Comparative microbiome analysis reveals the variation in microbial communities between ‘Kyoho’ grape and its bud mutant variety

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Research Article

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Abstract

Background: Microbes are an important part of the vineyard ecosystem, which significantly influence the growth and development of grapevines. High-throughput microbiome sequencing can fully identify the microbial communities so as to help to guide viticulture and disease control. Previously, we identified a bud mutant variety, named 'Fengzao', from 'Kyoho' grapes. With both 'Fengzao' and 'Kyoho', we conducted high-throughput microbiome sequencing and investigated their microbial communities in different tissues.

Methods: Samples of fruit, stem and leaf were separately collected from 'Fengzao' and 'Kyoho'. After microbiome sequencing, analysis of OTU (Operational Taxonomic Unit) and taxonomy were conducted. The species diversity among different samples were analyzed by performing alpha and beta diversity analysis.

Results: A total of 34 OTUs were identified from the different tissues of 'Fengzao' and 'Kyoho'. There were obvious differences in the microbial communities between 'Fengzao' and 'Kyoho'. The fruit and the stem are the tissues with relatively higher abundance of microbes, while the leaves contained less microbes. The fruit and stem of 'Kyoho' and the stem of 'Fengzao' had relatively higher species diversity based on the alpha diversity analysis. *Proteobacteria*, *Enterobacteriaceae* and *Rhodobacteraceae* had significantly high abundance in 'Fengzao'. *Firmicutes* and *Pseudomonas* were highly abundant in the stems of 'Kyoho', and family of *Spirochaetaceae*, *Anaplasmataceae*, *Chlorobiaceae*, and *Sphingomonadaceae*, and genera of *Spirochaeta*, *Sphingomonas*, *Chlorobaculum* and *Wolbachia* were abundant in the fruits of 'Kyoho'.

Conclusion: The fruit and the stem (but not leaf) of grapevines are important hosts for microbes, and there are significant differences in microbial compositions between 'Fengzao' and 'Kyoho'. These identified microbes will be significant resources for the future researches on the quality regulation and disease control of grapevines.

Background

Grape is an important cash crop, and China is one of the most important grape-growing countries with the production and area of table grapes ranked top around the world for a long time [1]. Microbes are an important part of the vineyard ecosystem, which participate in multiple physiological and biochemical processes during the grapevine cultivation [2, 3]. It is of great significance for grapevines to study the microbiome composition and microbial diversity [4].

The microbial compositions are various for different tissues of the grapevines. In the rhizosphere (the area around the root), microbes are more numerous and complex due to their direct contact with the soil [5–7]. Some important microbes have been identified from the grapevine rhizosphere, such as *Clostridium*, *Bacillus*, *Rhizobium*, *Acinetobacter*, *Streptococcus*, *Paenibacillus* and other bacteria, as well as some fungi, such as *Filobasidium capsuligenum*, *Aureobasidium pullulans* and *Hanseniaspora* [4, 8–10]. Rhizosphere microbes are affected by plant uptake, root exudates, and soil activities. At the same time, rhizosphere microbes also directly affect nutrient uptake, nutrient utilization, growth, development, and disease occurrence for the grapevines [11–13]. For example, *Proteobacteria*, with high abundance in grapevines, is involved in the cycling process of major nutrient elements, which can improve nitrogen utilization efficiency [14, 15]. Microbial diversity in the phyllosphere (or leaf surface) is also one of the focuses of current researches. Leaves are the main dynamic habitats for microbes. Phyllospheric microbes mainly affect the fixation of carbon and nitrogen, thereby affecting plant growth and development [3, 7]. In addition, some harmful microbes in the phyllosphere are also the main sources of some diseases [6, 16, 17]. Grape berries are also important habitats for microbes, which directly affect the economic value and nutritional value of

grapes, especially for wine grapes, and the microbes inhabiting wine grapes have a direct impact on the aroma, color and quality of wine [18, 19]. The microbes on grape berries can also cause some serious diseases, resulting in a decrease in yield and quality. For example, *Alternaria* sp., a bacterium on grape berries, can produce a variety of toxic metabolites, which cause the disease of black spot, and even giving rise to the poisoning and cancer after human ingestion [20, 21]. Beyond rhizosphere, phyllosphere and berry, some studies have focused on the microbes in the other tissues of the grapevines, like *Xanthobcter*, *Xanthomonas*, *Cellulomonas*, and *Xylella* from the stems, and *Pseudomonas* sp. and *Bacillus* ssp. from the flowers [13, 15].

The diversity of microbial community has been the focus for the researchers in microbiology, ecology and phytopathology in recent years [5, 22]. By studying the dynamic changes of microbial community, we can understand the ecological functions of microbes and optimize community structure, contributing to the control and prevention of plant diseases. The current research approaches on microbial diversity has extended from traditional microorganism culture to high-throughput sequencing methods [23]. It has been very easy to understand all the microbial species and compositions of plants through high-throughput sequencing, which is commonly referred to as microbiome [24, 25]. Through the comparative study of microbiome, we can systematically analyze the effects of different varieties, different ecological environments, and different treatment factors on the microbial community of fruit trees, so as to better guide the production and disease control for the orchard.

Previously, we identified a bud mutant from the ‘Kyoho’ grape, named as ‘Fengzao’, which is typically characterized by early-ripening, with a maturity period of 30 days earlier than ‘Kyoho’ [26]. We have also compared the developmental process, the fruit physiology, and the transcriptome between the two cultivars [27–29], but the microbiome differences between them have not been investigated. Therefore, in this study, we systematically compared the microbiome in different tissues of ‘Kyoho’ and ‘Fengzao’ in order to reveal the regulatory mechanisms of microbes in grape fruit ripening.

Results

Sequencing statistics

To understand the microbial community of grapevines, we conducted high-throughput microbiome sequencing with samples of different tissues of ‘Kyoho’ and ‘Fengzao’, with ‘FF’, ‘FL’, ‘FS’ representing samples of fruit, leaf, stem in ‘Fengzao’, and ‘KF’, ‘KL’, ‘KS’ representing samples of fruit, leaf, stem in ‘Kyoho’. A total of 1,390,484 pairs of Reads were sequenced from the 6 samples, and 1,154,430 clean tags were generated after splicing and filtering, with an average of 192,405 clean tags generated per sample. The data quality was evaluated by statistical data processing, mainly by statistics of sequence number, sequence length, GC content, Q20 and Q30 quality value, effective value and other parameters in each sample (Table S1). After quality control, the data was used for subsequent analysis. The length distribution of obtained clean tags was counted in the corresponding length range of each sample, and the widest distribution of clean tags length is 440 to 450 nt (nucleotide) for all samples (Figure S1).

