Succession of endophytic bacterial community and its contribution to cinnamon oil production during cinnamon shade-drying process

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ARTICLE INFO

ABSTRACT

Cinnamon oil is a blend of secondary metabolites and is widely used as spice. Endophytic bacteria are always related to the secondary metabolites production. However, the potential of endophytic bacteria communities for cinnamon oil production during cinnamon shade-drying process is still not clear. In this study, we investigated the composition and metabolic function of endophytic bacterial community during 80-day shade-drying process. The temporal dynamics of essential oil content and its dominant constituents were analyzed. The succession of endophytic bacterial community from d0 to d80 was identified. The influence of endophytic bacterial community evolution on cinnamon oil is significant positive. Predictive functional analysis indicated that shade-drying process was rich in *Saccharopolyspora* that produce enzymes for the conversion of phenylalanine to cinnamaldehyde. These findings enhance our understanding of the functional bacterial genera and functional genes involved in the production of cinnamon oil during cinnamon shade-drying process.

1. Introduction

Cinnamon oil is an essential oil obtained from genus *Cinnamomum*. It is used as an ingredient in several food products to provide flavor and aroma (Nwanade et al., 2021; Xue et al., 2021). Chemically, cinnamon oil is a blend of secondary metabolites composed of phenylpropanoids, with cinnamaldehyde present as major component (Ding et al., 2011; Yang et al., 2022).

Cinnamon oil is conventionally obtained by hydro-distillation of cinnamon leaves. Once cinnamon leaves have been collected, they have to be shade-drying, which can significantly increased the contents of cinnamon oil. And this regulation has been widely recognized. Drying is usually considered as a method for inhibiting microbial growth and forestalls biochemical changes but, at the same time, it can change the composition of endophytic community (Bernard, Kwabena, Osei, Daniel, Elom, & Sandra, 2014). However, the succession of endophytic bacterial community during cinnamon leaves shade-drying process have not been

characterized. The absence of this knowledge hinders our understanding of the microbial activity on the production of cinnamon oil during shade-drying process.

Previous reports have demonstrated that the cinnamon oil is strongly correlated with endophytic fungus (Chowdhury, Islam, Islam, & Khan, 2019; Strobel, 2018). One of the most important endophyte obtained from cinnamon tree (*Cinnamon zeylanicum*) is *Muscodor Albus* (Stobel, Dirkse, Sears, & Markworth, 2001). It has the potential to make a plethora of volatile organic compounds such as alcohols, esters, and lipids. Despite a few papers have dealt with endophytic fungi in cinnamon, none of them identified the endophytic bacteria responsible for cinnamon oil production. Actually, in recent studies, researchers found that internal parts of the plants could be colonized by bacteria as well. Similarly, endophytic bacteria also play a significant role in modulating plant metabolite synthesis (Singh, Kumar, Singh, & Pandey, 2017). Firstly, the induction of secondary metabolite production by endophytic bacteria was aimed to strongly influence the stress tolerance of plants.

This phenomenon is usually occurred in aromatic and medicinal plants. Secondly, some secondary metabolites were produced by a plant in combination with associated endophytic bacteria. In this respect, the rich metabolic repertoire of endophytic bacteria is usually shown in diverse actinobacterial strains. In addition, endophytic bacteria exhibited high species diversity and adaption to harsh environments (Wallace & May, 2018). The endosymbiont between endophytic bacteria and hosts was stable due to the change in bacterial community structure (Chintan Kapadia, Trupti, Vyas, & Patel, 2021). These characters were helpful for endophytic bacteria surviving in dried plants. Thus, endophytic bacterial community may still play a key role for the metabolic potential of dried plants. In other words, the functions of secondary metabolites synthesis based on endophytic bacteria may continue in dried plants. Zhang et al. measured the diversity and functions of the endophytic bacterial community of fresh and dry *Panax notoginseng* by high-throughput sequencing (Zhang et al., 2020). Results showed that the plant sample exhibited a higher abundance of the endophyte-associated metabolic function of terpenoids synthesis after drying. Their reports highlights the metabolic function of endophytic bacteria community in dry plants. And, the excellent metabolic function of endophytic bacteria in drying plants should be taken into consideration in further studies investigating cinnamon oil. Hitherto, there is no evidence to provide a comprehensive understanding of the composition and metabolic function of endophytic bacterial community during cinnamon leaves shade-drying process. Furthermore, the effect and mechanism of endophytic bacterial community on the production of cinnamon oil is unclear, and the key bacteria that are responsible for cinnamon oil production are still unknown.

The objective of this work was to uncover the effect of endophytic bacterial communities on the production of cinnamon oil during cinnamon leaves shade-drying process. For this objective, the temporal dynamics of essential oil content and its dominant constituents were investigated. The endophytic bacterial communities were monitored throughout the shade-drying process using the Illumina MiSeq platform for high-throughput sequencing. And, the association analysis was performed between the content of cinnamon oil and endophytic bacterial communities, including the bacterial diversity and abundance of marker taxa. Finally, the possible endophyte-associated metabolic pathway was predicted by PICRUSt analysis, and the critical endophytic bacteria responsible for cinnamon oil production were also identified.

