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Diversity and Function of Strawberry Endophytic Bacterial Communities Associated with Host Genotype and Niche

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Abstract

Strawberry (*Fragaria*×*ananassa*) is the most widely cultivated small berries in the world. They are not only delicious, juicy, and nutritious, but also have important economic value. However, current research on endophytic bacteria related to strawberry is limited. This work provides a comprehensive description of the composition and diversity of bacterial communities in three niches (root, stem, and leaf) of three strawberry cultivars (White Elves, Tokun, and Akihime). This study indicated that the diversity and composition of strawberry bacterial communities differ significantly between the belowground niche (roots) and aboveground compartments (stems and leaves). The bacterial diversity and richness varied between niches for all three cultivars; and it significantly decreased from root to stem to leaf. The richness and alpha diversity of Akihime bacterial community were significantly lower than that of White Elves in the stems and leaves. Beneficial bacterial genera, such as *Ochrobacter*, *Bradyrhizobium*, *Sphingomonas*, and *Pseudolobrys*, were more abundant in White Elves and Tokun than in Akihime, especially in roots and stems. The results of this study provide an important reference for discovering new species or genetic variations to improve host fitness and stress tolerance. Further research is needed to uncover the interactions between plants and endophytic bacteria, as well as the potential for extracting bioactive compounds from these bacteria.

Introduction

Plants are complex microecosystems that contain various endophytic bacteria that can colonize different organs and tissues of plants, including roots, stems, leaves, flowers, fruits, and seeds [1–3]. These endophytic bacteria establish mutualistic relationships with plants, influencing plant growth, phosphate solubilization, nitrogen fixation, siderophore production, and protecting plants from environmental stress and plant pathogens [4–7]. Endophytic bacteria live within plants and are rarely affected by environmental stress and nutrient competition. However, there are several factors that can affect their taxa and distribution, such as plant genotype, plant developmental stage, plant life history strategy, geographical location, and soil chemical properties [8–12]. Understanding the composition of endophytic bacterial communities in plants is crucial for developing valuable bioactive metabolites, promoting plant growth, and protecting plant against pathogens in the context of sustainable agriculture.

The cultivated strawberry (*Fragaria*×*ananassa*) is the most-cultivated small berries in the world and a high-value specialty crop that is popular for its color, taste, and health benefits such as vitamins, phenolics, polyphenols, fiber, and micronutrients [13, 14]. The cultivation of strawberry is challenging as it is susceptible to various diseases such as gray mold, root rot, anthracnose, wilt, and powdery mildew [15–20]. The cultivar of strawberry is the most important factor that determines the yield and quality of strawberry. White Elves is a domestically cultivated variety in China, with white fruits and good resistance to diseases such as powdery mildew and anthracnose. Tokun is a Japanese variety with a light pink fruit shaped like a peach and a faint peach aroma. Akihime is also a Japanese variety, with orange red fruit and weak disease resistance [11]. During the strawberry cultivation process, it was found that the White Elves are less susceptible to pathogens, followed by Tokun, and Akihime is the least tolerant to diseases [11]. Therefore,

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elucidating the microbial differences among the three strawberry cultivars is of great significance. However, to date, the differences in endophytic bacteria of roots, stems, and leaves among these three cultivars remain unknown.

Endophytic bacteria have recently received attention for their role in host growth, development, and as a source of novel bioactive compounds [21, 22]. However, current research on endophytic bacteria associated with different cultivars of strawberry is still limited. Therefore, in this study, we used 16S rRNA amplicon sequencing to evaluate bacterial differences in the root, stem, and leaf for three strawberry genotypes (White Elves, Tokun, and Akihime). The aim of this research was to analyze the composition, diversity and potential functions of endophytic bacteria in the roots, stems, and leaves of three different cultivars of strawberry, which could be useful for providing new insights into the application of endophytic bacteria from strawberry. We hypothesized that (1) due to differing microbial inoculum sources, different niches (roots, stems, leaves) would affect the diversity and composition of strawberry bacteria, and that (2) due to differences in tolerance of different strawberry cultivars to pathogens, the bacterial communities would differ across the three strawberry genotypes (White Elves, Tokun, and Akihime).

Materials and Methods

Experimental Design and Sample Collection

The samples were collected from a strawberry greenhouse in Jurong (32°02′N, 119°26′E, Jiangsu Agricultural Expo Park, Jiangsu Province, China), during the flowering period of strawberry. The cultivars (genotypes) were White Elves, Tokun, and Akihime. We randomly chose ten healthy plants from each cultivar. We dug out the whole strawberry plant, the roots, stems, and leaves (three niches) of each plant were placed separately in sterile plastic bags. These bags were immediately transported to the laboratory on ice. A total of 90 samples were analyzed: 3 niches (root, stem, and leaf) × 3 cultivars (White Elves, Tokun, and Akihime) × 10 plants.

Sample Surface Sterilization

In order to eliminate the interference of epiphytic microbes, the collected samples were surface sterilized according to our previous protocol [16]. The method is as follows: First, the surface of each strawberry plant was rinsed with tap water to remove soil residue and dust. Cut each part of the root, stem, and leaf samples of each cultivar into small pieces, and mix each part thoroughly separately, retaining 2 g of each sample. Adding 100 mL of sterile water and two drops of Tween 20, the mixture was shaken at 220 rpm for 20 min at room temperature. The plant tissues were treated with sterile water for 20 s, washed each tissue sequentially with 70% (v/v) ethanol for 30 s, and soaked in 2.5% (v/v) sodium hypochlorite solution for 2 min. Finally, the samples were rinsed three times with sterile water to obtain surface sterilized samples for extracting endophytic bacterial DNA [11, 23, 24].