OTU (operational taxonomic unit) analysis

OTU is artificially-assigned taxon (strain, species, genus, group, etc.) for the convenience of analysis in phylogenetic studies or population genetics studies [20]. In general, if the similarity between sequences is higher than 97%, it can be defined as an OTU, and each OTU corresponds to a representative sequence [30]. Accordingly, at 97% similarity level, we conducted OTU analysis with the obtained sequences, and performed taxonomic annotation on OTU based on Silva (bacteria) and UNITE (fungi) taxonomic databases. A total of 34 OTUs were obtained from the six samples

(Table 1, Table S2, Fig. 1a), with 27 OTUs in FF, 30 OTUs in FL, 33 OTUs in FS, 34 OTUs in KF, 30 OTUs in KL, and 32 OTUs in KS (Fig. 1a). The Venn diagrams showed that 28 OTUs were commonly present in different tissues of 'Kyoho' (KF, KL, KS), and 26 OTUs were commonly present in different tissues of 'Fengzao' (FF, FL, FS). Only a few OTUs are tissue specific (6 in 'Kyoho' and 8 in 'Fengzao') (Fig. 1b, c).

Species annotation and taxonomic analysis

In order to analyze the community composition of each sample, we compared the representative sequence of OTU with the microbial reference database to obtain the corresponding species classification information for each OTU, and then obtained the classification information of each OTU at various levels (phylum, class, order, family, genus) (Table 1). By statistics, at the level of phylum, the microbial community with a relatively higher abundance in the six samples were *Cyanobacteria* and *Proteobacteria* (Figure S2A). At the level of class, *Chloroplast* and *Alphaproteobacteria* had higher abundance in the six samples (Figure S2B). And at the level of order and family, *Rickettsiales* and *mitochondria* with known functions had relatively higher abundance in the six samples, while much more microbial communities with highest abundance were unknown (Figure S2C, D). At the level of genus, eight OTUs had annotation, like OTU477 (*Spirochaeta*), OTU3488 (*Wolbachia*), OTU3748 (*Chlorobaculum*), OTU4313 (*Tetragenococcus*), OTU4701 (*Sphingomonas*), OTU6854 (*Incertae_Sedis*), OTU7563 (*Pseudomonas*), and OTU7879 (*Enterobacter*) (Table 1).

To specify and compare the bacterial compositions in the six samples, we used heatmaps to show the relative abundance in each sample at different levels (Fig. 2). At the level of phylum, *Proteobacteria* had the highest abundance in the sample of FF; *Chlorobi* and *Spirochaetae* had the highest abundance in KF; *Cyanobacteria* had the highest abundance in KL; *Firmicutes* and *Synergistetes* had the highest abundance in KS (Fig. 2a). At the level of class, *Chloroplast* and *Alphaproteobacteria* had the highest abundance in KL and FF, respectively; *Bacilli* and *Synergistia* had the highest abundance in KS; *Chlorobia* and *Spirochaetes* had the highest abundance in KF; *Clostridia*, *Betaproteobacteria* and *Gammaproteobacteria* had the highest abundance in FS (Fig. 2b). At the level of order, *Lactobacillales*, *Pseudomonadales* and *Synergistales* had the highest abundance in KS; *Rhizobiales*, *Sphingomonadales*, *Rhodobacterales*, *Gerbera_hybrid_cultivar* and *Rickettsiales* had relatively higher abundance in FF and KF, indicating their abundance in fruit samples; *Chlorobiales* and *Spirochaetales* had the highest abundance in KF; *Clostridiales*, *Burkholderiales* and *Enterobacteriales* had the highest abundance in FS (Fig. 2c). At the level of family, the microbial communities with the highest abundance were *Enterococcaceae*, *Pseudomonadaceae*, and *Synergistaceae* in the sample of KS, *Ruminococcaceae*, *Enterobacteriaceae*, and *Oxalobacteraceae* in FS, *Spirochaetaceae*, *Anaplasmataceae*, *Chlorobiaceae*, and *Sphingomonadaceae* in KF, *Rhodobacteraceae* and *mitochondria* in FF (Fig. 2d). At the level of genus, *Enterobacter* and *Incertae_Sedis* had the highest abundance in FS; *Tetragenococcus* and *Pseudomonas* had the highest abundance in KS; *Spirochaeta*, *Sphingomonas*, *Chlorobaculum* and *Wolbachia* had the highest abundance in KF (Fig. 2e).

Additionally, we also returned our sequenced OTU information to the taxonomic system of NCBI database, so as to comprehensively understand the evolutionary relationships and abundance differences of all microbes from the samples. The evolutionary tree showed the relationship of all microbial communities and the differences of their relative abundance between the samples of 'Kyoho' and 'Fengzao' (Fig. 3). The results indicated obvious abundance differences between 'Kyoho' and 'Fengzao'. For example, *Cyanobacteria*, with highest abundance among all microbial communities, had higher bacterial abundance in 'Fengzao' than 'Kyoho'; *Ruminococcaceae*, *Rhizobiales*, *Rhodobacteraceae*, *Oxalobacteraceae*, and *Enterobacter* all had relatively higher abundance in 'Fengzao' than 'Kyoho'; while *Chlorobaculum*, *Tetragenococcus*, *Wolbachia*, *Pseudomonas*, and *Synergistaceae* had relatively higher abundance in 'Kyoho' than 'Fengzao' (Fig. 3).

Table 1 Information of the 34 OUTs from each sample

OTU ID	Sequence count						Taxonomy
	FF	FL	FS	KF	KL	KS	
OTU477	0	28	5	47	29	5	k__Bacteria; p__Spirochaetae; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Spirochaeta
OTU623	18	20	16	21	18	6	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU893	15	14	12	8	9	4	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__uncultured_bacterium;
OTU1418	120	29	59	77	26	27	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Gerbera_hybrid_cultivar;
OTU1664	291	162	245	238	94	148	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria
OTU2296	0	4	32	31	2	6	k__Bacteria; p__Spirochaetae; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__uncultured; s__uncultured_bacterium
OTU2554	48	36	59	36	33	32	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__uncultured_bacterium;
OTU2622	92	50	13	85	14	4	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales
OTU2892	10	10	15	12	12	6	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU2939	19	21	15	14	11	16	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU3079	35	2	7	17	6	1	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae
OTU3218	32	15	20	19	19	19	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU3488	4	7	7	295	22	37	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__Anaplasmataceae; g__Wolbachia
OTU3613	21	20	7	11	12	11	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU3748	0	0	20	130	5	0	k__Bacteria; p__Chlorobi; c__Chlorobia; o__Chlorobiales;

							f__Chlorobiaceae; g__Chlorobaculum
OTU4063	9	11	16	13	8	11	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU4313	359	0	0	287	0	441	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Enterococcaceae; g__Tetragenococcus
OTU4701	68	22	14	81	6	8	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Sphingomonas; s__uncultured_Sphingomonas_sp.
OTU4736	36	32	43	53	33	20	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria
OTU5662	30	24	19	25	27	16	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU5889	0	0	22	1	0	50	k__Bacteria; p__Synergistetes; c__Synergistia; o__Synergistales; f__Synergistaceae; g__uncultured; s__uncultured_bacterium
OTU6041	13	10	11	14	7	8	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU6515	49	49	35	40	24	25	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria
OTU6717	85	70	86	115	62	65	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Gerbera_hybrid_cultivar;
OTU6854	0	44	47	6	0	1	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Incertae_Sedis; s__uncultured_bacterium
OTU6916	16	15	12	16	11	5	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Gerbera_hybrid_cultivar;
OTU6922	54	2	1	42	41	24	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__uncultured_bacterium;
OTU7563	0	0	15	20	0	30	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas
OTU7879	11	27	285	14	4	0	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Enterobacter

