage, disease-free status, and with attached petioles, as described in our previous study [10].

Reagents and consumables:

DNA Extraction: TIANamp Bacteria DNA Kit (TIANGEN Biotech, Beijing Co., Ltd.)

PCR Product Purification: GeneJET Gel Extraction Kit (Thermo Scientific™)

Library Preparation: TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina Inc.)

2.2 Pretreatment of *B. schreberi* samples

Mature leaves, petioles, and tender leaves samples of *B. schreberi* were collected and immediately transported back to the laboratory using dry ice. The samples were first treated with sterile water to remove surface mud and other impurities. Subsequently, they were cleaned in an ultrasonic cleaner at 50 W power for 1 minute (10 seconds of cleaning followed by 10 seconds of interval). The washing solution collected during this process was used as the raw material for epiphytic microorganisms. After cleaning, the leaves were rinsed three times with sterile water. The samples were then immersed in 10% hydrogen peroxide for 40 minutes, followed by washing three times with sterile water. Subsequently, the samples were treated with 70% alcohol for 1 minute and rinsed three times with sterile water. The cleaned leaf samples were then placed on filter paper to dry. After drying, the samples were wrapped in aluminum foil, cryopreserved in liquid nitrogen for 30 seconds, and then ground in a pre-chilled mortar while adding liquid nitrogen. The resulting powder was used as the raw material for endophytic microorganisms.

The samples were divided into six groups, each with three replicates:

Leaves 1, 2, 3: Epiphytic bacteria from mature leaves (YP1-3)

Leaves 4, 5, 6: Endophytic bacteria from mature leaves (YP4-6)

Leaves 11, 12, 13: Epiphytic bacteria from tender leaves (YP11-13)

Leaves 14, 15, 16: Endophytic bacteria from tender leaves (YP14-16)

Leaves 21, 22, 23: Epiphytic bacteria from petioles (YP21-23)

Leaves 24, 25, 26: Endophytic bacteria from petioles (YP24-26)

2.3 Extraction and sequencing of total genomic DNA of *B. schreberi* samples

The DNA extraction steps for all samples were performed following the instructions of the TIANamp Bacteria DNA Kit for bacterial genomic DNA extraction. The quality of the extracted genomic DNA was detected using 1% (w/v) agarose gel electrophoresis, and the concentration and purity of the DNA were measured using the NanoDropTM 200. After quality control, the DNA samples were amplified using primers 319F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') to target the V3-V4 region of the bacterial 16S rRNA gene. The PCR reaction system (50 μL) was as follows: 2 μL of DNA template (20 ng/μL), 1 μL of forward primer (5 μmol/L), 1 μL of reverse primer (5 μmol/L), 25 μL of 2×Taq PCR Master Mix, and ddH2O to a final volume of 50 μL. The PCR reaction conditions were: 95℃ for 5 min; 95℃ for 30 s, 55℃ for 45 s, 72℃ for 1 min, repeated for 30 cycles; and a final extension at 72℃ for 7 min. The PCR products were purified, quantified, and normalized to construct the sequencing library. After library quality control, the qualified libraries were sequenced using the Illumina Novaseq 6000 platform with paired-end sequencing (PE250). The sequencing of bacterial genomic DNA from the *B. schreberi* samples was completed by Beijing Novogene Bioinformatics Technology Co., Ltd.

2.4 Processing and analysis of sequencing data

First, the raw sequencing data were subjected to quality control using Trimmomatic (v0.33) [24]. Subsequently, Cutadapt (v1.9.1) was used to identify and remove primer sequences, resulting in clean reads that did not contain primer sequences [25]. Based on the clean reads, the QIIME2 (v2020.6) platform was employed to perform denoising using the divisive amplicon denoising algorithm 2 (DADA2), and sequences with a frequency count less than 5 were filtered out [26, 27]. This process yielded the final amplicon sequence variants (ASVs) [28], which were analogous to operational taxonomic units (OTU) representative sequences. For the obtained ASVs, two main analyses were conducted: (1) taxonomic annotation and classification of the representative sequences of each ASV to obtain species information and abundance distribution at the species level; and (2) using R software (v1.4.17), analyses of ASV abundance, alpha diversity (α diversity), and beta diversity (β diversity) were performed. Additionally, dimensionality reduction analysis and sample clustering trees were conducted using methods such as principal component analysis (PCA), and principal co-ordinates analysis (PCoA) to investigate differences in community structures among different samples. To further explore differences in community structures among different groups, statistical methods such as *t-test* and LEfSe were used to perform significance tests on the species composition and community structure of grouped samples. Finally, PICRUSt2 software was used to predict the functional profiles of bacterial communities in the sample [29].

2.5 Statistical analysis

The data was presented as mean ± standard error. All statistical analyses were conducted using R software (version 1.4.17), with multigroup comparisons assessed via one-way ANOVA.

3. Results

3.1 Sequence assembly and quality control

As shown in Table 1, a total of 1,549,823 epiphytic and endophytic bacterial raw sequences were obtained from 18 *B. schreberi* samples. Following quality control and assembly, 1,385,408 high-quality sequences of epiphytic and endophytic bacteria were retained. After removing the chimeras, a total of 1253,339 sequences were finally obtained for subsequent analysis.

Table 1
Basic information of high-throughput sequencing of bacteria 16S rRNA gene epiphytic and endophytic with *B. schreberi*

Group	Sample ID	Raw Reads	Clean Reads	Denoised Reads	Merged Reads	Non-chimeric Reads
CYF	YP1	87039	81493	81151	78857	70903
	YP2	83883	79384	79038	75841	66653
	YP3	85024	78992	78483	73406	59844
CYN	YP4	84382	74781	74728	73943	73298
	YP5	86212	76394	76300	74693	73841
	YP6	88168	71852	71738	70547	69933
YYF	YP11	90879	83285	83008	79466	68966
	YP12	91196	85345	85017	81383	71129
	YP13	86934	78559	78117	73367	62861
YYN	YP14	87415	69300	69260	68365	67609
	YP15	87832	80296	80269	79598	78648
	YP16	84661	76783	76734	75800	74088
YBF	YP21	85601	79497	79146	77103	72442
	YP22	89069	83673	83291	80207	73138
	YP23	84484	74851	74558	69914	65112
YBN	YP24	89360	71499	71389	69926	68955
	YP25	80285	72102	72064	71323	70598
	YP26	77399	67322	67241	66283	65321

3.2 Gene sequencing depth analysis

The rarefaction curve analysis showed that when the bacterial sequencing depth of *B. schreberi* samples reached approximately 10000 reads, the rarefaction curve plateaued (**Fig. S1**). With the increase of sequencing depth, the number of observed bacteria no longer increases, indicating that the sequencing depth was sufficient and the sample sequencing results could be used for all subsequent analyses.