DNA Extraction

DNA was extracted from strawberry roots, stems, and leaves samples of each cultivars using the DNAsecure Plant Kit (Tiangen, Beijing, China) following the instructions, and stored at − 20 °C prior to further analysis. The DNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

PCR Amplification and Sequencing

The V5–V7 regions of the bacterial 16S rRNA gene were amplified using the forward primer 799F (5′-AACMGGA TTAGATACCCKG-3′) and reverse primer 1193R (5′-ACG TCATCCCCACCTTCC-3′) [23, 25]. Subsequently, PCR amplification was performed, with the PCR amplification system and conditions following the previous method [16]. The PCR products were detected using 2% agarose gel electrophoresis. All the samples were submitted for the Illumina NovaSeq sequencing platform with PE250 (Shanghai Personal Biotechnology, Shanghai, China).

Bioinformatic Analysis

After the sequencing was completed, we used FLASH v.1.2.11 to merge the resulting sequences [26] and performed quality filtering using fastp v.0.19.6 software [27]. High-quality sequences were denoised using the DADA2 [28] or Deblur [29] plugins in the Qiime2 v. 2020.0 software [30] to obtain the amplicon sequence variants (ASVs). Taxonomy assignments for the ASVs of bacteria were performed using the classify-sklearn naive Bayes taxonomy classifier in the feature-classifier plugin [31] with reference to the SILVA database v.138 [32].

ASVs classified as “Cyanobacteria” or “Mitochondria” were excluded from the ASV table of bacteria. According to the minimum total number of sequences among all samples, 45,572 sequence were rarefied per sample for the 16S rRNA gene. Alpha diversity indices, namely the Chao1 and Shannon diversity indices, were calculated from the ASV table in QIIME2. Principal coordinate analysis (PCoA) of the Bray–Curtis distances was performed in QIIME and visualized using R v.3.3.1. Potential functions of the bacterial communities among different cultivars in different

strawberry niches were estimated using PICRUSt2 (http://www.genome.jp/kegg/) [33–35].

Statistical Analysis

The relative abundances of bacteria (phylum and genus), α-diversity indices, the relative abundance of bacterial metabolic function among genotypes (cultivars) for each plant compartments (niches) were compared using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test ($P < 0.05$). The relative abundances of the most abundant higher bacterial taxa in each plant compartment were also compared using one-way ANOVA followed by Tukey's HSD test ($P < 0.05$). All statistical analyses were performed using SPSS ver. 20.0 (IBM, Armonk, NY, USA).

Raw sequence reads were deposited at the Sequence Read Archive of the National Center for Biotechnology Information (accession ID: PRJNA1116712).

Results

Bacterial Community Diversity and Structure were Associated with Host Niche and Genotype

To examine the associations of host niche and genotype on the strawberry microbiome, we analyzed the bacterial communities in three niches (root, stem, and leaf) of three strawberry cultivars (White Elves, Tokun, and Akihime) by sequencing the V5–V7 region of the 16S rRNA gene. Through quality filtering, denoising, merging, and removing chimeras, we obtained a total of 4, 413, 008 sequences, which could be assembled into 45, 719 bacterial ASVs. There were 14, 103 ASVs in White Elves, 16, 406 ASVs in Tokun, and 15, 210 ASVs in Akihime (Table S1).

The Shannon alpha diversity index and Chao1 community richness index suggested that bacterial diversity and richness varied between niches for all three cultivars; and it significantly decreased from root to stem to leaf (Fig. 1a, b). In the roots, the three cultivars exhibited similar diversity and richness (Fig. 1c, d). In the stem, the three cultivars showed similar diversity; and the overall richness of the Akihime bacterial communities was significantly lower than those of the other cultivars (Fig. 1c, d). In the leaves, the Akihime bacterial community was significantly less diverse than that of White Elves, while the three cultivars showed similar the richness (Fig. 1c, d).

PCoA combined with an analysis of similarities (ANOSIM) indicated that niche had a stronger association than genotype in relation to the prevalences of bacterial communities (niche $R = 0.216$, $P < 0.001$; genotype $R = 0.090$, $P < 0.001$) (Fig. S1). The bacterial community structure of the three strawberry cultivars differed the most in the root ($R = 0.642$, $P < 0.001$), followed by the leaf ($R = 0.291$, $P < 0.001$) and then the stem ($R = 0.285$, $P < 0.001$) (Fig. 2).

