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Shifts in diversity and function of the bacterial community during the manufacture of Fu brick tea

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ABSTRACT

To better understand the effects of bacteria on the characteristics of Fu brick tea, we investigated bacterial community structure as well as the predicted functions of identified bacteria and their correlations with chemical compounds during the manufacturing process. Overall, *Klebsiella* species dominated during the initial stage of processing, but were quickly replaced by *Pseudomonas*, *Lactococcus*, *Stenotrophomonas*, *Enterococcus*, and *Bacillus* species, which remained stable until the end of the manufacturing process. Network analysis identified 11 bacterial genera as keystone taxa, which contributed to the stabilization of the microbial community in the co-occurrence network. Bacterial taxa were grouped into eight modules, with the dominant genera mainly distributed amongst modules I and VI, which were involved in metabolism of carbon and flavor compounds in the Fu brick tea ecosystem. Using bidirectional orthogonal partial least squares analysis, 19 bacterial genera were identified as core functional genera linked to the metabolism of chemical compounds during the manufacturing process, while three genera, namely *Klebsiella*, *Lactococcus*, and *Bacillus*, also dominated the Fu brick tea fermentation process. These findings provide new insights into Fu Brick tea bacterial community variation and increased our understanding of the core functional bacterial genera involved in the manufacture of Fu brick tea.

1. Introduction

Fu brick tea, one of the major brands varieties of dark tea, has received increasing attention in recent years owing to its special flavor and many health benefits, including its anti-hyperlipidemic, anti-obesity, and antimicrobial properties (Zhang et al., 2013).

The main steps in the Fu brick tea manufacturing process are steaming, piling, pressing, fermentation, and drying (Mo et al., 2008), with microbial fermentation considered the key step in the development of special characteristics of Fu brick tea (Wang et al., 1991). Fungi, including those belonging to the genera *Aspergillus*, *Cyberlindnera*, *Candida*, *Penicillium*, *Eurotium*, *Beauveria*, *Debaryomyces*, *Pestalotiopsis*, *Pichia*, *Rhizomucor*, and *Verticillium*, have been identified using culture-dependent and culture-independent methods as important members of the microbial community for the production of Fu brick tea (Qi and Sun, 1990; Wen and Liu, 1991; Xu et al., 2011a; Li et al., 2017). For example, both culture-dependent and multilocus sequence typing methods identified *Eurotium cristatum* as the predominant fungal species during the processing of Fu brick tea (Qi and Sun, 1990; Xu et al., 2011). In contrast, very little is known about the bacterial community present during the manufacturing process. One of the few available studies found that *Lactococcus* was the predominated bacterial genus in Fu brick tea (Fu et al., 2016).

Microorganisms within a specific ecological niche form complex interaction webs (Faust and Raes, 2012; Chen et al., 2016). Using high-throughput sequencing technology, broad and deep surveys of microbial communities have been used to reveal ecological linkages among microorganisms in complex ecosystems, such as soil, activated sludge, and chinese liquor (Barberán et al., 2012; Hu et al., 2016; Ju et al., 2014; Luo et al., 2017a; Yu et al., 2018). However, as far as we know,

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this type of analysis has not been used to xamine direct and indirect interactions among microbial taxa in Fu brick tea.

Therefore, we investigated changes in bacterial community structure as well as the predicted functions of predominant bacteria and correlations with chemical compounds during the manufacture of Fu brick tea. We hypothesized that 1) the dominant bacterial community shifts during the manufacturing process, and 2) that the special characteristics of Fu brick tea could be attributed to a core group of functional genera rather than the whole community. This study provides new insights into the dynamic shift in bacterial community structure and the different functions of the core genera during the Fu brick tea manufacturing process, providing valuable information that can be used to improve Fu brick tea production.

2. Materials and methods

2.1. Sample preparation and sampling process

Fu brick tea samples were collected in our previous study (Li et al., 2017). Briefly, samples were collected at 10 different stages of the manufacturing process: primary dark tea (S1), piling-fermentation of tea materials (S2), fermentation of brick tea in the fermentation room for 0 days (S3), 3 days (S4), 6 days (S5), 9 days (S6), 12 days (S7), 15 days (S8), and 18 days (S9), and finished tea products (22 day) (S10). Samples were collected from three independent, parallel batches and used as replicates. Three samples were collected from each batch at each time point and pooled for use as a single sample for analysis. The 30 samples were packaged in sterile polyethylene bags, transported to the laboratory, and stored at −80 °C until required.