3.3 Community structure analysis of endophytic and epiphytic bacteria in three tissues of *B. schreberi*

3.3.1 ASV distribution analysis

There are significant differences in the number of ASVs in different parts and developmental stages of *B. schreberi* (Fig. 1). A total of 578 epiphytic and endophytic bacterial ASVs were identified in 18 *B. schreberi* samples. The number of epiphytic bacterial ASVs in the three tissues of *B. schreberi* was significantly higher than that of endophytic bacteria (Fig. 1A). The Venn diagram showed that a total of 560 epiphytic bacterial ASVs and 118 endophytic bacterial ASVs were detected in the *B. schreberi* samples (Fig. 1B & 1C). There were 396 shared epiphytic bacterial ASVs and 40 shared endophytic bacterial ASVs in all groups, which may represent the core taxa in the mature leaves, young leaves, and petioles of *B. schreberi.* As shown in Fig. 1B, the number of epiphytic bacterial ASVs detected in the mature leaves, young leaves, and petioles of *B. schreberi* was 522, 431, and 526, respectively. Among these, 5, 11, and 21 ASVs were unique to each tissue, accounting for 0.96%, 2.55%, and 3.99% of the corresponding total ASVs, respectively. The number of epiphytic bacterial ASVs in the mature leaves and petioles of *B. schreberi* was the largest. This may be attributed to the mature leaves floating on the water surface, while the petioles are submerged. Submerged petioles had closer contact with the aquatic environment, likely leading to a higher diversity of epiphytic bacteria shared between the plant and the surrounding water. As shown in Fig. 1C, the number of endophytic bacterial ASVs detected in the mature leaves, young leaves, and petioles of *B. schreberi* was 84, 68, and 73, respectively. Among these, 23, 15, and 13 ASVs were unique to each tissue, representing 26.19%, 22.06%, and 17.81% of the total ASVs, respectively. The phylogenetic diversity of endophytic bacterial ASVs in mature leaves was the largest among the three tissues of *B. schreberi*, indicating that the longer the developmental duration, the richer the endophytic bacterial community. The above results showed that the diversity of epiphytic and endophytic bacteria in *B. schreberi* leaves was closely related to their developmental stage and the microenvironment of each tissue.

3.3.2 Changes in alpha diversity of epiphytic and endophytic bacteria in *B. schreberi* leaves

The Shannon diversity index rarefaction curve showed that as sequencing depth increased, the curve plateaued (**Fig. S2**). With the increase of sequencing depth, the Shannon diversity index basically remained unchanged, indicating that the amount of sequencing data was sufficient, and new taxa would not increase with the increase of sequencing volume. In addition, the Shannon index of epiphytic bacteria in the three tissues of *B. schreberi* was significantly greater than that of endophytic bacteria, indicating that the species of epiphytic bacteria were more diverse and abundant.

The alpha diversity indices of bacterial communities in *B. schreberi* leaf samples were summarized in Table 2. Overall, the epiphytic bacterial community exhibited higher richness and diversity compared to the endophytic community. Significant differences were observed in OTUs, ACE, Chao1, Simpson, Shannon, and other indices between the mature leaves/petioles and the young leaves ($P < 0.05$), with higher values in the former, which indicates greater richness of epiphytic bacteria in mature tissues. Among the endophytic bacteria, the Simpson and Shannon indices of the bacterial community in mature leaves were significantly higher than those of petioles and young leaves ($P < 0.05$), and there was no significant difference in other indicators. The diversity of the endophytic bacterial community in mature leaves was higher than that in petioles and young leaves. These results further proved that the diversity of epiphytic and endophytic bacteria in *B. schreberi* leaves was related to the tissues and development stage.

Table 2
Alpha-diversity index statistics of epiphytic and endophytic bacteria from three different parts of *B. schreberi*

Microbiome	Group	OTUs	ACE	Chao1	Simpson	Shannon	PD_whole_tree
	CYF	453.33 ± 11.02a	453.52 ± 10.98a	453.40 ± 10.95a	0.99 ± 0.00a	7.22 ± 0.05a	22.89 ± 0.43a
Epiphytes	YYF	341.67 ± 21.55b	341.76 ± 21.58b	341.67 ± 21.55b	0.96 ± 0.01b	6.38 ± 0.09b	19.53 ± 0.15b
	YBF	466.67 ± 13.58a	467.04 ± 13.46a	467.83 ± 13.34a	0.99 ± 0.00a	7.37 ± 0.08a	23.53 ± 0.80a
	CYN	53.33 ± 12.9a	54.33 ± 12.09a	55.00 ± 12.29a	0.58 ± 0.18a	2.71 ± 0.89a	8.93 ± 1.86a
Endophytes	YYN	41.00 ± 5.29a	41.32 ± 5.26a	41.33 ± 4.93a	0.17 ± 0.04b	0.83 ± 0.16b	7.33 ± 1.15a
	YBN	40.33 ± 7.51a	40.49 ± 7.37a	40.33 ± 7.51a	0.22 ± 0.06b	1.08 ± 0.28b	7.45 ± 1.47a

Note: Values followed by different lowercase letters in a column are significantly different ($P < 0.05$).

3.3.3 β-diversity analysis of c epiphytic and endophytic bacteria in *B. schreberi* leaves

To evaluate the similarity and difference in their community composition, Principal Component Analysis (PCA) and Principal Coordinates Analysis (PCoA) were applied to assess the beta-diversity of epiphytic and endophytic bacteria in *B. schreberi* leaves. In addition, the Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test whether there were significant differences in beta-diversity among different groups. The results of PCA showed that the first two principal components (PC1 and PC2) explained 62.45% and 22.38% of the total variation of epiphytic and endophytic bacteria communities, respectively. At the OTU level, clustering patterns among epiphytic and endophytic bacterial communities from mature leaves, young leaves, and petioles were indistinct, suggesting no clear separation of community composition similarity (Fig. 2A). PCoA further demonstrated that the three groups of samples of epiphytic bacteria from mature leaves, young leaves, and petioles were close and concentrated, indicating high compositional similarity, while the community composition of endophytic bacteria was quite different (Fig. 2B).

PERMANOVA analysis of the epiphytic and endophytic bacteria in *B. schreberi* leaves was shown in Fig. 2C. The results indicated that the differences in the bacterial community structures of different tissues of *B. schreberi* leaves were explanatory ($R^2 = 0.758$), and there were significant differences in the community structures between the epiphytic bacteria and endophytic bacteria in the leaves, which indicated that different ecological niches had a significant impact on the community composition of the epiphytic and endophytic bacteria in *B. schreberi* leaves.

3.3.4 Taxonomic comparison of the epiphytic and endophytic bacterial community in B. schreberi leaves at the phylum and genus levels

According to the species annotation results, the top 10 most abundant taxa at the phylum and genus levels were selected from each *B. schreberi* sample, and a relative abundance bar chart was generated. These results illustrated the dominant taxa within epiphytic and endophytic bacterial communities across mature leaves, young leaves, and petioles at distinct taxonomic resolutions. The taxonomic richness (number of taxa) at each classification level is summarized in Table 3.