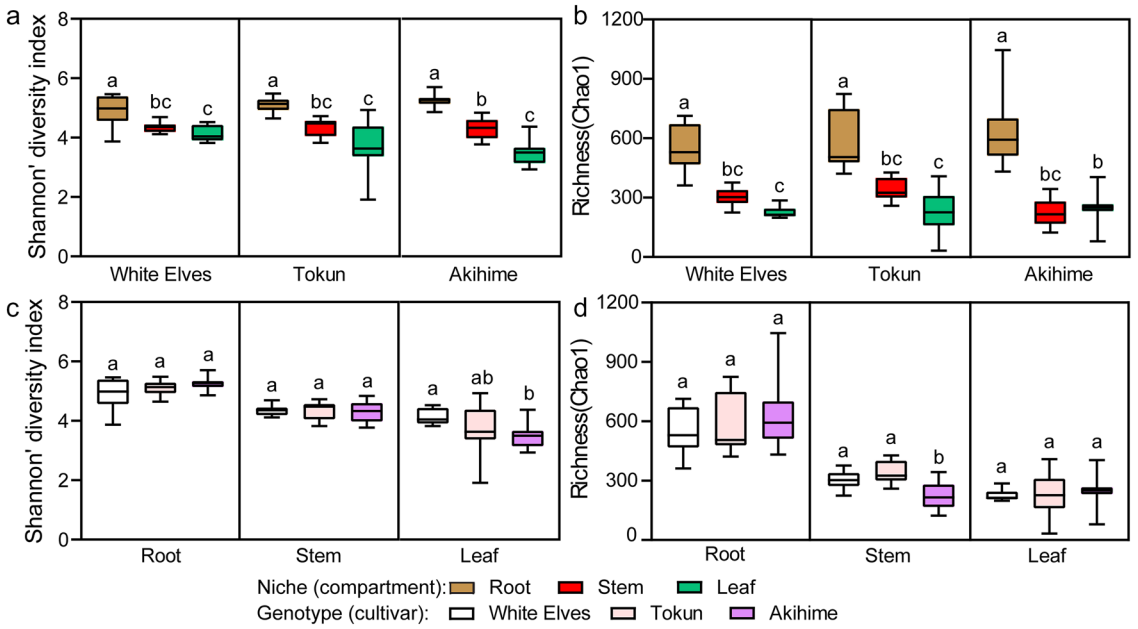


Fig. 1 Alpha diversity and richness of bacterial communities among three strawberry cultivars in roots, stems, and leaves (n = 10). **a**, **b** Diversity (Shannon's index) and richness (Chao1) of the bacterial community among three strawberry cultivars. **c**, **d** Diversity (Shannon's index) and richness (Chao1) of the bacterial community among three strawberry niches. Different letters above the bars indicate statistically significant differences according to Tukey's HSD test ($P < 0.05$, one-way ANOVA)

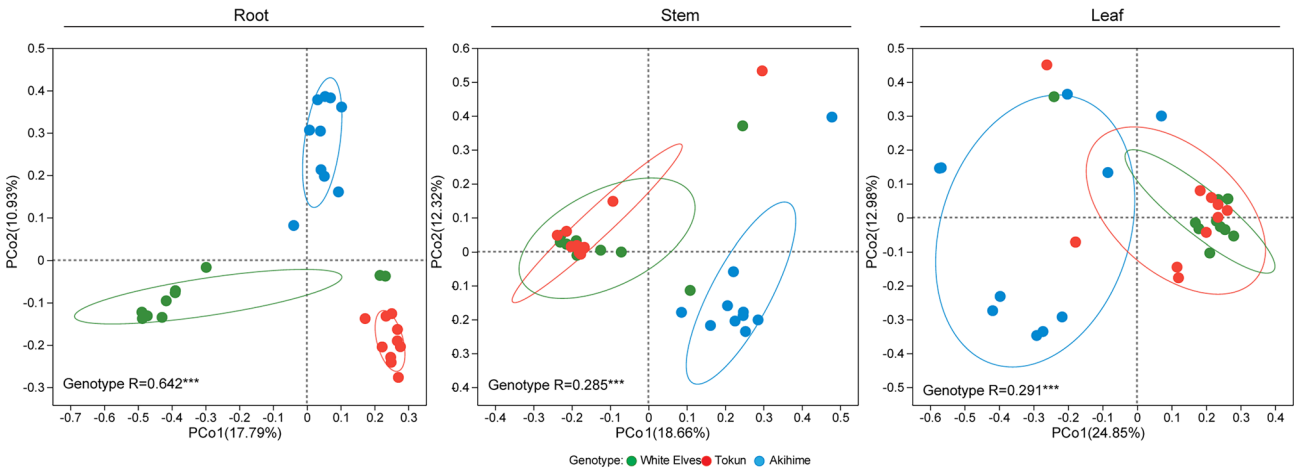


Fig. 2 PCoA based on Bray–Curtis differential analysis indicating the association of genotype with bacterial communities in strawberry roots, stems, and leaves ($n=10$). ANOSIM conducted to test for differences in community composition resulting from genotype. *R* values labeled with asterisks: $^{***}P<0.001$

