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Dynamic succession of substrate-associated bacterial structure and function during *Ganoderma lucidum* growth

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Background *Ganoderma lucidum*, a valuable medicinal fungus, is widely distributed in China. Its growth is combined with a complex microbial ecosystem in the substrate. As sequencing technology advances, it's possible to reveal the structure and function of *G. lucidum*-associated bacterial communities. **Methods** We analyzed the microbiota in cultivation mixtures during different growth stages of the fungus using next-generation sequencing technology. **Results** In total, we obtained 598,771 sequences from 12 samples and assigned them to 22 bacterial phyla. Bacterial genus *Agrobacterium* dominated in the substrate during the hyphal and budding stages, while it almost disappeared at the elongation and mature stages. *Lactococcus*, a less dominant genus, increased its abundance during the growth of *G. lucidum*. Moreover, other genera such as *Pseudomonas*, *Acinetobacter* and *Brevundimonas* exhibited less richness in the hyphal and budding stages but occupied obvious dominance in the elongation and mature stages, demonstrating their potentially significant roles in the growth of *G. lucidum*. Functional pathways prediction uncovered changes in abundance of the ten most prevalent pathways. Three types of metabolism (energy, lipids, and cofactors and vitamins) increased during different growth stages of *G. lucidum*, while the abundance of membrane transport pathways decreased in the elongation and mature stages. **Discussion** Our findings shed light on the relationship between bacterial communities and the growth mechanism of *G. lucidum*, which should aid the elucidation of the medicinal benefits of this valuable fungus.

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Introduction

Ganoderma lucidum belongs to the class *Basidiomycetes*, and its growth mainly depends on lignin as a carbon source (*Mizuno et al., 1995*). The fruiting bodies and spores of *G. lucidum* are highly appreciated as health products in China for their richness in polysaccharides and triterpenoids, which are proposed to strengthen the immune system and inhibit tumor formation (*Wang, 2002*; *Sakamoto, 2016*). Because of its high medicinal value, the planting area of *G. lucidum* is expanding. *G. lucidum* has become the main economic pillar in some places due to the advantages of its cost-effective production management, e.g. requiring a smaller investment, having a shorter life cycle and yielding benefits faster (*Boh et al., 2007*). Like other edible fungi, the growth of *G. lucidum* depends on many environmental factors (e.g. temperature, enzyme activity and microbial community), which likely induce changes in the content of nutrients such as polysaccharides and microelements in its fruiting bodies (*Stajic et al., 2002*;*Tanaka et al., 2016*). *Li et al. (2014)* attributed the mechanism of changes in substrate mass during *G. lucidum* growth to the extracellular enzyme activities in a preliminary study. Significant growth-related differences were present in crude polysaccharides and triterpenes in the fruiting bodies of most of the *G. lucidum* strains that were tested (*Fu et al., 2008*). A greater number of trace elements and heavy metals were found in the mycelia than in the fruiting bodies or spores of *G. lucidum* (*Xing et al., 2001*). Studies have characterized the subtle changes of various substances in the body of *G. lucidum* during its growth stages; however, changes in microbial community in the surrounding cultivating environment or in the substrate have seldom been studied.

As previously reported, bacteria in the surrounding soil or the culture media were likely to change into endophytic bacteria, and play an important role in the growth of edible fungi (*Gagne et al., 1987*; *Elvira & Van, 2000*). The endophytic bacteria assist their host with nitrogen fixation, growth promotion and disease resistance (*Mano & Morisaki, 2008*; *Wang, Chen & Peng, 2016*; *Zhang, 2010*). *Qiu et al. (2011)* revealed that a variety of microorganisms existed in the mushroom substrate and significantly affected the host development. In particular, tiny changes in microbial communities in the culture substrates may impact the growth and development of edible fungi (*Cho et al., 2003*). On the other hand, the particular bacterial community structure of the culture substrate could indicate the health status of the mushrooms. *Ma, Chen & Chen (2016)* characterized the signficant allelopathic effects of the dominant microbes (mainly molds and bacteria) on the growth of *G. lucidum* in a continuous cropping soil using culture-based methods and demonstrated that bacteria such as *Clostridium*, *Alkaligenes* and *Bacillus* had stronger allelopathic effects on *G. lucidum*. In addition, pollution rates were associated to changes in microbial communities in the industrial production of *Pleurotus eryngii* (*Lin et al., 2010*).

