Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Changes of active substances and antioxidant activity as well as microbial diversity and community structure during the natural fermentation of Dendrobium officinale Jiaosu

Lihong Jiang
Nankai University
Shuo Wang
18371305897@126.com

Nankai University
Min Zhang
Hefei University of Technology
Xuefeng Wu
Hefei University of Technology
Fuhuai Jia
Ningbo Yufangtang Biological Science and Technology Co., Ltd
Du Pan
Nankai University

Research Article

Keywords: antioxidant activity, Dendrobium fermentation, high-throughput gene sequencing, microbial diversity, superoxide dismutase

Posted Date: November 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11306644/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Changes of active substances and antioxidant activity as well as microbial diversity and community structure during the natural fermentation of *Dendrobium officinale* Jiaosu

Lihong Jiang[a], Shuo Wang[a]*, Min Zhang[b], Xuefeng Wu[b], Fuhuai Jia[c], Du Pan[a]

[a]College of Life Science, Nankai University, Tianjin, 300071, China;

[b]School of Food and Biological Engineering, Hefei University of Technology, Hefei, Anhui, 230009, China;

[c]Ningbo Yufangtang Biological Science and Technology Co., Ltd., Ningbo, Zhejiang, 315012, China

*Corresponding author: Shuo Wang. College of Life Science, Nankai University, Tianjin 300071, China. E-mail: 18371305897@126.com

Abstract: Dendrobium officinale has long been valued for its medicinal properties. This study investigated the alterations in bioactive compounds, antioxidant activity, and microbial communities during the 50-day natural fermentation process of Dendrobium jiaosu. The antioxidant activity fluctuated, reaching its peak around days 20-25. The total antioxidant capacity and superoxide dismutase activity showed a positive correlation, reaching peak levels around day 10. The correlation analysis showed positive relationships between superoxide dismutase activity and both sugars and phenols. An analysis of 16S rRNA sequencing revealed that unclassified Enterobacteriaceae and *Sporolactobacillus* were the main taxa in the initial stages, but were subsequently replaced by a diverse range of genera. The correlation analysis showed a positive relationship between Klebsiella and antioxidant markers. *Phenylobacterium showed a positive correlation with 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) scavenging, while Weissella and Sporolactobacillus exhibited negative correlations. Chitinophaga* exhibited an inverse correlation with amino acids. An anal

ysis of the metabolic pathway showed heightened activity in carbohydrate, amino acid, and lipid metabolism in the later stage. Significant changes in phytochemicals, antioxidants, and microbiota were observed during extended fermentation. The findings offer valuable insights for improving fermentation processes to enhance the quality and functionality of Dendrobium jiaosu.

Keywords: antioxidant activity, *Dendrobium* fermentation, high-throughput gene sequencing, microbial diversity, superoxide dismutase

Introduction

Dendrobium officinale Kimura et Migo belongs to the Orchidaceae family, which encompasses over 1400 species globally (Tang et al. 2017), and has been treasured in Asia and Northern Australia for its medicinal properties as a herbal remedy (Tao et al. 2019). The phytochemical profile of Dendrobium is extensive, encompassing polysaccharides, polyphenols, flavonoids, alkaloids, amino acids, and vitamins (He et al. 2022), among which polysaccharides predominate (Xing et al. 2014). Contemporary pharmacological research has demonstrated the multifaceted bioactivities of Dendrobium and its polysaccharide components, including anticancer, hepatoprotective, hypolipidemic, gastric mucosal protective (Ke et al. 2020), and antihypertensive properties (Tang et al. 2017).

High-throughput sequencing allows the concurrent sequencing of thousands to millions of DNA molecules in a sample without isolation and purification. It enables rapidly identifying microbial species and their relative abundances in a sample (Wu et al. 2017). Li et al. (2021) investigated microflora dynamics during the natural fermentation of three sufu variants using

high-throughput sequencing. Their study revealed 153 microbial species common to all three sufu types, with *Tetragonococcus*, *Bacillus*, *Acinetobacter,* and *Staphylococcus* being the most prevalent (Li et al. 2021).

Edible Plant Jiaosu (EPJ), or fermented juice, is a novel food product created by fermenting various vegetables, fruits, grains, seaweeds, and mushrooms with naturally occurring or added bacteria. It contains several bioactive components, such as polysaccharides, proteins, peptides, amino acids, and phenols (Jiang et al. 2021). EPJ production predominantly uses spontaneous fermentation (Zhang et al. 2022). Contemporary research on Jiaosu has focused on functional attributes (Han et al. 2019), antioxidant capacities, and practical applications. Although various Jiaosu products, such as watermelon, cantaloupe, and orange Jiaosu, have been produced (Jing et al. 2019). Investigations into Dendrobium Jiaosu are scarce. Qin et al. (2019) found attenuated antioxidant activity in naturally fermented Jiaosu compared to mixed fermentation Jiaosu, substantiating the primacy of mixed fermentation over single and spontaneous methods. However, few studies have verified this conclusion by examining the bacterial diversity and stability during Jiaosu's natural fermentation.

This study aims to investigate the various process variations that occur during the natural fermentation of Dendrobium jiaosu over different fermentation periods. To achieve this, we examine the composition and diversity of microbial communities in Dendrobium EPJ over a 0-50 day period using high-throughput sequencing. We examined the concentrations of particular bioactive compounds in Dendrobium EPJ and their corresponding antioxidant capacity. This study aims to establish guiding principles and insights for future production of Dendrobium and other edible and potable jiaosu (EPJ) products using natural fermentation, based on our investigation of

antioxidant capacity and other relevant analyses.

Materials and methods

Materials

All reagents used in the experiments were of analytical grade and required no further purification. Ethanol anhydrous (C_2H_6O) was purchased from Sinopharm Shanghai Co., Ltd. Potassium persulfate ($K_2S_2O_8$),1,1-diphenyl-2-picrylhydrazyl (DPPH),2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS), Potassium phosphate monobasic (KH_2PO_4) and Potassium phosphate dibasic anhydrous (K_2HPO_4) were purchased from Shanghai Macklin Biochemical Co., Ltd. Amino acid kit (BC1570-50T/48S), SOD activity kit (BC5165-100T/48S), Phytoflavonoids kit (BC1330-50T/24S), Total plant phenols kit (BC1340-50T/24S) and Total Antioxidant Capacity kit were purchased from Beijing Solarbio Science & Technology Co., Ltd.

Methods

Preparation of Dendrobium Jiaosu and sample collection

Dendrobium Jiaosu was produced in triplicate following the protocol described by Zou et al. (2018). Briefly, freshly harvested Dendrobium was air-dried after being washed with water. The processed Dendrobium was divided into three equal portions, and each portion was placed in a sterilized 250 mL glass fermenter manufactured by Changde BKMAM Biotechnology Co., Ltd., Changde, China. The three fermenters served as biological triplicates. The Dendrobium Jiaosu was fermented under anaerobic conditions at 37 °C for 50 days in fermenters supplemented with 100 mL of water and 10 g of brown sugar. Specimens were collected periodically at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 days throughout the fermentation process. The collected samples were stored at -20 °C until further analysis. In order to investigate the changes in microbial

communities during different fermentation stages, samples were selected from day 5 (Feju_1, representing the initial fermentation stage), day 15 (Feju_2, intermediate stage), day 30 (Feju_3, mid-late fermentation stage), and day 45 (Feju_4, final stage) for high-throughput sequencing analysis.

Determination of anti-oxidant activity

DPPH scavenging assay

The DPPH radical scavenging assay was performed following the method of Park et al. (2020) with minor modifications. A 2 mL aliquot of Dendrobium Jiaosu extract was diluted 10-fold with distilled water to obtain the test solution. The test solution (2 mL) was mixed with 2 mL of 0.2 mmol/L DPPH ethanol solution, vortexed thoroughly, and kept in the dark for 30 min. The absorbance at 517 nm was measured and recorded as A_1. An equal volume of absolute ethanol was used instead of the sample for the blank control, and the resulting absorbance was denoted as A_2. An equal volume of absolute ethanol was used as a reference control instead of the sample, and the corresponding absorbance was recorded as A_0. The DPPH radical scavenging activity was calculated using Equation 1.

$$DPPH\ radical\ scavenging\ rate\ /\ \% = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (1)$$

ABTS scavenging assay

The ABTS radical scavenging assay was conducted following the method of He et al. (2019)with minor modifications. The ABTS solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to achieve an absorbance of 0.7 at 734 nm. A 1 μL sample was added to 5 mL of the pre-diluted ABTS solution and incubated at 30 °C for 5 min. The absorbance at 734 nm was measured using deionized water as a blank (A_1). The ABTS solution was replaced with an equal

volume of PBS to control for any potential effects of the ABTS solution. The resulting absorbance was recorded as A_2. Similarly, to account for any background effects, the sample was substituted with an equal volume of PBS and the resulting absorbance was measured as A_0. The ABTS radical scavenging activity was then calculated using Equation 2.

$$ABTS\ \ scavenging\ rate\ /\ \% = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (2)$$

Total antioxidant capacity (T-AOC) assay

The Dendrobium EPJ's total antioxidant capacity (T-AOC) was measured as described by T-AOC assay kit (Solarbio, Beijing, China). The ability of antioxidants to reduce Fe^{3+}- 2,4,6-tripyridyl-s-triazine (Fe^{3+}-TPTZ) to produce blue Fe^{2+} - 2,4,6-tripyridyl-s-triazine (Fe^{2+}-TPTZ) reflects the total antioxidant capacity (Pellegrini et al. 2006).

Superoxide dismutase (SOD) activity assay

The Dendrobium EPJ's superoxide dismutase (SOD) activity was measured as described by SOD activity assay kit (Solarbio, Beijing, China). The reaction solution produces superoxide anion (O_2^-) through the xanthine and xanthine oxidase reaction system. O_2^- can reduce azotetrazolium to produce blue formazan, which absorbs at 560 nm; SOD scavenges O_2^-, thus inhibiting the formation of formazan; the darker the blue colour of the reaction solution, the lower the activity of SOD (Spitz and Oberley 1989).