Table 3
Statistics of epiphytic and endophytic bacterial species in three different parts of *B. schreberi*

Group	Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
CYF	YP1	1	14	18	55	94	162	186
	YP2	1	14	18	56	97	168	192
	YP3	1	14	19	57	102	173	197
CYN	YP4	1	9	11	18	25	28	28
	YP5	2	13	16	29	40	49	50
	YP6	2	10	13	24	34	45	46
YYF	YP11	1	14	17	49	80	142	159
	YP12	1	14	17	48	81	142	158
	YP13	1	14	18	48	79	137	153
YYN	YP14	1	9	12	19	29	33	34
	YP15	1	9	10	21	28	31	32
	YP16	2	9	12	24	34	40	41
YBF	YP21	1	14	19	53	93	159	184
	YP22	1	14	19	56	100	172	197
	YP23	1	14	19	56	99	173	198
YBN	YP24	2	10	13	23	32	38	38
	YP25	1	9	10	17	25	30	30
	YP26	1	10	12	22	26	29	29
	Total	2	17	23	64	117	199	226

At the phylum classification level, epiphytic and endophytic bacteria in *B. schreberi* leaves comprised 17 phyla (Table 3, Fig. 3A). The dominant phyla of epiphytic bacteria in the mature leaves, young leaves, and petioles of *B. schreberi* were *Proteobacteria* and *Bacteroidota*. Endophytic bacterial communities in *B. schreberi* shared the dominant phyla (e.g., *Proteobacteria*, *Firmicutes*) with aquatic environmental microbes, suggesting host-environment coadaptation[29], and their proportions were 89.25%, 93.34%, 75.18% and 5.28%, 1.99%, 4.50%, respectively. Minor phyla included *Firmicutes* and *Actinobacteriota* (each 1%) in mature leaves, and *Verrucomicrobiota* (1%) in young leaves. Notably, epiphytic bacteria in petioles exhibited higher diversity, with *unclassified bacteria* (9.82%), *Verrucomicrobiota* (4.33%), *Actinobacteriota* (1.86%), and *Myxomycota* (1.04%). The above results indicated that epiphytic bacterial phyla on *B. schreberi* petioles exhibit higher diversity and abundance. For endophytic bacteria, *Proteobacteria* and *Firmicutes* were dominant across tissues: *Proteobacteria* accounted for 88.05%, 96.90%, and 95.22% in mature leaves, young leaves, and petioles, respectively, while *Firmicutes* represented 4.56%, 1.33%, and 1.45%. In addition, the endophytic bacteria in the petioles also contained 1.67% *Actinobacteriota*. Mature leaves exhibited the highest phylum richness, with *Actinobacteriota* (2.38%), *Halobacterota* (1.74%), *Chloroflexi* (1.40%), and *unclassified bacteria* (0.94%). These findings revealed that endophytic bacterial phyla in mature leaves were both more diverse and abundant compared to other tissues.

From the genus level, the epiphytic bacteria in *B. schreberi* leaves belonged to 199 genera (Table 3, Fig. 3A). The dominant genera of epiphytic bacteria in the mature leaves, young leaves, and petioles of *B. schreberi* were *unclassified_Comamonadaceae*, *Aquitalea*, *Curvibacter*, *unclassified Sphingomonadaceae, Novosphingobium*, *unclassified Acetobacteraceae*, and *Duganella*. The proportions of their abundances were 17.08%, 14.78%, 15.66%; 3.63%, 18.40%, 1.58%; 7.69%, 6.17%, 4.57%; 6.00%, 1.94%, 2.45%; 4.80%, 3.02%, 3.11%; 3.55%, 2.16%, 3.45% and 4.49%, 1.58%, 3.20%. Notably, the proportion of *Aquitalea* in young leaves was significantly higher than that in the other two groups, suggesting its potential role in mucilage formation. In addition, *unclassified_Methylophilaceae* was prominent in mature leaves (3.77%) and petioles (3.87%), while *unclassified_Bacteria* was unique to petioles. For endophytic bacteria, the dominant genera in the mature leaves of *B. schreberi* were only *unclassified_Comamonadaceae* and *unclassified_Sphingomonadaceae*, with abundance proportions of 4.45% and 1.42%, respectively. The abundance proportions of endophytic bacterial genera in young leaves were all less than 1%. In petioles, only u*nclassified_Comamonadaceae* (4.45%) had an abundance proportion above 1%. These results highlighted that epiphytic bacterial communities exhibited substantially higher genus richness compared to endophytic communities. The limited enrichment of specific endophytic genera suggested that epiphytic taxa might play a more critical role in *B. schreberi* development.

2.3.5 Analysis of differentially expressed species

LEfSe analysis ($LDA > 4$, $P < 0.05$) identified representative taxa within epiphytic and endophytic bacterial communities across three tissues of *B. schreberi*, and the results revealed distinct community structures from phylum to species level, with 81 significantly differentiated taxa (Table 4, Fig. 4). For epiphytic bacteria, 28, 17, and 17 taxa were enriched in the mature leaves, young leaves, and petioles of *B. schreberi*, respectively. The significantly enriched epiphytic bacteria in the mature leaves of *B. schreberi* were 4 orders, namely *Acetobacterales*, *Burkholderiales*, *Cytophagales*, and *Sphingomonadales*; 6 families, namely *Acetobacteraceae*, *Comamonadaceae*, *Oxalobacteraceae*, *Rhodocyclaceae*, *Spirosomaceae*, and *Sphingomonadaceae*; 9 genera, including

Aquabacterium, *Curvibacter, Duganella*, *Emticicia*, *Novosphingobium*, *unclassified_Acetobacteraceae*, *unclassified_Sphingomonadaceae*, *unclassified_Comamonadaceae*, and *unclassified_ Rhodocyclaceae*, as well as 9 species. The epiphytic bacteria in the young leaves were significantly enriched in 3 orders, namely *Burkholderiales*, *Rhizobiales*, and *Rhodospirillales*; 5 families, namely *Chromobacteriaceae*, *Comamonadaceae*, *Magnetospirillaceae*, *Rhizobiaceae*, and *Rhodocyclaceae*; 5 genera, namely *Aquitalea*, *Allorhizobium/Neorhizobium/Pararhizobium/Rhizobium*, *Curvibacter*, *Uliginosibacterium*, and *unclassified_Rhizobiaceae*, as well as 5 species. The epiphytic bacteria on the petioles were significantly enriched in 4 orders, namely *Burkholderiales*, *Caulobacterales*, *Salinisphaerales*, and *unclassified_Bacteria*; 4 families, namely *Caulobacteraceae*, *Methylophilaceae*, *Solimonadaceae*, and *unclassified_Bacteria*; 3 genera, namely *Nevskia*, *unclassified_Bacteria*, and *unclassified_ Methylophilaceae*, as well as 3 species.