The DNA-based community-fingerprinting methods, such as DGGE and T-RFLP (*Székely AJ et al., 2009*), are cost-effective to explore the changes in microbial community structure in the environment. Nevertheless, these methods lack a clear description on the microbial taxonomy and tend to underestimate microbial diversity with a relatively

low resolution. Currently, next-generation sequencing technology has overcomed these issues and is widely utilized to explore the distribution of microorganisms in diverse ecological conditions, including freshwater lakes, marine water, agriculture soil, forest soil, thermal vents and even in some precious herbs (*Patel & Jain 2015*; *Meier, 2016*; *Ávila et al., 2016*; *Su et al., 2017*; *Gigliotti et al., 2015*; *Xia et al., 2016*).

Previous studies on *G. lucidum* were mainly related to the cultivation technology and the active components in fruiting bodies. There have been few reports on the dynamic changes in microbial communities in the substrate at different growth stages of *G. lucidum*. In this study, next-generation sequencing of the V3–V4 region of bacterial 16S rRNA gene was used to determine the composition and diversity of bacterial communities and to predict the potential functions of the dominant microorganisms in the substrate during the four growth stages of *G. lucidum*.

Materials and methods

Cultivation of *Ganoderma lucidum*

The *Ganoderma lucidum* cultivar Chuan Yuanzhi No. 1 provided by the Soil and Fertilizer Institute at the Sichuan Academy of Agriculture Science, has been deposited in China General Microbiological Culture Collection Center (CGMCC) with the strain number of CGMCC 13174 on October 21st, 2016. The substrate was composed of cottonseed hull (90%), wheat bran (5%), corn flour (4%) and gypsum (1%), all of which were fresh, dry and unspoiled. The substrate was put into polypropylene cultivation bags (size: 17 cm×20 cm×0.005 cm) and autoclaved at 121 ℃ for 2 hours. After sterilization, the bags were cooled to room temperature and placed in a laminar flow cabinet for inoculation of *G. lucidum*. After inoculation, they were planted in the cultivation site at Zhaojia, Jintang, China. The space of the cultivation site had been ventilated, cleaned and disinfected before the experiment was carried out.

The sampling of *G. lucidum* was done at the four growth stages: hyphal stage, budding stage, elongation stage and mature stage. After inoculation, the mycelia of *G. lucidum* began to germinate. The first sampling was done at the hyphal stage (approximately 35 days after the inoculation) when the mycelia of *G. lucidum* spread and subsequently filled the whole culture medium. The mycelia twisted constantly. The second sampling was done at the budding stage (approximately 46 days after the inoculation) when the primordia formed and started to differentiate. The third sampling was done at the elongation stage (approximately 56 days after inoculation) when the promordia grew longer and the stipe was formed. Cap differentiation began after elongation. The last sampling was done at the mature stage (approximately 66 days after the inoculation) when the spores appeared on the pileus surface and gradually covered the yellow edges. Disposable disinfected gloves, sterilized tweezers and knives were used for sampling. Three duplicate samples of substrate were taken at each growth stage of *G. lucidum*. Subsamples of substrate materials taken from different parts of each cultivation bag were pooled together and homogenized. No less than 500 mg of substrate per sample was collected for DNA extraction. The fresh samples were stored at −20°C in 2 mL Eppendorf tubes prior to DNA extraction.

Chemical analysis of substrate materials

Substrate materials at different growing stages of *G. lucidum* were collected and the chemical properties were determined including total nitrogen, total phosphorus and total potassium. The samples were digested with sulfuric acid hydrogen peroxide at first, then the treatment solution was analyzed by conventional method according to *Thomas RL, Sheard RW & Moyer JR (1967)*.

DNA extraction, PCR amplification and MiSeq sequencing

Three replicates of samples taken at each growth stage were treated independently to ensure the methodological reproducibility. The E.Z.N.A.® Soil DNA kit (OMEGA Bio-Tek, USA) was used to isolate DNA from the substrate following the manufacturer's protocol. DNA concentration was measured using a UV spectrophotometer (Eppendorf, Bio Photometer). The quality and size of the extracted DNA was checked by 0.8% agarose gel electrophoresis.