Core Bacterial Taxa and Community Composition Among Genotypes

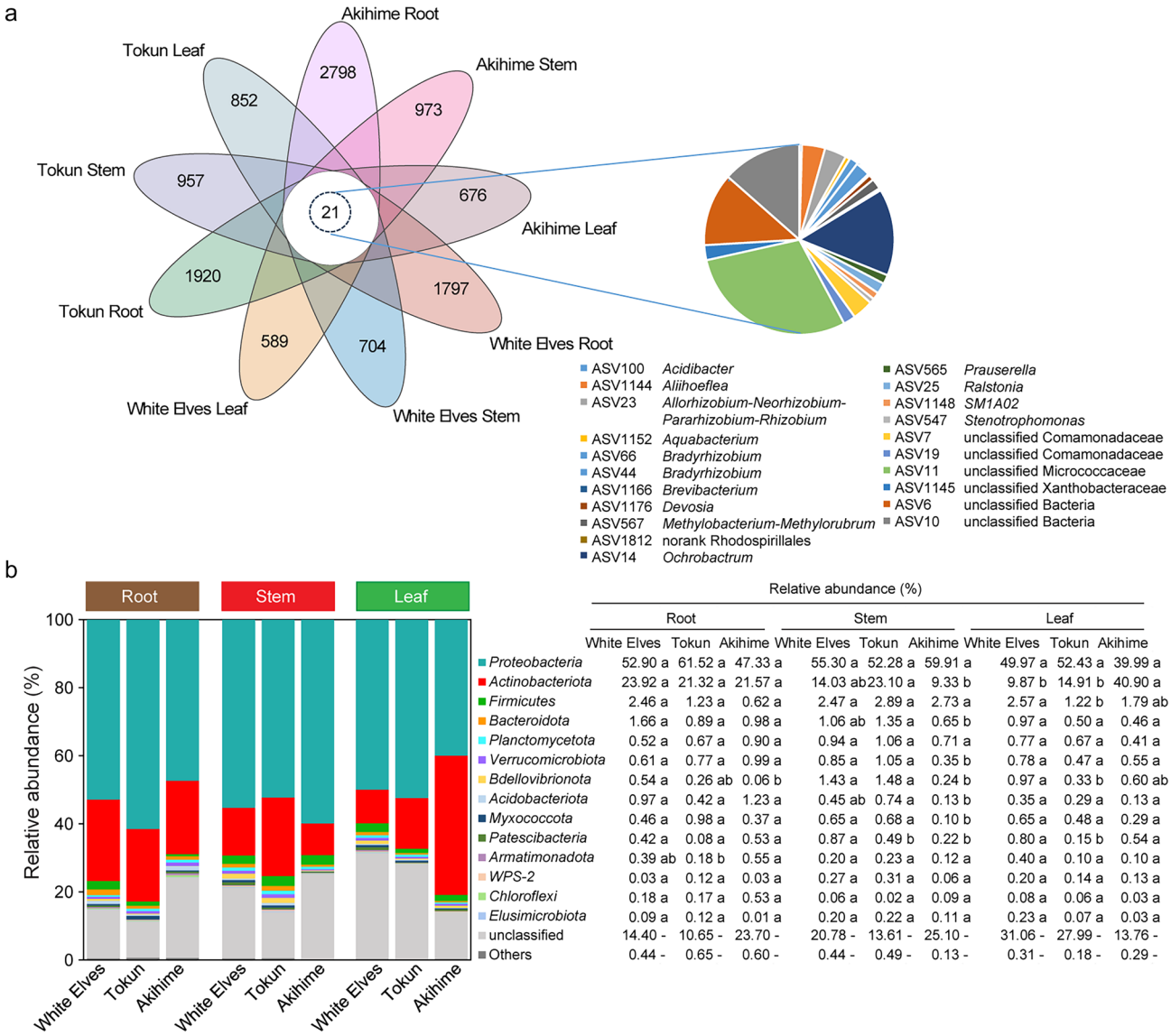
The number of unique bacterial taxa from root to stem to leaf decreased sequentially among the three cultivars. Among them, the number of unique ASVs in roots and stems was highest in Akihime, followed by Tokun, and the lowest in White Elf (Root: Akihime 2798 ASVs, Tokun 1920 ASVs, and White Elf 1797 ASVs, respectively; Stem: Akihime 973 ASVs, Tokun 957 ASVs, and White Elf 704 ASVs, respectively) The number of unique ASVs in leaves was highest in Tokun, followed by Akihime, and the least in White Elf (Tokun 852 ASVs, Akihime 676 ASVs, and White Elf 589 ASVs, respectively) (Fig. 3a). A total of 21 common core ASVs in the bacterial community were identified. These core taxa mainly belong to the genera *Ochrobactrum*, *Bradyrhizobium*, *Aliihoeflea*, and Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium (Fig. 3a).

At the phylum level, fourteen dominant (≥ 0.1% relative abundance) bacterial phyla were detected in this study (Fig. 3b). In the roots, Bdellovibrionota was more abundant in White Elves than in Akihime, whereas Armatimonadota was more abundant in Akihime than in Tokun ($P<0.05$, Tukey's HSD). In the stems, Actinobacteriota, Bacteroidota, and Acidobacteriota were more abundant in Tokun than in Akihime ($P<0.05$, Tukey's HSD). Verrucomicrobiota, Bdellovibrionota, and Myxococcota were more abundant in White Elves and Tokun than in Akihime, whereas Patescibacteria had the greatest abundance in White Elves than in all other cultivars ($P<0.01$, Tukey's HSD). In the leaves, Actinobacteriota had the greatest abundance in Akihime than in all other cultivars ($P<0.01$, Tukey's HSD). Firmicutes and Bdellovibrionota were more abundant in White Elves than in Tokun, whereas Patescibacteria was less abundant in Tokun than in the other cultivars (Firmicutes: $P<0.05$, Tukey's HSD; Bdellovibrionota and Patescibacteria: $P<0.01$, Tukey's HSD) (Fig. 3b).