Table 4
Bacterial communities differences in three different parts of *B. schreberi* based on Linear Discriminant Analysis E

Groups	Phylum	Class	Order	Family	Genus	Specie
CYF	*Bacteroidota*	*Bacteroidia*	*Cytophagales*	*Spirosomaceae*	*Emticicia*	*uncult*
CYF	*Bacteroidota*	*Bacteroidia*	*Cytophagales*	*Spirosomaceae*		
CYF	*Bacteroidota*	*Bacteroidia*	*Cytophagales*	*Spirosomaceae*	*Emticicia*	
CYF	*Bacteroidota*	*Bacteroidia*	*Cytophagales*			
CYF	*Bacteroidota*	*Bacteroidia*				
CYF	*Bacteroidota*					
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*	*Acetobacteraceae*	*unclassified_Acetobacteraceae*	
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*	*Acetobacteraceae*		
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*			
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*	*Acetobacteraceae*	*unclassified_Acetobacteraceae*	*unclas*
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Novosphingobium*	*unclas*
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Novosphingobium*	
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*unclassified_Sphingomonadaceae*	
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*unclassified_Sphingomonadaceae*	*unclas*
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*			
CYF	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*		
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Aquabacterium*	
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Aquabacterium*	*unclas*
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Rhodocyclaceae*	*unclassified_Rhodocyclaceae*	
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Rhodocyclaceae*	*unclassified_Rhodocyclaceae*	*unclas*
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Curvibacter*	*Curvib*
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Duganella*	
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*	*Duganella*	*unclas*
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Curvibacter*	
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Oxalobacteraceae*		
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*unclassified_Comamonadaceae*	*unclas*
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*unclassified_Comamonadaceae*	
CYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*		
CYN	*Actinobacteriota*	*Actinobacteria*	*Kineosporiales*	*Kineosporiaceae*		
CYN	*Actinobacteriota*	*Actinobacteria*	*Kineosporiales*			
CYN	*Actinobacteriota*	*Actinobacteria*	*Kineosporiales*	*Kineosporiaceae*	*unclassified_Kineosporiaceae*	*unclas*
CYN	*Actinobacteriota*	*Actinobacteria*	*Kineosporiales*	*Kineosporiaceae*	*unclassified_Kineosporiaceae*	
CYN	*Actinobacteriota*	*Actinobacteria*				
CYN	*Actinobacteriota*					
CYN	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Allobaculum*	*Alloba*
CYN	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*		
CYN	*Firmicutes*	*Bacilli*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Allobaculum*	
CYN	*Firmicutes*	*Bacilli*	*Erysipelotrichales*			
CYN	*Firmicutes*	*Bacilli*				
CYN	*Firmicutes*					
CYN	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*		

Groups	Phylum	Class	Order	Family	Genus	Specie
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Allorhizobium_Neorhizobium_ Pararhizobium_Rhizobium*	*unclas _Parar*
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Allorhizobium_Neorhizobium_ Pararhizobium_Rhizobium*	
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*unclassified_Rhizobiaceae*	
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*unclassified_Rhizobiaceae*	*unclas*
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*		
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*			
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhodospirillales*	*Magnetospirillaceae*		
YYF	*Proteobacteria*	*Alphaproteobacteria*	*Rhodospirillales*			
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Rhodocyclaceae*	*Uliginosibacterium*	*unclas*
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Rhodocyclaceae*	*Uliginosibacterium*	
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	*Curvibacter*	*Curvib*
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Rhodocyclaceae*		
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Chromobacteriaceae*	*Aquitalea*	*Aquita*
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Chromobacteriaceae*	*Aquitalea*	
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Chromobacteriaceae*		
YYF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*			
YYF	*Proteobacteria*	*Gammaproteobacteria*				
YYN	*Proteobacteria*	*Alphaproteobacteria*	*Rickettsiales*	*Mitochondria*	*Brasenia_schreberi*	*unclas*
YYN	*Proteobacteria*	*Alphaproteobacteria*	*Rickettsiales*			
YYN	*Proteobacteria*	*Alphaproteobacteria*	*Rickettsiales*	*Mitochondria*		
YYN	*Proteobacteria*	*Alphaproteobacteria*	*Rickettsiales*	*Mitochondria*	*Brasenia_schreberi*	
YYN	*Proteobacteria*	*Alphaproteobacteria*				
YYN	*Proteobacteria*					
YBF	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*		
YBF	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*			
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Methylophilaceae*	*unclassified_Methylophilaceae*	
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Methylophilaceae*	*unclassified_Methylophilaceae*	*unclas*
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Burkholderiales*	*Methylophilaceae*		
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Salinisphaerales*			
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Salinisphaerales*	*Solimonadaceae*	*Nevskia*	*unclas*
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Salinisphaerales*	*Solimonadaceae*		
YBF	*Proteobacteria*	*Gammaproteobacteria*	*Salinisphaerales*	*Solimonadaceae*	*Nevskia*	
YBF	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	
YBF	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*		
YBF	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclas*
YBF	*unclassified_Bacteria*	*unclassified_Bacteria*	*unclassified_Bacteria*			
YBF	*unclassified_Bacteria*	*unclassified_Bacteria*				
YBF	*unclassified_Bacteria*					
YBF	*Verrucomicrobiota*	*Verrucomicrobiae*				
YBF	*Verrucomicrobiota*					

For endophytic bacteria, 13 and 6 taxa were significantly enriched in the mature leaves and young leaves of *B. schreberi*, respectively. In contrast, no significantly enriched taxa were detected in petioles (Table 4, Fig. 4). The endophytic bacteria in the mature leaves were significantly enriched in 3 orders, namely *Erysipelotrichales*, *Kineosporiales*, and *Rhizobiales*; 3 families, namely *Erysipelotrichaceae*, *Kineosporiaceae*, and *Xanthobacteraceae*; 2 genera, namely *Allobaculum* and *unclassified_Kineosporiaceae*, and 2 species. The endophytic bacteria in the young leaves were significantly enriched in *Rickettsiales*.

Based on tissue-specific differences and the phylogenetic tree (Fig. 4), the epiphytic bacteria in the mature leaves of *B. schreberi* had the largest number of significantly enriched taxa, followed by petioles, young leaves, endophytic bacteria in mature leaves, and young leaves. Notably, no taxa met the enrichment threshold (*LDA* > 4) in petiole endophytes. The results indicated that significant divergence in epiphytic and endophytic bacterial community structures occurred across mature leaves, young leaves, and the petiole of *B. schreberi*.

3.4 Functional prediction of epiphytic and endophytic bacteria in *B. schreberi*

The PICRUSt2 was used to perform functional annotation and relative abundance analysis of the epiphytic and endophytic bacteria in three parts of *B. schreberi*. The results showed that a total of 300 KEGG pathways were identified across all samples, with annotations at Levels 1, 2, and 3 comprising 6, 44, and 300 pathways, respectively (**Supplementary Table S1)**. At the KEGG level 1, metabolism-related pathways dominated, accounting for 76.28% (mature leaf epiphytes), 76.18% (young leaf epiphytes), 76.59% (petiole epiphytes), 76.87% (mature leaf endophytes), 76.45% (young leaf endophytes), and 77.37% (petiole endophytes), respectively. Environmental information processing and genetic information processing ranked second (Fig. 5A). At the KEGG level 2, the "Global and overview maps" pathway was the KEGG pathway with the highest abundance proportion (> 40%), followed by carbohydrate metabolism, amino acid metabolism, energy metabolism, membrane transport, metabolism of cofactors and vitamins, signal transduction, nucleotide metabolism, xenobiotic biodegradation and metabolism, and lipid metabolism (Fig. 5B). At the KEGG level 3, metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in diverse environments, and biosynthesis of antibiotics were dominant. The average relative abundance proportions of these four functions in the epiphytic bacteria of mature leaves, young leaves, petioles, endophytic bacteria of mature leaves, young leaves, and petioles were 32.23%, 32.33%, 32.71%, 32.79%, 32.64%, and 32.94%, respectively (Fig. 5C). PCA analysis was performed based on the abundance statistics of database functional annotations. The results showed that at the three KEGG levels, there were significant differences in the relative abundances of metabolic pathways of epiphytic and endophytic bacteria in different parts of *B. schreberi* (Fig. 5D-E). This result was consistent with the analysis trend of the bacterial community composition differences, indicating significant functional differences among the epiphytic and endophytic bacteria in different parts of *B. schreberi*.