PCR amplification was performed using the bacterial 16S rRNA gene-specific primers 338F (5'-ACTCCTACGGGAGGCAGCA -3') and 806R (5'- GGACTACHVGGGTWTCTAAT -3') with the following conditions: 98°C for 2 min (initial denaturation), 25-27 cycles of 98°C for 15 s (denaturation), 55°C for 30 s (annealing) and 72°C for 30 s (extension), and 72°C for 5 min (final extension) (*Langenheder & Szekely, 2011*). The PCR products were quantified using a Quant-iT Pico Green dsDNA Assay Kit with a M[illegible] plate reader (Bio Tek, FLx800) and were mixed based on the concentration of each sample. Amplicon sequencing was performed on Illumina's MiSeq platform (Personalbio, Shanghai, China). All raw data were submitted to the Sequence Read Archive (SRA) database with the accession numbers SRR5801759-SRR5801768 and SRR5801783-SRR5801784.

Sequence and statistical analysis

Reads containing ambiguous 'N' or with length <120 nt or >140 nt were discarded. The barcodes and adapters were trimmed with FASTX Toolkit. High-quality sequences with 97% or greater similarity were clustered into OTUs using UCLUST (*Edgar, 2010*), a sequence alignment tool, using QIIME pipeline version 1.7.0 (*Caporaso et al., 2010*). The most abundant sequence of each OTU was selected as the representative sequence of this OTU. The OTUs with abundance lower than 0.001% of the total sequences across all samples were removed (*Bokulich & Mills, 2013*). The multivariate statistical analysis of the OTU data was done in R environment (*R Core Team, 2016*). An unconstrained ordination (non-metric multidimensional scaling NMDS) was used to visualize the broad pattern of the distribution of bacterial communities. PERMANOVA was used to test the significance of the difference in bacterial communities between different growth stages of *G. lucidum* based on 999 permuations. Both nMDS and PERMANOVA analysis were performed based on weighted UniFrac distance using the R vegan package (*Oksanen et al., 2007*). The numbers of shared OTUs were presented in a Venn diagram (Venn Diagram package). Bacterial alpha diversity indices including observed OTUs, Chao1, ACE, Shannon and Simpson were rarefied and calculated based on the smallest library size of the samples. A heatmap was drawn to cluster hierarchically and analyze the changes (the amount by which each genus deviated in a specific sample from the genus's average across all samples) of the 50 most abundant genera using R heatmap package (*Kolde R, 2012*). LEfSe analysis (*Segata et al., 2011*) was used to reveal the bacterial taxa that showed differential abundance between different growth stages of *G. lucidum* at all taxonomic levels. PICRUSt software (*Langille et al., 2013*) was used to predict the metabolic functions of

bacterial communities based on the microbial metabolic function categories in the KEGG database. All significant differences were concluded at $P \leqslant 0.05$.

Results

Chemical analysis of substrate materials

Total nitrogen, phosphorus and potassium of substrate materials showed differences in four growing phases of *G. lucidum* (Table S1). For total nitrogen there was no big change during the growth of *G. lucidum*. However, great alteration occurred in total phosphorus and potassium of substrate materials. The content of both had similar decreasing trend, and both were in higher content in hyphal and budding stage but in lower content in elongation and mature stage.

Bacterial alpha diversity

In total, 598,771 sequences from the 12 sample were clustered into 1200 OTUs at 97% similarity. 15.7% OTUs were unclassified at the phylum level. Between the four growth stages of *G. lucidum*, 295 bacterial OTUs were shared in the substrate (Fig. S1). Bacterial alpha diversity indices significantly differed between the four growth stages of *G. lucidum* (Table 1). The richness indices (e.g. observed OTUs, Chao1 and ACE) were significantly higher at the late growth stages (e.g. elongation and mature stages) than at the early growth stages (e.g. hyphal and budding stages). The Shannon diversity was signficantly higher at the elongation stage than at the hyphal stage. There was no difference in the Simpson index between the different growth stages of *G. lucidum*.

