Dendrobium officinale Endophytes May Colonize the Intestinal Tract and Regulate Gut Microbiota in Mice

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Research Article

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Abstract

Dendrobium officinale is a traditional Chinese medicine for treating gastrointestinal diseases by nourishing “Yin” and thickening stomach lining. To study whether *D. officinale* endophytes can colonize the intestinal tract and regulate gut microbiota in mice, we used hot-pressure and ^{60}Co-γ radiation to eliminate *D. officinale* endophytes from its juice. Then, high-throughput ITS1-ITS2 rDNA and 16S rRNA gene amplicons were sequenced to analyze the microbial community of *D. officinale* endophytes and fecal samples of mice after administration of fresh *D. officinale* juice. Sterilization of *D. officinale* juice by autoclaving for 40 min (ASDO40) could more effectively eliminate the *D. officinale* endophytes and decrease their interference on the gut microbiota. *D. officinale* juice could increase beneficial gut microbiota and metabolites including short-chain fatty acids. *D. officinale* endophytes *Pseudomonas mosselii, Trichocladium asperum*, *Titata maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae* could colonize the intestinal tract of mice and modulate gut microbiota after oral administration of the juice for 28 days. Thus, the regulatory effect of *D. officinale* juice on gut microbiota was observed, which provides a basis for inferring that *D. officinale* endophytes might colonize the intestinal tract and participate in regulating gut microbiota to treat diseases. Thus, this study further provides a new approach for the treatment of diseases by colonizing plant endophytes in the intestinal tract and regulating gut microbiota.

1. Introduction

Dendrobium officinale Kimura et Migo (*D. officinale*), recorded in Chinese Pharmacopoeia, is used as herbal medicine and novel food material in China (China Pharmacopoeia Committee, 2020). It is widely used as a traditional medicine to strengthen “Yin”, which can tonify the five viscera, relieve fatigue, thicken stomach lining, lighten the body, and prolong life span (Chen et al., 2021 a). According to modern pharmacological studies, *D. officinale* exhibits various biological functions, including balancing gut microbiota (Li et al., 2020), immune modulation (Huang et al., 2018), anti-tumor (Wei et al., 2018 b), gastrointestinal-protective (Shi et al., 2020), cardio-protective (Xiao et al., 2018), and anti-diabetes effects (Zheng et al., 2017). Several recent studies have shown the role of gut microbiota in mediating the health and disease of the host (Lai et al., 2021). Hence, balancing the effect of gut microbiota should be paid more attention to in the prevention and treatment of diseases (Li et al., 2020).

The gut microbiota consists of trillions of bacteria and fungi and is profoundly important in maintaining human health because of its role in nutrient acquisition and energy regulation (Liu et al., 2020).

Microbes that colonize inner plant tissues are designated endophytes. Endophytes exist widely in host plants and are important components of plant micro-ecosystems (Zhu et al., 2021). Endophytes have increasingly become the research hotspot of scholars worldwide because they can produce active components, promote host plant growth, and enhance the host plant resistance against biotic and abiotic stresses (Jia et al., 2016; Wu et al., 2021 a). Recent research has unearthed a network of endophytic-enteric-soil-endophytic microbes that process animal feces to serve as natural microbial inoculants for

plants. These function to serve as bacterial sources for animal gut systems (Martínez et al., 2021). Gut microbiota contains hundreds to thousands of bacteria obtained from a specific diet (Rothschild et al., 2018). Li et al. (2012) found that *Paenibacillus* sp. strain Aloe-11 had excellent intestine colonization ability and could significantly promote forage fiber degradation, thus producing antibiotic activity against many pathogenic bacteria and fungi (Li et al., 2012). Likewise, Zheng et al. (2020) found that a proportion of the intestinal microbes of potato tuber moth might be derived from bacterial endophytes in potatoes (Zheng et al., 2020). In another report, it was shown that fungal endophytes in grass eaten by sheep could reach the gut and reduce fecal degradation rates (Crippls et al., 2013).

D. officinale can act as a prebiotic agent to promote short-chain fatty acids (SCFAs)-producing genera and avoid gut dysbiosis in dogs (Liu et al., 2020). *D. officinale* can also inhibit the growth of pathogenic bacteria by increasing the SCFAs-producing beneficial bacteria, showing anti-inflammatory activity, and improving the human intestinal environment (Qin et al., 2020). While potato endophytic bacteria can colonize and transform into intestinal microbes in potato tuber moths (Zheng et al., 2020), little is known whether *D. officinale* endophytes can colonize and transform the intestinal tract and play a similar role in regulating gut microbiota in mice. In this study, we investigated the endophytic microbes (fungi and bacteria) of *D. officinale* that can colonize the intestinal tract and regulate gut microbiota in mice. This work will provide an important basis for studying the colonization of *D. officinale* endophyte in the intestinal tract of mice.

2. Materials And Methods

2.1 Plant Material

D. officinale plant material was artificially cultivated in the base of Lin'an (30°23' N, 119°72' E), and was identified as *D. officinale* Kimura & Migo by Professor Qiaoyan Zhang of the Zhejiang Chinese Medicine University. The collected samples of *D. officinale* were packed in sterile plastic bags and brought back to the laboratory.

The collected samples of fresh *D. officinale* stems (DO) were cleaned under running water, surface-sterilized with 75% ethanol for 3 min, 5% NaClO for 3 min, 75% ethanol for 30 s, and finally washed five times with sterile distilled water (Chen et al., 2020). Fresh DOs were treated with doses of 5, 10, 15, 20, and 25 kGy ^{60}Co-γ irradiation (CIDO5, CIDO10, CIDO15, CIDO20, CIDO25, respectively). Fresh DOs were sterilized by autoclaving for 20 min (ASDO20) and 40 min (ASDO40) at 121 ℃. Three parallel copies of each sample were prepared using 2 g of each sample and placed in a DNA-Be-Locked reagent (Majorbio Bio-pharm, Shanghai, China) to immobilize DNA to study the endophytic bacteria and fungi.

2.2 Animal Experiments

All animal experiments were performed following the guidelines approved by the Committee on the Ethics of Animal Experiments of Zhejiang Chinese Medical University (SYXK—2018—0012). Eighteen 6-week-old C57BL/6 male mice were purchased from Shanghai Laboratory Animal Center (Shanghai, China). The

animals were kept in three individual cages with free access to food and sterile drinking water in a temperature-controlled room (22 ± 2°C), relative humidity (50 ± 10%), and 12 h/12 h light/dark cycle.

After an adaption period of one week, the mice were randomly categorized into three groups, with six mice in each group. The mouse in DO and ASDO groups were administrated with fresh *D. officinale* juice and autoclaved fresh *D. officinale* juice (121 ℃ for 40 min), respectively, at a dose of 1 g/kg daily for 28 days (Liang et al., 2017), whereas the control groups were orally administered sterile drinking water.

Fresh fecal samples of control (sterile water), DO, and ASDO (121 ℃ for 40 min) groups were collected in sterile cryovial tubes for 0 (C0, DO0, ASDO0) and 28 days (C28, DO28, ASDO28). After 28 days, feces were stored at -80 ℃ for the analysis of SCFAs and high throughput sequencing.