Differential analysis of KEGG metabolic pathways revealed functional adaptations of bacterial communities in different parts of *B. schreberi* to environmental changes. Significant differences ($P < 0.05$) were observed in epiphytic bacteria between mature and young leaves for pathways including membrane transport, metabolism of amino acids/terpenoids/polyketides, energy metabolism, lipid metabolism, cofactor/vitamin metabolism, and replication/repair (Fig. 6, **Supplementary Table S2**). However, endophytic bacteria showed minimal functional divergence, with only the "bacterial infectious diseases" pathway differing significantly ($P = 0.031$) between mature and young leaves (**Supplementary Table S3**). The above results indicated that there were differences in the metabolic functions of epiphytic and endophytic bacteria in *B. schreberi* leaves at different development stages, especially between mature leaves and mucilage-rich young leaves. This difference was closely related to the growth and metabolic processes of *B. schreberi*.

4. Discussion

The mucilage of *B. schreberi* has multiple effects and it can also protect young buds from damage. The mechanism behind the mucilage formation in *B. schreberi* is primarily explained by two hypotheses. One suggests that the mucilaginous substance originates from the Golgi vesicles within the secretory glands of plants and then projects into the intercellular spaces of the organs, which is closely related to the plants themselves [14]. Ai et al. revealed the potential factors for mucilage disappearance in *B. schreberi* through proteomics analysis and found that mucilage accumulation was positively correlated with tryptophan metabolism, with high levels of indole-3-acetic acid (IAA) contributing to mucilage accumulation [8]. One of our previous multi-omics approaches combining metabolomics, transcriptomics and proteomics also identified the metabolite, gene and protein markers associated with the generation or disappearance of mucilage [10]. The other proposes that the mucilage is excreted by symbiotic bacteria residing in *B. schreberi*, specifically belonging to the genus *Acinetobacter* (Feng, Ning, et al., 2019). Xu et al. revealed that the mucilage polysaccharides of *B. schreberi* leaf surface contained abundant gum-producing bacteria (e.g., strain 5242). The polysaccharide composition of the fermentation products from these bacteria closely resembled that of *B. schreberi*'s mucilage polysaccharides, thus demonstrating an intimate relationship between the formation of *B. schreberi* polysaccharides and polysaccharide-producing bacteria [21]. Zhang et al. explored the endophytic bacterial resources of *B. schreberi* and found that the diversity of endophytic bacteria in the stems of *B. schreberi* was greater than that in other tissues, and each tissue had its specific bacterial genus [22]. Currently, the exact mechanism of the epiphytic and endophytic bacteria responsible for the mucilage production or disappearance in *B. schreberi* remains unclear.

In this study, the Illumina MiSeq high-throughput sequencing technology was used to sequence the V3-V4 region of the 16S rRNA gene of epiphytic and endophytic bacteria in the mature leaves, young leaves, and petioles of *B. schreberi*, and then to explore the community structure and functions of epiphytic and endophytic bacteria, aiming to reveal the changes in *B. schreberi* mucilage from the perspective of bacterial metabolism. This study found that mature leaves and petioles harbored significantly more ASVs than young leaves, with epiphytic bacteria showing greater richness than endophytic communities. This likely stems from prolonged developmental time and larger surface areas in mature tissues and petioles, enhancing microbial colonization and resource acquisition. Meanwhile, diversity index analysis revealed that the epiphytic bacterial communities exhibited higher diversity than the endophytic bacterial communities. Significant differences were observed in the OTU number, ACE, Chao1, Simpson, and Shannon indices between mature leaves/petioles and young leaves of *B. schreberi*, with mature leaves and petioles showing significantly higher values than young leaves ($P < 0.05$). This indicated that the epiphytic bacterial communities of mature leaves and petioles have greater richness and diversity. Among the endophytic bacteria, the Simpson and Shannon indices of the bacterial community in mature leaves were significantly higher than those in petioles and young leaves ($P < 0.05$), while there was no

significant difference in other indicators. These results highlighted tissue-specific variations in epiphytic and endophytic bacterial diversity, driven by distinct ecological niches, environmental exposures, and developmental demands in *B. schreberi*.

In addition, to further understand the community structure composition of epiphytic and endophytic bacteria in different tissues of *B. schreberi*, we analyzed the relative abundance and differential taxa of epiphytic and endophytic bacterial communities. Dominant bacterial phyla showed no significant differences between epiphytes and endophytes. At the phylum classification level, the types of epiphytic bacteria and endophytic bacteria were more diverse and abundant in the petioles and mature leaves of *B. schreberi*, respectively. The dominant phyla of epiphytic bacteria were *Proteobacteria* and *Bacteroidota*, with minor contributions from *Firmicutes*, *Actinobacteriota*, and *Verrucomicrobiota*. In terms of the endophytic bacterial community structure, the results of our study were consistent with those of Zhang et al. [22], and the dominant phyla of endophytic bacteria in *B. schreberi* were *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, etc. Bacteria within the phylum *Proteobacteria* are all Gram-negative, and most species are facultative or obligate anaerobes, exhibiting heterotrophic or chemolithoautotrophic metabolisms. Studies have indicated that a higher abundance of *Proteobacteria* correlates with increased levels of organic matter and nutrients (C, N) in their environment [30, 31], which may be related to the formation of mucilage in *B. schreberi*.

LEfSe analysis revealed that the significantly different epiphytic bacteria on the young leaves of *B. schreberi* were *Rhodospirillales*, *Rhizobiales*, and *Burkholderiales* under the phylum *Proteobacteria* (LDA > 4, $P < 0.05$). *Rhodospirillales* bacteria are typical facultative phototrophic bacteria containing bacteriochlorophyll a and rhodobactinal-like carotenoids, which perform non-oxygenic photosynthesis. Under anaerobic conditions, members of the order *Rhodospirillales* may produce polysaccharides by metabolizing by-products through photosynthesis [32]. *Rhizobium* and *Bradyrhizobium* are typical genera of *Rhizobiales*, and their biological nitrogen fixation process is often accompanied by the synthesis of capsular polysaccharides, which are used for interaction with plant roots and environmental adaptation [33]. The mucilage in the *B. schreberi* leaves is rich in polysaccharides and proteins. It is speculated that the synthesis of polysaccharides and mucilage in *B. schreberi* is closely related to the bacteria of the *Proteobacteria* phylum in the epiphytic bacteria of the leaves. The dominant genera of epiphytic bacteria in mature leaves, young leaves, and petioles of *B. schreberi* included *unclassified_Comamonadaceae*, *Aquitalea*, *Curvibacter*, *unclassified_Sphingomonadaceae*, *Novosphingobium*, *unclassified_Acetobacteraceae*, and *Duganella*. It is worth noting that the relative abundance of *Aquitalea* in young leaves is 5-fold and 11-fold higher than in mature levels and petioles, respectively. *Aquitalea* plays crucial roles in organic matter degradation, nitrogen cycling, bioremediation, plant-microbe interactions, and secondary metabolite synthesis, exerting broad impacts on ecosystems and biotechnological applications. In a study on *Aquitalea magnusonii H3* colonizing duckweed, Ishizawa et al. found that genes related to bacterial motility, lipopolysaccharide synthesis, and type IV pili were critical for host adaptation. These structures likely facilitate surface colonization by secreting adhesive polysaccharides [34]. Moreover, research by Xu et al. revealed that the mucilage polysaccharides of *B. schreberi* contained abundant exopolysaccharide-producing bacteria, such as the mucilage-producing strain 5242. The polysaccharide composition of the bacterial fermentation products closely resembled that of *B. schreberi* mucilage polysaccharides, demonstrating a strong correlation between *B. schreberi* polysaccharide formation and exopolysaccharide-producing bacteria [21]. Further studies are needed to identify additional functional bacteria associated with *B. schreberi* mucilage production.