2.2. DNA extraction, polymerase chain reaction amplification, and sequencing

Microbial DNA was extracted from the samples using an E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) as per manufacturer's instructions. The V3-V4 region of the 16S rRNA gene was amplified from the extracted DNA using forward primer 338F (5′-ACTCCTACGG GAGGCAGCA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWT-CTAAT-3′) (Srinivasan et al., 2012). Following purification and quantification, the mixed sample amplicons were sequenced using the Illumina Miseq system by Majorbio Bio-Pharm Technology Co., Shanghai, China.

2.3. Sequence processing, taxonomic assignment, and statistical analyses

Raw reads were demultiplexed and quality-filtered using Trimmomatic (version 0.36, http://www.usadellab.org/cms/?page=trimmomatic) (Bolger et al., 2014). Resulting sequences were clustered into operational taxonomic units (OTUs) with a cut-off 97% sequence identity using Mothur (version 1.25.0, http://www.mothur.org) (Schloss et al., 2009). The alpha diversity (Shannon and Simpson indexes) and richness (ACE and Chao1 indexes) along with Good's coverage estimates and rarefaction curves were also analyzed or generated using Mothur. In addition, R package vegan (version 3.4.0, https://mirrors.tuna.tsinghua.edu.cn/CRAN/) was used to generate rank abundance curves and to carry out principal components analysis (PCA). All results are presented as the mean value ± standard error. Differences between groups were considered significant at $P < 0.05$.

2.4. Co-occurrence network and bidirectional orthogonal partial least squares (O2PLS) analysis

The co-occurrence patterns of within the Fu brick tea bacterial community were analyzed using the Molecular Ecological Network Analyses Pipeline (MENAP) (http://129.15.40.240/mena/) (Deng et al., 2012). OTUs with a relative abundance greater than 0.01% were used to calculate the pairwise Pearson correlation coefficient, for which a proper threshold was identified based on the random matrix theory approach. The default cutoff of 0.99 was used to construct co-occurrence networks for the bacterial community present in Fu brick tea. Network topological properties were also calculated using MENAP. O2PLS analysis was performed using SIMCAP 14 (version 14.1.0.2047), as described previously (Li et al., 2018). The X-matrix represented the bacterial community dataset, while the Y-matrix represent the chemical compound datasets. Finally, the co-occurrence network was visualized using Cytoscape (version 3.5.1).

2.5. Predictive function analysis

To investigate the functions of predominant genera or the entire bacterial community in Fu brick tea, PICRUSt (http://huttenhower.sph.harvard.edu/galaxy) was used to predict the functional capabilities of the microbial community based on 16S rRNA gene-based phylogenies (Langille et al., 2013). The seq.fasta files were used to assign OTUs using a closed reference OTU approach against the GreenGenes database (version 13.5) at a cut-off of 97% identity. The resultant OTU table was then normalized, predicted, and categorized according to online PICRUSt protocols using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

3. Results

3.1. Dynamic shift in bacterial communities during the Fu brick tea manufacturing process

In total, 1044,157 high-quality sequence reads were obtained across all samples and were clustered into 777 OTUs after trimming and filtering. The Good's coverage estimator, rarefaction curve, rank abundance curve, Shannon and Simpson index, and Chao1 and ACE index values are shown in Table S1 and Figs. S1 and S2. Shannon and Simpson diversity index analyses indicated that bacterial diversity significantly increased between sampling points S1 and S2, but remained relatively constant from S2 until the end of the manufacturing process. The Chao1 and ACE index values indicated that species richness gradually increased over the course of the Fu brick tea manufacturing process, reaching a peak at sampling point S8.