2.3 Determination of SCFAs by Gas Chromatography-mass Spectrometry (GC—MS)

The SCFAs were analyzed by GC-MS as previously described (Zhang et al. 2020). The analysis was carried out using an Agilent 8890B-5977B system equipped with HP FFAP capillary column (30 m × 0.25 mm × 0.25 μm; Agilent J&W Scientific, Folsom, CA, USA). The initial oven temperature was kept at 80 ℃ for 0.5 min and then raised to 120 ℃ at the rate of 40 ℃/min and 200 ℃ at the rate of 10 ℃/min. The temperature of injection was 230 ℃ for 3 min. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The injected sample volume for GC analysis was 1 μL. Electron bombardment ion source (EI) was used at a temperature of 230 ℃, four-stage rod temperature of 150 ℃, transmission line temperature of 230 ℃, and electron energy of 70 eV. The scanning mode was ion scanning mode (SIM). Calibration curves were constructed in the range of 0.2–400 μg/mL for acetic acid, propionic acid, and n-butyric acid, and 0.1–200 μg/mL for isobutyric acid, valeric acid, isovaleric acid, hexanoic acid, and isohexanoic acid (eight concentration levels, three replicates for each level), by adding known amounts of the analytes to the blank.

2.4 Genomic DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Sequencing

Two plant samples (DO and ASDO40) and six fecal samples (C0, DO0, ASDO0, C28, DO28, and ASDO28) were selected to prepare the DNA of *D. officinale* endophytes and mice intestinal microorganisms (Chen et al., 2020). In brief, total genomic DNA was extracted using the FastDNA 2 mL SPIN Kit for Soil (50 preps., Cat. No. 116560200, MP Biomedicals GmbH, Eschwege, Germany) and evaluated using a NanoDrop Spectrophotometer Qubit2.0.

The V5-V7 regions of the 16S rRNA genes of plant and bacteria in fecal samples were PCR amplified using universal primers for Illumina deep sequencing (Zheng et al., 2020). Primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') outperforms all other primer pairs in our study in the elimination of non-target DNA and retrieval of bacterial OTUs (Beckers et al., 2016). In the plant and fecal samples, fungal internal transcribed spacer region 1 (ITS1 region) of

ribosomal RNA was amplified using ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) primers (Zhu et al., 2021). The PCR was carried out according to a protocol as described in our previous publication (Chen et al., 2020). Library construction and sequencing were performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd, (Shanghai, China).

2.5 Bioinformatic Analysis

The plant endophytic bacterial and fungal communities and fecal bacteria and fungi were subjected to the same analytical procedures. First, paired-end Illumina MiSeq sequences were merged using FLASH (v1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml) to obtain the raw tags (Magoc and Salzberg, 2011). These raw tags were then filtered and clustered using the Quantitative Insights into Microbial Ecology (QIIME) software v1.9.1 (http://qiime.org/install/index.html) (Caporaso et al., 2010). Primers, short reads, low complexity reads, and low-quality sequences were removed using PRINSEQ v0.20.4 (Schmieder and Edwards, 2011). Sequencing and PCR-induced errors were corrected with the pre-cluster function of the software Mothur v1.30.2 (https://www.mothur.org/wiki/Download-mothur) (Schloss et al., 2009). High qualified tags with ≥ 97% similarity were clustered into operational taxonomic units (OTUs) based on using software USEARCH v7 to the Greengenes v135 database for endophytic bacteria and fungi (Edgar, 2010; K ~ oljalg et al., 2013). Bacterial and fungal OTUs were classified by searching against the SILVA databases (Release 119, http://www.arb-silva.de), using the Ribosomal Database Project (RDP) classifier v2.11 (https://sourceforge.net/projects/rdp-classifier/) within QIIME (Quast et al., 2013; Wang et al., 2007). The analysis of variance (ANOVA), Multi-Dimension, and Venn diagram with R software v3.3.1 based on OTUs were applied to compare the bacterial and fungal communities of each sample (Veach et al., 2019).

2.6 Statistical analysis

All results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by a Tukey's post-hoc test using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). P-values less than 0.05 were considered of statistical significance.

A heatmap was employed to exhibit the relative abundances of the 50 predominant genera in each sample. The distance matrices of community composition of endophytic fungi and bacteria were evaluated by calculating dissimilarities using the Bray-Curtis method (Faith et al., 1987). Beta diversity was estimated by calculating the weighted UniFrac distances between samples (Chen et al. 2012) using beta diversity by a program in QIIME. The data analysis for all correlations was finished in the online “i-sanger” (http://www.-majorbio.com/) developed by Majorbio Bio-Pharm Technology Co. Ltd (Zhu et al., 2020).

3. Results

3.1. Hot-pressure and ^{60}Co-*γ* Treatment Eliminated the *D. officinale* Endophytes

To eliminate the *D. officinale* endophytes and decrease their interference on the gut microbiota in mice, *D. officinale* was treated with hot-pressure and ^{60}Co-*γ*. After quality control, denoising, and removal of chimera sequences, 265,440 and 961,560 high-quality 16S and ITS1-ITS2 sequences were obtained. A total of 1,339 and 1,083 OTUs with 97% identity cutoffs of bacteria and fungi of *D. officinale* were found (Fig. 1). The number of bacterial and fungal OTUs of *D. officinale* was reduced significantly after ^{60}Co-*γ* irradiation and hot pressure treatment; hot pressure treatment (121 ℃ for 40 min) decreased the OTUs most. Concurrently, the number of the phylum, class, order, family, genus, and species of bacteria (**Fig. S1 A**) and fungi (**Fig. S1 B**) of DO and ASDO40 decreased by 53.33%, 65.38%, 61.02%, 61.36%, 70.78%, and 64.97%, and 33.33%, 42.86%, 51.35%, 46.77%, 49.35%, and 44.68%, respectively. Overall, the sorts of *D. officinale* endophytic fungi and bacteria decreased the most significantly after 40 min hot pressure treatment.

Analysis of alpha diversity indicated that the community richness (Chao) and community diversity (Shannon) of endophytic bacteria and fungi in samples under hot pressure treatment (ASDO40) was lower than DO (**Fig. S2 A-D**). However, ASDO40 treatment did not change the dominant bacterial genera compared to DO samples (Fig. 2A). The top three dominant bacterial genera (*Pseudomonas*, *Ochrobactrum*, and *Rhodococcus*) in DO and ASDO samples were accounting for 69.45% and 95.48% of the relative abundance, respectively. However, in ASDO40, there was a decrease in the relative abundance of *Burkholderia*, *Caballeronia*, *Paraburkholderia*, *Alloprevotella*, *Prevotella*, *Neisseria*, and *Streptococcus*, compared with those in DO (Fig. 2A). In addition, ASDO40 treatment changed the dominant fungal genera. The top three dominant fungal genera in DO were *Fusarium*, unclassified-f-Didymellaceae, and *Occultifur*, accounting for 62.68% of the relative abundance, while those in ASDO were *Fusarium*, *Cutaneotrichosporon*, and *Simplicillium*, accounting for 63.65% of the relative abundance. Furthermore, ASDO reduced the relative abundance of *Occultifur*, *Rhodotorula*, *Pyrenochaetopsis*, *Sporidiobolus*, and *Sordaria*, compared with those in DO (Fig. 2B). Thus, ASDO40 treatment reduced the diversity and richness of *D. officinale* endophytes and participated in eliminating the interference of *D. officinale* endophytes.