To reveal the potential functions of epiphytic and endophytic bacteria in different tissues of *B. schreberi*, this study employed PICRUSt2 for functional prediction. At the KEGG level 1, metabolic pathways dominated the functional profile, followed by environmental information processing and genetic information processing. At the KEGG level 2, the "Global and overview maps" category dominated the functional profile, followed by carbohydrate metabolism, amino acid metabolism and energy metabolism. At the KEGG level 3, metabolic pathways and biosynthesis of secondary metabolites were dominant. These results suggest that the dominant functions of epiphytic and endophytic microbial communities on *B. schreberi* leaves mainly focus on anabolic metabolism of carbon and nitrogen. Further comparative analysis revealed significant differences in the abundance of KEGG functional categories between epiphytic bacteria on mature and young leaves of *B. schreberi* ($P < 0.05$). These divergences were prominent in metabolic pathways including membrane transport, amino acid metabolism, metabolism of terpenoids and polyketides, and energy metabolism. This suggests that these pathways may be functionally associated with mucilage production in *B. schreberi*. The mucilage of *B. schreberi* contains abundant polysaccharides, terpenoids, phenolic compounds, and polyketides. This compositional profile suggests that epiphytic bacteria on *B. schreberi* leaves participate in the biosynthesis of proteins, terpenoids, and polyketide metabolites within the mucilage.

5. Conclusion

This study suggested that epiphytic bacterial diversity in *B. schreberi* was correlated with its developmental stage and environment. Significant differences existed in bacterial community structure and function across the young leaves, mature leaves and petioles of *B. schreberi*, suggesting potential involvement of epiphytic bacteria in *B. schreberi* mucilage biosynthesis. This study provides preliminary insights into the mechanism of *B. schreberi* mucilage biosynthesis from a bacterial metabolic perspective. However, current research on epiphytic microorganisms associated with *B. schreberi* is still in its early stages. Future research can further explore the significantly enriched families and genera of epiphytic microorganisms in young leaves, identify core bacterial taxa driving *B. schreberi* polysaccharide metabolism, and validate functional links between microbial metabolism and mucilage biosynthesis via *in vitro* synthetic communities. Integrative analysis of metabolomics, transcriptomics, and proteomics can unravel cross-kingdom regulatory networks between *B. schreberi* and its microbiota, providing targets for synthetic biology applications. This approach may enable innovative applications of *B. schreberi* resources while informing conservation strategies for this threatened species.

Declarations

Data Availability Statement

Data will be made available on request.

Author Contributions

WL: Supervision, Resources, Writing – review & editing. **PL:** Investigation, data analysis, Writing –original draft. **YQ:** Investigation, data analysis. **ML:** Investigation, data analysis. **YH:** Methodology, Supervision, Resources, Writing – review & editing. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no competing interests.

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Figures

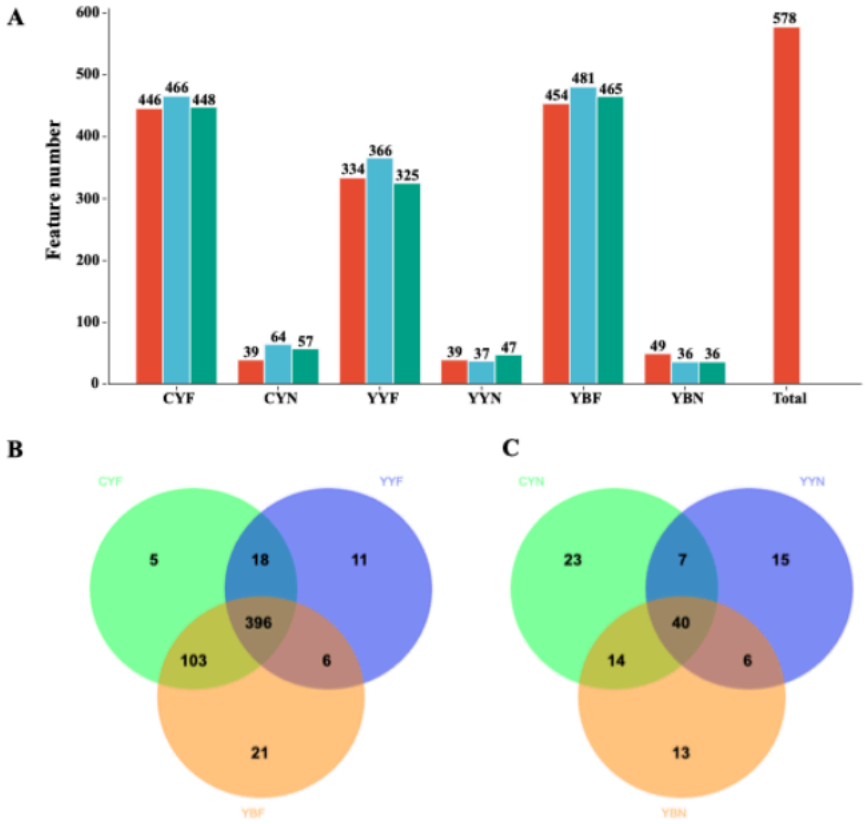


Figure 1

ASV of epiphytic and endophytic bacteria in different parts of *B. schreberi*. **(A)** Number distribution of ASV of epiphytic and endophytic bacteria in different parts of *B. schreberi*. **(B)** ASV Venn diagram analysis of epiphytic bacteria in different parts of *B. schreberi*. **(C)** ASV Venn diagram analysis of endophytic bacteria in different parts of *B. schreberi*.

Note: CYF: Mature leaf epiphytes. CYN: Mature leaf endophytes. YYF: Young leaf epiphytes. YYN: Young leaf endophytes. YBF: Petiole epiphytes. YBN: Petiole endophytes. The same as below.