Determination of bioactive substances

Determination of total phenols content

The Dendrobium EPJ's total phenols content was tested by means of a total phenols assay kit (Solarbio, Beijing, China). Under alkaline conditions, the phenolics reduce the tungsten-molybdenum acid to produce a blue compound with a characteristic absorption peak at 760 nm

(Wang et al. 2020). The absorbance value at 760 nm can be used to calculate the total phenolic content of the sample.

Determination of plant flavonoids

The Dendrobium EPJ's plant flavonoids were tested by means of a plant flavonoids assay kit (Solarbio, Beijing, China). In alkaline nitrite solution, plant flavonoids form a red complex with aluminium ions with a characteristic absorption peak at 470 nm (Tattini et al. 2004). The absorbance value at 470 nm can be used to calculate the plant flavonoid content of the sample.

Determination of amino acids

The amino acid content of Dendrobium EPJ was determined using an amino acids assay kit (Solarbio, Beijing, China). The α-amino group of the amino acid reacts with hydrated ninhydrin to produce a blue-purple compound with a characteristic absorption peak at 570 nm. the absorbance at 570 nm is measured to calculate the amino acid content (Lee et al. 2003). The analysis was performed according to the instructions of the manufacturer.

Determination of total sugar content

The total sugar content of Dendrobium EPJ was quantified using a total sugar assay kit (Solarbio, Beijing, China). The category of total sugars primarily comprises reducing sugars such as glucose, fructose, pentose, lactose, sucrose, maltose and partially hydrolysed starch. These compounds have the potential to undergo hydrolysis, resulting in the production of reducing monosaccharides within the specified assay conditions. Upon hydrolysis of total sugars into reducing sugars, the reducing sugars have the potential to be converted into amino compounds. This reaction results in the substance exhibiting a reddish-brown colour when subjected to heat with DNS reagents under alkaline conditions.

The intensity of the reddish-brown colour generated is directly correlated with the quantity of reducing sugar present. This colorimetric reaction is frequently employed for the determination of the overall sugar content in a sample.

DNA extraction and high-throughput 16S rRNA gene sequencing

The total genomic DNA of the community was extracted using the E.Z.N.A.™ MagBind Stool DNA Kit manufactured by Zhejiang Yuxiang Biotechnology Co., Ltd., Jiaxing, China. The presence of genomic DNA was verified through 2.0% (w/v) agarose gel electrophoresis. To examine the microbiological community, the V3-V4 hypervariable regions of the 16S rRNA gene were amplified using the 2× Hieff Robust PCR Master Mix (Yeasen, 10105ES03, China). The process involved the utilisation of forward and reverse amplicon PCR primers (CCTACGGGNGGCWGCAG). Sequencing was conducted utilising the Illumina MiSeq system (Illumina MiSeq, USA) in accordance with the manufacturer's guidelines.

Statistical analysis

The data pertaining to the antioxidant activity and bioactive substances of Dendrobium EPJ were analysed and presented using Origin 2021. All determinations were performed in triplicate ($n = 3$) and are expressed as the mean ± standard deviation (SD). Differences among fermentation time points were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test, with $P < 0.05$ considered statistically significant. In Figs. 1 and 2, error bars represent the SD of three independent replicates, and means sharing different lowercase letters indicate significant differences among fermentation time points ($P < 0.05$). The Operational Taxonomic Unit (OTU) richness was used to evaluate diversity indices, such as the Chao, Simpson, and Shannon indices.

MetaCyc, accessed at MetaCyc.org, serves as a comprehensive repository of data about metabolic pathways and enzymes found in all domains of life. The database comprises 2,749 pathways extracted from over 60,000 publications, establishing it as the most comprehensive curated collection of metabolic pathways presently accessible. The data in MetaCyc is obtained from evidence and carefully curated, establishing it as a reliable reference source for metabolism (Caspi et al. 2019).

The Mothur software was utilised to evaluate all diversity indices. Furthermore, coverage indices of 0.9995 to 1.0000 indicated successful identification of the communities, and rarefaction curves were generated to evaluate the adequacy of the samples in terms of the observed OTU numbers. SparCC was used to compute correlation coefficients between communities and OTUs, and the R corrplot program was employed to produce correlation matrix heatmaps. Network graphs were created utilising the ggraph package in the R programming language (https://cran.r-project.org/).

Function prediction

Functional prediction analysis of bacteria and archaea using PICRUSt v.1.1.4 software, by comparing existing 16S rRNA gene sequencing data with a microbial reference genome database of known metabolic functions, enabling the prediction of bacterial and archaeal metabolic functions.

Results

Anti-oxidant activity in fermented Dendrobium

The stable free radical known as DPPH is frequently employed for in vitro assessment of the free radical scavenging activity of natural or standard compounds (La et al. 2021). The trend of the

DPPH scavenging rate is illustrated in Fig. 1, showing a gradual decrease until day 15, when it reached a minimum value of 64.52%. Subsequently, there was a progressive increase, reaching a peak of 97.40% on day 25. After day 25, the DPPH scavenging rate showed consistent fluctuations between 88.91% and 93.20%.

The assessment of the antioxidant efficiency of peptides in various systems often involves the use of the ABTS methods to measure radical scavenging capacity (Yang et al. 2020). Fig. 1 depicts that the observed trend for the ABTS scavenging rate is similar to that of the DPPH scavenging rate. The ABTS scavenging rate exhibited a decreasing trend, followed by an increasing trend until day 20, reaching its lowest value of 74.06% on day 15. Subsequently, it attained its maximum value of 87.75% on the 20th day, after which it experienced a slight decrease and remained relatively stable, fluctuating around 80%.

The total antioxidant capacity (T-AOC) of Dendrobium EPJ consists of a variety of antioxidant compounds and enzymes. As depicted in Fig. 1, the T-AOC value demonstrated a rising trajectory until day 10, reaching a peak of 0.522 μmol/mL. Following this, it exhibited a decreasing trend before reaching a stable level of approximately 0.2 μmol/mL after day 25.

Superoxide dismutase (SOD) facilitates the transformation of superoxide anion radicals (O_2^-) into H_2O_2 and O_2, thereby protecting oxygen-metabolizing cells from the harmful effects of superoxide radicals (Xikeranmu et al. 2019). Fig. 1 demonstrates that SOD activity exhibits a similar trend to T-AOC. Before day 25, the activity of SOD exhibited an initial increase followed by a subsequent decrease, reaching its peak at 209.43 U/mL on the 10th day. Following day 25, the activity of SOD fluctuated irregularly between 20.08 and 74.26 U/mL.

Phenols may have a protective role in reducing cardiovascular disease (CVD) by improving

endothelial function and exerting antiplatelet and anti-inflammatory effects (Wisnuwardani et al. 2022). As shown in Fig. 2, the total phenol content decreased over the fermentation period, reaching 5.80 mg/mL by day 50.

Flavonoids are diverse specialized metabolites found in many plant species. In addition to providing UV radiation protection, they also scavenge reactive oxygen species and participate in microbial interactions (Lei et al. 2018). According to Fig. 2, the concentration of plant flavonoids decreased from 4.15 mg/mL on day 0 to a minimum of 0.78 mg/mL on day 10, then rose sharply to a maximum of 17.20 mg/mL on day 20, after which it fluctuated between 2.20 and 8.67 mg/mL until the end of fermentation.

Amino acids are essential building blocks for protein synthesis. As shown in Fig. 2, there is a noticeable pattern of fluctuating amino acid content, peaking at 54.39 μmol/mL on day 50.

Sugar is the main carbon source for microorganisms in the fermentation process, and its different forms are closely linked to microbial metabolism. As shown in Fig. 2, the sugar content initially peaked at 337.2 mg/mL on day 5, then rapidly dropped to 164.2 mg/mL by day 20. After the 20th day, the concentration fluctuated around 160 mg/mL.

Correlation analysis between antioxidant activity and bioactive substances

Previous findings have shown that Dendrobium Jiaosu exhibits strong antioxidant properties. We used Pearson's correlation coefficient to analyse the interrelationships among antioxidant parameters during fermentation. The study examined the DPPH and ABTS radical scavenging activities, T-AOC, SOD activity, amino acid, flavonoid, total sugar, and total phenolic content of fermented Dendrobium Jiaosu. Fig. 3 depicts significant positive correlations between DPPH and

ABTS radical scavenging activities ($P < 0.05$), as well as between T-AOC and SOD activity ($P < 0.05$). A significant positive correlation was observed between total sugar and phenolic content ($P < 0.05$). SOD activity displayed significant positive correlations with total sugar and phenolic content ($P < 0.05$), showing that the antioxidant capacity of naturally fermented Dendrobium Jiaosu is associated with these phytochemical levels.

Sequencing sample data analysis

The sample sequences were bioinformatically assessed at a similarity level of 97%. The valid sequence data were obtained through high-throughput sequencing of Dendrobium EPJ samples (Feju_1/Feju_2/Feju_3/Feju_4) collected at four distinct time points.

Rarefaction curve

The rarefaction curve is graphed with the number of reads on the horizontal axis and the number of OTUs at the Sobs level on the vertical axis. This curve is contingent upon the series randomly selected from the sample. Furthermore, this method can be utilised not only to evaluate the adequacy of the quantity of sequenced samples but also to assess the diversity of species in samples with differing levels of sequencing data. A flat curve suggests that the existing sequencing data is adequate, and further sequencing efforts would not lead to a substantial increase in Operational Taxonomic Units (OTUs) at the Sobs level. Fig. 4A illustrates that the OTU index at the Sobs level exhibits a significant increase with the number of sequences when the number of sampled reads is less than 10,000. Conversely, as the number of sampled reads surpasses 10,000, the OTU index increases only slightly with the number of sequence reads beyond 10,000, and the curve shows a tendency to plateau, suggesting that the quantity of sequencing data is adequate (Online Resource 5).

Furthermore, Fig. 4B illustrates the dilution curve for the Shannon index. As depicted in the figure, the curve demonstrates a rapid flattening, indicating that the quantity of sequencing data is adequate for the accurate representation of bacterial information within the Dendrobium EPJ (Online Resource 6).

α-Diversity index analysis

Alpha diversity analysis refers to the assessment of species diversity within the same biological community or sample, focusing on the species richness within a single ecosystem and the relative evenness of these species. Put simply, Alpha diversity aims to quantify how rich or evenly distributed the biological species are within a local area. Common metrics of Alpha diversity include species richness, the Shannon index, and the Simpson index, among others.