Taxonomy-based analysis of bacterial community

A total of 22 phyla were detected in the substrate at the four growth stages of *G. lucidum*. As shown in Table S2, the most abundant phylum was *Proteobacteria*, which accounted for 41.47-72.86% (average 57.23%) of all the bacterial sequences, followed by *Firmicutes* (22.50-40.33%, average 34.12%). Together, these two phyla represented 80.12-95.93% of the bacterial species. The less dominant phyla (average abundance >1%) included *Bacteroidetes* (3.30%), *Acidobacteria* (2.75%) and *Actinobacteria* (2.03%).

A total of 195 genera were identified in the samples. Of these genera, 100 were shared in the substrate between the four growth stages, accounting for 71.27% of the classified sequences. Most of the 20 most abundant genera belonged to *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, with 91.35% of the genera belonging to the first two phyla (Fig. S2). *Agrobacterium* and *Lactococcus* were the two dominant genera, accounting for 12.68% and 10.49% of the classified sequences on average, respectively. Other abundant genera include *Burkholderia* (average 8.18%), *Bacillus* (average 6.62%), *Enterococcus* (average 4.97%), *Ralstonia* (average 3.42%), *Staphylococcus* (average 3.21%), *Sediminibacterium* (average 3.10%), and *Geobacillus* (average 3.09%) and 3 unidentified genera (in total 18.15%).

Bacterial beta diversity

The NMDS analysis was used to visualize the broad distribution patterns of the bacterial community samples at a specific distance scale by reducing the dimension and reordering the samples in a lower-dimensional coordinate system. The bacterial community samples taken at the two early growth stages of *G. lucidum* were clearly separated from those taken at the two late growth stages on the first axis of the NMDS ordination (Fig. 1). According to PERMANOVA test (Table 2), no signficant difference in bacteria community structure occurred between the hyphal, budding and mature stages during *G. lucidum* growth. However, the bacteria community structure in the substrate at the elongation stage was significantly different from that at the other three growth stages.

Biomarker discovery

LEfSe analysis was used to reveal the bacterial taxa that showed differential abundance between different growth stages of *G. lucidum* at all taxonomic levels (Fig. 2). LEfSe analysis revealed a significantly higher abundance of the order *Rhizobiales* (e.g. genus *Rhodoplanes*) and the genus *Sphingobium* at the budding stage than at the other three growth stages of *G. lucidum*. The family *Frankiaceae* and the genera of *Alkaliphilus* and *Erwinia* were significantly enriched in the substrate at the hyphal stage, with regard to other growth stages. A large number of bacterial taxa exhibited a significantly higher abundance at the elongation stage. Those taxa include the phyla of *Bacteroidetes* (e.g. genera *flavobacterium* and *Sediminibacterium*), *Acidobacteria* (e.g. genus *Candidatus Koribacter*), *Nitrospirae* (e.g. genus *Nitrospira*), *Cyanobacteria* (e.g. order *Streptophyta*) and other taxa such as the orders of *Xanthomonadales* and *Rhodospirillales* and the family of *Comamonadaceae*. The phylum of TM7 and the orders of *Clostridiales* (e.g. families *Peptostreptococcaceae* and *Lachnospiraceae*) and *Pseudomonadales* (e.g. genera *Acinetobacter* and *Pseudomonas*) were significantly more abundant at the mature stage than at other growth stages.

Heatmap analysis

The heat map was further used to visualize the abundance changes of top 50 bacterial OTUs in the substrate during the growth stages of *G. lucidum* (Fig. S3). A total of 50 genera in 6 phyla were grouped into four clusters. *Proteobacteria* was the dominant phylum in the Cluster I, II, and IV. Half of the genera in Cluster III belonged to *Firmicutes*. Other phyla such as *Bacteroidetes*, *Corynebacterium* and *Fusobacteria* were present in clusters II and III.

The bacteria of Cluster I and III were more abundant at the elongation and mature stages, whereas other bacteria of Cluster II were more abundant at the hyphal and budding stages. Interestingly, the bacterial genera (e.g. *Agrobacterium*, *Paenibacillus*, and *Alkaliphilus*), which were highly abundant at the two early growth stages, were particularly less abundant at the two late growth stages. Instead, the bacterial genera such as *Ralstonia*, *Rhodococcus* and *Flavobacterium* were more abundant at the elongation and mature stages than at the hyphal and budding stages. The bacterial genera in Cluster IV were highly abundant at the mature stage. Besides considering the abundance changes of the genera, the clustering analysis could also infer the microbial succession in the substrate during the growth stage of *G. lucidum*.