At the genus level, 27 dominant bacterial groups with a relative abundance of ≥ 0.5% were detected (Fig. 4). In the roots, unclassified Micrococcaceae, *Halomonas*, and *Methylobacterium-Methylorubrum* were more abundant in White Elves than in Akihime ($P<0.05$, Tukey's HSD). *Ochrobactrum*, unclassified Gammaproteobacteria, *Aliihoeflea*, *Sphingomonas*, unclassified Xanthobacteraceae, *Prauserella*, and *Pseudolabrys* were more abundant in White Elves and Tokun than in Akihime ($P<0.05$, Tukey's HSD). *Bradyrhizobium* was more abundant in White Elves than other cultivars ($P<0.05$, Tukey's HSD), whereas *Burkholderia-Caballeronia-Paraburkholderia* was more abundant in Akihime than in the other cultivars ($P<0.01$, Tukey's HSD). In the stems, *Ochrobactrum*, *Bradyrhizobium*, *Sphingomonas*, unclassified Xanthobacteraceae, and *Pseudolabrys* were more abundant in White Elves and Tokun than in Akihime, whereas *Comamonadaceae* and *Brevundimonas* were more abundant in Akihime than other cultivars ($P<0.01$, Tukey's HSD). In the leaves, *Halomonas* was more abundant in White Elves than in Akihime ($P<0.01$, Tukey's HSD). *Methylobacterium-Methylorubrum* and *Sphingomonas* were more abundant in White Elves than in Tokun (*Methylobacterium-Methylorubrums*: $P<0.05$, Tukey's HSD; *Sphingomonas*: $P<0.05$, Tukey's HSD) (Fig. 4).

Potential Bacterial Metabolic Function of the Three Strawberry Genotypes

The potential functional profiles of the bacterial community of the three cultivars in the three niches of strawberry



	Root			Stem			Leaf		
	White Elves	Tokun	Akihime	White Elves	Tokun	Akihime	White Elves	Tokun	Akihime
Proteobacteria	52.90 a	61.52 a	47.33 a	55.30 a	52.28 a	59.91 a	49.97 a	52.43 a	39.99 a
Actinobacteriota	23.92 a	21.32 a	21.57 a	14.03 ab	23.10 a	9.33 b	9.87 b	14.91 b	40.90 a
Firmicutes	2.46 a	1.23 a	0.62 a	2.47 a	2.89 a	2.73 a	2.57 a	1.22 b	1.79 ab
Bacteroidota	1.66 a	0.89 a	0.98 a	1.06 ab	1.35 a	0.65 b	0.97 a	0.50 a	0.46 a
Planctomycetota	0.52 a	0.67 a	0.90 a	0.94 a	1.06 a	0.71 a	0.77 a	0.67 a	0.41 a
Verrucomicrobiota	0.61 a	0.77 a	0.99 a	0.85 a	1.05 a	0.35 b	0.78 a	0.47 a	0.55 a
Bdellovibrionota	0.54 a	0.26 ab	0.06 b	1.43 a	1.48 a	0.24 b	0.97 a	0.33 b	0.60 ab
Acidobacteriota	0.97 a	0.42 a	1.23 a	0.45 ab	0.74 a	0.13 b	0.35 a	0.29 a	0.13 a
Myxococcota	0.46 a	0.98 a	0.37 a	0.65 a	0.68 a	0.10 b	0.65 a	0.48 a	0.29 a
Patescibacteria	0.42 a	0.08 a	0.53 a	0.87 a	0.49 b	0.22 b	0.80 a	0.15 b	0.54 a
Armatimonadota	0.39 ab	0.18 b	0.55 a	0.20 a	0.23 a	0.12 a	0.40 a	0.10 a	0.10 a
WPS-2	0.03 a	0.12 a	0.03 a	0.27 a	0.31 a	0.06 a	0.20 a	0.14 a	0.13 a
Chloroflexi	0.18 a	0.17 a	0.53 a	0.06 a	0.02 a	0.09 a	0.08 a	0.06 a	0.03 a
Elusimicrobiota	0.09 a	0.12 a	0.01 a	0.20 a	0.22 a	0.11 a	0.23 a	0.07 a	0.03 a
unclassified	14.40 -	10.65 -	23.70 -	20.78 -	13.61 -	25.10 -	31.06 -	27.99 -	13.76 -
Others	0.44 -	0.65 -	0.60 -	0.44 -	0.49 -	0.13 -	0.31 -	0.18 -	0.29 -

Fig. 3 **a** The flower diagram showing the number of bacterial ASVs shared among the three strawberry cultivars in roots, stems, and leaves ($n = 10$). **b** Relative abundance of most abundant (> 0.1%) bacterial phyla among three strawberry cultivars in roots, stems, and leaves ($n = 10$)

roots, stems, and leaves were predicted by applying the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway with Phylogenetic Investigation of Communities by Reconstruction of Unobserved States [35]. The results indicated that most of the predicted bacterial protein sequences in the strawberry samples fell into metabolism (76.25%), environmental information processing (6.60%), genetic information processing (5.93%), cellular processes (5.01%), and organismal systems (1.98%) clusters. A total of 12, 10, 5, 4, and 3 pathways were identified for the metabolism, organismal systems, cellular processes, genetic information processing, and environmental information processing and cellular processes clusters, respectively (Fig. 5).