Fig. 2

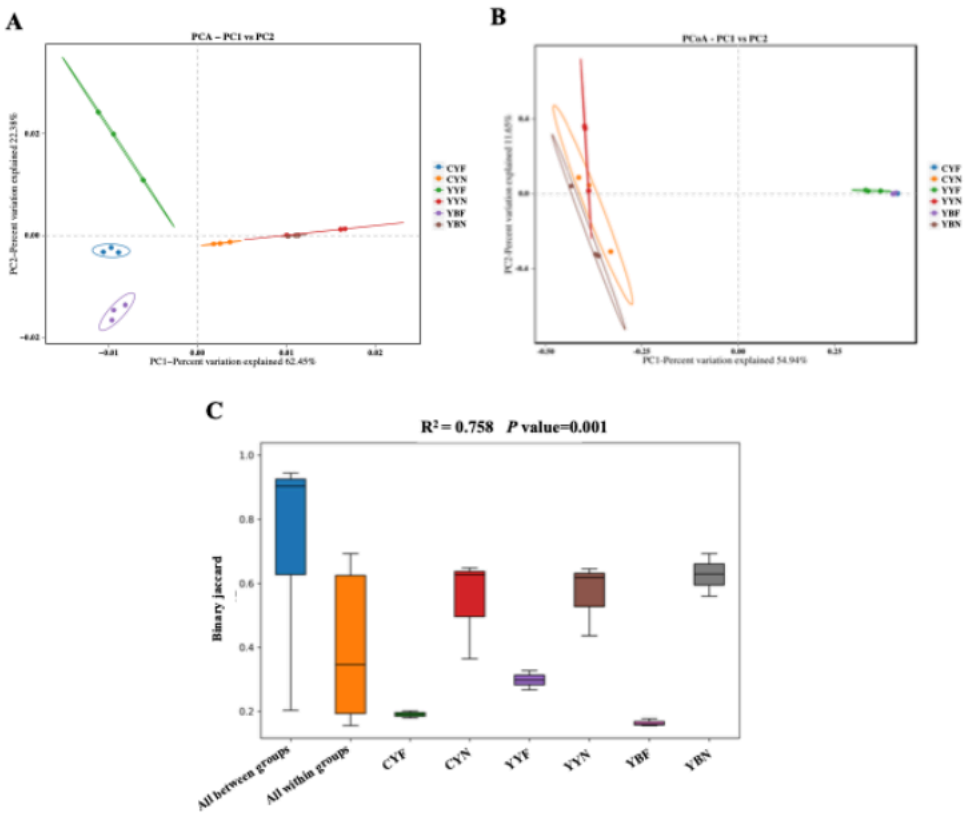


Figure 2

β-diversity analysis of epiphytic and endophyticbacteria in *B. schreberi*. **(A)** PCA analysis of epiphytic and endophytic bacteria in *B. schreberi*. **(B)** PCoA analysis of epiphytic and endophytic bacteria in *B. schreberi*. **(C)** Box plot of Permanova analysis of epiphytic and endophytic bacteria in *B. schreberi*.

Fig. 3

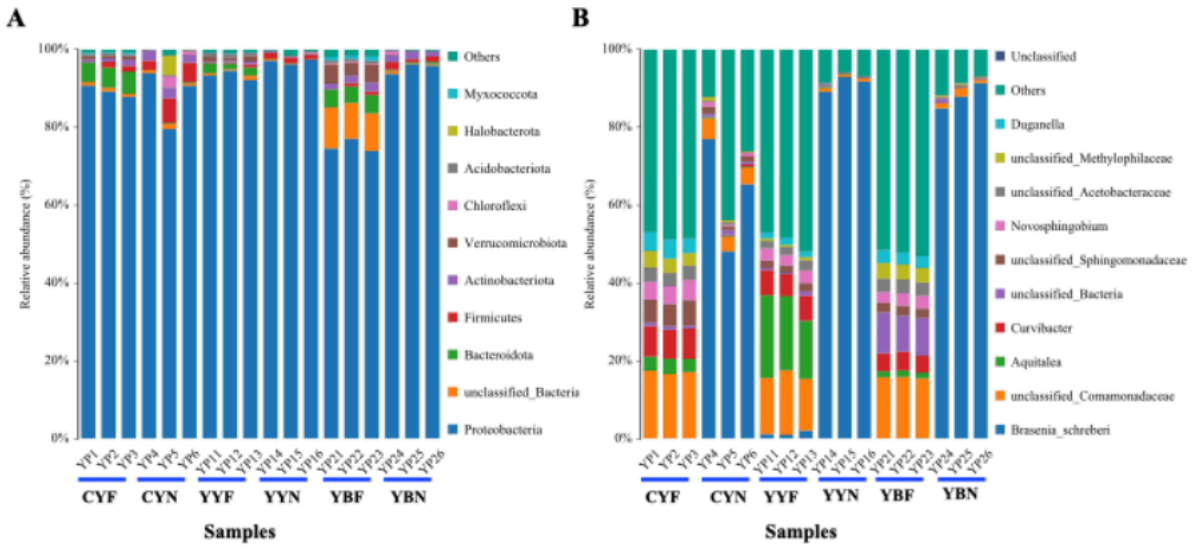


Figure 3

Relative abundance of epiphytic and endophytic bacteria in leaves of *B. schreberi* at phylum (A) and genus (B) levels.