Functional prediction of bacteria community

A total of 7 enzyme function types including 39 KEGG pathways were identified in the study (Table 3). Of these KEGG pathways, 48.93% were related to metabolism, 17.24% to environmental information processing and 14.23% to genetic information processing. The ten most prevalent pathways (Table 3) were related to four function types: metabolism, environmental information processing, genetic information processing and unclassified. Membrane transport was the predominant KEGG pathway predicted by PICRUSt, accounting for an average of 15.93% during the growth of *G. lucidum*. Carbohydrate metabolism (on average 10.03%) and amino acid metabolism (on average 9.81%) were the second the third most abundant pathways during the growth of *G. lucidum*. In general, the ten most abundant pathways changed in abundance between different growth stages of *G. lucidum*. The metabolism of energy, lipids, cofactors and vitamins, as well as cellular process and signaling pathways increased in abundance with time, whereas the membrane transport pathway diminished at the elongation and mature stages (Fig. S4). Surprisingly, the pathway of xenobiotic biodegradation and metabolism, which were abundant at the budding, hyphal and mature stages, was not present at the elongation stage, whereas the pathway of translation, which was absent at the budding, hyphal and mature stages, was present with high abundance at the elongation stage (Table 3).

Discussion

The growth of *G. lucidum* significantly impacted the diversity and structure of bacterial community in the substrate. In this study the bacterial community in the substrate at late growth stages (e.g. elongation and mature stages) had richer diversity than at the early growth stages (e.g. hyphal and budding stages). Few studies reported about microbial community compositions and their dynamic changes in the substrate of *G. lucidum*. Even though frequent reports are about active substances in the fruiting bodies and the cultivation of *G. lucidum* (*Peksen & Yakupoglu, 2009*; *Bao et al., 2002*; *Fang & Zhong, 2002*) and more work has focused on new molecularly modified cultivars and the transcriptome of *G. lucidum* based on the development of bio-technology (*Liu et al., 2017*; *Qian et al., 2013*; *Syed et al., 2013*). Only a few recent studies characterized the bacterial community dynamics during the growth of edible fungi (e.g. *Ye et al., 2014*).

G. lucidum has unique morphological structures and growth characteristics in each growth stage; in these stages, various enzymes and microbes help the fungus accomplish its growth so that it can degrade lignin and synthesis substances (*Coelho et al., 2010*). Eventually, bacteria and enzymes interact with each other, causing dynamic change in the bacterial communities (*Tischer, Blagodatskaya & Hamer, 2015*). In addition, the substrate, with the involvement of various microbes, provides adequate nutrients for *G. lucidum* growth. The physicochemical properties of the *G. lucidum* substrate change during the growth process, resulting in a direct effect on microbial constitution. A previous study demonstrated that indicators such as organic C and total N are closely related to the abundances of most bacterial groups (*Zhang, 2010*). In particular, bacterial community structures changed with time and their environment, including the chemical properties of the substrate (Table S1). These changes can affect bacterial communities that are associated with *G. lucidum*, while similar bacterial community structures occurred throughout the adjacent stages because of close environmental conditions.

There was a big difference in bacterial distribution at different growth stages of *G. lucidum*. Most bacterial taxa were in higher abundance at the elongation stage, while less quantity of bacterial taxa exhibited obvious dominance at other three growth stages. In detail, the abundance of *Proteobacteria* varied irregularly in different stages: the highest and lowest average contents occurred in the fourth (72.86%) and third (41.47%) stages of *G. lucidum* growth. *Proteobacteria* were more adaptive toward the complex substrates in the mature stage, exhibiting rich diversity. Similarly, the abundance of the less dominant phylum *Firmicutes* decreased to 22.50% in the last growth stage. As was reported, *Firmicutes* can become a potential indicator of process performance in anaerobic conditions (*Chen et al., 2016*). Interestingly, the samples became extraordinarily rich in the less abundant phyla during the elongation stage, reaching approximately 20% in abundance; this behavior was likely due to either inhibitory factors that prevented the reproduction of *Proteobacteria* and *Firmicutes* or promoting factors that stimulated the activities of other phyla. At the phylum level, bacterial communities varied with the growth stages, whereas dominant bacteria had significant effects on the growth and development of *G. lucidum*.