3.2 Change in the Gut Microbiota Structure and Metabolite SCFAs after the Intake of D. officinale Juice

To analyze how *D. officinale* juice modulates the gut microbiota structure in mice, we carried out 16S and ITS sequencing on fecal samples of mice administered with sterile water (C), *D. officinale* juice (DO), and autoclaved *D. officinale* juice (ASDO) on days 0 and 28. In all fecal samples, *Lactobacillus*, *Bifidobacterium*, *Desulfovibrio* were the three top dominant bacterial genera, while *Aspergillus*, *Penicillium*, *Acaulium* were the three top dominant fungal genera. Our experiments showed that oral administration of *D. officinale* juice for 28 days (DO28) could effectively increase the diversity of gut microbiota and the relative abundance of beneficial endophytes and decrease the relative abundance of harmful endophytes. The results indicated that the number of bacterial and fungal OTUs in the C0 group

and C28 group were not different but increased in the DO28 group in contrast to the DO0 group (**Fig. S3 A-B**). Meanwhile, the number of bacterial OTUs in the ASDO28 group increased compared with the ASDO0 in bacteria, but that of fungi decreased (**Fig. S3 B**). According to the Kruskal Wallis rank-sum test, there was no significant difference in the relative abundance of *Lactobacillus*, *Ruminococcus*, *Alistipes*, *Aerococcus*, *Bacteroides*, *Lachnoclostridium*, *Anaerostipes*, *Parasutterella*, *Pyxidiophora*, *Cladosporium*, *Talaromyces*, *Rhodotorula*, *Filobasidium*, *Aspergillus*, *Mortierella*, *Penicillium*, *Cutaneotrichosporon*, and *Candida* in C0, ASDO0 and DO0 groups ($P < 0.05$); in addition, the relative abundance of *Bifidobacterium* of DO0 was significantly lower than that of C0 and ASDO0 groups ($P > 0.05$). However, DO28 increased the relative abundance of bacterial genera, including *Lactobacillus*, *Bifidobacterium*, *Ruminococcus*, *Alistipes*, whereas decreased *Aerococcus*, *Bacteroides*, *Lachnoclostridium*, *Anaerostipes*, and *Parasutterella* compared with those in C28 and ASDO28 (Fig. 3A). DO28 increased the relative abundance of fungal genera, including *Pyxidiophora*, *Cladosporium*, *Talaromyces*, *Rhodotorula*, *Filobasidium*, and decreased *Aspergillus*, *Mortierella*, *Penicillium*, *Cutaneotrichosporon*, *Candida*, compared with those in C28 and ASDO28 groups (Fig. 3B). Our results also indicated that DO28 altered the gut microbiota structure in mice by increasing the beneficial bacteria *Lactobacillus murinus* (Wu et al., 2021 b), *Lactobacillus johnsonii* (Olnood et al., 2015), *Bifidobacterium pseudolongum* (Tatsuoka et al., 2021), and *Lactobacillus reuteri* (Singh et al., 2021), and reducing harmful bacteria *Ochrobactrum anthropi* (Grabowska-Markowska et al., 2019), *Aerococcus urinaeequi* (Heidemann et al., 2018), and *Clostridium* sp. cTPY-12 (Wang et al., 2019), compared with those in C28 and ASDO28 groups (**Fig. S4 A**).

SCFAs are one of the index components to evaluate gastrointestinal function (Shi et al., 2020); we found that the *D. officinale* juice could effectively increase the content of SCFAs in mouse guts. Compared with the C28 and ASDO28 groups, the concentration of total SCFAs in the DO28 group was increased (Fig. 4). Among them, acetic acid, propanoic acid, and butanoic acid contents of the DO28 group improved remarkably ($P < 0.05$), suggesting that DO28 may affect the intestinal environment to some extent by increasing SCFAs-producing bacteria, compared with those in C28 and ASDO28 groups. Taken together, *D. officinale* juice can effectively increase the contents of SCFAs.

These findings cumulatively suggest that *D. officinale* juice can effectively regulate gut microbiota by improving their diversity, increase the relative abundance of beneficial endophytes and the contents of SCFAs, and reduce the relative abundance of harmful endophytes while these were not observed in sterilized (autoclaved) *D. officinale* juice.

3.3 *D. officinale* Endophytes may Colonize in the Intestinal Tract of Mice and Modulate Gut Microbiota

To examine which *D. officinale* endophytes may colonize in the intestinal tract of mice, we compared the microbiological community in the control mice and those administered with *D. officinale* juice and autoclaved *D. officinale* juice. We found three bacterial and 22 fungal genera in DO28 that were not found in DO0, C28, and ASDO28 groups. In addition, seven endophytic bacterial species were shared both in the DO and gut microbiota of normal mice. In addition, 24 kinds of endophytic fungal species were shared by

DO and gut microbiota in normal mice. Among them, *Pseudomonas mosselii, Trichocladium asperum*, *Titaea maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae* were found only in DO and DO28 groups while not in ASDO40, DO0, C0, C28, ASDO0, ASDO28 mice (Fig. 5). Therefore, we hypothesize that the increase in beneficial gut microbiota after the administration of *D. officinale* fresh juice may be related to the *D. officinale* endophytes, including *Pseudomonas mosselii*, *Trichocladium asperum*, *Titaea maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae*. These strains may colonize in the intestinal tract of mice and modulate gut microbiota.

4. Discussion

Hot-pressure and ^{60}Co-*γ* treatment could effectively reduce the OTU number and diversity of *D. officinale* endophytes and played a role in eliminating the interference of *D. officinale* endophytes. However, the results indicated that the relative abundance of *Ochrobactrum anthropi*, *Dokmaia monthadangi*, *Sporidiobolus pararoseus*, *Cladosporium delicatulum*, *Papiliotrema flavescens*, and *Rhodotorula babjevae* from ClDO25 increased, while that of *Ochrobactrum anthropi*, *Rhodococcus erythropolis*, *Lycoperdon utriforme*, *Cutaneotrichosporon cutaneum*, *Monascus pilosus*, and *Vishniacozyma* sp from ASDO40 increased. Previous studies have reported the radioresistance of *Ochrobactrum anthropi* and *Rhodotorula babjevae* (Ibrahim et al., 2016; Pereira et al.,2013) and heat resistance of *Ochrobactrum anthropi* and *Rhodococcus erythropolis* (Varano et al., 2016; Wu et al.,2011). Therefore, we suggest that *Ochrobactrum anthropi* and *Rhodotorula babjevae* are potentially radiation-resistant while *Ochrobactrum anthropi* and *Rhodococcus erythropolis* are heat-resistant.

D. officinale can regulate gut microbiota and is closely related to the treatment of diseases (Chen et al., 2021 a). Previous studies suggest that *D. officinale* can balance gut microbiota by improving the relative abundance of intestinal bacteria, such as *Ruminococcus*, *Clostridium*, and *Parabacteroides*, and decreasing the relative abundance of *Prevotella* and *Bacteroides* (Liu et al., 2020). At the phylum level, Bacteroidota and Firmicutes were the predominant bacterial phyla, and Ascomycota were the predominant fungal phylum in all fecal samples. The predominant bacterial phyla were the same as those reported in a previous study (Qin et al., 2020). *Lactobacillus* was the dominant bacterial genus in all the fecal samples, but the percent of relative abundance in DO28 was significantly higher than in ASDO28 and C28. Previous studies have reported the probiotic, exhibited immunomodulating (Zegarra et al., 2019), gastrointestinal protection (Hasannejad et al., 2020), and anti-tumor (Chen et al., 2021 b) activities of *Lactobacillus*. The results of this study suggest that mice might produce more *Lactobacillus* to exert immunological, gastrointestinal protective, and anti-tumor effects after intragastric administration of DO. Interestingly, the relative abundance of *Lactobacillus johnsonii* from DO28 increased significantly compared with that in other samples. *Lactobacillus johnsonii* can promote T cell differentiation into T helper (Th)1/Th2/ regulatory T (Treg) cells and play an important role in improving the balance between these cells (Zheng et al., 2021). Therefore, we suggest that *Lactobacillus johnsonii* might improve the bioaccessibility and bioavailability of functional components of *D. officinale* through

the microbial-host metabolic pathway, thus maximizing its anti-immune function. However, future research is needed to determine whether these dominant gut microbial species can promote the utilization of effective components in *D. officinale*.