2. Materials and methods

2.1. Shade drying experiment and samples preparation

Fresh leaves of cinnamon (Cinnamomum cassia Presl.) were harvested in August 2020 from Dongxing (China) which is one of the most significant origin of cinnamon. This field features typical subtropical monsoon climate with an annual average temperature of 23.2 °C, annual average rainfall of 2738 mm, and annual sunshine hours of 1500 h. For shade drying, the leaves were spread uniformly on a single layer sample tray and were placed in a dark and dry room with appropriate ventilation (Sefidkon, Abbasi, & Khaniki, 2006). The temperature of the room was 26 ± 2 °C, and the relative air humidity varied within the range of 30 ± 5%. About 300 g well-mixed samples were collected every twenty days from day 0, and the further analysis were performed at day 0, 20, 40, 60 and 80 respectively. The collected samples were randomly divided into different sets. Firstly, three sets of 5 g each were used for the measurement of moisture content. To measure the moisture content, the samples were dried using an oven (105 ± 2 °C) until there was no change in the weight between the two consecutive measurements (Baiqi, Utami, Anugrah, Fauzan, & Rusydi, 2019). Secondly, three sets of 40 g each were used for cinnamon oil extraction. To extract volatile oil, the samples were powdered to a homogeneous size, and submerged in 200 mL of water, followed by submitting to water distillation for 5 h (Wang et al., 2012). The weight of oil was determined using a laboratory scale with readability to 0.01 mg. Lastly, about 20 g plant samples (six biological replicates) were used for endophytic bacterial analysis. In addition, the levels of polysaccharides and shikimate dehydrogenase activity in collected samples were measured using commercially available kits according to the manufacturers' instructions.

2.2. Cinnamon oil analysis

The content of cinnamon oil is calculated based on the dry weight of leaves. The extracted cinnamon oil diluted 10 times with EtOAc was injected for GC–MS and GC analysis. GC/MS analysis was performed on a 7890A gas chromatograph (Agilent, California, USA) equipped with a 5975C plus mass spectrometer (Agilent, California, USA). Identification of compounds was based on comparisons of their mass spectra with those recorded in the National Institute of Standards and Technology libraries data (NIST05.LIB). Area percentage of each essential oil component was calculated using a peak area normalization measurement. Quantitative analyses were performed on a GC-2014AF gas chromatography (Shimadzu, Kyoto, Japan). A cocktail of three aldehyde standards, including *trans*-cinnamaldehyde, *o*-methoxy cinnamaldehyde, and benzaldehyde was prepared for the calibration curves. The ranges of calibration curves were 0.7–428.6, 0.7–424.6, and 0.6–374.6 ng, respectively ($n = 7$). Significant difference was evaluated by one-way analysis of variance (ANOVA). All of the statistical analyses were performed using SPSS software (v. 16.0).

2.3. DNA extraction and Illumina MiSeq sequencing

The collected leaves were washed with running water to remove the surface soil, soaked in alcohol (70% *v/v*) for 3 min, and finally rinsed thoroughly with sterile water. Microbial DNA was extracted from the leaf samples using the MP-soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturers' instructions (Xu, Ling, Zhao, Wang, & Wang, 2020). The final DNA concentration was determined by UV spectrophotometer. The V5-V7 regions of gene were amplified with primers 799F (5′-AACMGGATTAGATACCCKG-3′)-1193R(5′-ACGT-CATCCCCACCTTCC-3′) by PCR (GeneAmp 9700, ABI, USA) (Bulgarelli et al., 2015). The amplified PCR products were further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Generally, we obtained 641,733 valid sequences with an average length of 376. The well sequences were then further proceeded using Mothur (v. 1.30.2) to create operational taxonomic units (OTUs) (Xu et al., 2020).

2.4. Endophytic bacterial community analysis

Rarefaction curves were done using the nonnormalized SVs table. α-diversity was calculated with ace, shannon, and simpson using R software (v. 3.3.1) with the vegan package. The operational OTU sequences were assigned taxonomy by Ribosomal Database Project (RDP) classifier (v. 2.11). Principal component analysis (PCA) was performed using the SIMCA software (v. 14.1) (Zhao et al., 2021). Linear discriminant analysis (LDA) effect Size (LEfSe) based on a non-parametric factorial Kruskal-Wallis (KW) sum-rank test were applied to the clusters (Zhong, Li, Zeng, Wang, Sun, & Tang, 2020). The identification of significantly different taxa from d0 to d80 was performed in R software (v. 3.3.1) with the stats package and Python software with the scipy package. Linear regression analysis and redundancy analysis (RDA) were used to link cinnamon oil content and bacterial community composition (Chen, Hsu, Kumar, Budzianowski, & Ong, 2017; Wang, Liu, Xia, & Chen, 2019). The analyses were performed in R software (v. 3.3.1) with the vegan package. The Spearman's correlation coefficient values between the marker taxa and the main component of cinnamon oil were calculated by R software (v. 3.3.1) with the psych package.

2.5. Functional prediction analysis

Phylogenetic investigation of communities by reconstruction of un-observed states (PICRUSt) on galaxy cloud server was used to predict the potential function of bacterial community (Mohd Nor, Steeneveld, Mourits, & Hogeveen, 2015). According to the database of EggNOG (evolutionary genealogy of genes) and KEGG (Kyoto Encyclopedia of Genes and Genomes), the KEGG Ortholog (KO) based on OUT matrix were predicted. At the same time, their abundances were obtained. The EC number involved in the possible metabolic pathways was manually categorized based on the KEGG databases.

3. Results and discussion

3.1. Change in cinnamon oil

Shade drying is accompanied by the loss of water (Ebadi, Azizi, Sefidkon, & Ahmadi, 2015). At d20, the moisture content of cinnamon leaves was reduced by as much as 40% of the initial content (Fig. 1A). From d20 to d80, the cinnamon leaves had no differences in their moisture content, which suggested an equal dry weight. This was similar to the reported results from other plant materials, showing a rapid moisture removal from the leaves in the initial drying period. On the contary, cinnamon oil exhibited a smaller content (0.7%) at d0 and it remained nearly constant until d40 (Fig. 1B). At d60, the highest oil

content (1.61%, P < 0.01) was obtained. It suggested that shade-drying couldn't cause significant variation of the cinnamon oil in a short time. The time required to improve cinnamon oil content through shade-drying was 60 days.