In total, bacteria belonging to 26 phyla, 53 classes, 112 orders, 216 families and 448 genera were identified across all stages of the manufacturing process. As shown in Fig. 1A, *Proteobacteria* and *Firmicutes* were the predominant phyla in all samples, accounting for 81.12–96.65% of the total number of sequences. The order *Enterobacteriales* was predominant in the S1 sample, but was quickly replaced by the orders *Pseudomonadales*, *Lactobacillales*, *Xanthomonadales* and *Bacillales* in the subsequent samples, which remained stable until the end of the manufacturing process (Fig. 1B).

At the genus level, the 14 most dominant bacterial genera constituted 69.49–93.28% of all sequences in each sample (Fig. 1C). While *Pseudomonas* was the predominant genus across the entire fermentation process, its relative abundance dramatically increased from 1.98% in the initial sample (S1) to 20.91% in S2, but decreased to 16.0% by the end of the process. *Klebsiella* dominated in S1 (82.65%) but sharply decreased to 0.87% in S2. The abundance then increased again to 27.08% in S3 but decreased to 3.25% by the end of the process. *Lactococcus* and *Stenotrophomonas* accounted for 1.80% and 1.15% of all sequences, respectively, in sample S1 and then significantly increased to 14.40% and 14.51% of all sequences, respectively, in S2. In both cases, the relative abundance then gradually decreased, with *Lactococcus* and *Stenotrophomonas* accounting for 12.94% and 10.36% of all sequences, respectively, at the end of the fermentation process. *Enterococcus* and *Bacillus* only accounted for 0.89% and 0.61% of all sequences, respectively, in sample S1, but dramatically increased to 9.03% and 6.06%, respectively, in S2. The abundance of the two genera

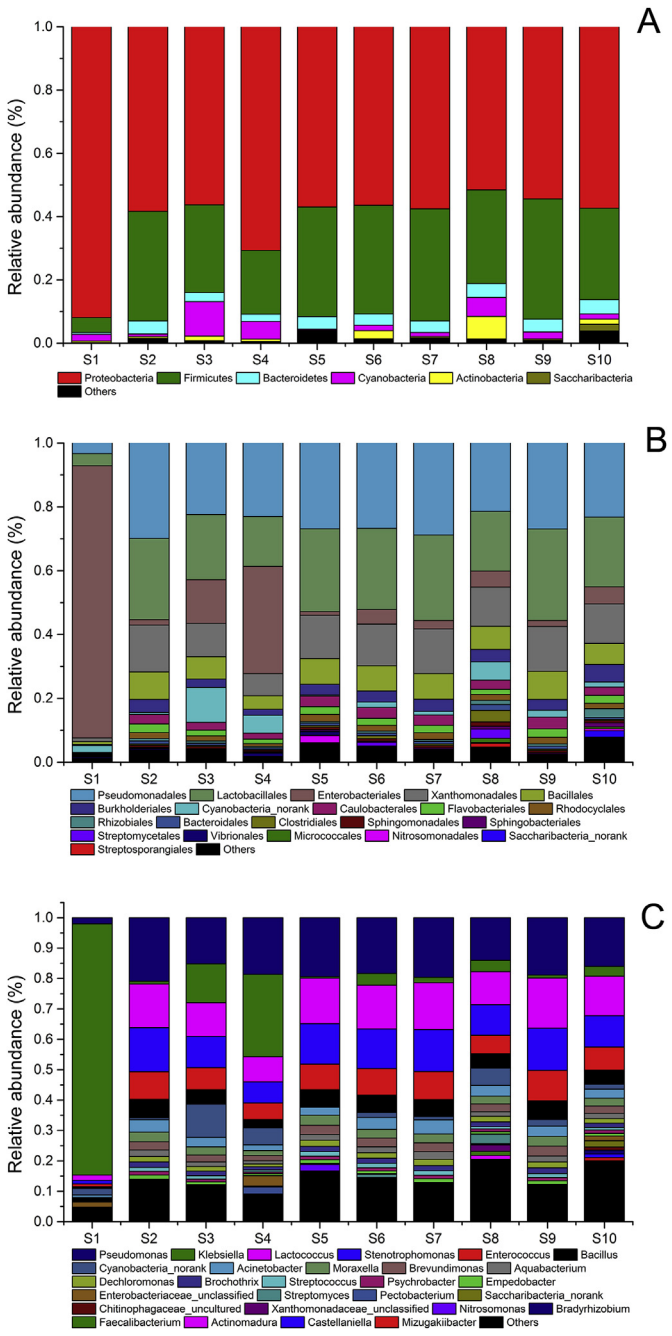

Fig. 1. Bacterial taxonomic compositions showing the bacterial successions at phylum (A), order (B) and genus (C) level during manufacturing process of Fu brick tea. The taxonomic abundance < 1% were classified into "others".