Fig. 4

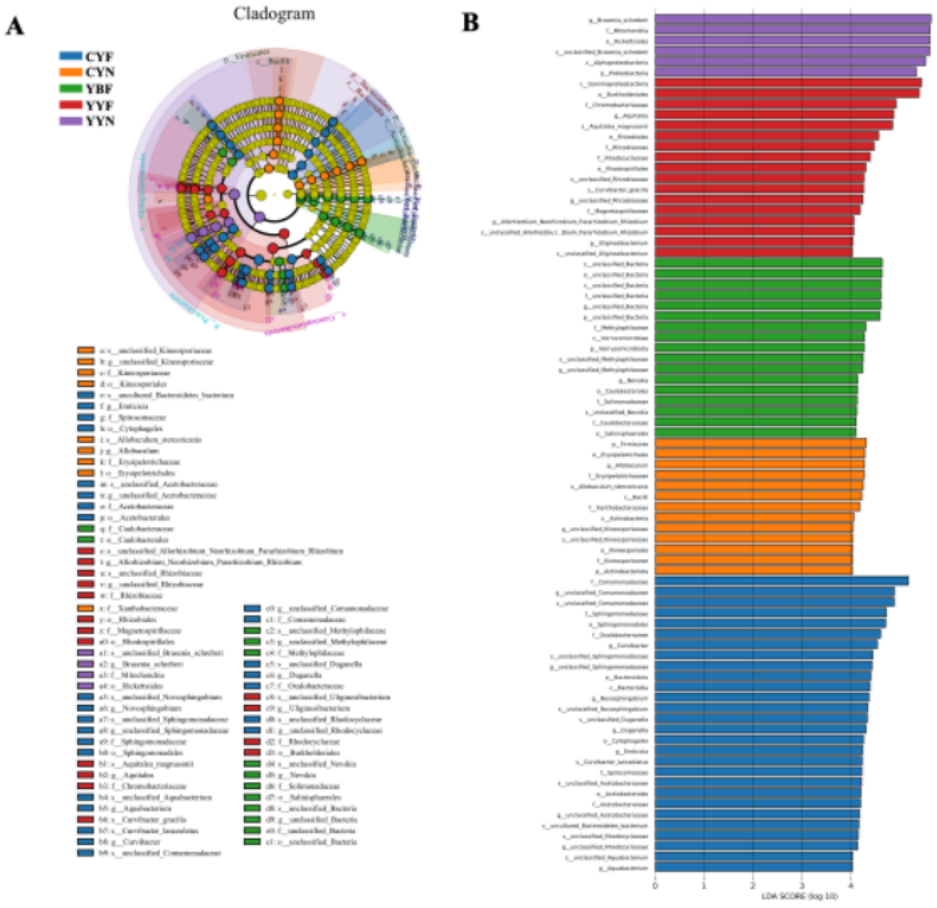


Figure 4

Representative core bacteria and their LDA scores of epiphytic and endophytic bacteria in different parts of *B. schreberi*. **(A)** The phylogenetic trees of the representative core bacteria in different parts of *B. schreberi*. **(B)** LDA scores of the representative core bacteria in different parts of *B. schreberi*.

Fig. 5

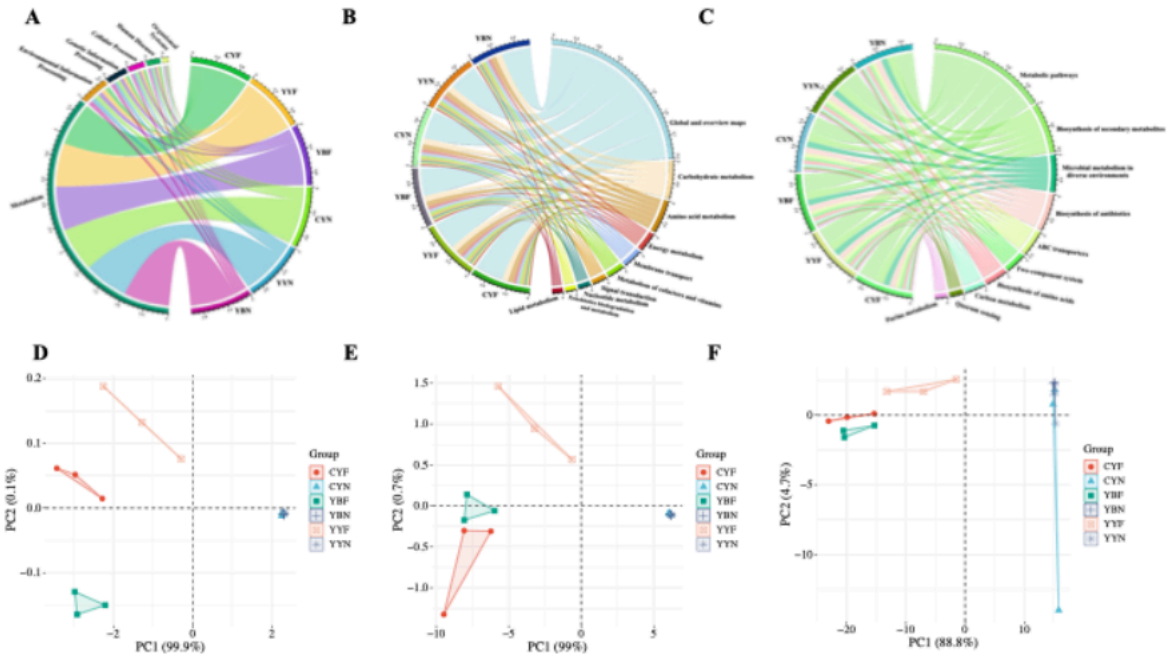


Figure 5

KEGG function analysis and principal component analysis of epiphytic and endophytic bacteria in different parts of *B. schreberi*. (A-C) KEGG function analysis in Level 1, Level 2 and Level 3, respectively. **(D-E)** PCA analysis of epiphytic and endophytic bacteria function Level 1, Level 2 and Level 3, respectively.

Fig. 6

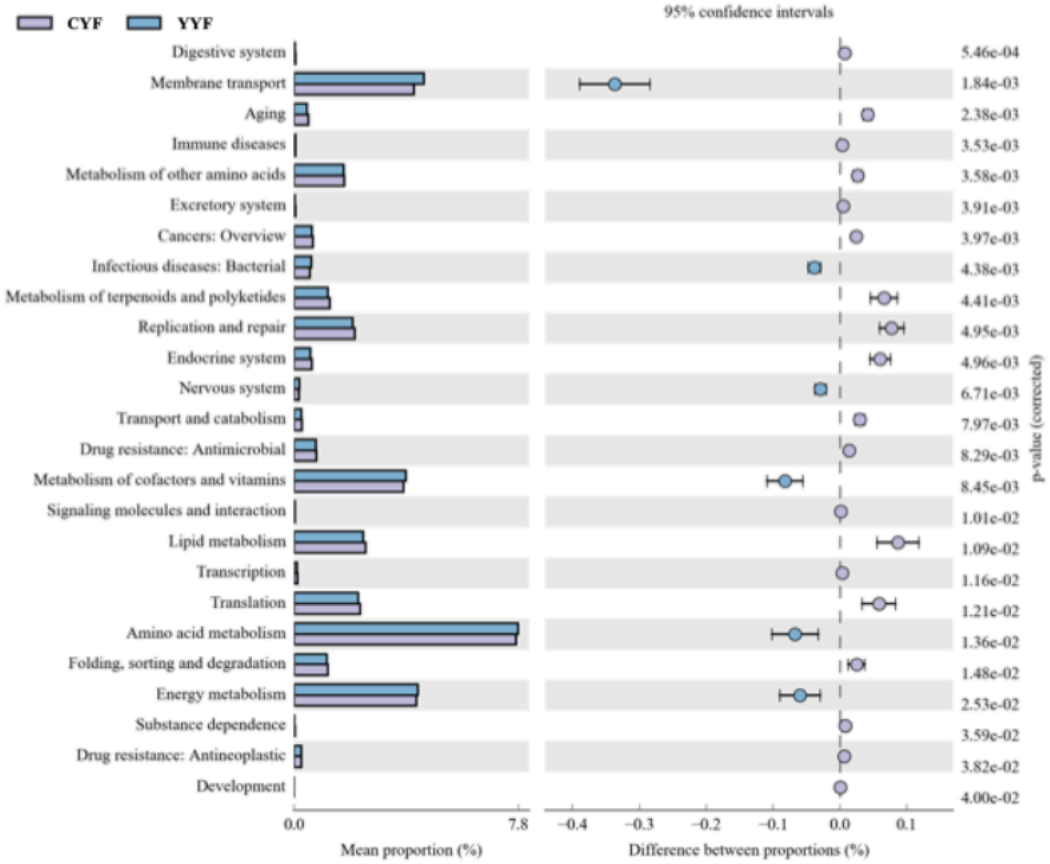


Figure 6

Comparison of KEGG functional classification differences of epiphytic bacteria between mature and young leaves of *B. schreberi*.

Supplementary Files

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- FigS1S2.docx