In the roots, the relative abundance of sequences related to cell growth and death and nucleotide metabolism was significantly higher in White Elves than in Akihime ($P < 0.05$, Tukey's HSD). The relative abundance of sequences related to membrane transport was significantly higher in Tokun than in White Elves or Akihime, whereas the opposite was the case for the relative abundance of the sequences related to energy metabolism ($P < 0.05$, Tukey's HSD). The relative abundance of the sequences related to the endocrine system was significantly higher in Akihime than in other cultivars ($P < 0.01$, Tukey's HSD). In the stems, the relative abundances of the sequences related to cellular community-prokaryotes, signal transduction, glycan biosynthesis and

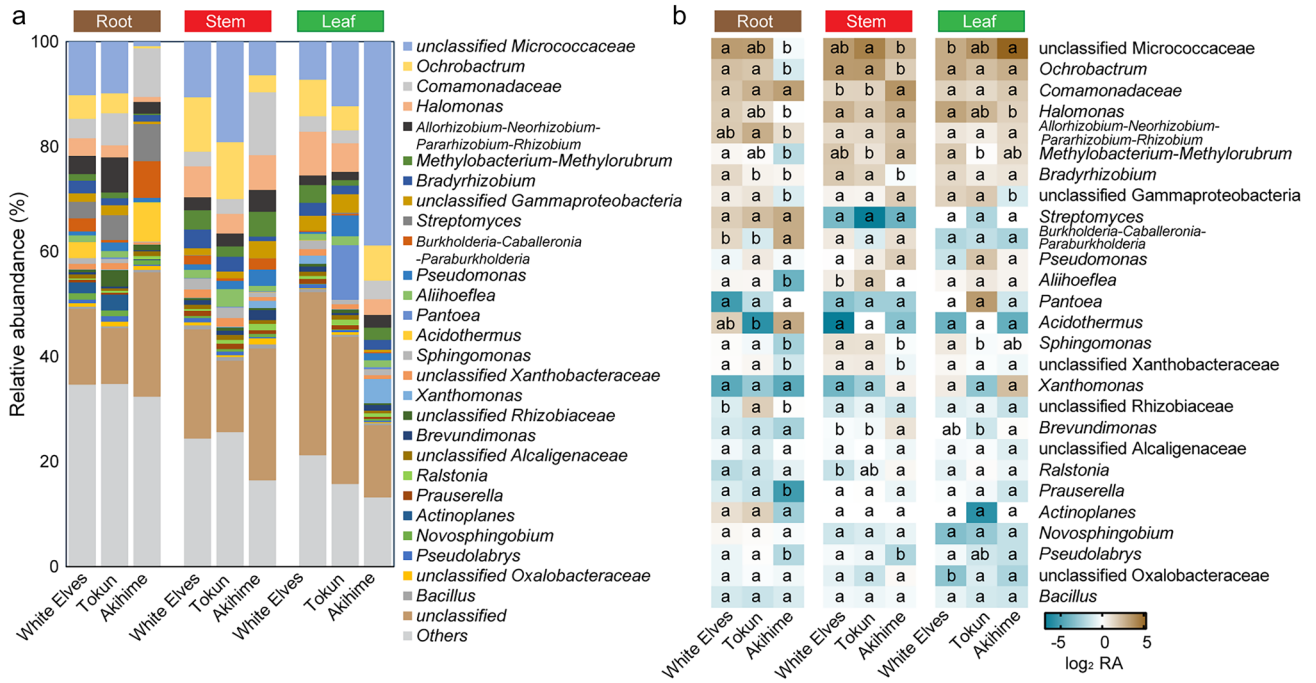


Fig. 4 Bacterial taxa distribution in strawberry. **a** Relative abundance of dominant bacterial genera among three strawberry cultivars in roots, stems, and leaves ($n=10$). **b** Heatmap of major bacterial genera in strawberry. Figure depicts bacterial genera with relative abundance of > 0.5%. Cell colors represent the $\log_2$ fold change in relative abundance compared with control treatment; brown and cyan indicate increasing and decreasing trends, respectively. Different letters above the bars indicate statistically significant differences according to Tukey's HSD test ($P<0.05$, one-way ANOVA)

metabolism, the circulatory system, the digestive system, environmental adaptation, and the immune system were significantly higher in Akihime than in other cultivars ($P<0.01$, Tukey's HSD). The relative abundances of the sequences related to energy metabolism and development and regeneration were significantly lower in Tokun than in other cultivars, whereas the relative abundances of the sequences related to carbohydrate metabolism, global and overview maps, and the nervous system were significantly higher in Tokun than in Akihime ($P<0.05$, Tukey's HSD). In the leaves, the relative abundances of the sequences related to transport and catabolism, amino acid metabolism, xenobiotic biodegradation and metabolism, and the endocrine system were significantly higher in Akihime than in other cultivars, whereas the opposite was the case for the relative abundances of the sequences related to energy metabolism, glycan biosynthesis and metabolism, and the metabolism of cofactors and vitamins ($P<0.01$, Tukey's HSD). The relative abundances of the sequences related to cell growth and death and cell motility were significantly higher in White Elves than in Akihime ($P<0.05$, Tukey's HSD) (Fig. 5).