then decreased to 7.64% and 4.64%, respectively, at the end of the process. Sequences identified as *Cyanobacteria_norank* decreased in abundance from 2.13% in S1 to 0.65% in S2, before increasing to 10.93% in S3 and then gradually decreasing to 1.56% by the end of the fermentation. Small changes in the abundance of other genera, including *Acinetobacter* (0.78%–3.03%), *Moraxella* (0.34%–2.56%), *Brevundimonas* (0.23%–2.39%), *Aquabacterium* (0.15%–1.70%), *Dechloromonas* (0.20%–1.55%), *Brochothrix* (0.17%–1.37%), and *Streptococcus* (0.18%–0.90%) were also observed during the manufacturing process.

To assess β-diversity, PCA and PCoA analyses were used to investigate overall differences in bacterial community structure among the samples across the Fu brick tea manufacturing process. The results indicated that the samples could be separated into three periods based on euclidean distance and Bray-Curits dissimilarity analyses, designated stage I (S1), stage II (S2-S4), and stage III (S5-S10) (Fig. 2).

3.2. Co-occurrence network analysis

The bacterial network generated for the Fu brick tea community consisted of 213 nodes (OTUs) and 400 edges (246 positive and 154 negative correlations), for which significant topological properties were calculated to describe the inter-relationship patterns between the OTUs in the network (Fig. 3A, Table S2). Overall, the genera *Colwellia* (OTU 508) and *Pseudofulvibacter* (OTU 588) showed the highest degree of interaction with other nodes (28 correlations each). The modularity index value for the network was 0.600 (> 0.4), suggesting that the network had a typical modular structure (Newman, 2006).

The nodes in the network were grouped into eight major modules (Fig. 3B, Table S3, and Fig. S3). Newman (2006) suggested that the nodes in different modules perform different functions. Based on the within-module connectivity (Zi) and among-module connectivity (Pi) values, the roles of the different nodes were classified into four categories: peripherals ($Zi \leq 2.5$, $Pi \leq 0.62$), connectors ($Zi \leq 0.25$, $Pi > 0.62$), module hubs ($Zi > 2.5$, $Pi \leq 0.62$) and network hubs ($Zi > 2.5$, $Pi > 0.62$) (Deng et al., 2012). The majority of OTUs (85.45%) were peripherals, with the majority of links inside their own modules. A total of 21 nodes (9.85%) were connectors, eight nodes (3.76%) were module hubs, and two nodes (0.94%) were network hubs (Fig. S4). These ten hubs were identified as keystone genera, including *Candidatus Brocadia*, *Ruegeria, Vibrio, Colwellia, Parasutterella*, *Pseudofulvibacter*, *Marmoricola*, *Synechococcus*, norank_order_Solirubrobacterales and *Aquitalea*. These keystone genera served as gatekeepers for the ecological functions of the bacterial community, implying that they may play critical roles in maintaining the structural and functional stability of Fu brick tea communities.