OTU7914	30425	11094	20369	23534	6629	7682	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria
OTU8110	0	1	68	9	3	2	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae
OTU8121	203992	193188	163517	191117	142317	121669	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU8408	25	29	18	26	12	14	k__Bacteria; p__Cyanobacteria; c__Chloroplast
OTU8592	14	15	9	11	9	6	k__Bacteria; p__Cyanobacteria; c__Chloroplast

Alpha diversity analysis

Alpha diversity reflects the species diversity within a single sample, which can be measured by multiple indexes, like Ace, Chao1, Simpson and Shannon [31]. A larger value of Chao1, Ace and Shannon, and a smaller value of Simpson indicate a higher species diversity of the sample. So, based on Ace and Chao1, the species diversity ranking for the six samples is KF, FS, KS, FL, KL, and FF. And based on Simpson and Shannon, this ranking is FF, KF, FS, KS, FL, and KL (Table 2). We also calculated the Coverage of the sample library, which indicated whether the sequencing results represent the true situation of the microbes in the sample, with a higher value representing a higher probability that all sequences in the sample has been measured. The results showed that the Coverage values of the six samples were all very high (more than 0.9999) (Table 2). In addition, we also plotted the Rarefaction curve and the Shannon Index curve to verify whether the amount of sequencing data was sufficient to reflect the species diversity in the samples (Figure S3). The two kinds of curves both tended to be flat for all the six samples, indicating that the sequencing data was reasonable and saturated, and no additional sequencing was needed.

Table 2 Alpha diversity index statistics

Sample_ID	OTU	Ace	Chao1	Simpson	Shannon	Coverage
FF	27	27	27	0.76447	0.437607	1
FL	30	30.570286	30	0.890567	0.245818	0.999995
FS	33	33.24112	33	0.792343	0.403951	0.999995
KF	34	34.377086	34	0.791338	0.414919	0.999995
KL	30	30	30	0.908121	0.217048	1
KS	32	32.554171	32.5	0.874068	0.288689	0.999985

(Sample_ID: the sample name; OTU: the number of OTUs; Ace, Chao1, Simpson and Shannon represent each index respectively; Coverage indicates the coverage of the sample library.)

Beta diversity analysis

We employed two methods, PCoA (Principal coordinates analysis) and NMDS (Non-metric multi-dimensional scaling), to conduct beta diversity analysis, in order to further investigate the differences of microbial communities among the six samples. PCoA and NMDS exhibited similar results, both showing that samples of ‘Kyoho’ and

‘Fengzao’ were roughly divided into two classes (Fig. 4), indicating the diversity of microbial communities between ‘Kyoho’ and ‘Fengzao’.

Functional analysis of microbial community

In order to analyze the differences of functional genes of microbial communities in different samples, we mapped the obtained OTUs to the corresponding COG (Clusters of orthologous groups of proteins) database to calculate the abundance of each COG, and performed pairwise tests for significant differences between different samples at the genus level. The different tissues (fruit, leaf and stem) of ‘Kyoho’ and ‘Fengzao’ were analyzed, respectively. The COG pathway with most obviously differences between ‘Kyoho’ and ‘Fengzao’ was ‘Translation, ribosomal structure and biogenesis’ (Fig. 5, Figure S4-S5). Totally speaking, The results from fruit, leaf and stem were similar, with some pathways identified in all the three tissues, like ‘Translation, ribosomal structure and biogenesis’, ‘Cell motility’, ‘Energy production and conversion’, ‘Function unknown’, ‘Carbohydrate transport and metabolism’, ‘Inorgenic ion transport and metabolism’, ‘Intracellular trafficking, secretion, and vesicular transport’, ‘General function prediction only’, and so on (Fig. 5).

Discussion

Microbes are an important part of the grape ecosystem, which directly affect the yield, quality, stress resistance, growth and development of grapes. Especially for wine grapes, the microbial composition and content directly determine the quality of wine product [32]. ‘Kyoho’ is mainly used as table grapes, but recent studies have also shown that the microbes inside table grapes can also significantly affect grape quality and disease resistance [33]. However, studies on the microbial composition of table grapes are far behind that of wine grapes, and most of them focused on the rhizosphere, with less attention paid to leaves, fruits, and stems [34, 35]. Therefore, in this study, we investigated the microbiome in leaves, stems and fruits of ‘Kyoho’, a representative of table grapes. In addition, we have previously identified a bud mutant derived from ‘Kyoho’, namely ‘Fengzao’ [26]. We have conducted deep studies on the comparisons of maturity period, fruit quality, physiological and molecular mechanisms of fruit development between ‘Fengzao’ and ‘Kyoho’ [26–28], but the microbiome differences between them are unknown. Therefore, in this study, the differences between the microbiomes of ‘Kyoho’ and ‘Fengzao’ in fruits, leaves, and stems were investigated using high-throughput sequencing. Our results will provide important reference for related researches on the influence of microbes on table grape quality and the effect of bud mutant on microbiome.

We identified 34 OTUs from the different grape tissues of ‘Kyoho’ and ‘Fengzao’ (Fig. 1a). Although the identified microbial communities are not so rich, many representative microbes (such as *Proteobacteria*, *Firmicutes*, *Synergistetes*, *Pseudomonadales*, etc.) were identified, which are common in the horticultural plants as revealed by the previous studies [36–38]. The microbes we identified were mainly endogenous microbes (or named endophyte) which existed in the interior of plants. The endophyte of plants are less abundant than the epiphyte on the surface of the plant bodies (especially the rhizosphere) [22]. Previous studies mainly focused on the epiphyte [39], but in fact, the endophyte might play a more significant role for plants [25]. In recent years, more and more studies have started to investigate the endogenous microbes of plants, including *Arabidopsis* [25, 40, 41], apples [42] and grapes [19]. Lundberg et al. [25] rigorously defined the rhizosphere and the endophytic compartment (within the root) in *Arabidopsis*, and revealed the important functions of endophytic microbiome for plant-microbe interactions. So, while continuing to pay attention to rhizosphere microbes, we should also strengthen the researches on the endogenous microbes of other plant tissues (like leaf, fruit, stem, flower, etc.).