A total of 31 compounds was identified in the cinnamon oil under different drying time, that represented 99% of the oils (Table S1). Fig. 1C shows the relative proportions of major classes of cinnamon oil. During shade-drying process, aldehydes formed the group with the most peak area (>60%), especially at d60. Esters (9–25%) are the second important class of compounds after aldehyde, followed by acid (2.3–4.7%), ketone (1.3–4.7%), alcohol (1.0–2.0%) and terpenes (0.4–2.0%). In the case of aldehydes, *trans*-cinnamaldehyde, o-methoxy cinnamaldehyde and benzaldehyde are major compounds, which were detected over the whole drying process. As seen in Fig. 1D, the content of *trans*-cinnamaldehyde represented approximately 3.43‰ at the beginning of shade-drying, Whereas, drying for 60 days resulted in the highest content doubled to 8.81‰. Similar results were found in *o*-methoxy cinnamaldehyde and benzaldehyde. Therefore, shade-drying process had a significant effect on the content but not the chemical composition of the cinnamon oil. The major composition in cinnamon oil belong to aldehyde, which is closely associated with endophytic bacteria. We hypothesize that the mechanism of this significantly improved cinnamon oil is due to the unique endophytic bacterial community of d60. In-depth MiSeq sequencing for endophytic bacteria in cinnamon leaves is needed to prove the inference.
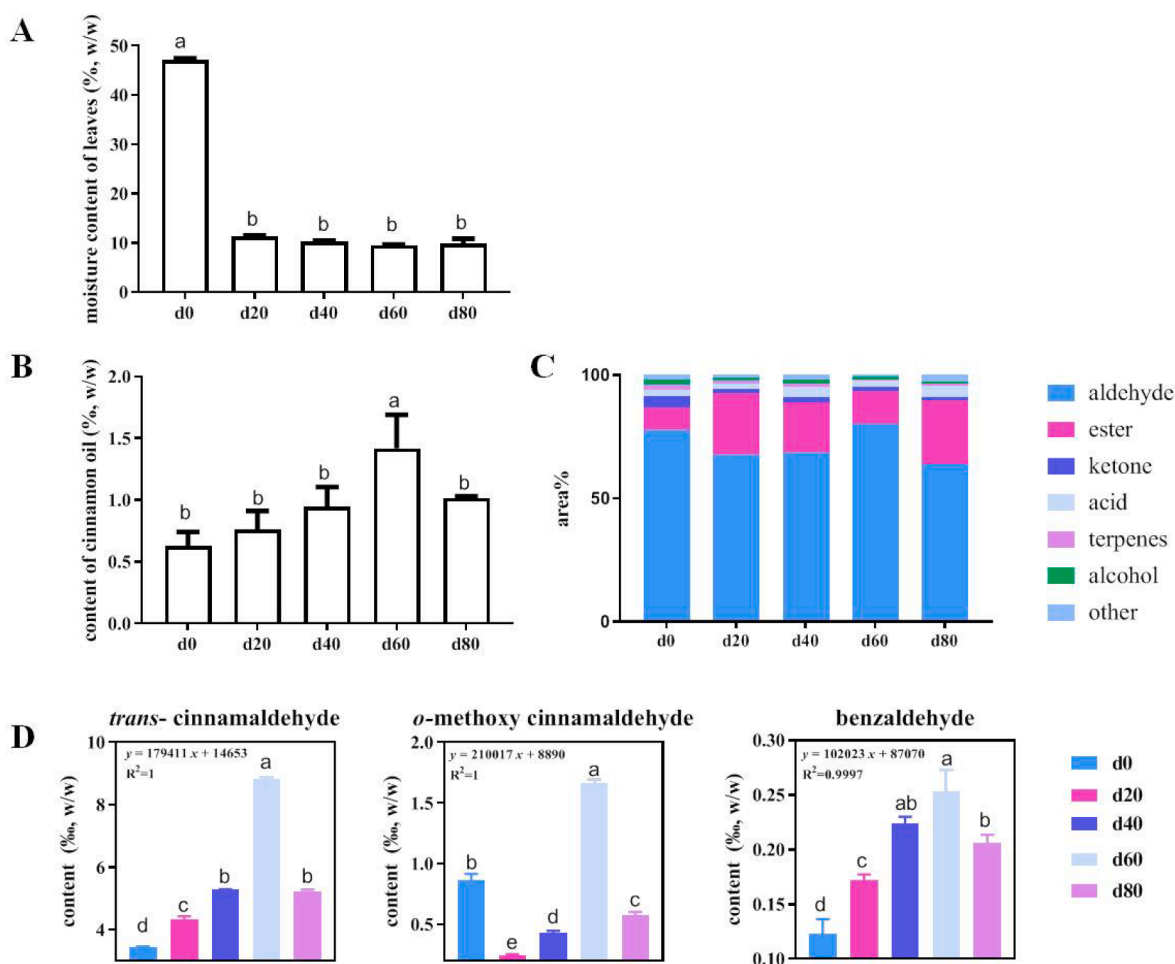

Fig. 1. Analysis of cinnamon oil and moisture content during cinnamon leaves shade-drying process. (A) Moisture content of cinnamon leaves. (B) Cinnamon oil content (%, w/w, dry basis) obtained by water distillation. (C) Area percentage of major constituents in cinnamon oil based on GC–MS. (D) Content (%, w/w, dry basis) of *trans*-cinnamaldehyde, *o*-methoxy cinnamaldehyde, and benzaldehyde calculated using the respective calibration curves. The content of cinnamon oil is calculated based on dry weight of leaves. Data refer to mean ± standard deviation (n = 3). Different letters indicate statistically significant differences at p < 0.05. d0, d20, d40, d60, and d80 mean drying time in days.