3.3. Predictive functional profiling

The functional profiles of the bacterial communities present during the three periods of Fu brick tea production (Stage I: S1; Stage II: S2-S4;

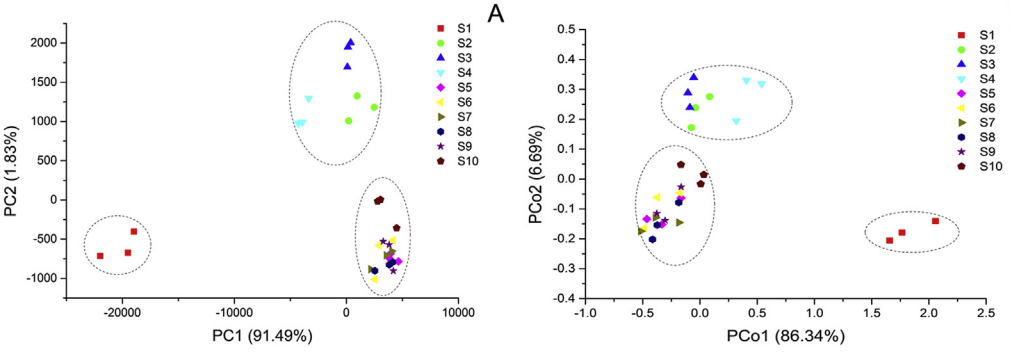

Fig. 2. PCA (A) and PCoA (B) analysis of bacterial communities in sample during manufacturing process of Fu brick tea. Primary dark tea (S1), piling-fermentation tea materials (S2), fermenting brick-tea in the fermentation room for 0 days (S3), 3 days (S4), 6 days (S5), 9 days (S6), 12 days (S7), 15 days (S8), 18 days (S9) and finished tea products at day 22 (S10).