Microbes exist everywhere and participate in the life activities of organisms (*Singh, 2015*). Most likely, due to environmental bacteria slipping into the substrate and reproducing, the number of OTUs increased as *G. lucidum* grew. Bacterial communities were rich in diversity in every growth stage, and the bacterial diversity of the substrate tended to become more abundant over time. Former studies clearly report that bacterial communities with distinct functions vary at different composting stages, with diverse enzymes and substances forming a specific ecosystem (*Zhang et al., 2014*). In this study, the abundance of *Agrobacterium*, *Lactococcus*, *Burkholderia*, *Bacillus* and *Enterococcus* was obviously greater in the hyphal and budding stages, accounting for 65-70% of genera. Having particular structures and adaptive capacities makes the genera that are higher in abundance more likely to survive.

Differentially abundant taxa were uncovered in the substrate at different growth stages and they were connected with different enzyme function types. Pathways in functional prediction were present at one stage while absent at other stages, and it's probably a suggestion that specific bacteria have specific functions at a certain stage. The differences in bacterial communities at the phylum level were determined in this study, revealing that *Proteobacteria* was the most abundant phylum in all the samples and had its own characteristics and functions. Similarly, this phylum is typically observed in soils with diverse morphologies, physiologies and metabolisms and is considered to be advantageous in global carbon, nitrogen and sulfur cycling, which establishes the important status of its high abundance among all the phyla (*Janssen, 2006*; *Spain, Krumholz & Elshahed, 2009*; *Kersters et al., 2006*). Furthermore, different microbes interact with biological systems, and even agronomic practices are related to the changes in soil microbial diversity (*Gigliotti et al., 2015*). Moreover, the data of *Durrer et al. (2016)* reported biogeographical patterns of bacterial communities across agricultural soils and suggested a role of biological connections that was based on a co-occurrence/mutual-exclusion analysis; in their data, *Proteobacteria* and *Acidobacteria* co-occurred, but both were negatively correlated with the presence of *Actinobacteria*. In other words, competition among various bacteria may cause a decline in abundance of some genera. Particular structures and the adaptive capacities of various genera determined their inhabitation in substrates at different periods, each performing their respective essential functions. In general, stable bacterial community structures in the environment are

dedicated to the better growth of crops and mushroom, which is a fact that should be considered more often (*Vajna et al., 2010*).

Of the identified KEGG pathways, 17.24% were related to environmental information processing. The interaction between bacteria and *G. lucidum* growth was closely related to the substrate. As the main material of *G. lucidum* culture substrate, cottonseed hull has high water-holding capacity and nitrogen content and it is also thought to be a potential medium in the cultivation of aerobic bacteria (*Lu & Qu, 1984*; *Slifkin & Pouchet G, 1975*). Previous studies investigated compositional changes in a substrate with cottonseed hull, which included cellulose, hemicelluloses and lignin, during the growth period of mushrooms (*Li, Pang & Zhang, 2001*; *Ni et al., 2002*). The nutrients transformation produced an effect on enzyme activities as well as microbial communities. Ultimately, the microbes cooperated and played roles in supplying nutrients or resisting viruses for the sake of a stable organic system that provided suitable growing environments for mushrooms (*Li et al., 2015*; *Saha, Pipariya & Bhaduri, 2016*).