It is worth mentioning that our study found very significant differences in microbial composition between grape and its bud mutant variety (Fig. 6). Likewise, Portillo Mdel et al. [9] also found differences in the microbial communities of fruit surface between two grape varieties, Grenache and Carignan. Bodenhausen et al. [40] studied the influence of host on the microbiome of *Arabidopsis* and found that different genotypes significantly affected microbial communities. From these studies, we can deduce that plant microbiome is genome- or genotype- specific, which can be further investigated in more plant varieties [43, 44]. In addition, our study also found that different grape tissues harbored significantly different microbial communities. Fruits and stems were rich in microbes, while leaves contained very few microbes, with almost no microbes detected in 'Fengzao' leaves (Fig. 6). Of course, the microbes inside the roots should be the most abundant. Previous studies have conducted detailed microbiome studies on grape roots, so our study did not replicate the study of the root. Martins et al. [19] examined the microbiome in the roots, barks, leaves and fruits of grapes, and found that the roots were the most abundant in microbes, followed by barks, fruits and leaves. The tissue specificity of microbial compositions reminds us that a whole understanding of the microbiome for a plant requires analysis of different tissues, and although microbes can move within a plant, their final hosts may be relatively stationary.

Many microbes identified in this study have been proved as key regulators in plant growth and development [18, 36–38, 45]. For example, *Proteobacteria* was found significantly enriched in the fruits of 'Fengzao'. The phylum of *Proteobacteria* can be further categorized as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammarproteobacteria* and *Deltaproteobacteria*, which were also identified in 'Fengzao' with a high abundance in fruits and stems (Fig. 6). *Rhodobacteraceae*, one of the major subdivisions of *Alphaproteobacteria* [45], was also identified in the fruits of 'Fengzao' with a high abundance (Fig. 6). Studies have found that *Proteobacteria* can promote growth in polluted farmland [38]. Interestingly, *Proteobacteria* and its subphylum were only identified and enriched in the fruits and stems of 'Fengzao', with a significantly lower abundance in 'Kyoho' (Fig. 2 and Fig. 6), suggesting that bacterial abundance of *Proteobacteria* may have an impact on the differences of fruit development between 'Fengzao' and 'Kyoho'. *Firmicutes* were also the main phyla at the early fruit enlargement stage and in the rhizosphere soil in vineyards [37], which were identified from the stems of 'Kyoho' in this study. Zhang et al. [37] found that *Firmicutes* were sensitive to abiotic stresses, especially drought. The *Pseudomonas* was found most significantly enriched in the stems of 'Kyoho'(Fig. 6). The content of *Pseudomonas* was high especially on the surface of wine grapes [36]. The *Pseudomonas* can produce extracellular polysaccharide, which is conducive to the formation of microbial membranes and affects the colonization of microbes on the fruit surface [36]. The *Enterobacteriaceae* family or the *Enterobacter* genus was identified with a significantly higher abundance in the stems of 'Fengzao' (Fig. 6). The *Enterobacteriaceae* is thought to be beneficial for vineyards, as it can produce glucanases, chitinases and proteases to provide host resistance [18]. Additionally, some other identified microbes, like *Ruminococcaceae* family, *Oxalobacteraceae* family and *Rhodobacteraceae* genus from 'Fengzao', *Tetragenococcus* genus, *Sphingomonas* genus and *Chlorobaculum* genus from 'Kyoho', can be further investigated to understand their roles in grapevines .

Conclusion

In this study, we systematically analyzed the microbiomes of the 'Kyoho' grape and its bud mutant variety (named 'Fengzao'). A total of 34 OTUs were identified from stems, leaves and fruits. There were obvious differences in the microbial communities between 'Fengzao' and 'Kyoho'. The microbes in different grape tissues also showed remarkable differences, and the fruits and stems are the tissues with relatively higher abundance of microbes, while the leaves contained less microbes. *Proteobacteria* phylum, *Enterobacteriaceae* family and *Rhodobacteraceae* family were significantly enriched in 'Fengzao', which are beneficial bacteria and can promote the growth of grapevines. The *Firmicutes* phylum and the *Pseudomonas* genus were highly abundant in the stems of 'Kyoho', and family of

Spirochaetaceae, *Anaplasmataceae*, *Chlorobiaceae*, and *Sphingomonadaceae*, and genera of *Spirochaeta*, *Sphingomonas*, *Chlorobaculum* and *Wolbachia* were abundant in the fruits of 'Kyoho'. These identified microbes will be significant resources for the future researches on the grapevine microbiology.

Methods

Plant materials

Grapevines of 'Kyoho' and 'Fengzao' were planted in the experimental fields of Henan University of Science and Technology (Luoyang, China) under the same viticulture management practices. Samples of fruit, stem and leaf were collected on April 15, 2020. Three trees were selected for 'Fengzao' and 'Kyoho', and a bunch of berries, 5 leaves, and 10 cm-length stem segments were respectively taken from each vine. Samples of each tissue from 'Fengzao' and 'Kyoho' were mixed together, immediately frozen in liquid nitrogen, and stored in a -80 ℃ freezer for further use.

Library construction and sequencing

After extraction of total DNA from each sample, primers were designed according to the conservative regions of bacteria. After connecting with the adaptor, PCR amplification was performed. The products were purified, quantified and homogenized to form sequencing libraries. The constructed libraries were subjected to library-quality inspection, and the qualified libraries were sequenced by Illumina HiSeq 2500 platform. Raw image data files obtained by high-throughput sequencing are converted into raw sequenced reads by base calling analysis. The results are stored in FASTQ file format, which contained the detailed sequence information of reads and their corresponding sequencing quality information. The generated data are available in the NCBI SRA repository under the BioProject ID: PRJNA939915 (accession numbers SRX19531416-SRX19531421).

Data preprocessing

According to the overlaps of the reads, the paired-end sequence data obtained by Hiseq were merged into sequence tags, which were filtered by quality control according to the following three steps: (1) paired-end reads were spliced using FLASH v1.2.7 software (http://ccb.jhu.edu/software/FLASH/) based on a criterion: minimum overlapping length is 10 bp and maximum mismatching ratio of overlapping regions is 0.2, to obtain the raw tags data. (2) The raw tags data was filtered using Trimmomatic v0.33 software (http://www.usadellab.org/cms/?page=trimmomatic) with the parameter set as a window of 50 bp. If the average quality value in the window was lower than 20, the base at the back end would be cut from the window, and the tags with length less than 75% would be eliminated. After this, the high-quality tags data (clean tags) were obtained. (3) Using UCHIME v4.2 software (http://drive5.com/usearch/manual/uchime_algo.html), the chimeric sequences were identified and removed to obtain the final effective clean tags.

OTU (Operational Taxonomic Unit) analysis

All obtained tags were divided into different OTUs. Generally, if the similarity between sequences is higher than 97%, it can be defined as an OTU, and each OTU corresponds to a representative sequence. Each OTUs were obtained with the UCLUST in QIIME (version 1.8.0) software [46] at a 97% similarity level.

Species annotation and taxonomic analysis

The representative sequences of OTUs were aligned with the microbial reference database to obtain the classification information, and then the community composition of each sample was counted at each level (phylum, class, order,

family, genus, species). QIIME (version 1.8.0) software [46] was used to generate figures showing species abundance at different taxonomic levels, and R package tools were used to draw maps showing the bacterial community structure at each taxonomic level.