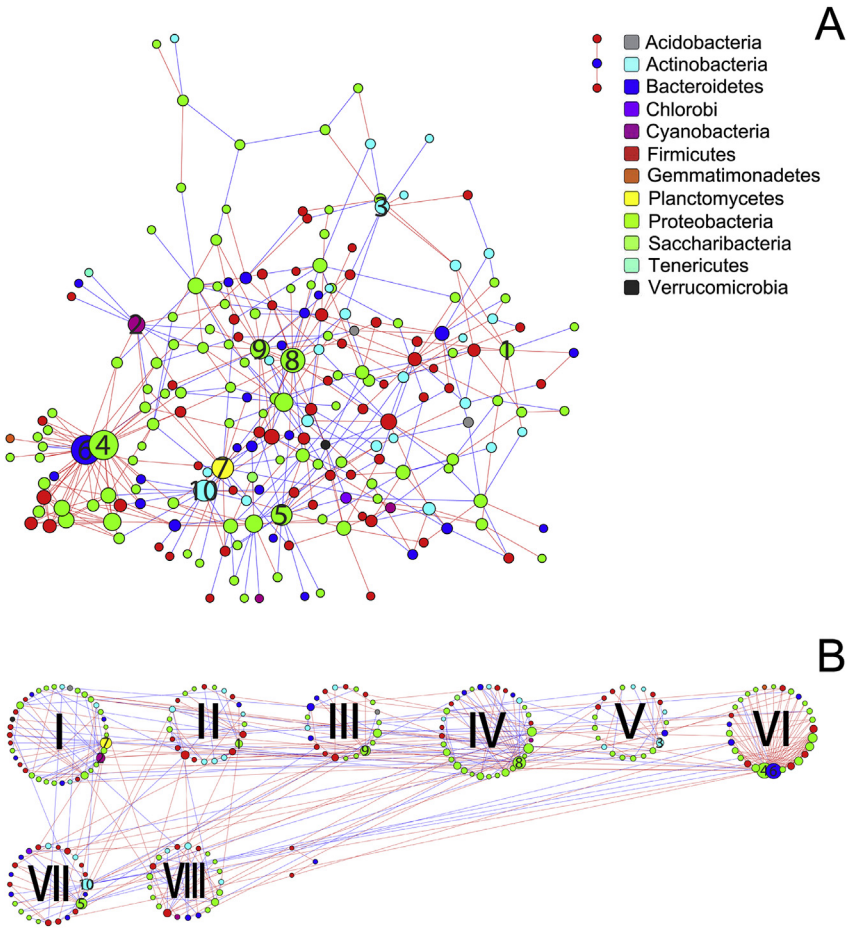

Fig. 3. Interaction networks in the bacterial communities of Fu brick tea (A). The nodes of network were group by modules (B). The co-occurring networks are colored by phylum. The size of each node is proportion to the number of connections (that is, degree), and the red edge indicates a positive interaction and the blue edge indicated a negative interaction between two nodes. The numbers are indicated as follows: 1–8 the module hubs OTU208, OTU240, OTU270, OTU508, OTU511, OTU588, OTU596 and OTU753.9–10 the network hubs OTU93 and OTU427. I ~ VIII indicated the number of modules. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Stage III: S5-S10) were analyzed using PICRUSt based on the KEGG database. The different KEGG pathways predicted for each bacterial community are shown in Table S4. Unsurprisingly, metabolism was the most abundant functional category throughout the entire production process, although abundance was decreased by 23.13% by the end of the manufacturing process (Fig. S5A). Within the metabolism category, the relative abundance of pathways associated with amino acid metabolism, carbohydrate metabolism, energy metabolism, and metabolism of cofactors and vitamins significantly decreased between stages I and II, and was slightly decreased between stages II and III. Furthermore, the relative abundance of pathways involved in biodegradation and metabolism of xenobiotics, nucleotide metabolism, metabolism of terpenoids and polyketides, lipid metabolism, glycan biosynthesis and metabolism, and biosynthesis of other secondary metabolites also significantly decreased between stages I and II but then remained stable until the end of the manufacturing process (Fig. S5B). It is worth noting that pathways associated with terpenoid backbone synthesis (KEGG level-3) and limonene, pinene, and geraniol metabolism in the metabolism of terpenoids and polyketides (KEGG level-2) were enriched during the manufacturing process (Fig. S5C). In addition, pathways involved in the biosynthesis of penicillin, cephalosporin, butirosin, neomycin, stilbenoids, diarylheptanoid, and gingerol (KEGG level-3) in the biosynthesis of other secondary metabolites (KEGG level-2) increased in abundance during the manufacturing process (Fig. S5D).

3.4. Core functional bacteria in Fu brick tea production

O2PLS was used to analyze associations between the bacterial community and chemical compounds during the production of Fu brick tea. The concentrations of major chemical compounds that significantly contribute to the unique flavors of the tea (Scharbert and Hofmann, 2005; Scharbert et al., 2004; Yang et al., 2018) were analyzed in our previous study (Li et al., 2017) (Supplementary material 1 and Table S5). The O2PLS model had R2 and Q2 values of 0.934 and 0.871, respectively, suggesting that the model was a good fit for analysis and prediction. Three latent variables were identified, with microbiota predictive structures accounting for 92.9% of the total variation within the X dataset and the chemical compound predictive structures accounting for 99.8% of the total variation in the Y dataset. To identify core functional bacteria in the Fu brick tea manufacturing process, three conditions were considered: (a) VIP value ≥ 1; (b) correlation coefficient ≥ 0.7, $P < 0.05$; (c) number of microbes highly correlated ($|r| \geq 0.7$) with chemical compounds ≥ 1. Based on these criteria, 19 genera, including *Klebsiella*, *norank_phyla_Saccharibacteria*, *Alkaliphilus*, *norank_family_Chitinophagaceae*, *Escherichia-Shigella*, *Helcococcus*, *Blastomonas*, *Janthinobacterium*, *Aquabacterium*, *Psychrobacter*, *Dechloromonas*, *Opitutus*, *Chryseobacterium*, *Enhydrobacter*, *Bacillus*, *Brochothrix*, *Arcobacter*, *Lactococcus*, and *Streptomyces*, were identified as core functional bacteria for Fu brick tea production. Detailed information on the correlations between these core functional bacterial genera and chemical compounds is shown in Table 1.

4. Discussion

Microorganisms are critical in the fermentation step of Fu brick tea production. In the current study, we revealed that *Klebsiella* species dominated the bacterial community in the initial stage of production but were quickly replaced by *Pseudomonas*, *Lactococcus*, *Stenotrophomonas*, *Enterococcus*, and *Bacillus* species, which remained stable until the end of the manufacturing process. This dynamic shift in bacterial community structure during the production process could be caused by several factors: 1) the high-temperature steam treatment that

Table 1
The VIP value and correlation between the core functional genera and major compounds variables [a].