Discussion

Endophytic microbial community can colonize healthy plant tissues and organs, and are important sources of new microbial resources and natural bioactive products. they play a crucial role in promoting plant growth, stress resistance, the prevention and control of plant diseases [8, 10, 36, 37]. We confirmed hypothesis 1 that the diversity and composition of strawberry bacterial communities differ significantly between niches (roots, stems, and leaves), especially for the belowground niche (roots) compared with the aboveground compartments (stems and leaves). This is in line with previous findings that the aboveground tissues had a lower bacterial alpha diversity compared to belowground [10]. This indicates that tissue specificity is a strong selective force for endophytic bacterial communities. Endophytic bacteria likely derive from the soil, and they typically first enter the root tissue and can colonize within the roots before spreading to the aboveground parts. However, endophytic bacteria can also enter leaves through stomata [3]. This may be attributable

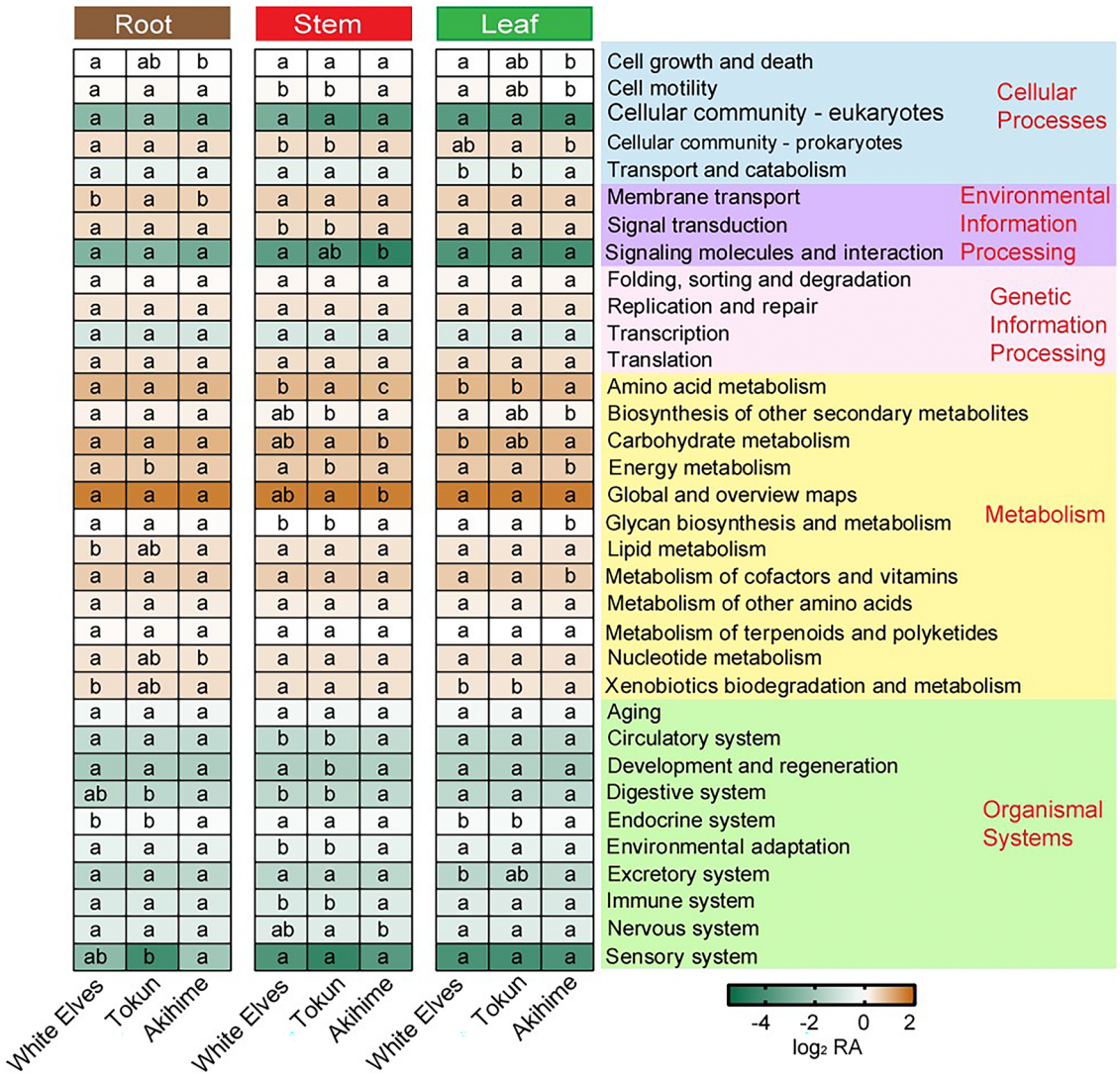


Fig. 5 Relative abundance inferred using PICRUSt2 of potential functional profiles of bacterial communities for three different cultivars in the three niches of strawberry roots, stems, and leaves ($n = 10$). Different letters above the bars indicate statistically significant differences according to Tukey's HSD test ($P < 0.05$, one-way ANOVA)

to the autonomous selection of microbial members within the organ niche [38].In addition, this study observed that although the three strawberry plant genotypes share a common core microbial community, plant genotypes have an impact on the composition of bacterial communities, which is consistent with previous research on strawberry [10, 11, 39, 40].