Alpha diversity analysis

Mothur (version v.1.30) software (http://mothur.org/) was used to conduct the Alpha diversity analysis. To compare diversity between samples, the number of sequences contained in the samples was normalized during analysis. The Alpha diversity was analyzed with four indicators, including Chao1, Ace, Shannon, and Simpson. The Rarefaction Curve and the Shannon Index Curve were drawn with Mothur software and R package to To verify whether the amount of sequencing data is sufficient to reflect the species diversity in the samples.

Beta diversity analysis

QIIME (version 1.8.0) software [46] was used for beta diversity analysis to compare the differences in species diversity among different samples. Based on the results of Beta diversity analysis, PCoA (Principal Coordinates Analysis) [47] and NMBS (Non-metric Multi-Dimensional Scaling) [48] maps were drawn respectively using R package tools.

Analysis of 16S functional genes

PICRUSt software [49] was used to infer the functional gene composition in the samples through 16S sequencing, so as to analyze the functional differences between different samples or groups. Firstly, the generated OTUs were standardized, as different genus or species have different 16S copy numbers. Then, through the greengene id corresponding to each OTU, the COG (Clusters of Orthologous Groups of proteins) family information of each OTU can be obtained. The COG abundance and the abundance of each functional category could be calculated by obtaining the KO, Pathway, EC information from the COG database. At the genus level, pairwise tests for significant differences between different samples were performed with two-sample T-TEST method in STAMP software (https://beikolab.cs.dal.ca/software/STAMP) (P-value was set to 0.05).

Abbreviations

OTU: Operational Taxonomic Unit; FF: The fruit of ‘Fengzao’; FL:The leaf of ‘Fengzao’; FS: The stem of ‘Fengzao’; KF: The fruit of ‘Kyoho’; KL: The leaf of ‘Kyoho’; KS: The stem of ‘Kyoho’; NCBI: National Center for Biotechnology Information; PCoA: Principal coordinates analysis; NMDS: Non-metric multi-dimensional scaling; COG: Clusters of orthologous groups of proteins; PCR: Polymerase Chain Reaction.

Declarations

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Availability of data and materials

The datasets generated during the current study are available in the NCBI SRA repository under the BioProject ID: PRJNA939915 (accession numbers SRX19531416-SRX19531421).

Authors' contributions

D.L.G. designed this project. T.L.W., Y.X.S and Z.H.W. conducted experiments. T.L.W., M.S.P. and H.N.L. performed the data analysis. Y.H.Y. and Q.F.S. assisted in the data analysis. T.L.W. wrote the manuscript. M.S.P., D.L.G. and Y.H.Y. revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

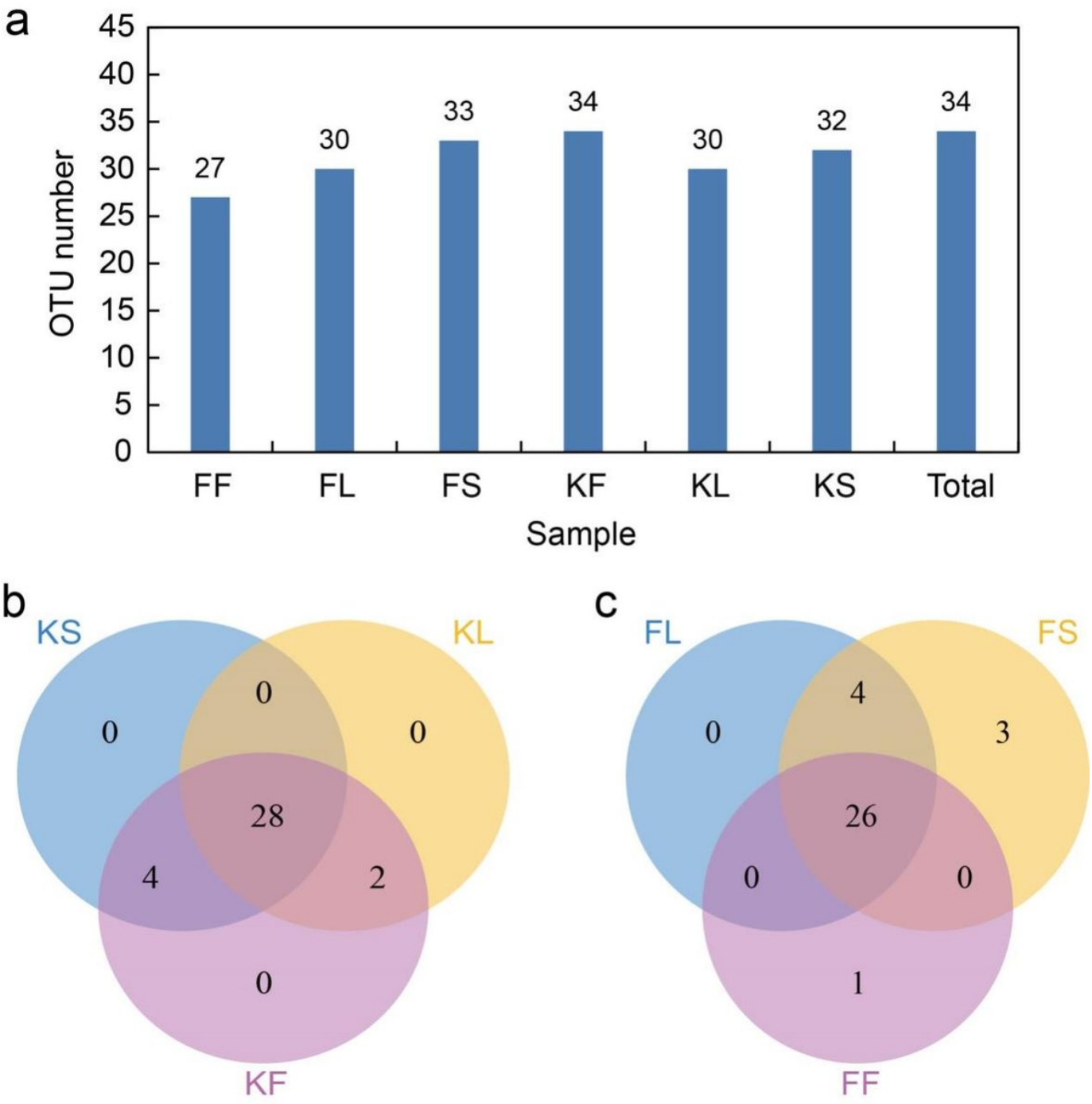

Figure 1

Statistics of OTU (operational taxonomic unit) in different samples. **a**OTU number in each sample and total number of the identified OTU. **b-c** Venn diagrams showing the OTU numbers among different samples of 'Kyoho' (b) and

'Fengzao' (c). 'FF', 'FL' and 'FS' indicate samples of fruit, leaf and stem in 'Fengzao'; 'KF', 'KL' and 'KS' indicate samples of fruit, leaf and stem in 'Kyoho'.