3.2. Succession of endophytic bacterial community

To gain insight into the dynamics of the bacterial community composition, the rarefaction and α diversity analysis were performed. Rarefaction analysis showed that all the test samples reached the plateau, which indicated sufficient sequencing depth (Fig. S1). Diversity index including ace, shannon, and simpson showed stable trends over time, which indicated similar richness among all the test samples (Fig. S1). Based on sufficient sequencing depth and stable diversity index, it's believed that any variation in the drying process may result primarily from the changes in the functional activities of metabolically active community (Klein, Bohannan, Jaffe, Levin, & Green, 2016). As shown in Fig. 2A, the endophytic bacterial community is relatively stable based on high diversity. *Proteobacteria* and *Actinobacteria* were the two most dominant groups, account for 93.5 to 98.5% of the totals, followed by *Acidobacteriota* and *Firmicutes*. *Proteobacteria* and *Actinobacteria* have been proposed as main taxa of bacterial community in plant (van Bergeijk, Terlouw, Medema, & van Wezel, 2020). The endophytic *Proteobacteria* currently comprises two classes, *α-Proteobacteria* and *γ- Proteobacteria*. *γ-Proteobacteria* is composed of the plant pathogenic xanthomonads, which were capable of producing diverse *exo*-enzymes and exopolysaccharides (del Barrio-Duque, Samad, Nybroe, Antonielli, Sessitsch, & Compant, 2020). The endophytic *Actinobacteria* have an unrivaled metabolic versatility and produce most of natural products with medical or agricultural applications. The relative abundance of *Proteobacteria* in cinnamon leaves significantly decreased from 64.1% at d0 to 41.8% at d60 and then significantly increased to 52.3% at d80. On the contrary, the relative abundance of *Actinobacteria* in cinnamon leaves significantly increased from 30.9% at d0 to 56.7% at d60 and then significantly decreased to 41.2% at d80. It is obvious to detect a transition from *Proteobacteria* to *Actinobacteria* during cinnamon leaves shade-drying process. The variation of bacterial community composition suggested that (1) the initial phase of cinnamon leaves shade-drying is mainly conducted by the *Proteobacteria*, releasing *exo*-enzymes to hydrolysis polysaccharides. (2) At d60, *Actinobacteria* became the most dominant phyla, which might direct the carbon flow from glycolysis to phenypropanoid metabolism.

After analysis of bacterial taxonomic composition, the sequencing data from d0 to d80 was fed to SIMCA-P for pattern recognition analysis. The square discriminant analysis (OPLS-DA) on OUT level was used to assess the alterations of overall endophytic bacteria among these five phases. As shown in Fig. 2B, the endophytic bacterial profiles of the five phases were separated from each other (R^2X: 0.726, R^2Y: 0.755, P: 0.005), indicating that shade-drying has obvious affection on the endophytic bacterial profile. The result of OPLS-DA suggests that each period of shade drying process has a unique microbial composition, thus potential biomarkers for each phase may exist. The unique biomarkers for each phase are probably an adaptation to the changing environment in cinnamon leaves. To identify the potential biomarkers in each phase, we conducted a linear discriminant analysis (LDA) effect size (LEfSe) analysis. According to the effect size (LDA > 2.0, P < 0.05), one hundred and eleven taxa were screened out with significant differences at all
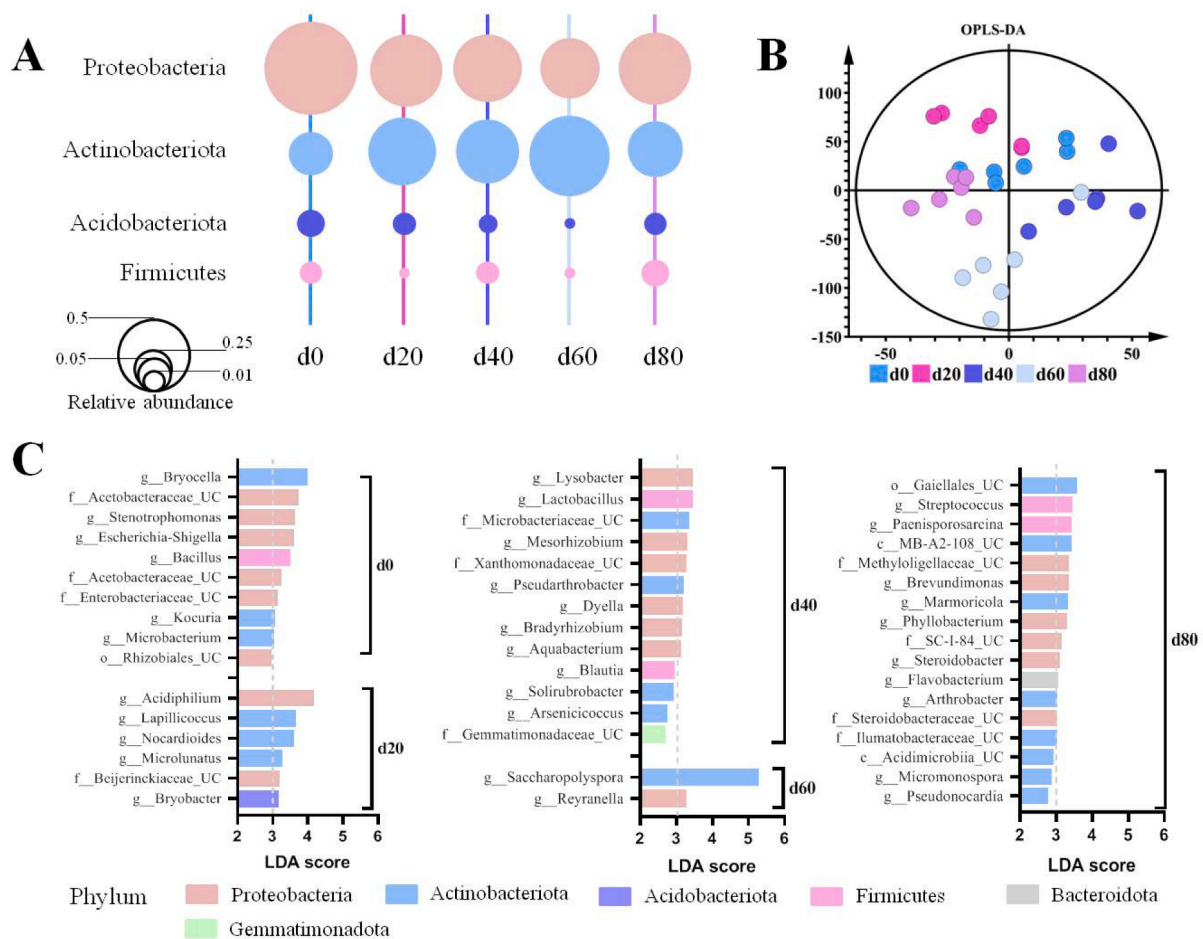