The analysis of Table 1 shows that all Dendrobium EPJ samples had coverage indices of 0.9995 to 1.0000, indicating a successful identification of all bacterial sequences and a low chance of non-detection. The Ace and Chao indices are commonly used to estimate overall species richness (Bai et al. 2015). The data in Table 1 present an overall increasing trend in the Chao index and a decreasing and then increasing trend in the Ace index. This indicates a decrease followed by an increase in the microbial community diversity of Dendrobium EPJ during fermentation. These trends are likely due to acid accumulation in the early stage, which inhibits acid-intolerant bacteria and subsequently leads to a decline in dominant acid-producing bacteria, weakening their antagonistic effects on other bacteria. Consequently, microbial community diversity increases during the later stages of fermentation.

Table 1. The diversity indices for four Dendrobium EPJ samples

Sample	Shannon	Chao	Ace	Simpson	Shannoneven	Coverage
FEJU_1	1.26	44	92	0.32	0.36	0.9996

FEJU_2	1.20	80	59	0.39	0.32	0.9995
FEJU_3	2.29	100	101	0.15	0.50	0.9998
FEJU_4	3.48	221	221	0.09	0.64	1.0000

The Simpson and Shannon indices assess community diversity (Soto del Rio et al. 2017). When using Simpson's index to assess microbial diversity in fermentation broth, a higher index value indicates a less diverse microbial community (Yao et al. 2020). The data in Table 1 show that the Shannon index decreased and then increased, while the Simpson index increased and then decreased. These trends further support the previously documented decrease and subsequent rise in microbial diversity of Dendrobium EPJ during fermentation.

Bacterial communities in Dendrobium EPJ

After consolidating the genera with a relative abundance of less than 1% in all samples into the "others" category, Fig. 5A depicts the relative abundance of different bacterial species at the genus level across all samples. Fig. 5B is a heatmap of bacterial species at the genus level in the four Dendrobium EPJ samples (Online Resource 1).

The findings depicted in Fig. 5A and Fig. 5B demonstrate that during the early stages of fermentation (Feju_1/Feju_2), the predominant genera in Dendrobium EPJ were *Sporolactobacillus* and unclassified_*Enterobacteriaceae*. During the middle and late stages of fermentation (Feju_3/Feju_4), there was a significant decrease in the presence of *Sporolactobacillus* and unclassified_*Enterobacteriaceae* while other genera swiftly substituted them. Meanwhile, the substantial turnover and variability of bacterial colonies during the middle and late stages of fermentation may have affected the fluctuating trend of the most active substances and antioxidant capacity in Dendrobium EPJ throughout this period. The heightened microbial diversity noted in the middle and late phases of fermentation may be ascribed to the reduction in *Sporolactobacillus* and unclassified *Enterobacteriaceae* populations as they transition

into the late stages of fermentation. As a result, the population of bacterial antagonists produced by these organisms, such as the *Sporolactobacillus* isolate (L2407) (Holzapfel and Botha 1988) or *Enterobactin* (Rohrbacher et al. 2022), decreased. This decrease facilitated the rapid proliferation of previously suppressed microorganisms, producing their bacterial antagonists or modifying the fermentation environment to become unfavorable for *Sporolactobacillus* and unclassified *Enterobacteriaceae*. Consequently, the prevalence of *Sporolactobacillus* and unclassified *Enterobacteriaceae* was further diminished and ultimately eradicated. At the same time, because *Sporolactobacillus* is an anaerobic bacterium, its rapid death in the later stages suggests that air enters the fermenter during the later stages of fermentation, which is unavoidable due to the long fermentation time (Abat et al. 2015).

Correlation analysis of Bacterial communities with antioxidant activity and bioactive substances

Pearson correlation analysis was employed to investigate the correlations between the identified bacteria at each stage of Dendrobium Jiaosu fermentation and the antioxidant activity indices and bioactive substances. The findings are shown in Fig. 6 as a correlation heatmap. The figure displays only the results with p-values less than 0.05 and absolute correlation values greater than 0.8. When the absolute value of the correlation coefficient (|r|) exceeds 0.8, the symbol '*' denotes a statistically significant correlation ($P < 0.05$). *Phenylobacterium* showed a significant positive correlation with the ABTS scavenging rate, whereas *Weissella* and *Sporolactobacillus* showed significant negative correlations with the ABTS scavenging rate. *Chitinophaga* showed a marked inverse relationship with amino acid content, while unclassified bacteria showed a significant positive correlation. *Klebsiella* showed significant positive correlations with SOD

activity and T-AOC.

PICRUSt functional predictive analysis

The KEGG database is a comprehensive bioinformatics resource that integrates genes, gene functions, metabolic pathways, and other relevant data. It allows for analysing metabolic pathways and the functional roles of biological systems, such as cells, at the molecular level (Tanabe and Kanehisa 2012).

The metabolic pathways of microorganisms involved in Dendrobium EPJ fermentation, among the top 50 in abundance, are annotated at pathway Level 1, primarily in Metabolism, Human Diseases, and Genetic Information Processing. The Feju_4 sample shows a significant dominance of genes related to metabolism, likely due to the high abundance of the microbial community at the end of the natural fermentation process of Dendrobium EPJ.

Fig. 7A shows the main KEGG metabolic pathways at pathway Level 2, including the top 50 classes. In the Dendrobium EPJ fermentation process, analysis across all stages revealed that the most prevalent genes for the entire fermentation process were linked to lipid metabolism, followed by carbohydrate metabolism, amino acid metabolism, and glycan biosynthesis and metabolism (Online Resources 3, 4).

The processes of carbohydrate, amino acid, energy, cofactor and vitamin, and lipid metabolism are essential for the growth and reproduction of microorganisms (Song et al. 2021). The active expression of these metabolic pathways enables the use of starch, cellulose, protein, and other macronutrients in the raw material and its metabolites in Dendrobium EPJ. Microorganisms convert these resources into organic acids, amino acids, or other bioactive substances through fermentation.

The distinct metabolic pathways of Dendrobium Jiaosu samples at different fermentation stages were annotated using MetaCyc, as depicted in Fig. 7B. A significant metabolic distinction was observed between late-stage Dendrobium Jiaosu samples (Feju_3, Feju_4) and early to mid-stage samples (Feju_1, Feju_2) about the pathway: aerobic respiration I (cytochrome c) (PWY-3781). Fermentation in sealed fermenters led to a restricted presence of oxygen during the initial to intermediate phases, causing a decrease in aerobic respiration. Over time, it is hypothesized that the fermenters became incapable of entirely preventing the ingress of external air, potentially resulting in a gradual rise in oxygen levels and the consequent demise of anaerobic bacteria near the liquid surface. This hypothesized phenomenon could have facilitated the proliferation of aerobic bacteria near the surface, leading to notable differences in the bacterial community composition between the early/mid-stage samples (Feju_1, Feju_2) and the late-stage samples (Feju_3, Feju_4). Furthermore, late-stage samples exhibited a significant rise in the prevalence of diverse lipid metabolism pathways (e.g. PWY-7663, FASYN-ELONG-PWY, PWY-5973), aligning with the observations at pathway Level 2. The prevalence of amino acid metabolism also exhibited a significant increase in the late-stage samples, consistent with the findings at pathway Level 2 and the previously noted elevation in Dendrobium Jiaosu amino acid content (Online Resource 2).

Discussion

This study investigated the variations of bioactive compounds and antioxidant activity during the natural fermentation of Dendrobium EPJ. Additionally, the study examined the variations in bacterial relative abundances at different fermentation stages of Dendrobium EPJ using high-throughput 16S rRNA gene sequencing and combined it with the KEGG database for functional

373 predictions of the bacterial community.

374 Using high-throughput 16S rRNA sequencing data as the foundation, clustering analyses and
375 taxonomic annotations are performed to classify microbial community types and their distinctions.
376 Functional gene compositions are predicted via mapping from OTUs to genes or proteins. These
377 inferred functional genes are then categorized according to the KEGG database, mapped to specific
378 pathways, and subjected to functional enrichment analysis to identify actively or notably altered
379 metabolic routes. Such findings serve as the basis for explaining the correlations between the diversity
380 of microbial communities and variations in environmental factors.

381 In the early stage of fermentation of Dendrobium EPJ fermentation, there is a consistent
382 reduction in the overall phenol content. This phenomenon can be linked to the dissolved oxygen
383 level in the fermentation medium and the microbial-driven breakdown of phenolic compounds,
384 leading to their conversion into alternative substances. Both the DPPH scavenging and ABTS
385 scavenging rates showed a pattern of initially decreasing and then increasing. This relationship
386 was demonstrated by Kumar (2013) and Henriquez (2004), who examined the connection between
387 the scavenging rates of DPPH and ABTS and the presence of phenols. However, the trends of the
388 DPPH scavenging rate and ABTS scavenging rate in this study differed from those of total
389 phenols. This suggests that the changes in the DPPH scavenging rate and ABTS scavenging rate of
390 Dendrobium EPJ were independent of total phenols, which aligns with the findings of Zhang
391 (2020).

392 Moreover, Dendrobium EPJ demonstrated a pattern of initial increase followed by a decrease
393 in both total antioxidant capacity and SOD activity, indicating a potential close relationship
394 between the two. The concentration of amino acids in Dendrobium EPJ exhibited an initial

increase followed by a subsequent decrease. As stated by Liu (2021), the free amino acids present in the fermentation broth originate from the degradation of proteins by proteases produced by microorganisms. The observed decrease in amino acids in Dendrobium EPJ can be attributed to the initial rapid increase in amino acids resulting from the breakdown of proteins by proteases produced by microorganisms. As the fermentation process advanced and the microorganisms in Dendrobium EPJ proliferated, an increased demand for amino acids resulted in a continual reduction in the amino acid content.

However, during the later stages of fermentation, Dendrobium EPJ showed oscillating trends in most antioxidant capacity indicators and active substances. These parameters encompass the DPPH radical scavenging rate, ABTS radical scavenging rate, total antioxidant capacity, and SOD activity, which is consistent with the results reported by Wu et al. (2017). The observed phenomenon is likely attributable to the diverse bacterial colony and the rapid fluctuations in bacterial community composition during the late stage of Dendrobium EPJ fermentation. These factors contribute to challenges regulating the various antioxidant capacity indicators and active substances, highlighting the inherent difficulty in consistently producing Dendrobium EPJ products with extended fermentation cycles using natural fermentation methods.