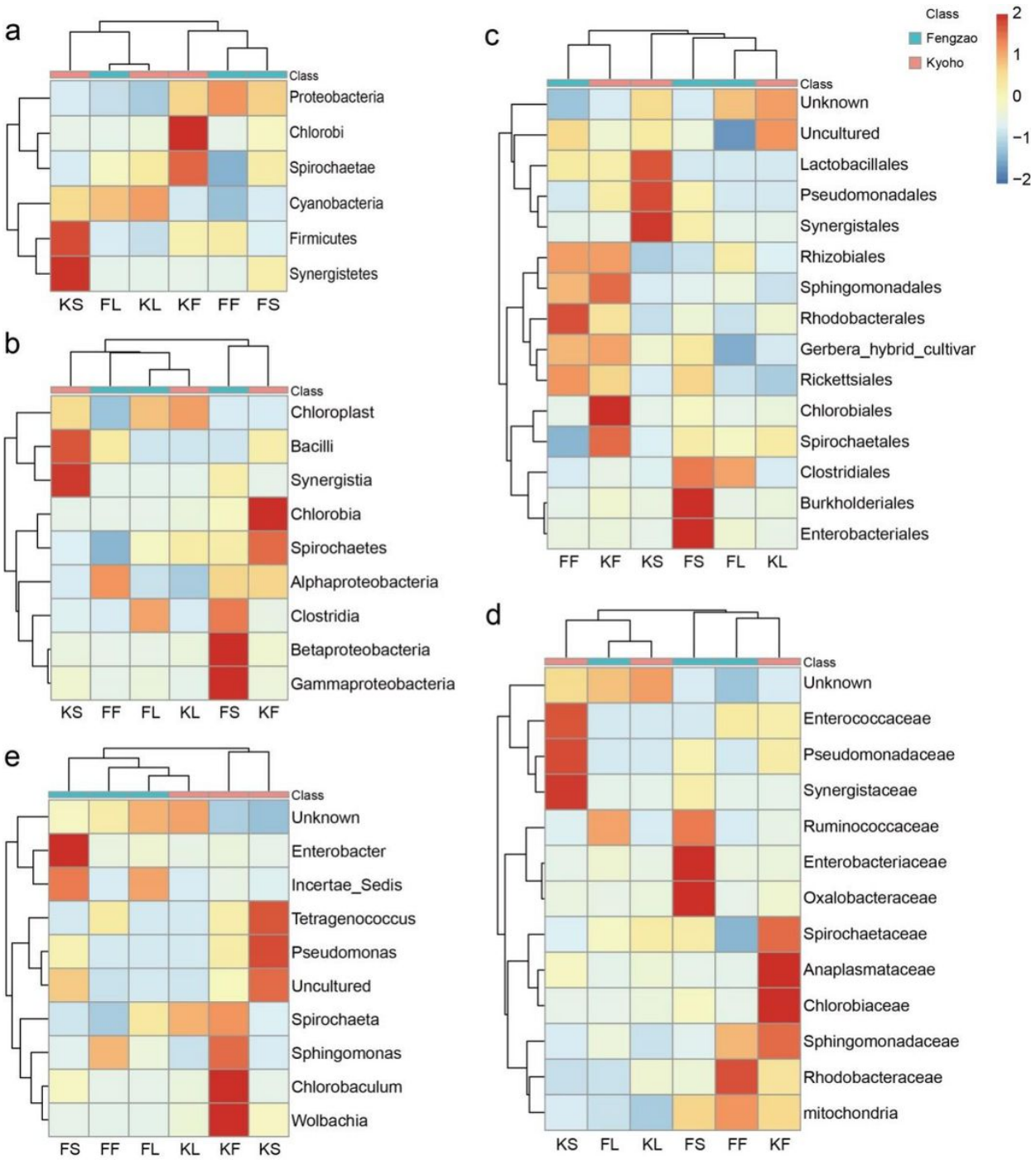

Figure 2

Clustering heat maps of bacterial abundance at all levels, including phylum (a), class (b), order (c), family (d), genus (e). Horizontal clustering refers to sample information and vertical clustering refers to bacterial information. Heatmap shows the bacterial abundance in each sample, with red and blue color representing high and low abundance, respectively.