Fig. 2. Analysis of endophytic bacterial community during cinnamon leaves shade-drying process. (A) Taxonomic classification of the endophytic microbe. Circle size represented the relative abundances (>1.0%) of phylum. (B) OPLS-DA scatter plot based on the operational OTU sequences. (C) LEfSe results of the endophytic bacterial structure (LDA score > 2.0, p < 0.05) Taxonomic cladograms and taxonomic assignment details were shown in E-Supplementary data. d0, d20, d40, d60, and d80 mean drying time in days.

classification levels (Fig. S2), including forty-eight clusters at the genus level (Fig. 2C). The genera *Bryocella*, *Stenotrophomonas*, and *Escherichia-Shigella*, were marker taxa at d0. The genus *Bryocella*, strictly aerobic chemoorganotrophs, which tend to be associated with exopolysaccharide-producing bacteria (Dedysh, 2018). Actually, the dominant genus at d0 is *Ralstonia*, which is typical exopolysaccharide-producing bacteria. Previous study has demonstrated that the *Stenotrophomonas* can increase the water permeability of plant, which enhancing the availability of water (Ryan et al., 2009). Thus, it is reasonable to observe the increased *Stenotrophomonas* abundance in the initial drying period. The genera *Acidiphilium*, *Lapillicoccus*, and *Nocardioides* were enriched at d20. Genus *Acidiphilium* is reported as acidophilic heterotrophic cluster with strong viability, which has an unrivalled hydrolysis activity of organic substances (Hiraishi & Imhoff, 2005). Thus, the Genus *Acidiphilium* was capable of making the substrates available to all the members of community, especially in extreme environments. The genera *Lysobacter*, *Lactobacillus*, and *Mesorhizobium* were marker taxa at d40. *Lysobacter* has lytic effects on many other microorganisms including fungi, oomycetes, nematodes, unicellular algae, and bacteria (de Bruijn et al., 2015). And, it grows best with microbial cells as a nutrient source. Thus, the enrichment of genus *Lysobacter* is probably linked to the invalidation of microorganisms from environment, as well as the degradation of sensitive bacteria in community. The genera *Saccharopolyspora* and *Reyranella* had significantly higher relative abundance at d60. This is consistent with previous studies which reported that genus *Saccharopolyspora* was present in 15% of freshly harvested plant samples, but in 33% of plant samples after drying (Kim & Goodfellow, 2015). Because genus *Saccharopolyspora* is described as thermophilic, alkaliphilic and halophilic taxa. It contained many sets of duplicated genes involved in defense and stress responses, which were used to support the saprophytic way of life (Sayed, Abdel-Wahab, Hassan, & Abdelmohsen, 2020). At d80, the marker taxa included the genera *Streptococcus*, *Paenisporosarcina*, and *Brevundimonas*. Seven unidentified genera belonging to the families *Methyloligellaceae* and order *Gaiellales*. Most of these genera were considered as plant pathogens that could keep robust and stable under harsh conditions (Shi et al., 2020). These results indicated that shade-drying process induced the succession of endophytic bacterial community in cinnamon leaves, which may make great contribution for cinnamon oil production.