The traditional EPJ fermentation process is a complex natural fermentation method that encompasses a variety of microorganisms (Alves et al. 2010). In the early stage of Dendrobium EPJ natural fermentation, unclassified *Enterobacteriaceae* consistently emerged as the predominant group of organisms. *Enterobacteriaceae*, which are frequently present in the gut flora, are prevalent human pathogens associated with a range of illnesses such as cystitis, pyelonephritis, septicemia, pneumonia, peritonitis, and meningitis (Nordmann et al. 2011). In

addition to *Enterobacteriaceae*, *Sporolactobacillus* was the second most prevalent group in the Dendrobium EPJ samples. *Sporolactobacillus* is a Gram-positive bacterium classified within the lactic acid bacteria group (Axelsson 2004). *Sporolactobacillus* is believed to offer various health advantages, including ameliorating lactose malabsorption (Jiang et al. 1996), lowering blood cholesterol levels (du Toit et al. 1998), preventing antibiotic-associated diarrhea and traveller's diarrhea (Salazar-Lindo et al. 2004), boosting the immune system (Gill 1998), and providing protection against pathogen invasion and infection (Asahara et al. 2004). Consequently, in the practical manufacturing process of Dendrobium EPJ, *Sporolactobacillus* can be introduced as the predominant strain to effectively suppress the proliferation of detrimental bacteria, while concurrently enhancing the production of flavor compounds and nutrients in Dendrobium EPJ.

In the later stages of Dendrobium EPJ fermentation, the dominance of *Enterobacteriaceae* and *Sporolactobacillus* waned as other bacteria, including the potentially pathogenic Pseudomonas, began to proliferate (Idigo et al. 2022). Moreover, Tamang (2016) demonstrated that fermented foods often exhibit elevated levels of pathogenic bacteria during fermentation, potentially resulting in health issues such as pneumonia and food poisoning. In the previously discussed metabolic pathways of microorganisms in Dendrobium EPJ fermentation at Pathway Level 1 (top 50 in abundance), there was an observation that human disease-related metabolic pathways exhibited higher abundance in the late fermentation period (Feju_3/Feju_4) compared to the early fermentation period (Feju_1/Feju_2). This may suggest the active expression of pathogenic genes during the later stages. This indicates that extended natural fermentation is challenging to control and may pose potential risks.

Nevertheless, a limitation of this study is that 16S rRNA gene sequencing is capable of

identifying bacteria only at the genus level and lacks the ability to differentiate further at the species level. Various species belonging to the same genus may exhibit significant differences in their structure and function, which cannot be differentiated solely through 16S rRNA gene sequencing (Jovel et al. 2016). For example, not all *Enterobacteriaceae* species are considered opportunistic pathogens. Our findings did not allow for the precise identification of the species.

Moreover, it has been noted that 16S rRNA gene sequencing might not be able to identify certain low abundance bacteria present in the samples (Wagner et al. 2016). The failure to detect and identify all bacterial species hinders our comprehension of microbial diversity and constrains our ability to make functional inferences. Future research endeavors should integrate whole-genome sequencing or other omics methodologies to enhance the understanding of the taxonomic compositions and functional capacities of bacteria during the process of Dendrobium Jiaosu fermentation at an increased level of detail (Franzosa et al. 2014).

In summary, although 16S rRNA gene sequencing has offered valuable insights into the dynamics of microbial communities, the technique is inherently limited in its ability to differentiate bacteria at the species level and to comprehensively capture both structural and functional information. Therefore, it is important to consider our findings in light of these limitations, and further research with improved phylogenetic and functional profiling is necessary. The bacterial dynamics during Dendrobium EPJ fermentation are complex and unpredictable, leading to substantial fluctuations in antioxidant capacity, active substances, and the proliferation of pathogenic bacteria over time. Different bacterial species may display varied dynamics as a result of factors such as osmotic pressure, acidity, and interactions among different microorganisms. Hence, an in-depth investigation of the microbial transformation process during

the fermentation of Dendrobium EPJ will be a crucial focus for future research.

Conclusion

This study explored the fermentation dynamics of Dendrobium Jiaosu, focusing on bioactive compounds, antioxidant activity, and microbial communities. Key findings include fluctuating antioxidant indices peaking around days 20-25, shifting microbial populations from Enterobacteriaceae to genera like Pseudomonas, and positive correlations between superoxide dismutase activity and sugars and phenolic compounds. Metabolic pathway analysis revealed heightened pathways related to carbohydrates, amino acids, lipid metabolism, and aerobic respiration in later fermentation stages. These insights inform the optimization of fermentation processes for enhancing the quality and functionality of Dendrobium Jiaosu.

Author Information

Author ORCIDs

Lihong Jiang https://orcid.org/0000-0002-3042-6817

Min Zhang https://orcid.org/0000-0002-1653-2082

Additional request

The following supplementary material is provided with this manuscript:

Online Resource 1: Relative abundances of bacterial taxa at the species level in the four sequenced fermentation samples (FEJU_1 to FEJU_4); (underlying data for Fig. 5).

Online Resource 2: PICRUSt2-predicted KEGG and MetaCyc pathway abundances across the fermentation stages; (underlying data for Fig. 7B).

Online Resource 3: PICRUSt2-predicted KEGG pathway level 2 abundances across the fermentation stages; (underlying data for Fig. 7A).

Online Resource 4: PICRUSt2-predicted KEGG pathway level 2 abundances, merged and sorted; (underlying data for Fig. 7A).

Online Resource 5: Rarefaction curve data at the Sobs level for the four samples; (underlying data for Fig. 4A).

Online Resource 6: Rarefaction curve data for the Shannon index for the four samples; (underlying data for Fig. 4B).

References

Abat C, Kerbaj J, Dubourg G, Garcia V, Rolain JM (2015) Human Infection with *Sporolactobacillus laevolacticus*, Marseille, France. Emerg Infect Dis 21:2106-2108. https://doi.org/10.3201/eid2111.151197

Alves JA, Lima LCdO, Dias DR, Nunes CA, Schwan RF (2010) Effects of spontaneous and inoculated fermentation on the volatile profile of lychee (*Litchi chinensis Sonn*) fermented beverages. International Journal of Food Science & Technology 45:2358-2365. https://doi.org/10.1111/j.1365-2621.2010.02409.x

Asahara T, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y (2004) Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing *Escherichia coli* O157:H7. Infect Immun 72:2240-2247. https://doi.org/10.1128/iai.72.4.2240-2247.2004

Axelsson L (2004) Lactic acid bacteria: classification and physiology. Food Science and Technology-New York-Marcel Dekker- 139:1-66.

Bai L, Cui J, Jie W, Cai B (2015) Analysis of the community compositions of rhizosphere fungi in

505 soybeans continuous cropping fields. Microbiological Research 180:49-56.
506 https://doi.org/10.1016/j.micres.2015.07.007
507 Caspi R, Billington R, Keseler IM, Kothari A, Krummenacker M, Midford PE, Ong WK, Paley S,
508 Subhraveti P, Karp PD (2019) The MetaCyc database of metabolic pathways and enzymes - a
509 2019 update. Nucleic Acids Research 48:D445-D453. https://doi.org/10.1093/nar/gkz862
510 du Toit M, Franz CMAP, Dicks LMT, Schillinger U, Haberer P, Warlies B, Ahrens F, Holzapfel WH
511 (1998) Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding
512 trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content.
513 International Journal of Food Microbiology 40:93-104. https://doi.org/10.1016/S0168-
514 1605(98)00024-5
515 Franzosa EA, Morgan XC, Segata N, Waldron L, Reyes J, Earl AM, Giannoukos G, Boylan MR, Ciulla
516 D, Gevers D, Izard J, Garrett WS, Chan AT, Huttenhower C (2014) Relating the
517 metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A 111:E2329-
518 2338. https://doi.org/10.1073/pnas.1319284111
519 Gill HS (1998) Stimulation of the Immune System by Lactic Cultures. International Dairy Journal 8:535-
520 544. https://doi.org/10.1016/S0958-6946(98)00074-0
521 Han Q, Zhao JM, Gao XQ, Yu M, Han ZY, Li XJ, Xiao ZG (2019) Development status of functional
522 fermented enzymes. Science and Technology of Food Industry 40:337-340.
523 https://doi.org/10.13386/j.issn1002-0306.2019.01.060
524 He S, Zhang Y, Sun H, Du M, Qiu J, Tang M, Sun X, Zhu B (2019) Antioxidative Peptides from
525 Proteolytic Hydrolysates of False Abalone (*Volutharpa ampullacea perryi*): Characterization,
526 Identification, and Molecular Docking. Marine Drugs 17:116.

https://doi.org/10.3390/md17020116

He Y, Li L, Chang H, Cai B, Gao H, Chen G, Hou W, Jappar Z, Yan Y (2022) Research progress on extraction, purification, structure and biological activity of *Dendrobium officinale* polysaccharides. Front Nutr 9:965073. https://doi.org/10.3389/fnut.2022.965073

Henriquez C, Aliaga C, Lissi E (2004) Kinetics profiles in the reaction of ABTS derived radicals with simple phenols and polyphenols. Journal of the Chilean Chemical Society 49:65-67. https://doi.org/10.4067/s0717-97072004000100011

Holzapfel WH, Botha SJ (1988) Physiology of Sporolactobacillus strains isolated from different habitats and the indication of in vitro antagonism against Bacillus species. International Journal of Food Microbiology 7:161-168. https://doi.org/10.1016/0168-1605(88)90008-6

Idigo AJ, Wells JM, Brown ML, Wiener HW, Griffin RL, Cutter G, Shrestha S, Lee RA (2022) Clinical risk factors for admission with *Pseudomonas* and multidrug-resistant *Pseudomonas* community-acquired pneumonia. Antimicrobial Resistance and Infection Control 11. https://doi.org/10.1186/s13756-022-01137-4

Jiang K, Zhao Y, Liang C, Xu Z, Peng J, Duan C, Yang G (2021) Composition and antioxidant analysis of jiaosu made from three common fruits: watermelon, cantaloupe and orange. CyTA - Journal of Food 19:146-151. https://doi.org/10.1080/19476337.2020.1865462

Jiang T, Mustapha A, Savaiano DA (1996) Improvement of Lactose Digestion in Humans by Ingestion of Unfermented Milk Containing *Bifidobacterium longum*. Journal of Dairy Science 79:750-757. https://doi.org/10.3168/jds.S0022-0302(96)76422-6

Jing XY, Li JX, Wang N, Yang HB (2019) Feasibility study on garbage enzyme for domestic wastewater treatment. Technology of Water Treatment 45:98-101.