G. lucidum co-evolves with bacteria that inhabit the substrate and display either direct host-microbiota interactions or indirect impacts that result from microbe-microbe interactions, building a subsequent complex relationship that may affect the physiology of the fungus. To display the essentiality of each genus and understand their genetic variation, gene function is being predicted with next-generation sequencing technology in an increasing number of studies (*Harrison, 2012*). The data that is presented in Table 3 reports genes with diverse physiological functions. The greatest number of genes in microbiomes in the *G. lucidum* substrate at the hyphal and budding stages are in pathways that are associated with environmental information processing and in particular, the membrane transport portion of the pathway (17.40 and 16.31% of their totals, respectively). Recognition of environmental information is very important for the survival of bacteria after they enter substrate, and membrane transport is probably the most significant mechanism in accomplishing this survival. Better and quicker adaptation to the environment lays the foundation for bacterial reproduction. However, pathways that are associated with metabolism and genetic information processing were maintained in greater abundance during the elongation and mature stages. During the vigorous growth of *G. lucidum*, more metabolites of the fungus are produced that change the physicochemical environment of the medium, which greatly influences the microorganisms. To minimize adverse external factors, bacteria change the environment through their energy and lipid metabolism and adjust themselves by replication and repair. In summary, diverse bacteria in the substrate play their roles during the growth stages of *G. lucidum* to overwhelmingly promoting its growth and development.

Conclusions

Different bacteria communities were detected at different growth stages of *G. lucidum* in the study, and it revealed a significant effect of periods on bacteria distribution in the substrate. Additionally, differentially abundant taxa existed at different stages, playing key roles in the growth and development of *G. lucidum*. However, we haven't recovered the exact relationship between various bacteria in the substrate and *G. lucidum* growth from the results. So more studies should be carried on to explore the mechanism.

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Table 1(on next page)

Bacterial alpha diversity indices

The index of the observed OTUs, was used to evaluate the observed OTU richness, whereas the Chao1 and ACE were used to estimate the total (observed and unobserved) OTU richness of the bacterial community.. The indices of Shannon and Simpson were used to access the richness and evenness of bacterial community, respectively. Different lower-case letters showed significant difference ($P<0.05$) of the diversity indices between different growth stages of *Ganoderma lucidum*.

Sample	Observed OTUs	Chao1	ACE	Simpson	Shannon
Hyphal stage	363±21a	197.00±21.66a	260.22±40.72a	0.85±0.10a	4.02±0.62a
Budding stage	357±13a	189.00±24.58a	270.17±35.29a	0.88±0.00a	4.08±0.09ab
Elongation stage	505±50b	372.00±70.55b	479.66±99.77b	0.90±0.06a	4.77±0.33b
Mature stage	491±33b	336.67±53.46b	422.42±56.42b	0.87±0.03a	4.21±0.13ab

Table 2(on next page)

PEMANOVA analysis of bacterial community between different growth stages based on weighted UniFrac distance

Signficance of the difference in bacterial communities between different growth stages were tested using 999 permuations. Signif. codes: '**', 0.001< P ≤ 0.01; '.', 0.05< P ≤ 0.1; ' ', 0.1< P ≤1.

		Significance
Hyphal stage	Budding stage	
	Elongation stage	**
	Mature stage	.
Budding stage	Elongation stage	**
	Mature stage	.
Elongation stage	Mature stage	**
Hyphal stage, Budding stage, Elongation stage, Mature stage		**

Table 3(on next page)

PICRUSt predicted KEGG pathways of bacterial community in the substrate at different growth stages of *G. lucidum*

Function type	KEGG pathway	Relative abundance (%)			
		Hyphal stage	Budding stage	Elongation stage	Mature stage
Environmental Information Processing	Membrane Transport	17.40	16.31	11.36	14.08
Metabolism	Carbohydrate Metabolism	10.07	10.09	10.68	9.94
	Amino Acid Metabolism	9.81	10.11	10.38	10.07
	Energy Metabolism	4.85	4.80	5.42	5.05
	Xenobiotics Biodegradation and Metabolism	4.37	4.61	0	4.86
	Lipid Metabolism	3.75	3.86	3.89	3.93
	Metabolism of Cofactors and Vitamins	3.58	3.58	3.94	3.74
Genetic Information Processing	Translation	0	0	4.11	0
	Replication and Repair	6.06	5.99	6.91	6.06
Unclassified	Poorly Characterized	4. 90	4.91	5.29	5.13
	Cellular Processes and Signaling	3.71	3.73	3.99	3.89

Figure 1(on next page)

Nonmetric Multidimensional Scaling ordination of bacterial communities based on weighted UniFrac distance

Each point in the diagram represents a bacterial community sample. The closer distance between two points in the ordination space indicates the lower dissimilarity between the microbial community structure of these two samples.