3.3. Relationship between bacterial community evolution and cinnamon oil

In order to investigate the relationship between endophytic bacteria and cinnamon oil, the PC1value of OPLS-DA (Fig. 2B) and cinnamon oil content (Fig. 1B) were performed for line regression analysis. The PC1 value of OPLS-DA is used for describing the data structure in terms of plots, which represent the characteristics of endophytic bacterial communities during cinnamon leaves shade-drying process. The regression line of endophytic bacterial communities and cinnamon oil was plotted
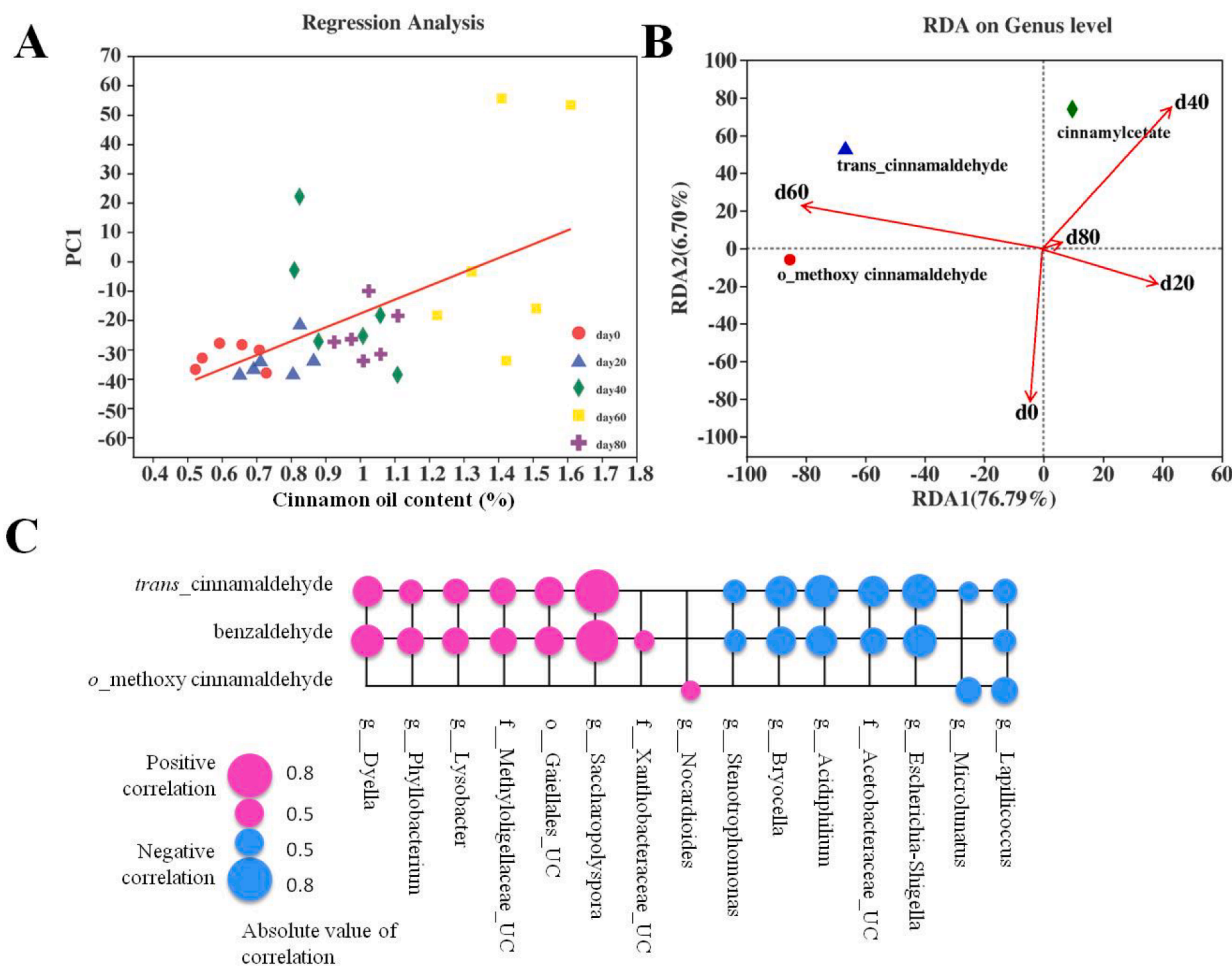

Fig. 3. Association analysis between endophytic bacterial community and cinnamon oil during cinnamon leaves shade-drying process. (A) Profiles and linear regression of endophytic bacterial structure versus cinnamon oil content. (B) Redundancy analysis of the link between bacterial community structures and the content of *trans*-cinnamaldehyde, o-methoxy cinnamaldehyde, and benzaldehyde. (C) Inter-correlation between marker genera and major compounds in cinnamon oil. The size of the nodes in the dot plot showed the Spearman's correlation coefficient (P < 0.05). d0, d20, d40, d60, and d80 mean drying time in days.

in Fig. 3A. The coefficient of determination (R^2) is 0.3216, elucidating the crude linear correlation between endophytic bacteria communities and cinnamon oil. It implies that there exists a linear relationship between evolution of bacterial community and oil production during cinnamon leaves shade-drying process. The slope of the regression line is 0.472, which reveals that the influence of the bacterial community evolution on the production of cinnamon oil is positive. Based on the endophytic bacterial community and cinnamon oil profile data, redundancy analysis (RDA) reflected bacterial community samples and cinnamon oil samples on the same two-dimensional ordination map, which was applied to visually describe the relationship between endophytic bacterial community and cinnamon oil (Gou, Wang, Zhang, Zhong, & Gao, 2021). As shown in Fig. 3B, RDA1 and RDA2 accounted for 83.49% of the variation of endophytic bacterial communities that characterized shade-drying process of cinnamon leaves. The correlation between the endophytic bacterial community and the major compounds of cinnamon oil was described according to the angle between the rays and sample points. The obtuse or right angles between the main components of cinnamon oil and most sample points (d0, d20, d40, and d80) were found, which represented negative or no correlation between endophytic bacterial communities and cinnamon oil at d0, d20, and d40. And, the distance between sample points (d0, d20, d40) was near, indicating that the bacterial community composition of those groups was similar. However, the difference between the d60 group and other groups was noticeable. At d60, the angle between the main components of cinnamon oil and most sample points was acute. Thus, the endophytic bacterial community at d60 displayed a significantly positive relationship with cinnamon oil.