549 Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, Perry T, Kao D, Mason AL, Madsen KL,
550 Wong GKS (2016) Characterization of the Gut Microbiome Using 16S or Shotgun
551 Metagenomics. Frontiers in Microbiology 7. https://doi.org/10.3389/fmicb.2016.00459
552 Ke Y, Zhan L, Lu T, Zhou C, Chen X, Dong Y, Lv G, Chen S (2020) Polysaccharides of Dendrobium
553 officinale Kimura & Migo leaves protect against ethanol-induced gastric mucosal injury via the
554 AMPK/mTOR signaling pathway in vitro and in vivo. Frontiers in Pharmacology 11:526349.
555 https://doi.org/10.3389/fphar.2020.526349
556 Kumar SA, Paul AD, Tanveer N (2013) Bioactivity guided extraction of Seabuckthorn (*Hippophae*
557 *rhamnoides* L. ssp *turkestanica*) leaves. Journal of Scientific & Industrial Research 72:307-311.
558 La J, Kim MJ, Lee J (2021) Evaluation of solvent effects on the DPPH reactivity for determining the
559 antioxidant activity in oil matrix. Food Science and Biotechnology 30:367-375.
560 https://doi.org/10.1007/s10068-020-00874-9
561 Lee SW, Lim JM, Bhoo SH, Paik YS, Hahn TR (2003) Colorimetric determination of amino acids using
562 genipin from *Gardenia jasminoides*. Analytica Chimica Acta 480:267-274.
563 https://doi.org/10.1016/S0003-2670(03)00023-0
564 Lei Z, Sumner BW, Bhatia A, Sarma SJ, Sumner LW (2018) UHPLC-MS Analyses of Plant Flavonoids.
565 Current Protocols in Plant Biology 4. https://doi.org/10.1002/cppb.20085
566 Li X, He Y, Yang W, Mu D, Zhang M, Dai Y, Zheng Z, Jiang S, Wu X (2021) Comparative analysis of
567 the microbial community and nutritional quality of sufu. Food Sci Nutr 9:4117-4126.
568 https://doi.org/10.1002/fsn3.2372
569 Liu Y, Mei B, Chen D, Cai L (2021) GC-MS metabolomics identifies novel biomarkers to distinguish
570 tuberculosis pleural effusion from malignant pleural effusion. J Clin Lab Anal 35:e23706.

https://doi.org/10.1002/jcla.23706

Nordmann P, Naas T, Poirel L (2011) Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases 17:1791-1798. https://doi.org/10.3201/eid1710.110655

Park MS, Park MS, Lee KG (2020) Antioxidative activities of volatile and non-volatile extracts of *Schisandra chinensis* Baill fruit. Flavour and Fragrance Journal 35:435-442. https://doi.org/10.1002/ffj.3582

Pellegrini N, Serafini M, Salvatore S, Del Rio D, Bianchi M, Brighenti F (2006) Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays. Molecular Nutrition & Food Research 50:1030-1038. https://doi.org/10.1002/mnfr.200600067

Qin Y, Xiong Y, Lu L, Bian J, Zhang Q (2019) Antioxidant activity of Rubus idaeus-Punica granatum compound juice enzyme prepared by different fermentation technologies. China Brewing 38:105-109. https://doi.org/10.11882/j.issn.0254-5071.2019.10.021

Rohrbacher C, Zscherp R, Weck SC, Klahn P, Ducho C (2022) Synthesis of an antimicrobial enterobactin-muraymycin conjugate for improved activity against Gram-negative bacteria. Chemistry - A European Journal 29:e202202408. https://doi.org/10.1002/chem.202202408

Salazar-Lindo E, Miranda-Langschwager P, Campos-Sanchez M, Chea-Woo E, Sack RB (2004) *Lactobacillus casei* strain GG in the treatment of infants with acute watery diarrhea: A randomized, double-blind, placebo controlled clinical trial [ISRCTN67363048]. BMC Pediatrics 4:18. https://doi.org/10.1186/1471-2431-4-18

Song BK, Li ZH, Li S, Zhang ZZ, Fu QT, Wang SJ, Li L, Qi ST (2021) Functional metagenomic and enrichment metatranscriptomic analysis of marine microbial activities within a marine oil spill

area. Environmental Pollution 274. https://doi.org/10.1016/j.envpol.2021.116555

Soto del Rio MdlD, Dalmasso A, Civera T, Bottero MT (2017) Characterization of bacterial communities of donkey milk by high-throughput sequencing. International Journal of Food Microbiology 251:67-72. https://doi.org/10.1016/j.ijfoodmicro.2017.03.023

Spitz DR, Oberley LW (1989) An assay for superoxide dismutase activity in mammalian tissue homogenates. Analytical Biochemistry 179:8-18. https://doi.org/10.1016/0003-2697(89)90192-9

Tamang JP, Watanabe K, Holzapfel WH (2016) Review: Diversity of Microorganisms in Global Fermented Foods and Beverages. Front Microbiol 7:377. https://doi.org/10.3389/fmicb.2016.00377

Tanabe M, Kanehisa M (2012) Using the KEGG Database Resource. Current Protocols in Bioinformatics 38. https://doi.org/10.1002/0471250953.bi0112s38

Tang H, Zhao T, Sheng Y, Zheng T, Fu L, Zhang Y (2017) *Dendrobium officinale* Kimura et Migo: A Review on Its Ethnopharmacology, Phytochemistry, Pharmacology, and Industrialization. Evid Based Complement Alternat Med 2017:7436259. https://doi.org/10.1155/2017/7436259

Tao S, Lei Z, Huang K, Li Y, Ren Z, Zhang X, Wei G, Chen H (2019) Structural characterization and immunomodulatory activity of two novel polysaccharides derived from the stem of *Dendrobium officinale* Kimura et Migo. Journal of Functional Foods 57:121-134. https://doi.org/10.1016/j.jff.2019.04.013

Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G (2004) Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. New Phytol 163:547-561. https://doi.org/10.1111/j.1469-8137.2004.01126.x

615 Wagner J, Coupland P, Browne HP, Lawley TD, Francis SC, Parkhill J (2016) Evaluation of PacBio
616 sequencing for full-length bacterial 16S rRNA gene classification. BMC Microbiology 16.
617 https://doi.org/10.1186/s12866-016-0891-4
618 Wang Y, Gao S, He X, Li Y, Zhang Y, Chen W (2020) Response of total phenols, flavonoids, minerals,
619 and amino acids of four edible fern species to four shading treatments. PeerJ 8:e8354.
620 https://doi.org/10.7717/peerj.8354
621 Wisnuwardani RW, De Henauw S, Forsner M, Gottrand F, Huybrechts I, Kafatos AG, Kersting M, Knaze
622 V, Manios Y, Nova E, Molnar D, Rothwell JA, Scalbert A, Sette S, Widhalm K, Moreno LA,
623 Michels N (2022) Adolescents' dietary polyphenol intake in relation to serum total antioxidant
624 capacity: the HELENA study. Int J Food Sci Nutr 73:71-81.
625 https://doi.org/10.1080/09637486.2021.1910631
626 Wu HC, Zhang SY, Ma YY, Zhou J, Luo HB, Yang JG (2017) Comparison of microbial communities in
627 the fermentation starter used to brew Xiaoqu liquor. Journal of the Institute of Brewing 123:113-
628 120. https://doi.org/10.1002/jib.388
629 Xikeranmu Z, Abdunasir M, Ma J, Tusong K, Liu X (2019) Characterization of two copper/zinc
630 superoxide dismutases (Cu/Zn-SODs) from the desert beetle Microdera punctipennis and their
631 activities in protecting E. coli cells against cold. Cryobiology 87:15-27.
632 https://doi.org/10.1016/j.cryobiol.2019.03.006
633 Xing X, Cui SW, Nie S, Phillips GO, Goff HD, Wang Q (2014) Study on *Dendrobium officinale* O-
634 acetyl-glucomannan (Dendronan®): Part I. Extraction, purification, and partial structural
635 characterization. Bioactive Carbohydrates and Dietary Fibre 4:74-83.
636 https://doi.org/10.1016/j.bcdf.2014.06.004

637 Yang Q, Cai X, Yan A, Tian Y, Du M, Wang S (2020) A specific antioxidant peptide: Its properties in
638 controlling oxidation and possible action mechanism. Food Chemistry 327:126984.
639 https://doi.org/10.1016/j.foodchem.2020.126984
640 Yao D, Xu L, Wang C (2020) Diversity of the microbial community and antioxidant activity during
641 fermentation of red raspberry Enzymes. Food Science & Nutrition 9:99-110.
642 https://doi.org/10.1002/fsn3.1961
643 Zhang M, Wu X, Mu D, Yang W, Jiang S, Sun W, Shen Y, Cai J, Zheng Z, Jiang S, Li X (2020) Profiling
644 the effects of physicochemical indexes on the microbial diversity and its aroma substances in
645 pit mud. Lett Appl Microbiol 71:667-678. https://doi.org/10.1111/lam.13380
646 Zhang Q, Zhu D, Niu G, Yan F, Wei W, Zhu L, Wang S (2022) Bacterial Diversity Analysis during Natural
647 Fermentation of Sea Buckthorn Jiaosu by High-throughput Sequencing. Food Science 43:158-
648 165. https://doi.org/10.7506/spkx1002-6630-20210128-326
649 Zou L, Sun Y, Lin B, Zhou Y, Lu FY, Nie CP (2018) Preparation and activity determination of
650 *Dendrobium officinale* enzyme (in Chinese). Food Research and Development 43:129-134.