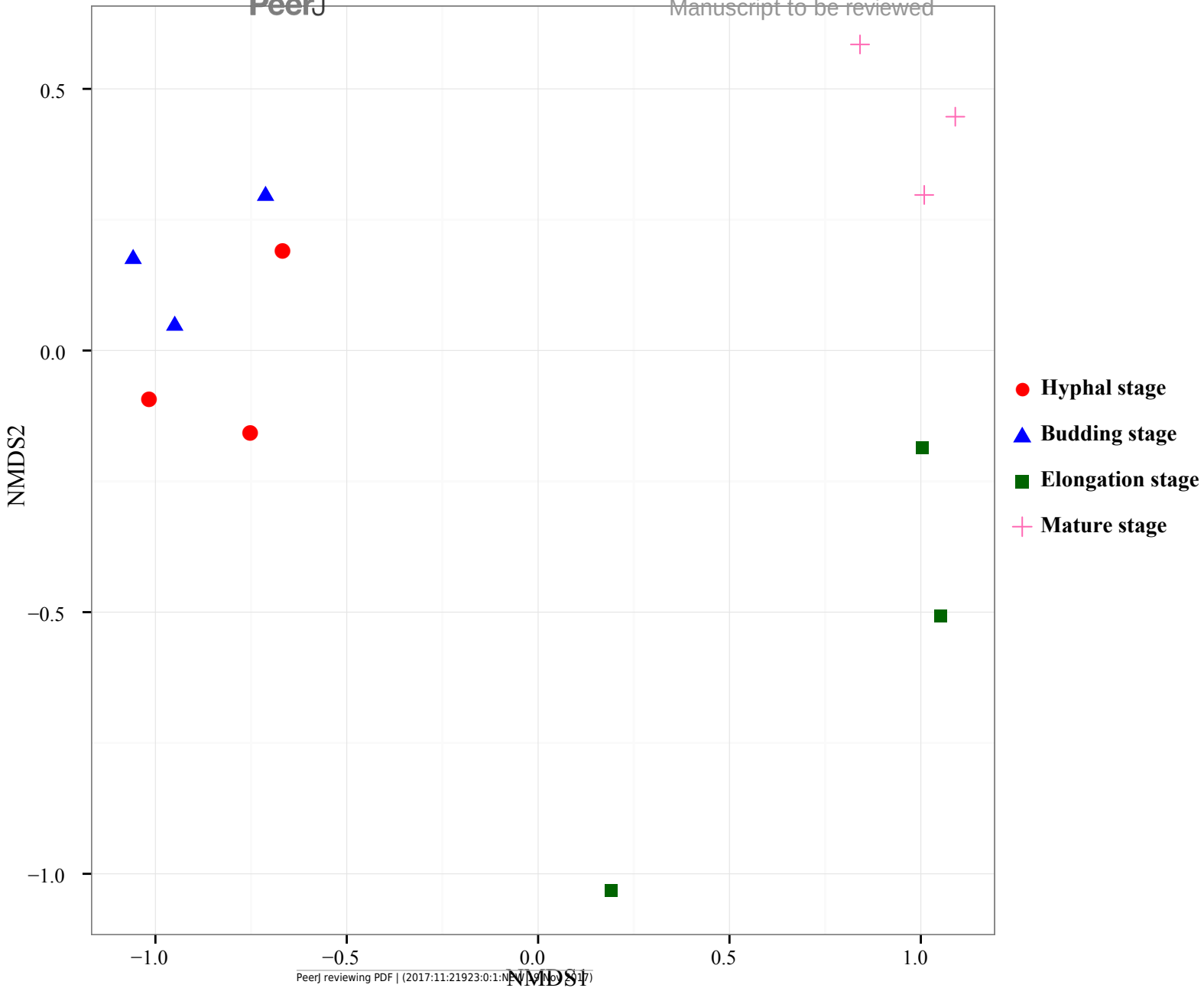
0.5
0.0
−0.5
−1.0
NMDS2
−1.0
−0.5
0.0
0.5
1.0
NMDS1
Hyphal stage
Budding stage
Elongation stage
Mature stage

Figure 2

Cladogram showing the differentially abundant bacterial taxa at each of the four growing stages of *G*. *lucidum* based on LEfSe analysis

(P < 0.05, LDA score > 2)