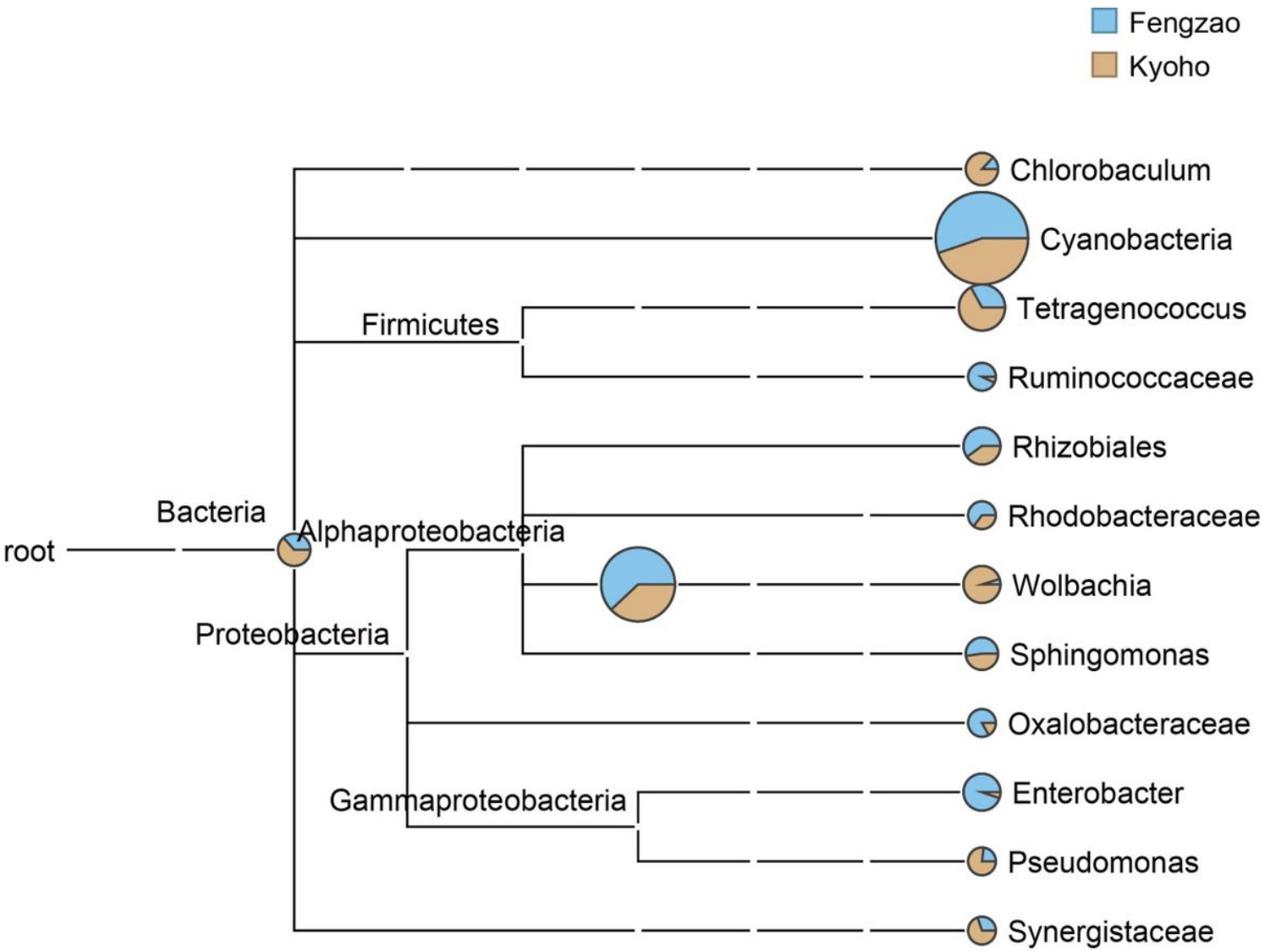

Figure 3

Tree diagram showing the bacterial abundance at each taxonomic level in ‘Fengzao’ and ‘Kyoho’. The bacterial abundance is compared between ‘Fengzao’ (blue) and ‘Kyoho’ (gray) with pie charts.

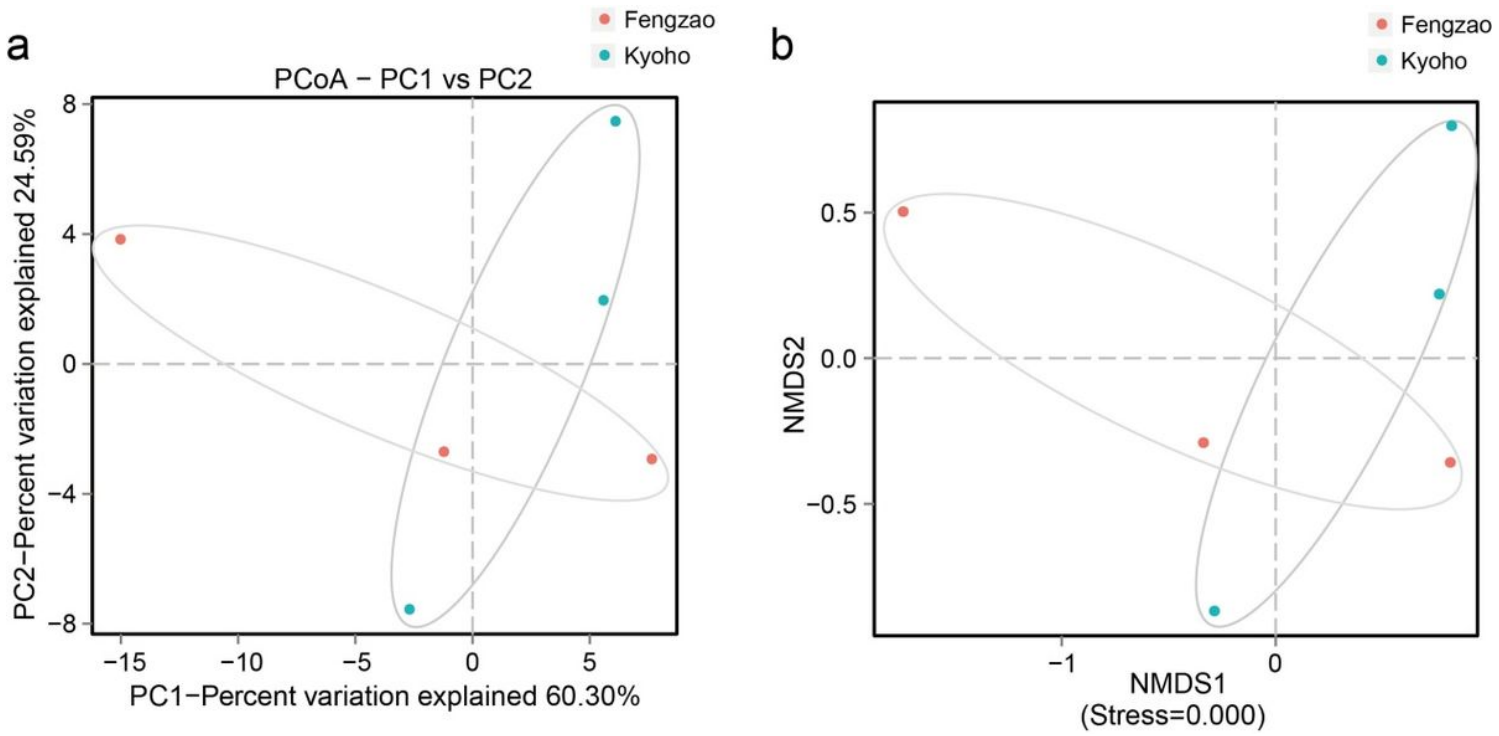

Figure 4

PCoA (principal coordinates analysis) (a) and NMDS (non-metric multi-dimensional scaling) diagrams (b) showing sample diversity in 'Fengzao' and 'Kyoho'. **a** The red and blue dots represent samples of 'Fengzao' and 'Kyoho', respectively. Horizontal and vertical coordinates are the two characteristic values that lead to the biggest difference between samples, and the influence degree is reflected by the percentage. **b** The red and blue dots represent samples of 'Fengzao' and 'Kyoho', respectively. When the Stress is less than 0.2, it indicates that the NMDS analysis has reliability.