651 Statements and Declarations

652 Author Contributions

653 Conceptualization: LJ, SW, MZ

654 Data curation: LJ, DP, SW, MZ

655 Formal analysis: LJ, MZ

656 Funding acquisition: SW, MZ, XW

657 Investigation: LJ, SW

658 Methodology: LJ, DP, SW, MZ

Project administration: MZ, SW, XW, FJ

Resources: MZ, XW, SW, FJ

Supervision: MZ, SW, XW

Validation: LJ, MZ

Writing – original draft: LJ, MZ

Writing – review & editing: DP, XW, FJ

Funding: This work was supported by Major Scientific and Technological Special Project of Anhui Province (201903a06020034, 202103b06020019) and Project of Natural Science Foundation of Anhui Province (2108085MC123).

Competing Interests: The authors have declared no conflicts of interest for this article.

Ethics Approval: Ethics approval was not required for this research.

Data Availability: The raw 16S rRNA gene amplicon sequences have been deposited in the NCBI Sequence Read Archive under BioProject accession PRJNA1537632 (SRA study SRP741554; BioSamples SAMN63698732–SAMN63698735; SRA run accessions SRR40956814–SRR40956817). All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Figure captions

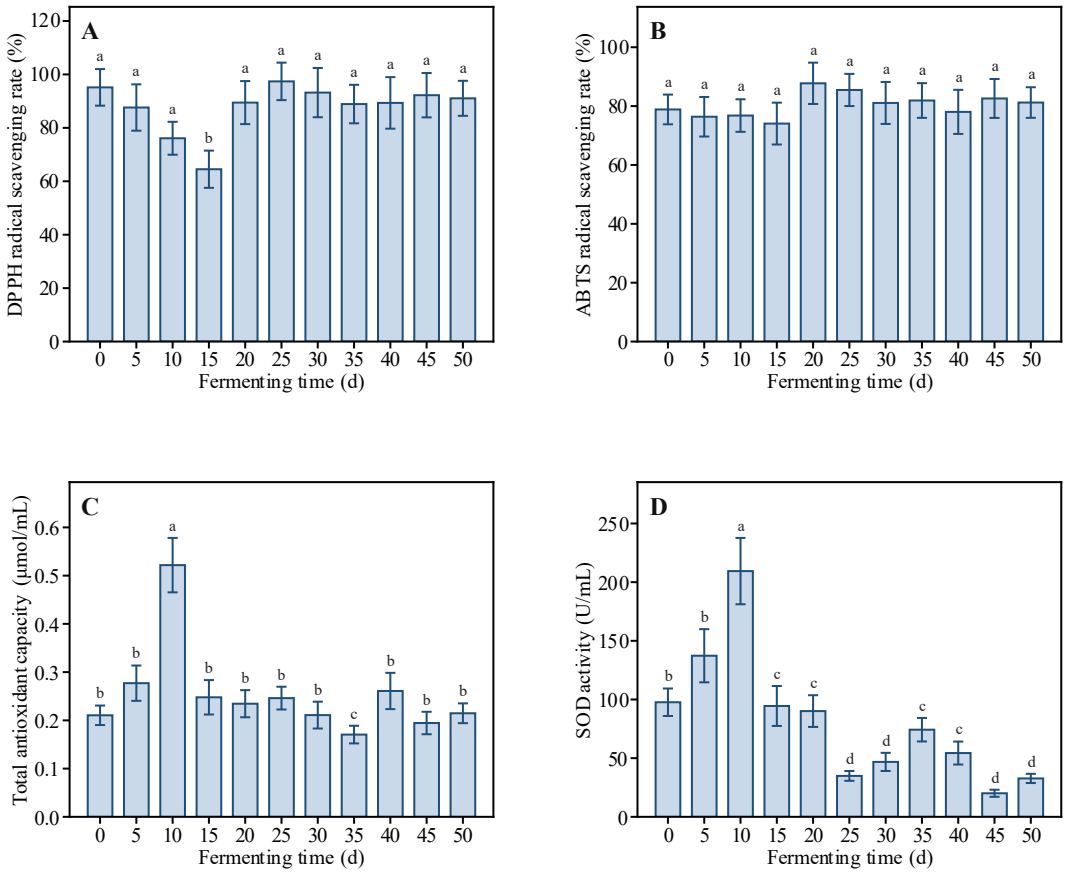
Fig. 1 Anti-oxidant activity in the fermentation of the Dendrobium EPJ (A) DPPH radical scavenging rate, (B) ABTS radical scavenging rate, (C) total antioxidant capacity (T-AOC) and (D) superoxide dismutase (SOD) activity. Each bar represents the mean of three independent

681 replicates (n = 3), and error bars indicate the standard deviation. Different lowercase letters above
682 the bars denote significant differences among fermentation time points (one-way ANOVA
683 followed by Tukey's HSD test, $P < 0.05$)
684 **Fig. 2** Bioactive substances in the fermentation of the Dendrobium EPJ (A) Amino acids content,
685 (B) plant flavonoids content, (C) total phenols content and (D) total sugar content. Each bar
686 represents the mean of three independent replicates (n = 3), and error bars indicate the standard
687 deviation. Different lowercase letters above the bars denote significant differences among
688 fermentation time points (one-way ANOVA followed by Tukey's HSD test, $P < 0.05$)
689 **Fig. 3** Pearson correlation analysis between antioxidant activity and bioactive substances during
690 fermentation (n = 11 time points). The colour scale indicates the direction and strength of the
691 correlation coefficient, and asterisks indicate statistically significant correlations (*$P < 0.05$)
692 **Fig. 4** Rarefaction curve of Dendrobium EPJ samples at the Sobs level (A) and the Shannon index
693 (B)
694 **Fig. 5** Genus-level relative abundances (A) and heatmap (B) of bacterial species in four
695 Dendrobium EPJ samples
696 **Fig. 6** Correlation analysis of bacterial communities with antioxidant activity and bioactive
697 substances. The horizontal axis depicts the antioxidant activity indices and bioactive substances,
698 while the vertical axis identifies the genera. The colour red shows a positive correlation, whereas
699 the colour blue signifies a negative correlation. The upper dendrogram illustrates the hierarchical
700 clustering of the antioxidant capacity indices and bioactive substances, while the left dendrogram
701 depicts the hierarchical clustering of the genera. The lengths of the branches show the distance
702 between them, with those that are more similar being clustered closer together

703 **Fig. 7** Heatmap of the KEGG annotations at pathway level 2 (A) and the abundance statistics of

704 the MetaCyc pathways (Top 50) (B)

705



706

707 **Fig. 1** Anti-oxidant activity in the fermentation of the Dendrobium EPJ (A) DPPH radical

708 scavenging rate, (B) ABTS radical scavenging rate, (C) total antioxidant capacity (T-AOC) and

709 (D) superoxide dismutase (SOD) activity. Each bar represents the mean of three independent

710 replicates (n = 3), and error bars indicate the standard deviation. Different lowercase letters above

711 the bars denote significant differences among fermentation time points (one-way ANOVA

712 followed by Tukey's HSD test, $P < 0.05$)

713

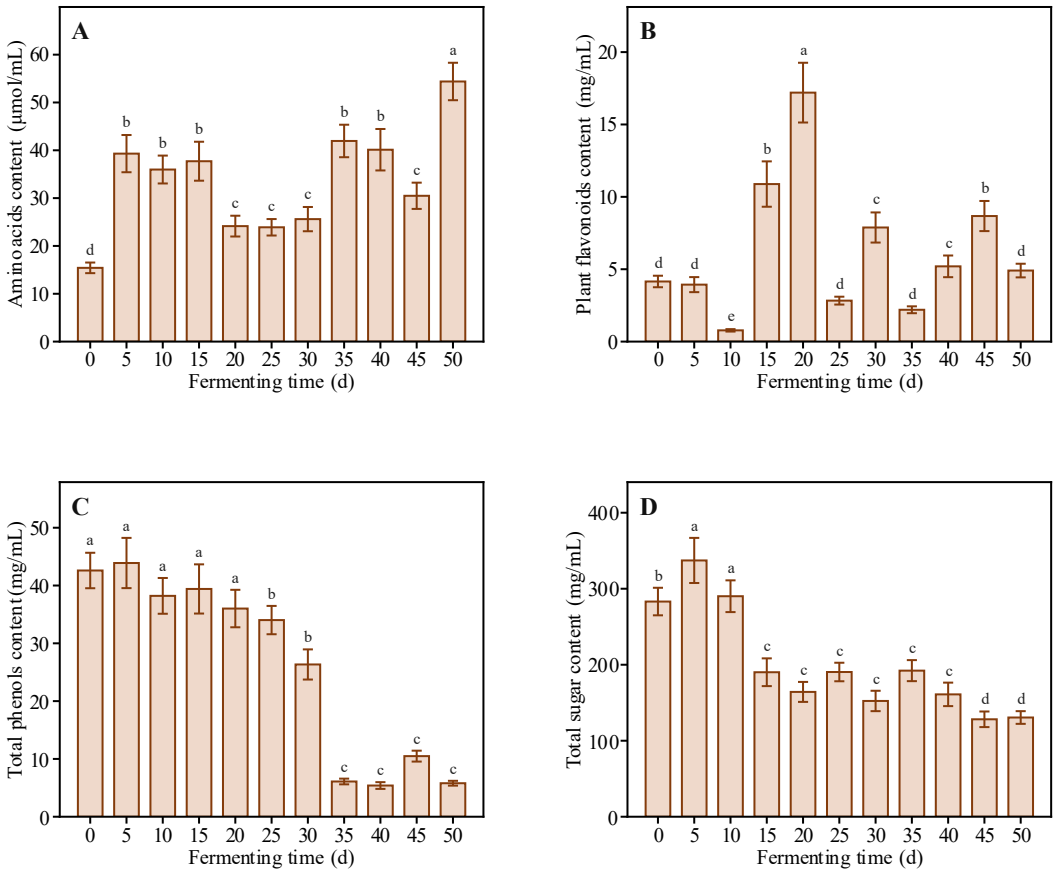


Fig. 2 Bioactive substances in the fermentation of the Dendrobium EPJ (A) Amino acids content, (B) plant flavonoids content, (C) total phenols content and (D) total sugar content. Each bar represents the mean of three independent replicates (n = 3), and error bars indicate the standard deviation. Different lowercase letters above the bars denote significant differences among fermentation time points (one-way ANOVA followed by Tukey's HSD test, $P < 0.05$)