Gut microbes are closely related to SCFA utilization. SCFAs are key bacteria metabolites, in particularly acetic acid, propanoic acid and butanoic acid (Li et al., 2020). Interestingly, butanoic acid induces the differentiation of colonic Treg cells (Arpaia et al., 2013). In our study, the SCFAs from the feces of mice were detected by GC-MS. As expected, DO-treated mice produced more butyrate ($p < 0.05$) and acetic acids ($p > 0.05$) than the control and ASDO groups. To know which are the primary butyrate-producing bacteria, the synthase that is responsible for butyrate synthesis should be investigated. This study indicated that DO caused an increase in the relative abundance of some SCFAs producing bacteria *Lactobacillus johnsonii* (Olnood et al., 2015), *Bifidobacterium pseudolongum* (Tatsuoka et al., 2021), and *Lactobacillus reuteri* (Singh et al., 2021), and decreased some pathobionts *Ochrobactrum anthropi* (Grabowska-Markowska et al., 2019; Saveli et al., 2010), *Aerococcus urinaeequi* (Heidemann et al., 2018), *Clostridium* sp. cTPY-12 (Wang et al., 2019). Therefore, we suggest that the increase in the relative abundance of *Lactobacillus johnsonii*, *Bifidobacterium Pseudolongum*, and *Lactobacillus reuteri* might be closely related to the utilization of SCFAs by the DO endophytes. The increase in the relative abundance of fungi of *Rhodotorula babjevae* exhibited antimicrobial activity against different bacteria species (Sen et al., 2017), and *Lycoperdon utriforme* performed antioxidant activities (Sezgin et al., 2020). However, there are no reports on SCFAs-producing fungal species, including *Rhodotorula Babjevae*, *Lycoperdon utriforme*, *Aspergillus Minisclerotigenes*, *Pyxidiophora arvernensis*, and other fungi.

Our results showed that the dominant bacterial and fungal genera of *D. officinale* were significantly different from gut microbiota in mice. However, *Fusarium* was a common dominant fungi genus identified both in *D. officinale* and fecal samples. Therefore, we provide evidence that a portion of gut microbiota in mice may be derived from *D. officinale* endophytes. Intriguingly, *Pseudomonas mosselii*, *Trichocladium asperum*, *Titaea maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae* were found only in DO and DO28 and not in ASDO40, C0, DO0, C28, ASDO0, ASDO28 groups. Therefore, we speculated that these strains might colonize in the intestinal tract of mice and modulate gut microbiota after oral administration of *D. officinale* fresh juice for 28 days. These findings have important implications for understanding the increase in beneficial gut microbiota and metabolite SCFAs after the intake of *D. officinale* fresh juice, which may be attributed to the *D. officinale* endophytes. *Paenibacillus* sp. strain Aloe-11 exhibits intestine colonization ability and can improve forage fiber degradation, thus producing antibiotic activity against many pathogenic bacteria and fungi (Li et al., 2012). Zheng et al. (2020) reported that a portion of the intestinal microbes of the potato tuber moth might be derived from potato endophytic bacteria. In addition, grass endophytic fungi could arrive at sheep guts and lower the fecal degradation rates (Crippls et al., 2013). However, the roles of *Pseudomonas mosselii*, *Trichocladium asperum*, *Titaea maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae* were mainly reported as antifungal (Sen et al., 2020), plant disease resistance (Wu et al., 2018; Yang et al., 2021; Wei et al., 2018 a) and promoting plant growth (De et al., 2016), while there have been only a few studies on the ability of *D. officinale* endophytes to colonize and transform host intestinal tract and a similar role in regulating gut

microbiota in mice. According to this research, *D. officinale* juice could increase beneficial gut microbiota and metabolize SCFAs and may be related to *D. officinale* endophytes. In the future, the red fluorescent protein can be applied to encode five target strains through CRISPR/cas9 and instill gastric bacterial or fungal suspension into specific pathogen-free-induced pseudosterile mice, and then the colonization of the target strains can be verified by *in vivo* imaging (Pareek et al., 2019; Sampson et al., 2016; Dollive et al., 2013; Bayer et al., 2019).

5. Conclusions

This study showed that ASDO40 was more suitable in eliminating the interference of *D. officinale* endophytes. *D. officinale* juice could increase beneficial gut microbiota and metabolite SCFAs, which might be related to *D. officinale* endophytes. Additionally, the *D. officinale* endophytes, *Pseudomonas mosselii, Trichocladium asperum*, *Titaea maxilliformis*, *Clonostachys epichloe*, and *Rhodotorula babjevae* of might colonize in the intestinal tract of mice and modulate gut microbiota after oral administration with DO for 28 days. Whether these strains can colonize in the mouse intestine and participate in the regulation of gut microbiota in the treatment of diseases, need experimental verification, and our results provide a new approach for the treatment of diseases by colonizing and transforming plant endophytes into gut microbiota.

Declarations

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

- Wenhua Chen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Yulong Li performed the experiments, prepared figures and/or tables, and approved the final draft.
- Bo Zhu conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Luping Qin conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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Data Availability

The following information was supplied regarding data availability:

The data has been uploaded to NCBI, Submission ID: SUB11220862, SUB1220752; BioProject ID: PRJNA819231, PRJNA819223.

References

1. Arpaia N, Campbell C, Fan X, Dikiy S, Veeken JVD, DeRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504(7480) :451-455. https://doi.org/10.1038/nature12726.
2. Bayer F, Ascher S, Pontarollo G, Reinhardt C (2019) Antibiotic treatment protocols and germ-free mouse models in vascular research. Front. Immunol 10: 2174-2180. https://doi.org/10.3389/fimmu.2019.02174.
3. Beckers B, Op DBM, Thijs S, Truyens S, Weyens N, Boerjan W, Vangronsveld J (2016) Performance of 16s rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. Front Microbiol 7: 650-664. https://doi.org/10.3389/fmicb.2016.00650.
4. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of highthroughput community sequencing data. Nat. Methods 7(5): 335–336. https://doi.org/10.1038/nmeth.f.303.
5. Chen G, Cao Z, Shi Z, Lei H, Chen C, Yuan P, Wu F, Liu C, Dong M, Song Y, Zhou J, Lu Y, Zhang L (2021 b) Microbiome analysis combined with targeted metabolomics reveal immunological anti-tumor activity of icariside I in a melanoma mouse model. Biomed. Pharmacother 140: 111542-111555. https://doi.org/10.1016/j.biopha.2021.111542.
6. Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, Collman RG, Bushman FD, Li H (2012) Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatic 28(16): 2106–2113. https://doi.org/10.1093/bioinformatics/bts342.
7. Chen W, Lu J, Zhang J, Wu J, Yu L, Qin L, Zhu B (2021 a) Traditional uses, phytochemistry, pharmacology, and quality control of *Dendrobium officinale* Kimura et. Migo. Front. Pharmacol 12: 726528-726551. https://doi.org/10.3389/fphar.2021.726528.
8. Chen WH, Wu SJ, Sun XL, Feng KM, Rahman K, Tan HY, Yu LY, Xu LC, Qin LP, Han T (2020) High-throughput sequencing analysis of endophytic fungal diversity in *Cynanchum* sp. S. Afr. J. Bot 134: 349-358. https://doi.org/10.1016/j.sajb.2020.04.010.
9. Cripps M G , Edwards G R (2013) Grass species and their fungal symbionts affect subsequent forage growth. Basic. Appl. Ecol 14(3):225-234. https://doi.org/10.1016/j.baae.2013.01.008.