Bacterial taxonomic composition varies greatly among the strawberry cultivars, thereby confirmed hypothesis 2. In the roots, Bdellovibrionota had a greater relative abundance in White Elves than in Akihime. For the stems, Actinobacteriota and Bacteroidota were more abundant in Tokun than in Akihime; Verrucomicrobiota, Bdellovibrionota, and Myxococcota were more abundant in White Elves and Tokun than in Akihime. In the leaves, Firmicutes and Bdellovibrionota were more abundant in White Elves than in Tokun. Actinobacteriota have the potential to promote plant growth and induce systemic resistance, and they can serve as effective biocontrol agents against plant pathogens [41]. Bdellovibrionota had positive effects on cucumber and pepper seedling growth [42]. Research has shown that members of the phylum Myxococcota have the potential for photosynthesis [43]. At the genus level, in the roots, *Halomonas*, and *Methylobacterium-Methylorubrum* were more abundant in White Elves than in Akihime. *Ochrobactrum*, *Sphingomonas*, and *Pseudolabrys* are more abundant in White Elves and Tokun than in Akihime, *Bradyrhizobium* is more abundant in White Elves than in other cultivars. In the stems, *Ochrobactrum*, *Bradyrhizobium*, *Sphingomonas*, and *Pseudolabrys* are

more abundant in White Elves and Tokun than in Akihime. In the leaves, *Halomonas* was more abundant in White Elves than in Akihime. *Methylobacterium-Methylorubrum* and *Sphingomonas* were more abundant in White Elves than in Tokun.

Our research shows that the relative abundance of *Sphingomonas*, and *Bradyrhizobium* in the roots and stems of White Elves was significantly higher than that of Akihime. This is consistent with previous findings that the relative abundances of *Sphingomonas*, and *Bradyrhizobium*, which are essential source of biocontrol bacteria, were significantly higher in the wild species *Fragaria nilgerrensis* than in the cultivated variety Akihime samples [11]. The abundance of *Halomonas*, and *Methylobacterium-Methylorubrum* and *Sphingomonas* in White Elves leaves was significantly higher than that of other varieties cultivars. Previous studies have shown that *Halomonas*, and *Methylobacterium-Methylorubrum* were associated with plant health and plant growth-promoting [44]. In addition, research has shown that *Ochrobactrum*, and *Pseudolabrys* are plant-beneficial bacteria; some of the strains can fix nitrogen, highlighting their close relationship with host plants. They can also improve germination rate, increase root elongation, and promote plant growth. Some also have the potential for biological control of phytopathogens [45–50]. These indicate that there may be more beneficial bacterial groups in the White Elves than in Akihime, and the White Elves may may have a greater ability to tolerate plant diseases than Akihime.

The PICRUSt functional prediction analysis of bacterial microbiome showed that plant genotype and niche have a significant impact on bacterial metabolic function, which is consistent with previous research findings in strawberry [11]. In the roots and leaves, the relative abundances of the sequences related to cell growth and death and nucleotide metabolism were significantly higher in White Elves than in Akihime. In the stems, the relative abundances of the sequences related to cellular community-prokaryotes, signal transduction, glycan biosynthesis and metabolism, circulatory system, digestive system, environmental adaptation, and immune system were significantly higher in Akihime than in other cultivars, whereas the relative abundances of the sequences related to carbohydrate metabolism, global and overview maps, and the nervous system were significantly higher in Tokun than in Akihime. In the leaves, the relative abundances of the sequences related to transport and catabolism, amino acid metabolism, xenobiotic biodegradation and metabolism, and endocrine system were significantly higher in Akihime than in other cultivars. Carbohydrate and amino acid metabolism may be related to the metabolism of plant pathogenic bacteria. Melatonin treatment downregulates the differentially expressed genes responsible for carbohydrate and amino acid metabolism in *Xanthomonas oryzae* pv. *oryzae*, inhibiting its proliferation [51]. The volatile organic compounds produced by *Bacillus amyloliquefaciens* SQR-9 also downregulate the carbohydrate and amino acid metabolism of the protein of *Fusarium oxysporum* [52].

Conclusion

In this study, 16S rRNA amplicon sequencing was used to comparatively study the bacterial communities in the root, stem, and leaf for three strawberry genotypes (White Elves, Tokun, and Akihime). We found that the diversity and composition of strawberry bacterial communities differ significantly between niches (roots, stems, and leaves), especially for the belowground niche (roots) compared with the aboveground compartments (stems and leaves). This indicates that tissue specificity is a strong selective force for endophytic bacterial communities. Additionally, significant differences in the bacterial taxonomic composition of different strawberry cultivars, which may be related to their tolerance to pathogens. Moreover, beneficial bacterial genera such as *Ochrobacter*, *Bradyrhizobium*, *Sphingomonas*, and *Pseudolobrys* were more abundant in White Elves and Tokun than in Akihime, especially in roots and stems. Our results provide theoretical guidance and data support for the biological control of strawberry diseases, the selection of beneficial microbes, and the realization of sustainable agriculture.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-025-04223-z.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest/competing interests.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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