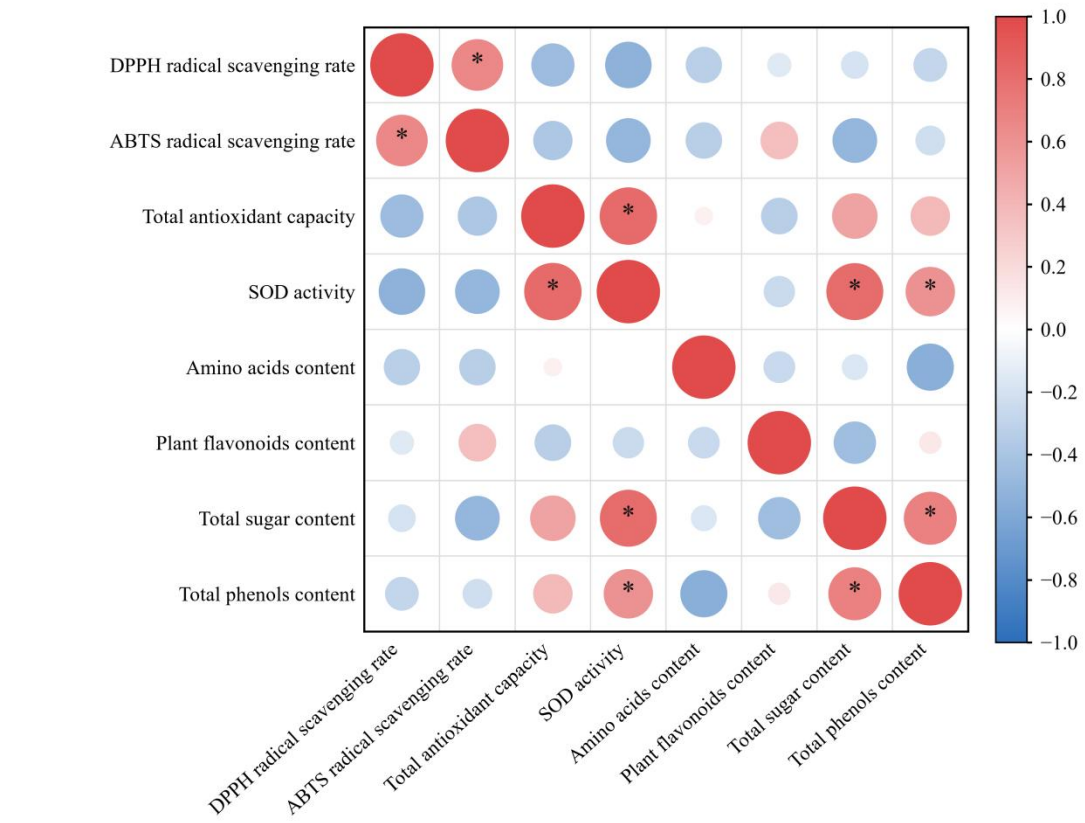
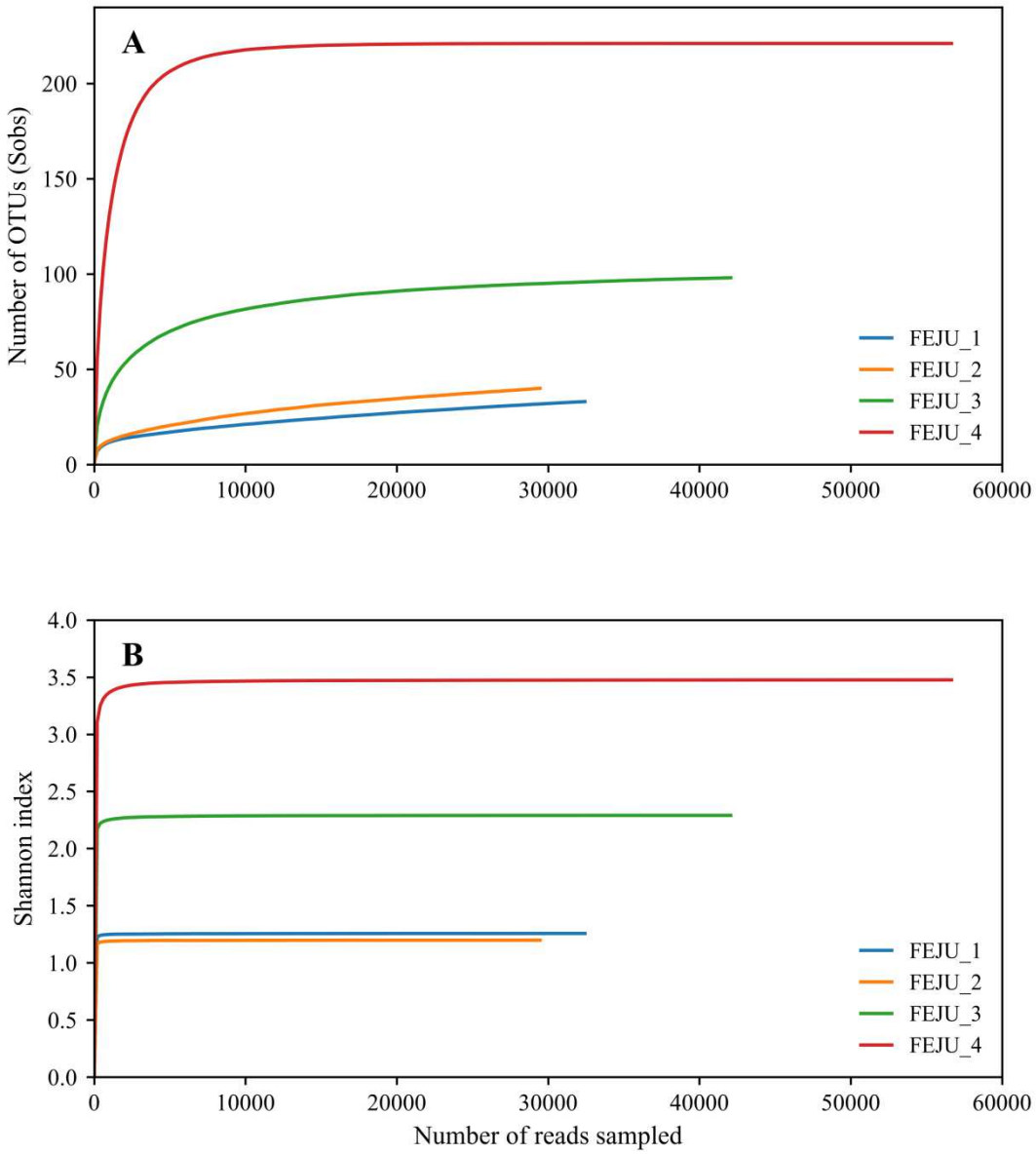


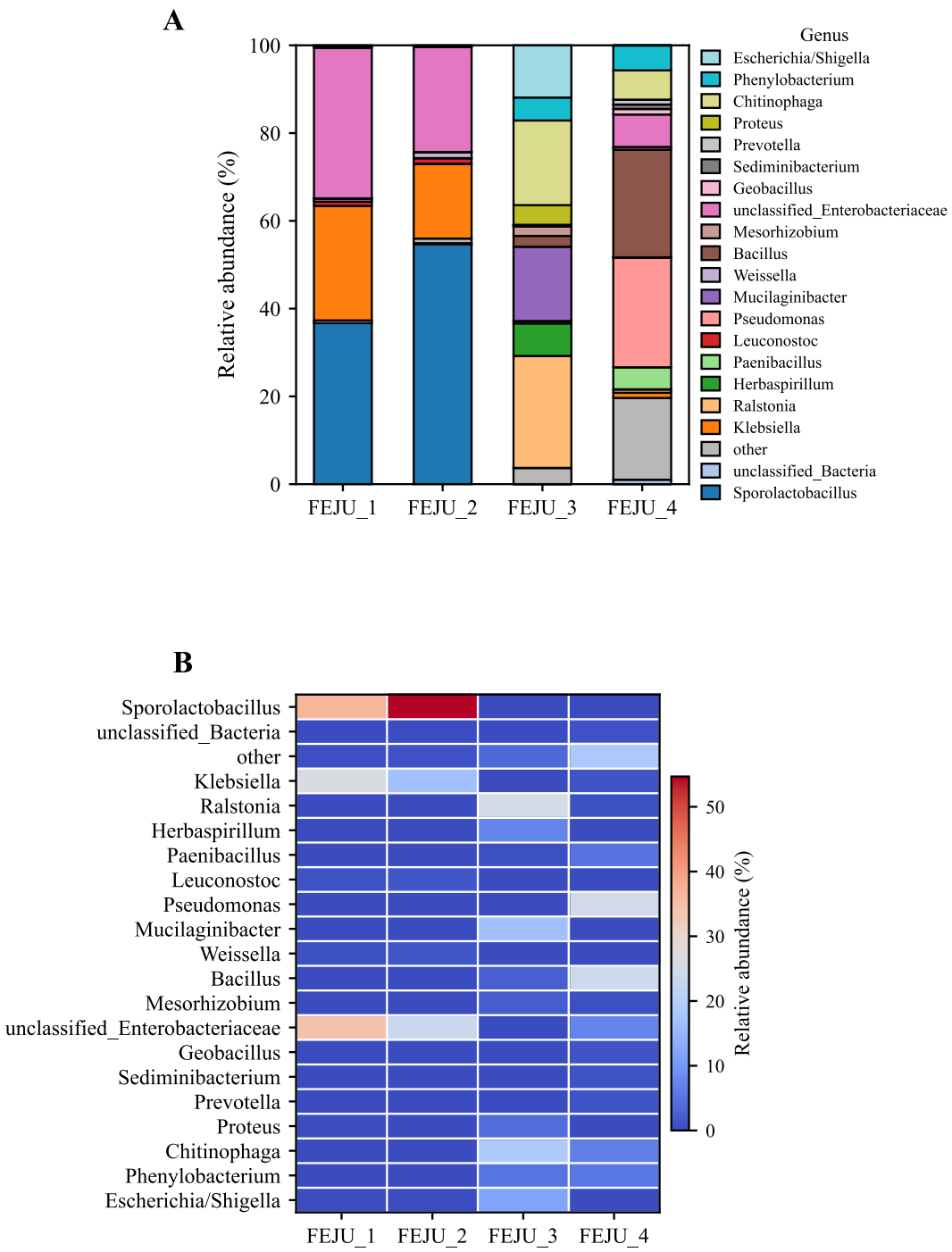
Fig. 3 Pearson correlation analysis between antioxidant activity and bioactive substances during fermentation (n = 11 time points). The colour scale indicates the direction and strength of the correlation coefficient, and asterisks indicate statistically significant correlations (*P < 0.05)