10. Dollive S, Chen YY, Grunberg S, Bittinger K, Hoffmann C, Vandivier L, Cuff C, Lewis JD, Wu GD, Bushman FD (2013) Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. PLoS One 8(8): 1-12. https://doi.org/10.1371/journal.pone.0071806.
11. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19): 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
12. Editorial Board of Chinese Pharmacopoeia. Chinese Pharmacopoeia (2020) China Medical Science Press, Beijing, China, 1: 295–296, 94–97.
13. Faith DP, Minchin PR, Belbin L (1987) Compositional dissimilarity as a robust measure of ecological distance. Vegetatio 69(1): 57–68. https://doi.org/10.1007/BF00038687.
14. Grabowska-Markowska J, Pawłowska I, Ziółkowski G, Wójkowska-Mach J (2019) Bacteraemia caused by *Ochrobactrum anthropi*-unusual behavior. Wiad. Lek 72(3): 489-492.https://doi.org/10.36740/WLek201903131.
15. Hasannejad BM, Mojtahedi A, Eshaghi M, Rohani M, Pourshafie MR, Talebi M (2020) The effect of selected *Lactobacillus* strains on dextran sulfate sodium-induced mouse colitis model. Acta Microbiol. Immunol 67(2): 138-142. https://doi.org/10.1556/030.2020.00834.
16. Heidemann OR, Christensen H, Kabell S, Bisgaard M (2018) Characterization of prevalent bacterial pathogens associated with pododermatitis in table egg layers. Avian. Pathol 47(3): 281-285. https://doi.org/10.1080/03079457.2018.1440066.
17. Huang YP, He TB, Cuan XD, Wang XJ, Hu JM, Sheng J (2018) 1,4-*β*-D-Glucomannan from *Dendrobium officinale* activates NF-*κ*B via TLR4 to regulate the immune response. Molecules 23(10): 2658-2672. https://doi.org/10.3390/molecules23102658.
18. Ibrahim HM (2016) Biodegradation of used engine oil by novel strains of *Ochrobactrum anthropi* HM-1 and *Citrobacter freundii* HM-2 isolated from oil-contaminated soil. 3 Biotech 6(2): 226-238. https://doi.org/10.1007/s13205-016-0540-5.
19. Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: A systematic review. Front. Microbiol 7: 906-950. https://doi.org/10.3389/fmicb.2016.00906.
20. Lai Y, Dhingra R, Zhang Z, Ball LM, Zylka MJ, Lu K (2021) Toward elucidating the human gut microbiota-brain axis: Molecules, biochemistry, and implications for health and diseases. Biochemistry 2021: 34910469. https://doi.org/10.1021/acs.biochem.1c00656.
21. Li M, Yue H, Wang Y, Guo C, Du Z, Jin C, Ding K (2020) Intestinal microbes derived butyrate is related to the immunomodulatory activities of *Dendrobium officinale* polysaccharide. Int. J. Biol. Macromol 149(2020): 717-723. https://doi.org/10.1016/j.ijbiomac.2020.01.305.
22. Li NZ, Xia T, Xu YL, Qiu RR, Xiang H, He D, Peng YY (2012) Genome sequence of *Paenibacillus* sp. Strain Aloe-11, an endophytic bacterium with broad antimicrobial activity and intestinal colonization ability. J. Bacteriol 194(8): 2117-2118. https://doi.org/10.1128/JB.00087-12.
23. Liang CY, Liang YM, Liu HZ, Zhu DM, Hou SZ, Wu YY, Huang S, Lai XP (2017) Effect of *Dendrobium officinale* on D-galactose-induced aging mice. Chin. J. Integr 2017: 1-9.

https://doi.org/10.1007/s11655-016-2631-x.

24. Liu CZ, Chen W, Wang MX, Wang Y, Chen LQ, Zhao F, Shi Y, Liu HJ, Dou XB, Liu C, Chen H (2020) *Dendrobium officinale* Kimura et Migo and *American ginseng* mixture: A Chinese herbal formulation for gut microbiota modulation. Chin. J. Nat. Med 18(6): 446-459. DOI 10.1016/S1875-5364(20) 30052-2
25. Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27(21): 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.
26. Martínez-Romero E, Aguirre-Noyola JL, Bustamante-Brito R, González-Román P, Hernández-Oaxaca D, Higareda-Alvear V, Montes-Carreto LM, Martínez-Romero JC, Rosenblueth M, Servín-Garcidueñas LE (2021) We and herbivores eat endophytes. Microb. Biotechnol 14(4): 1282-1299. https://doi.org/10.1111/1751-7915.13688.
27. Olnood CG, Beski SSM, Choct M, Iji PA (2015) Novel probiotics: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. Anim. Nutr 1(3): 184-191. DOI10.1016/j.aninu.2015.07.003.
28. Fujimoto K, Nii T, Ogawa T, Iida T, Bhandari N, Kida T, Nakamura S, Nair GB, Takeda K (2019) Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. NPJ. Biofilms. Microbiomes 5(1): 37-49. https://doi.org/10.1038/s41522-019-0110-9.
29. Pereira VJ, Ricardo J, Galinha R, Benoliel MJ, Barreto Crespo MT (2013) Occurrence and low pressure ultraviolet inactivation of yeasts in real water sources. Photochem. Photobiol. Sci 12(4): 626-630. https://doi.org/10.1039/c2pp25225b.
30. Qin K, Chen,W, Wang MX, Chen LQ, Liu HJ, Shi Y, Liu QY, Shi DS, Chen H (2020) Effects of Tin maple crystal on human intestinal microorganism. Chin. J. Trad. Chin. Med 35(4): 1718-1723.
31. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic. Acids. Res 41: 590–600. https://doi.org/10.1093/nar/gks1219.
32. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E (2018) Environment dominates over host genetics in shaping human gut microbiota. Nature 555(7695): 210–215. https://doi.org/10.1038/nature25973.
33. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V, Chesselet MF, Keshavarzian A, Shannon KM, Krajmalnik-Brown R, Wittung-Stafshede P, Knight R, Mazmanian SK (2016) Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. Cell 167(6): 1469-1480. https://doi.org/10.1016/j.cell.2016.11.018.
34. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: opensource, platform-independent, community-supported software for describing and

comparing microbial communities. Appl. Environ. Microbiol 75(23): 7537–7541. https://doi.org/10.1128/AEM. 01541-09.

35. Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27(6): 863–864. https://doi.org/10.1093/bioinformatics/btr026.
36. Sen S, Borah SN, Bora A, Deka S (2017) Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. Cell. Fact 16(1): 95-108. https://doi.org/10.1186/s12934-017-0711-z.
37. Sen S, Borah SN, Kandimalla R, Bora A, Deka S (2020) Sophorolipid biosurfactant can control cutaneous dermatophytosis caused by *Trichophyton mentagrophytes*. Front. Microbiol 11: 329-342. https://doi.org/10.3389/fmicb.2020.00329.
38. Sezgin S, Dalar A, Uzun, Y (2020) Determination of antioxidant activities and chemical composition of sequential fractions of five edible mushrooms from Turkey. J. Food Sci. Technol 57(5): 1866-1876. https://doi.org/10.1007/s13197-019-04221-7.
39. Shi XD, Yin JY, Cui SW, Wang Q, Wang SY, Nie SP (2020) Comparative study on glucomannans with different structural characteristics: Functional properties and intestinal production of short chain fatty acids. Int. J. Biol. Macromol 164: 826-835. https://doi.org/10.1016/j.ijbiomac.2020.07.186.
40. Singh M, Kumar S, Banakar PS, Vinay VV, Das A, Tyagi N, Tyagi AK (2021) Synbiotic formulation of *Cichorium intybus* root powder with *Lactobacillus acidophilus* NCDC15 and *Lactobacillus reuteri* BFE7 improves growth performance in Murrah buffalo calves via altering selective gut health indices. Trop. Anim. Health Prod 53(2): 291-300. https://doi.org/10.1007/s11250-021-02733-z.
41. Tatsuoka M, Osaki Y, Ohsaka F, Tsuruta T, Kadota Y, Tochio T, Hino S, Morita T, Sonoyama K (2022) Consumption of indigestible saccharides and administration of *Bifidobacterium pseudolongum* reduce mucosal serotonin in murine colonic mucosa. Br. J. Nutr 127(4): 513-525. https://doi.org/10.1017/S0007114521001306.
42. Varano M, Gaspari M, Quirino A, Cuda G, Liberto MC, Focà A (2016) Temperature-dependent regulation of the *Ochrobactrum anthropi* proteome. Proteomics 16(3): 3019-3024. https://doi.org/10.1002/pmic.201600048.
43. Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, Tschaplinski TJ, Schadt CW (2019) Rhizosphere microbiomes diverge among *Populus trichocarpa* plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome 7(1): 76-90. https://doi.org/10.1186/s40168-019-0668-8.
44. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol 73(16): 5261–5267. https://doi.org/10.1128/AEM.00062-07.
45. Wang S, Martins R, Sullivan MC, Friedman ES, Misic AM, El-Fahmawi A, De Martinis ECP, O'Brien K, Chen Y, Bradley C, Zhang G, Berry ASF, Hunter CA, Baldassano RN, Rondeau MP, Beiting DP (2019) Diet-induced remission in chronic enteropathy is associated with altered microbial community

structure and synthesis of secondary bile acids. Microbiome 7(1): 126-145. https://doi.org/10.1186/s40168-019-0740-4.

46. Wei JZ, O'Rear J, Schellenberger U, Rosen BA, Park YJ, McDonald MJ, Zhu G, Xie W, Kassa A, Procyk L, Perez Ortega C, Zhao JZ, Yalpani N, Crane VC, Diehn SH, Sandahl GA, Nelson ME, Lu AL, Wu G, Liu L (2018 a) A selective insecticidal protein from *Pseudomonas mosselii* for corn rootworm control. Plant. Biotechnol. J 16(2): 649-659. https://doi.org/10.1111/pbi.12806.
47. Wei Y, Wang L, Wang D, Wang D, Wen C, Han B, Ouyang Z (2018 b) Characterization and anti-tumor activity of a polysaccharide isolated from *Dendrobium officinale* grown in the Huoshan County. Chin. Med 13: 47-57. https://doi.org/10.1186/s13020-018-0205-x.
48. Wu L, Xiao W, Chen G, Song D, Khaskheli MA, Li P, Zhang S, Feng G (2018) Identification of *Pseudomonas mosselii* BS011 gene clusters required for suppression of rice blast fungus *Magnaporthe oryzae*. J. Biotechnol 282: 1-9. https://doi.org/10.1016/j.jbiotec.2018.04.016.
49. Wu W, Chen W, Liu S, Wu J, Zhu Y, Qin L, Zhu B (2021 a) Beneficial relationships between endophytic bacteria and medicinal plants. Front. Plant Sci 12: 646146-646158. https://doi.org/10.3389/fpls.2021.646146.
50. Wu X, Kobori H, Orita I, Zhang C, Imanaka T, Xing XH, Fukui T (2012) Application of a novel thermostable NAD(P)H oxidase from hyperthermophilic archaeon for the regeneration of both NAD and NADP. Biotechnol. Bioeng 109(1): 53-62. https://doi.org/10.1002/bit.23294.
51. Wu WY, Chou PL, Yang JC, Chien CT (2021 b) Silicon-containing water intake confers antioxidant effect, gastrointestinal protection, and gut microbiota modulation in the rodents. PLoS One 16(3): 0248508-0248524. https://doi.org/10.1371/journal.pone.0248508.
52. Xiao XC, Chen WH, Cao YY, Lou YJ, Liu Y, Wang J, Zhai XF, Li NS., Li Y (2018) Protective effect of *Dendrobium officinale* on isoproterenol induced cardiac hypertrophy in rats. China. J. Chin. Mater. Med 43(4): 800-804. https://doi.org/10.19540/j.cnki.cjcmm.2018.0021.
53. Yang RH, Li SZ, Li YL, Yan YC, Fang Y, Zou LF, Chen GY (2021) Bactericidal effect of *Pseudomonas oryziphila* sp. nov., a novel *Pseudomonas* species against *Xanthomonas oryzae* reduces disease severity of bacterial leaf streak of rice. Front. Microbiol 12: 759536-759550. https://doi.org/10.3389/fmicb.2021.759536.
54. Zheng H, Pan L, Xu P, Zhu J, Wang R, Zhu W, Hu Y, Gao H (2017) An NMR-based metabolomic approach to unravel the preventive effect of water-soluble extract from *Dendrobium officinale* Kimura & Migo on streptozotocin-induced diabetes in mice. Molecules 22(9): 1543-1556. https://doi.org/10.3390/molecules22091543.
55. Zegarra-Ruiz DF, El Beidaq A, Iñiguez AJ, Lubrano Di Ricco M, Manfredo Vieira S, Ruff WE, Mubiru D, Fine RL, Sterpka J, Greiling TM, Dehner C, Kriegel MA (2019) A Diet-sensitive commensal *Lactobacillus* strain mediates TLR7-dependent systemic autoimmunity. Cell Host Microbe 25(1): 113-127. https://doi.org/10.1016/j.chom.2018.11.009.
56. Zhang Y, Wu Z, Liu J, Zheng Z, Li Q, Wang H, Chen Z, Wang K (2019) Identification of the core active structure of a *Dendrobium officinale* polysaccharide and its protective effect against dextran sulfate

sodium-induced colitis via alleviating gut microbiota dysbiosis. Food Res. Int 137: 109641-109652. https://doi.org/10.1016/j.foodres.2020.109641.

57. Zheng D, Wang Z, Sui L, Xu Y, Wang L, Qiao X, Cui W, Jiang Y, Zhou H, Tang L, Li Y (2021) *Lactobacillus johnsonii* activates porcine monocyte derived dendritic cells maturation to modulate Th cellular immune response. Cytokine 144: 155581-155588. https://doi.org/10.1016/j.cyto.2021.155581.
58. Zheng Y, Xiao G, Zhou W, Gao Y, Li Z, Du G, Chen B (2020) Midgut microbiota diversity of potato tuber moth associated with potato tissue consumed. BMC Microbiol 20(1): 58-73. https://doi.org/10.1186/s12866-020-01740-8
59. Zhu B, Wu J, Ji Q, Wu W, Dong S, Yu J, Zhang Q, Qin L (2020) Diversity of rhizosphere and endophytic fungi in *Atractylodes macrocephala* during continuous cropping. Peer J 8: 8905-8922. https://doi.org/10.7717/peerj.8905.
60. Zhu J, Sun X, Zhang ZD, Tang QY, Gu MY, Zhang LJ, Hou M, Sharon A, Yuan HL (2021) Effect of ionizing radiation on the bacterial and fungal endophytes of the halophytic plant *Kalidium schrenkianum*. Microorganisms 9(5): 1050-1067. https://doi.org/10.3390/microorganisms9051050.