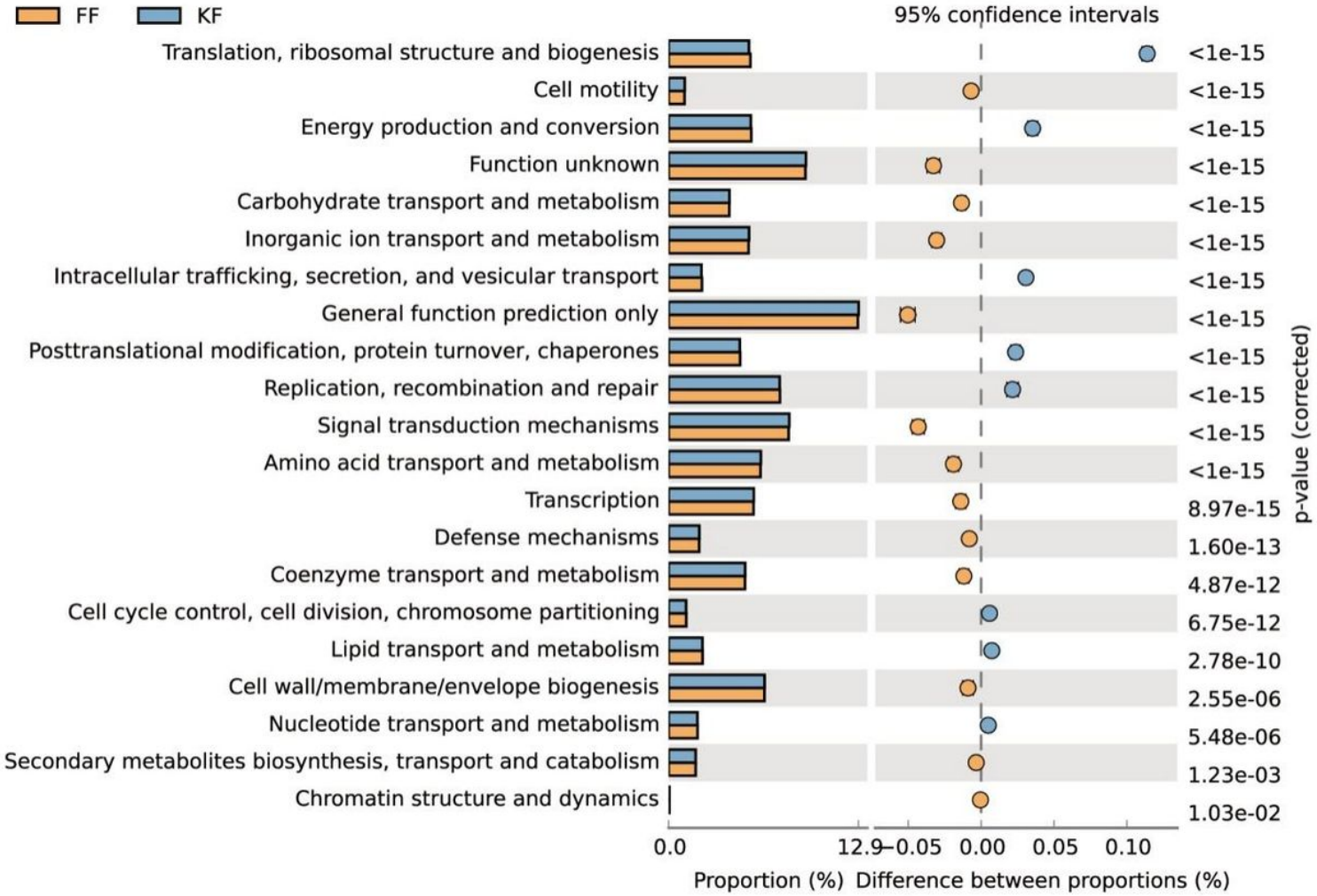

Figure 5

Analysis of COG metabolic pathways in fruits between ‘Fengzao’ (FF) and ‘Kyoho’ (KF). The figure shows the abundance ratio of different functions in the two cultivars. The middle bar plots and dot plots shows the difference ratio of different functions under the 95% confidence intervals, and the values on the right show the p values.

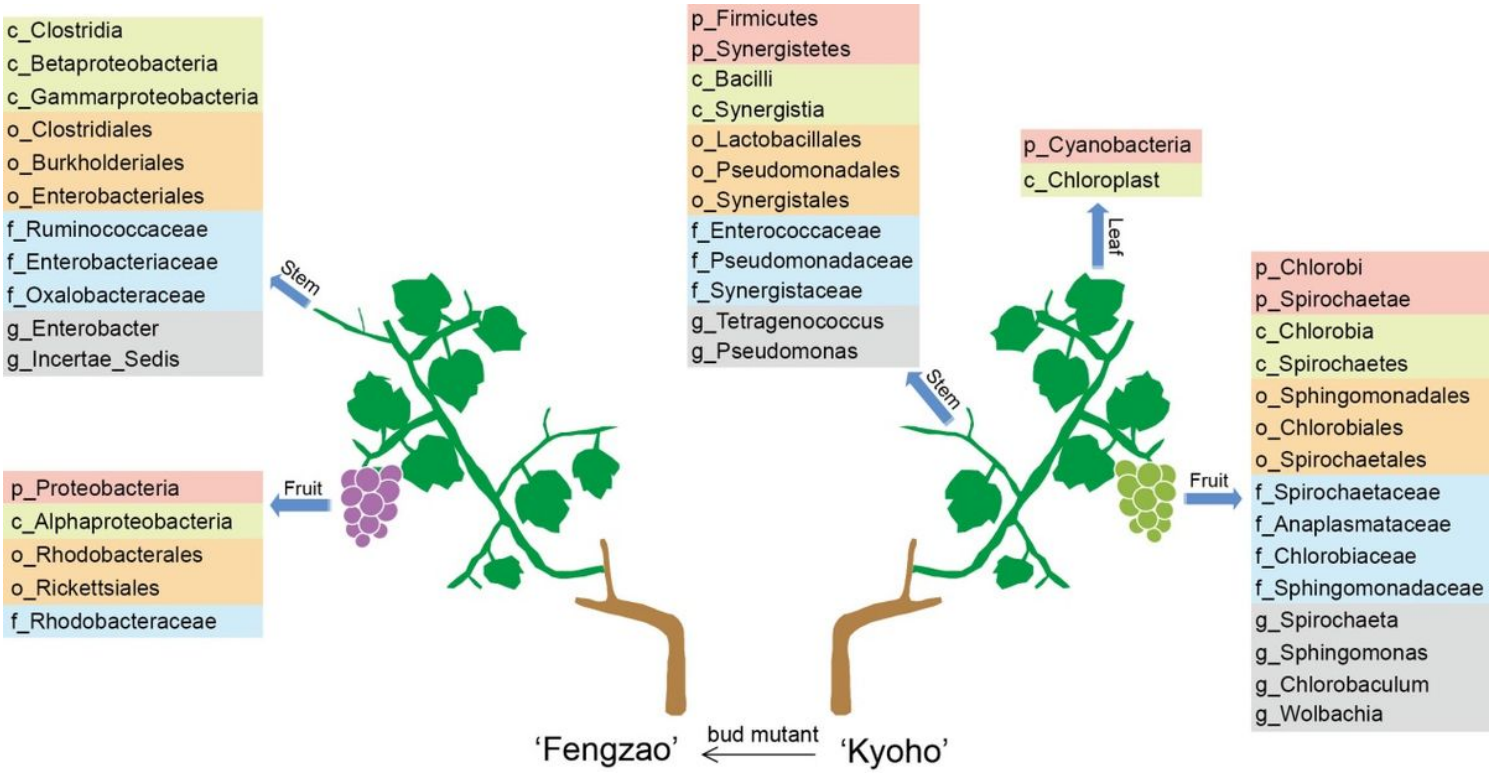

Figure 6

A model map showing the representative microbes in different samples of 'Fengzao' and 'Kyoho'. Only the microbial species with relatively high abundance are shown in the map, based on the results from Fig. 2. The different taxonomic levels (p: phylum, c: class, o: order, f: family, g: genus) are indicated with different colored background.

Supplementary Files

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- SupplementaryTablesandFigures.docx