Furthermore, Spearman's correlation between the marker taxa and the main components of cinnamon oil was visualized by a correlation plot (Fig. 3C). As a result, fifteen out of forty-eight marker genus showed a strong correlation with the main components of cinnamon oil, including eight positively correlated genera and seven negatively correlated genera. *Saccharopolyspora* was considered as most correlated genus, followed by two unidentified genera belonging to order *Gaiellales* and family *Methyloligellaceae*, genera *dyella, phyllobacterium*, and *lysobacter*. Genus *Saccharopolyspora* is not only described as robust and stable bacteria under harsh envieonment, but also characterized by its production of secondary metabolites with different chemical classes and diverse biological activities, such as macrolides, alkaloids, quinones and peptides (Wu, Zhang, Deng, Qian, Zhang, & Liu, 2011). It is clear that *Saccharopolyspora* genome contained at least 25 gene clusters for the production of known and predicted secondary metabolites (Kim & Goodfellow, 2015). Both *dyella* and *lysobacter* belong to family *Proteobacteria*, which play essential roles in the degradation of lignocellulose and polysaccharides for energy uptake (Spain, Krumholz, & Elshahed, 2009). Additionally, genus *lysobacter* has been demonstrated to harbor or express multiple sets of genes coding for antimicrobial compounds (de Bruijn et al., 2015). Genus *dyella* has been reported to possess genes encoding several enzymes involved in phenylalanine metabolism (Compant, Samad, Faist, & Sessitsch, 2019), which is an essential pathway for cinnamic acid biosynthesis. Genus *phyllobacterium* and two unidentified genera belonging to order *Gaiellales* and family *Methyloligellaceae* were marker genera screened from d80, which were also positively correlated with cinnamon oil. These genera can release extracellular enzymes for aromatic hydrocarbon degradation, which may lead to the reduce of cinnamon oil composition at d80 (Shi et al., 2020). The character of the marker taxa suggested that the improved cinnamon oil content during cinnamon leaves shade-drying process may be explained by unrivaled metabolic function of endophytic bacteria.

3.4. Predictive functional analysis of endophytic bacterial community

The functional profile of endophytic microbiota in cinnamon leaves was predicted by PICRUSt software. PICRUSt identified 16S rRNA gene sequences depending on KEGG pathway database, and infer the possible gene contents. The majority of predicted functional genes during cinnamon leaves shade-drying process were assigned into metabolism (77.8–80.2%), environmental information processing (5.4–5.9%), genetic information processing (4.7–5.5%), cellular processes (4.2–4.7%), human diseases (3.5–4.3%) and organismal systems (1.7–2.0%), respectively (Fig. S3). The relative abundance of metabolism increased from d0 to d60, which is in agreement with the trend of cinnamon oil content.

The temporal dynamic of functional genes that code for the enzymes involved in the cinnamon oil formation was shown in Fig. 4A, and the predicted genera that contribute to the functional genes were summarized in Fig. 4C. On basis of prediction, the metabolic pathway aimed at cinnamon oil formation during shade-drying process included glycolysis and shikimate pathway. In the glycolysis process, polysaccharides degradation was controlled by the key enzyme beta-glucosidase (EC: 3.2.1.21, n. 34) which was enriched at d20. The predicted main genera that contribute to the presence of beta-glucosidase were *Bifidobacterium* and *Caulobacter*, which were also enriched at d20 (Fig. 4C). The transformation of glucose to glucose-6P was mainly operated by *Bacillus* through the enzymes glucose-6-phosphate isomerase (EC:5.3.1.9, n. 20). The relative abundance of polysaccharides degradation function decreased after d20, suggesting the substrate of glycolysis is easily degradable polysaccharides such as exopolysaccharide. Because the easily degradable polysaccharides are rapidly consumed during the early stage and the degradation of refractory polysaccharides such as cellulose and lignin is very slow. This is in agreement with the enrichment of exopolysaccharide-producing bacteria at d0. PICRUSt results offer only a prediction, however, its combination with the temporal dynamics of enzyme activity and metabolic intermediate could corroborate the prediction. The decreased concentration of polysaccharides has been confirmed by phenol–sulfuric acid method (Fig. 4B). This result combined with the metabolic predictions corroborates the consumption of polysaccharides during the early stage of shade-drying process. The predicted relative abundance of the genes that encode key enzymes for shikimate biosynthesis (e.g. shikimate dehydrogenase, EC:1.1.1.25, n. 1) was high throughout the shade drying process. The predicted main genera that contribute to the presence of shikimate dehydrogenase were *Bacillus*, *Bryocella*, and *Saccharopolyspora* (Fig. 4C). These genera were enriched at d40 or d60. At the same time, the increasing shikimate dehydrogenase activity (Fig. 4B) from d0 to d60 combined with the metabolic predictions corroborate the existence of shikimate biosynthetic pathway. It is clear that the major components of cinnamon oil are derived from phenylalanine which is produced from the final product of the shikimate pathway. *Lapillicoccus*, *Microlunatus*, and *Saccharopolyspora* were the principal genera contributing to the conversion of shikimat to phenylalanine. Most of these genera were enriched after d40. The conversion of phenylalanine to cinnamon oil was dependent on histidinol-phosphate aminotransferase (EC:2.6.1.9, n.8) belonged to the genera *Lysobacter, Dyella*, and *Saccharopolyspora*. The only genus that contribute to the whole metabolic pathway was *Saccharopolyspora*. In addition, the correlation plot in Fig. 3C showed a significant positive correlation between cinnamaldehyde and *Saccharopolyspora*. Therefore, it indicated that *Saccharopolyspora* might act as a cinnamon oil-producing bacterium, which would contribute to explaining the enriched content of cinnamon oil during cinnamon leaves shade-drying process.