Figures

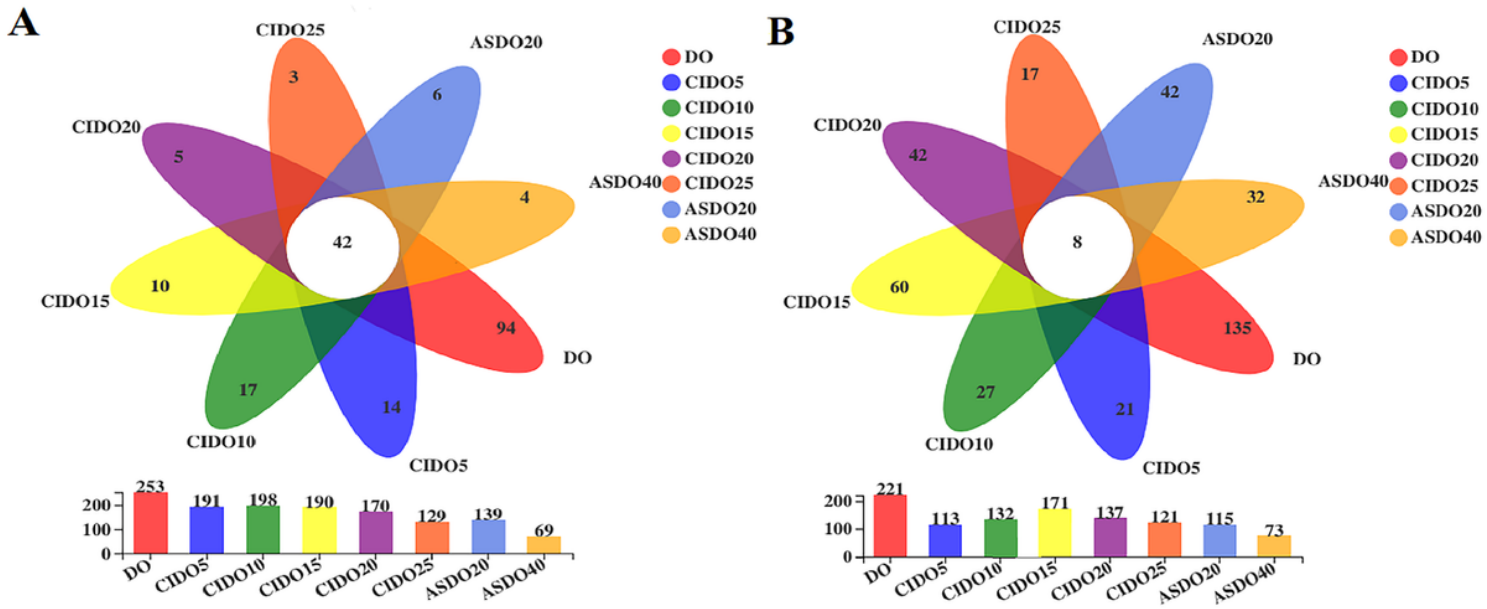

Figure 1

Venn diagram analysis for unique and shared operational taxonomic units among (A) bacteria and (B) fungi in *D. officinale* under different ^{60}Co-*γ* irradiation and hot pressure treatments.

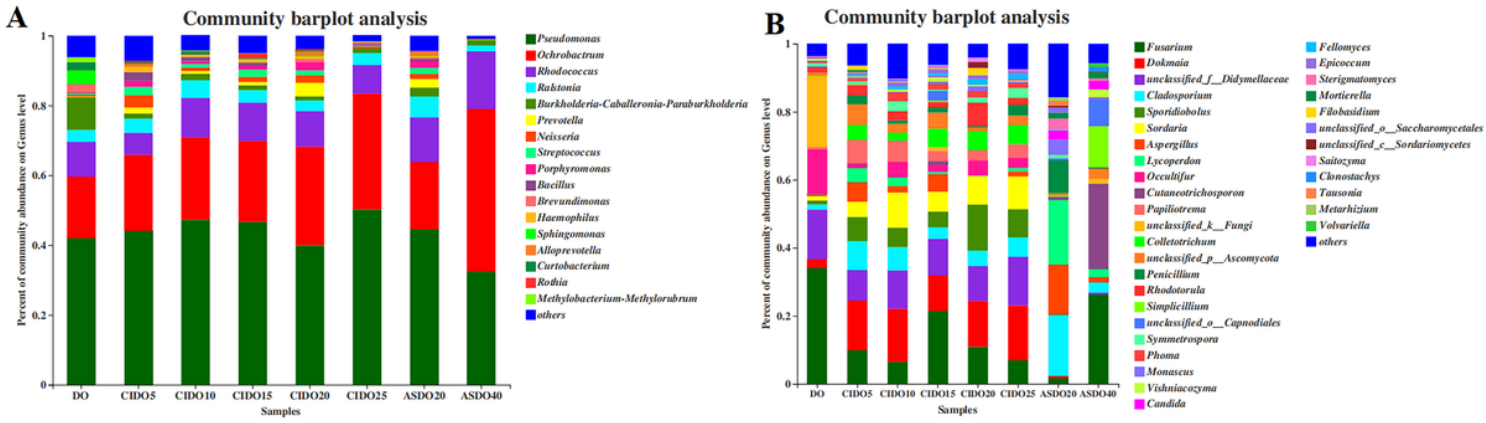

Figure 2

Relative abundance of the (A) bacterial and (B) fungal genus present in *D. officinale* with different ^{60}Co-*γ* irradiation and hot pressure.

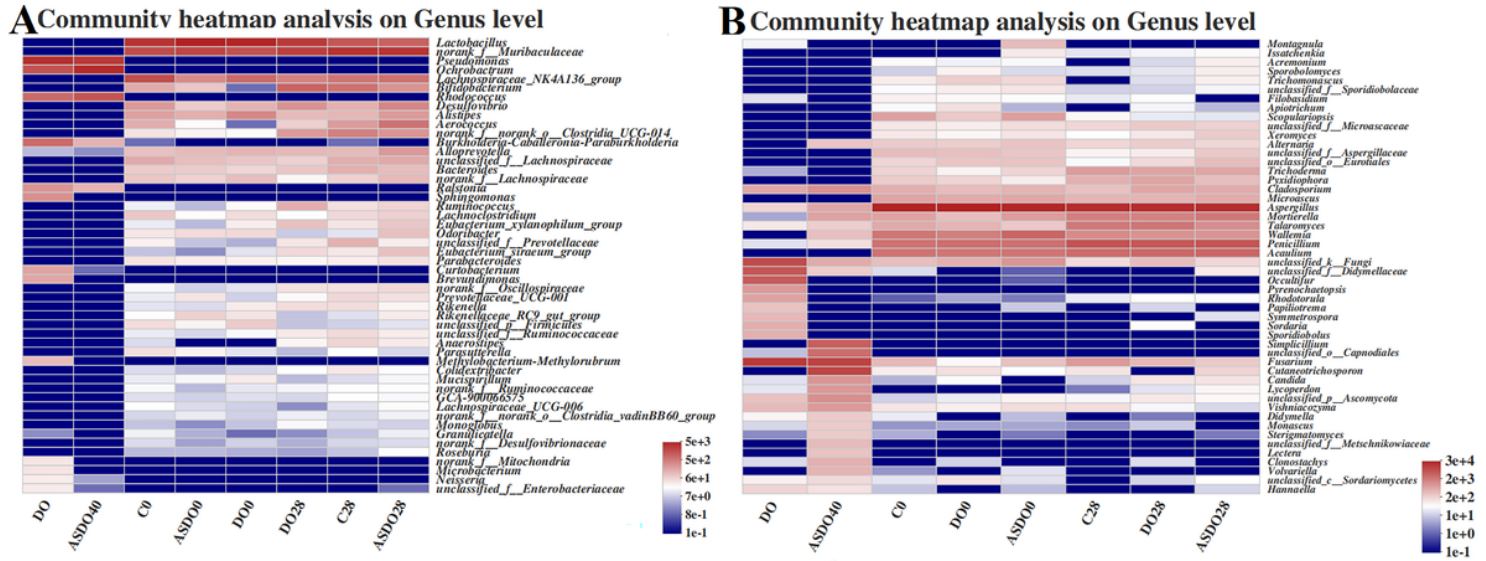

Figure 3

Community heatmap analysis of bacteria (A) and fungi (B) at the genus levels in fecal samples of mice.