	VIP Value	WE	FLA	TP	AA	SS	CAF	GA	OA	EGC	DL-C	EC	EGCG	GCG	ECG
norank_p_Saccharibacteria	1.55658	−0.65369[b]	0.28391	−0.80491[c]	−0.52017	−0.45808	−0.70128[b]	0.41764	−0.21054	−0.62947[b]	−0.17260	−0.4118	−0.66152[b]	−0.65107[b]	−0.63557[b]
Klebsiella	1.54609	0.62445[b]	−0.13652	0.71300[b]	0.47198	0.28498	0.87198[c]	−0.25687	0.20104	0.63160[b]	0.36478	0.532486	0.67914[b]	0.71603[b]	0.62085[b]
Alkaliphilus	1.46732	−0.50173	0.42886	−0.71692[b]	−0.45480	−0.32693	−0.70648[b]	0.49043	0.18768	−0.38511	−0.24312	−0.35104	−0.45267	−0.52220	−0.46916
norank_f_Chitinophagaceae	1.46122	−0.68047[b]	0.32328	−0.85487[c]	−0.55933	−0.48276	−0.75503[b]	0.45913	−0.10299	−0.62110[b]	−0.16566	−0.40551	−0.67234[b]	−0.68213[b]	−0.64823[b]
Escherichia-Shigella	1.43247	−0.69274[b]	0.03266	−0.67434[b]	−0.51357	−0.62217[b]	−0.58051	0.56945	0.08272	−0.36910	0.21904	−0.10477	−0.62002[b]	−0.65126[b]	−0.72688[b]
Helcococcus	1.29934	−0.63290[b]	0.39267	−0.74979[b]	−0.49240	−0.46584	−0.63598[b]	0.49006	0.01841	−0.44442	−0.14287	−0.36916	−0.56819	−0.60803[b]	−0.60863[b]
Blastomonas	1.28247	−0.37371	0.49938	−0.63537[b]	−0.20755	−0.16668	−0.71139[b]	0.29682	0.24071	−0.39744	−0.39433	−0.48402	−0.38580	−0.41136	−0.34443
Janthinobacterium	1.26021	−0.43880	0.42275	−0.67727[b]	−0.30163	−0.17095	−0.78006[b]	0.21442	0.14777	−0.56166	−0.48198	−0.61239[b]	−0.48685	−0.49031	−0.41401
Aquabacterium	1.14918	−0.47492	0.43999	−0.68127[b]	−0.33592	−0.25998	−0.72142[b]	0.37018	0.15292	−0.40415	−0.34226	−0.44429	−0.45094	−0.49126	−0.43919
Psychrobacter	1.11754	−0.43440	0.41794	−0.66253[b]	−0.30519	−0.21474	−0.76198[b]	0.32861	0.18170	−0.44618	−0.37203	−0.46849	−0.46238	−0.49714	−0.42948
Dechloromonas	1.11047	−0.49395	0.42271	−0.68136[b]	−0.33780	−0.27870	−0.74473[b]	0.36654	0.15787	−0.43476	−0.30659	−0.42027	−0.48379	−0.52686	−0.46125
Opitutus	1.11000	−0.69492[b]	0.25560	−0.83048[c]	−0.68621[b]	−0.45544	−0.72947[b]	0.51504	−0.21185	−0.63919[b]	−0.04845	−0.39377	−0.75288[b]	−0.74074[b]	−0.70602
Chryseobacterium	1.10037	−0.52819	0.42120	−0.71968[b]	−0.36272	−0.24684	−0.78352[b]	0.29114	0.13741	−0.56353	−0.35702	−0.55514	−0.56055	−0.56009	−0.49662
Enhydrobacter	1.08460	−0.45113	0.37331	−0.69965[b]	−0.32660	−0.25086	−0.74485[b]	0.33282	0.15247	−0.46480	−0.33064	−0.45344	−0.48440	−0.51757	−0.44177
Bacillus	1.06547	−0.43633	0.36589	−0.64851[b]	−0.32524	−0.24722	−0.71234[b]	0.34767	0.16342	−0.40498	−0.28889	−0.40946	−0.44737	−0.48771	−0.43731
Brochothrix	1.06200	−0.47030	0.41043	−0.66747[b]	−0.31636	−0.25509	−0.72177[b]	0.33562	0.22884	−0.43840	−0.30842	−0.46478	−0.46887	−0.48373	−0.43644
Arcobacter	1.06157	−0.29944	0.31478	−0.36222	−0.23817	0.09137	−0.73073[b]	0.04144	0.05106	−0.52366	−0.59213	−0.68184	−0.48308	−0.48290	−0.38487
Lactococcus	1.04327	−0.49448	0.40907	−0.71291[b]	−0.35434	−0.26103	−0.74426[b]	0.34139	0.09854	−0.44941	−0.37067	−0.48332	−0.48498	−0.52668	−0.46106
Streptomyces	1.02969	0.24406	0.29019	0.17630	0.19466	0.10132	0.09378	0.16018	0.76405[b]	0.30172	0.08698	0.15126	0.25800	0.22338	0.18671