4. Conclusion

In this work, we have first revealed the succession of endophytic bacterial community in cinnamon leaves and its association with cinnamon oil production during shade-drying process. Results showed that 60-day shade-drying had a significant effect on the content but not the chemical composition of the cinnamon oil. At the same time, shade-drying process changed the composition of endophytic bacterial community in cinnamon leaves, and forty-eight marker genera were
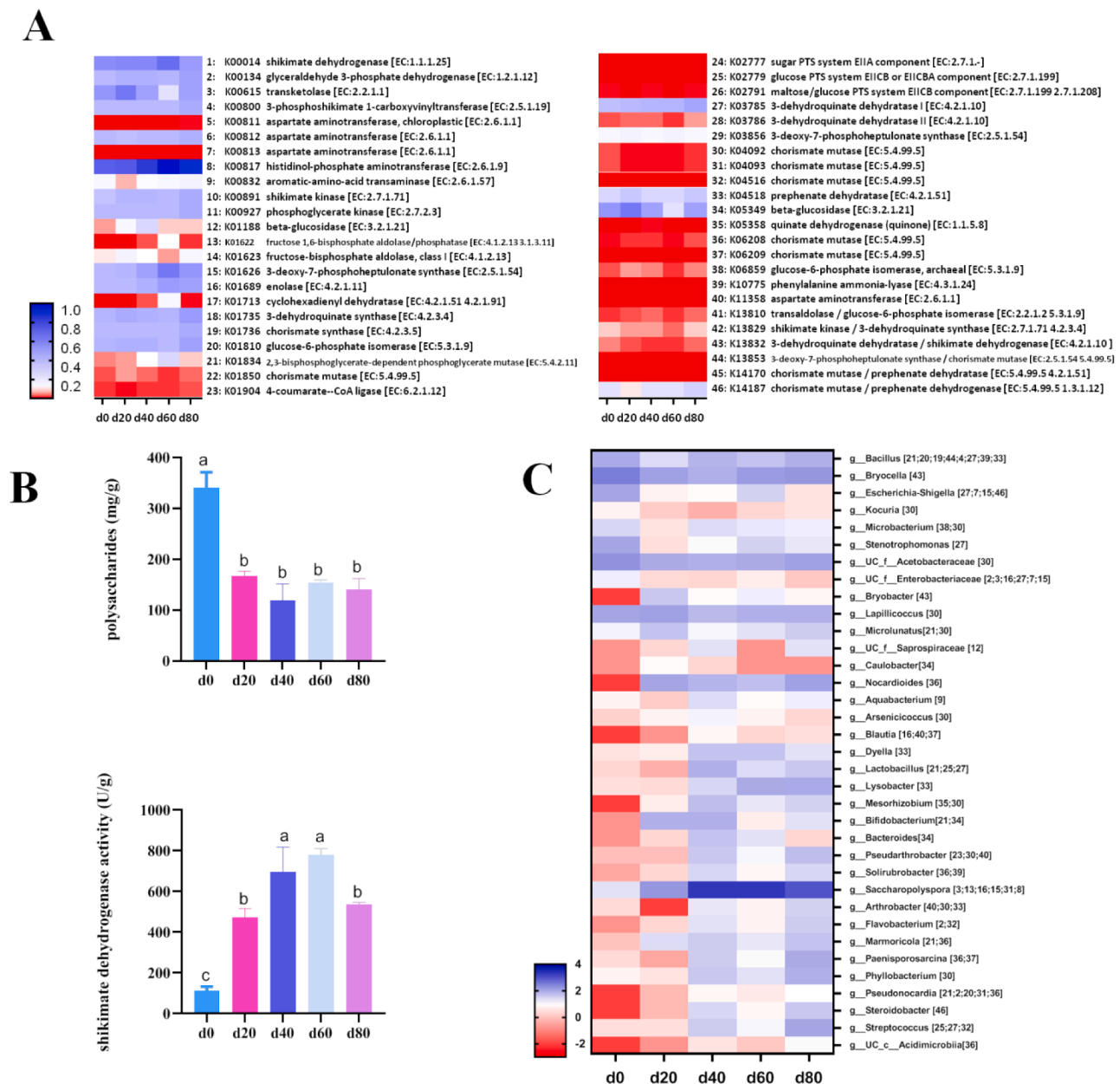
Fig. 4. Predictive functional analysis of endophytic bacterial community during shade-drying process. (A) The predicted genes that encode key enzymes involved in cinnamon oil production. Blue and red represented higher and lower relative abundance of genes respectively. (B) Concentration of polysaccharides and shikimate dehydrogenase activity in cinnamon leaves during shade-drying process. It is measured using commercially available kits and calculated based on dry weight of leaves. Data refer to mean ± standard deviation (n = 3). (C) Heat map of relative abundances of the predicted genera that contribute to genes encoding for key enzymes.

identified for different phases. The analysis of the correlation between endophytic bacterial community and cinnamon oil demonstrated that endophytic bacterial community was significantly positively correlated with cinnamon oil production during shade-drying process. PICRUSt suggested that *Saccharopolyspora* play key role for cinnamon oil production by improving the conversion of polysaccharides to cinnamaldehyde. These findings would help in understanding the endophytic bacterial genera responsible for cinnamon oil production and the bacterial functional genes that code for the enzymes involved in the cinnamon oil during cinnamon shade-drying process.

CRediT authorship contribution statement

Xian Cheng: Investigation, Writing – original draft. **Liang-Wu Bi:** Writing – review & editing. **Sheng-Nan Li:** Investigation, Validation, Formal analysis. **Yan-Ju Lu:** Validation, Formal analysis. **Jing Wang:** Validation, Formal analysis. **Shi-Chao Xu:** Validation, Formal analysis. **Yan Gu:** Validation, Formal analysis. **Zhen-Dong Zhao:** Conceptualization, Writing – review & editing. **Yu-Xiang Chen:** Conceptualization, Funding acquisition, Writing – review & editing.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100094.

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