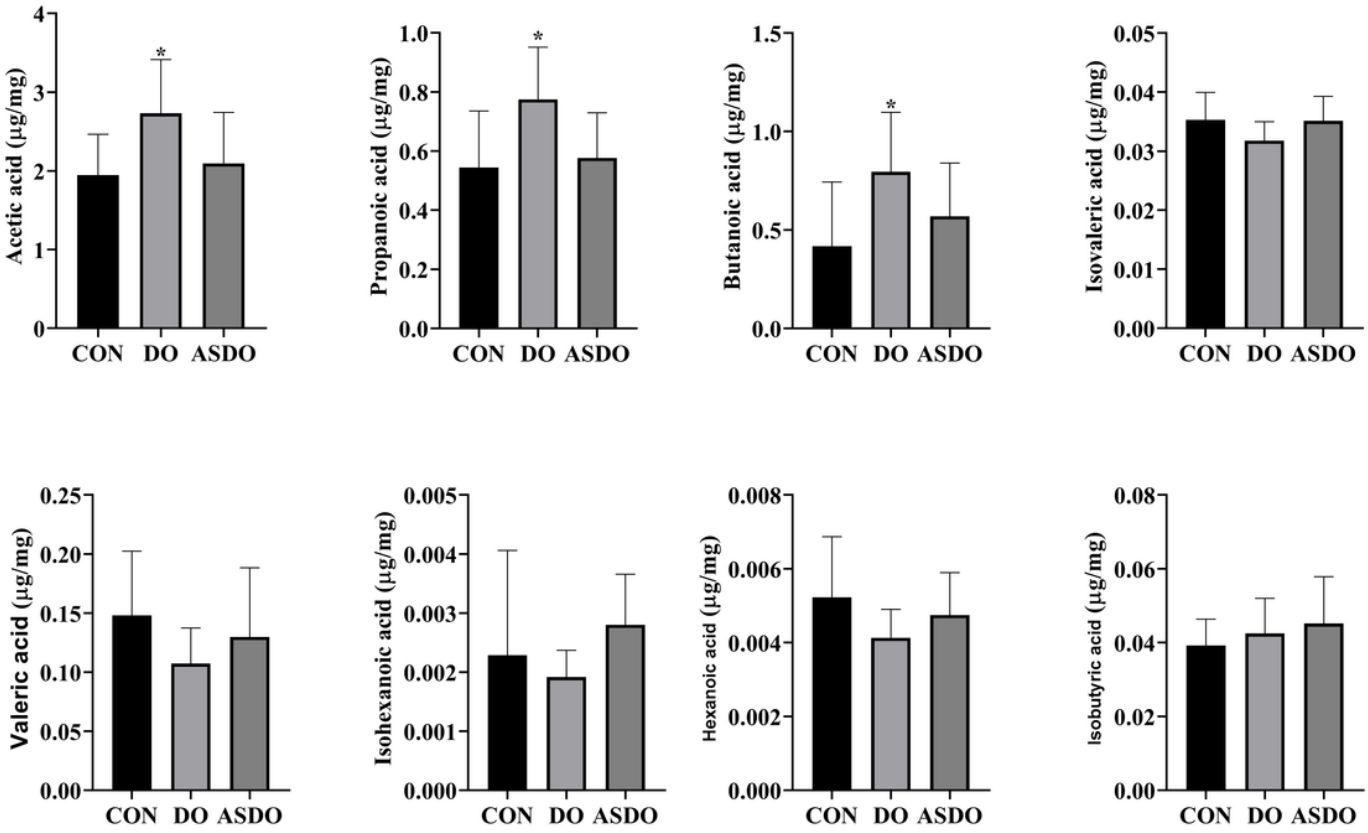

Figure 4

The concentration of eight SCFAs in fecal samples. * P ≤ 0.05

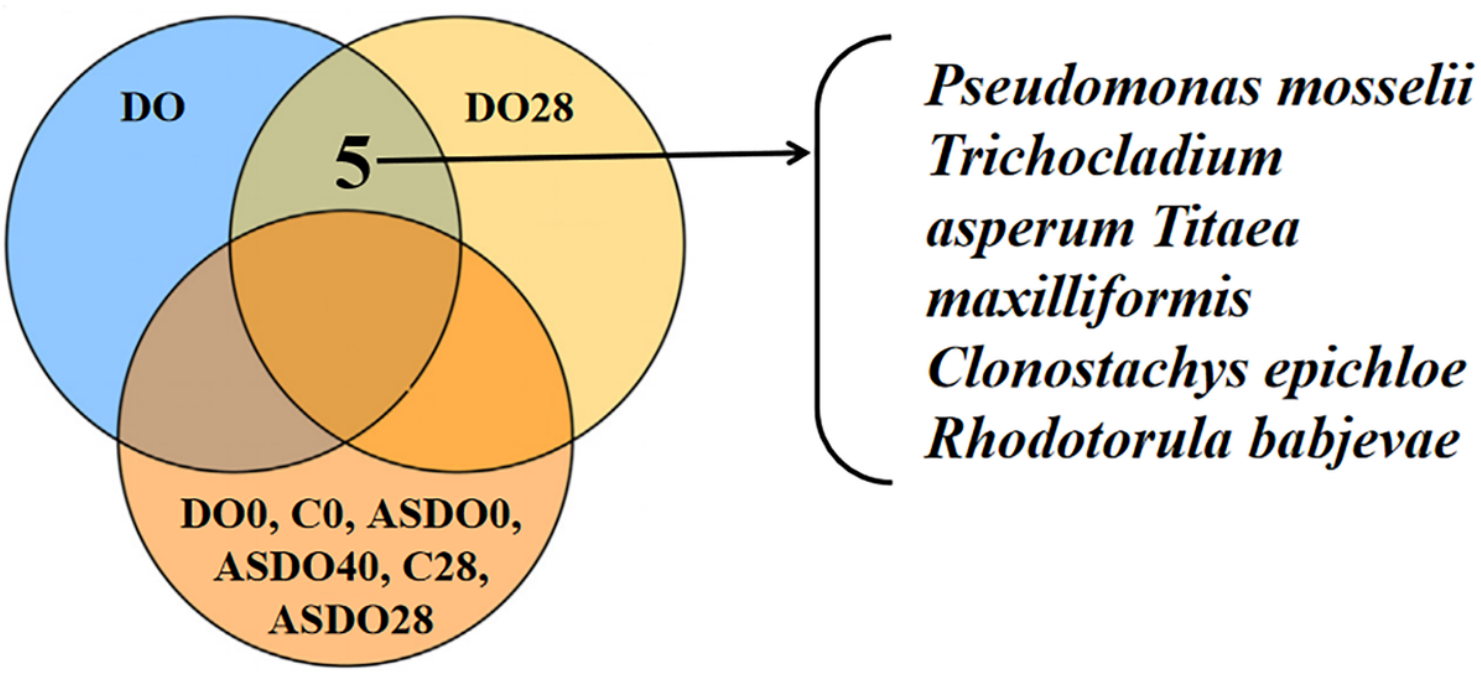

Figure 5

D. officinale endophytes may colonize in the intestinal tract of mice and modulate gut microbiota.

Supplementary Files

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