729

730 **Fig. 4** Rarefaction curve of Dendrobium EPJ samples at the Sobs level (A) and the Shannon index

731 (B)



732

733 **Fig. 5** Genus-level relative abundances (A) and heatmap (B) of bacterial species in four

734 Dendrobium EPJ samples

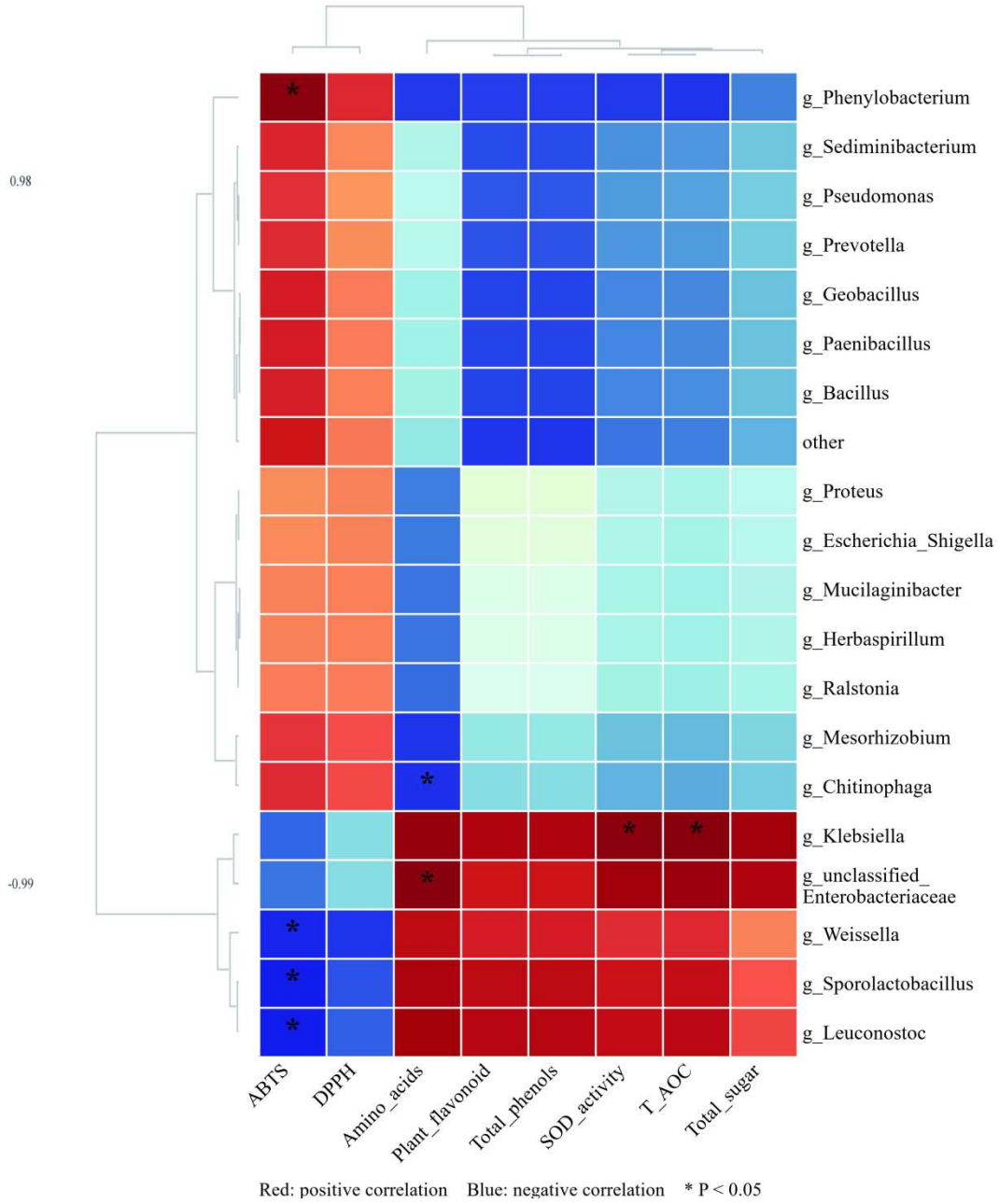
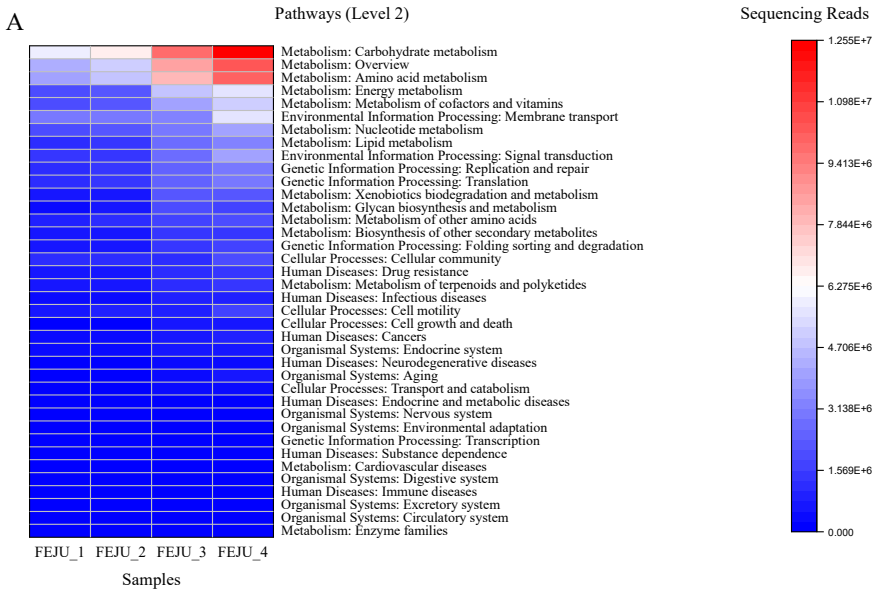
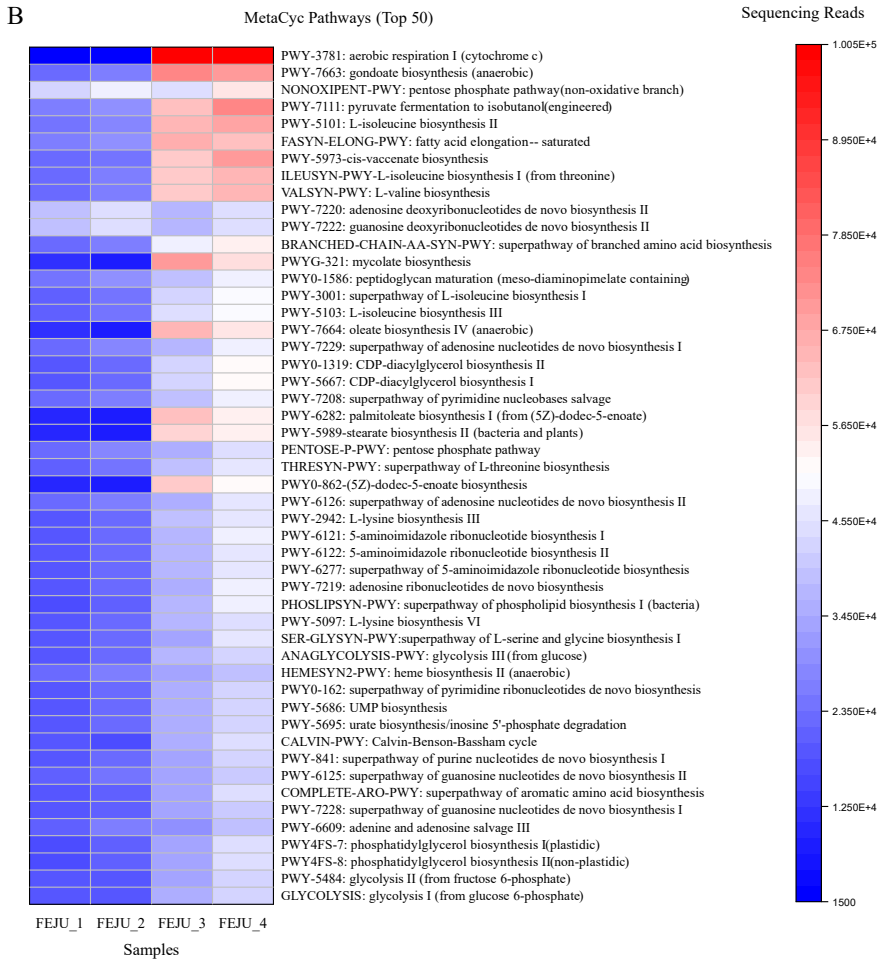


Fig. 6 Correlation analysis of bacterial communities with antioxidant activity and bioactive substances. The horizontal axis depicts the antioxidant activity indices and bioactive substances, while the vertical axis identifies the genera. The colour red shows a positive correlation, whereas the colour blue signifies a negative correlation. The upper dendrogram illustrates the hierarchical clustering of the antioxidant capacity indices and bioactive substances, while the left dendrogram

741 depicts the hierarchical clustering of the genera. The lengths of the branches show the distance

742 between them, with those that are more similar being clustered closer together





743

744 **Fig. 7** Heatmap of the KEGG annotations at pathway level 2 (A) and the abundance statistics of

745 the MetaCyc pathways (Top 50) (B)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarydata.rar