[a] Water extract (WE), soluble sugar (SS), flavonoid (FLA), organic acid (OA), tea polyphenol (TP), amino acid (AA), gallic acid (GA), caffeine (CAF), epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin gallate (GCG), epicatechin (EC), catechins (C).
[b] Indicated that the significance < 0.05.
[c] Indicated that the significance < 0.01.

occurs between sampling points S1 and S2, or 2) the inhibitory effects of the dominant fungal community, which is in the logarithmic growth phase from sampling points S5-S10 (Li et al., 2017; Wang et al., 1991). The succession of different bacterial genera during the manufacturing process also reflects their functions. In general, most of the functional pathways predicted by PICURSt decreased in abundance during the manufacturing process. However, several KEGG level-3 pathways involved in the metabolism of terpenoids and polyketides and in the biosynthesis of other secondary metabolites were enhanced during the process. This implies that a specific subset of species in the bacterial community contributes to the formation of the characteristics aroma compounds and beneficial secondary metabolites, such as geraniol and gingerol.

Co-occurrence analysis revealed that most of the dominant genera were distributed amongst modules I and VI (Fig. S3), indicating a significant role for these genera in the Fu brick tea ecosystem. In module I, *Klebsiella* was the dominant genus, accounting for > 90% of all sequences in the module. It was also identified as the core functional bacterial genus, with functions mainly relating to the metabolism of tea polyphenol (TP), caffeine (CAF) and gallocatechin gallate (GCG) during the production of Fu brick tea. These findings indicated that module I may be related to carbon metabolism. Several studies have reported that *Klebsiella* species can ferment a wide range of substrates, including pentoses (xylose and arabinose), hexoses (glucose, mannose, and galactose), and disaccharides (sucrose, lactose, and cellobiose), producing ethanol, butanediol and propanediol (Akbas and Stark, 2016; Cho et al., 2012; Du et al., 2006). These substrates are readily used by various microorganisms as carbon sources during growth (Luo et al., 2017a, 2017b). In addition, *Klebsiella* was identified during the fermentation of Pu-erh tea, soy sauce, and other fermented foods (Puerari et al., 2015; Qin et al., 2016; Yang et al., 2016; Zhao et al., 2015).

The predominant genera in module VI were *Pseudomonas, Stenotrophomonas*, *Enterococcus*, *Lactococcus* and *Bacillus* (Fig. S3), all of which are involved in the formation of flavor compounds in Fu brick tea. Since the early 1960s, *Pseudomonas* species have been investigated for their potential as biocatalysts in the industrial-scale production of value-added flavor compounds from terpenes (Molina et al., 2013). This genus is also used to produce natural flavors for foods and beverages, including ethyl butyrate (Medeiros et al., 2000), ethyl hexanoate (Cormier et al., 1991), perillyl alcohol (Mirata et al., 2009), verbenol (Divyashree et al., 2005), and perillic acid (Di Gioia et al., 2011). *Stenotrophomonas* species produce glutaminase to degrade peptides and amino acids during fermentation, and are strongly associated with the distinctive sensory characteristics of fermented foods (Kim et al., 2016). *Enterococcus* is a large genus of lactic acid bacteria that contribute to flavor promotion and beverage preservation (Puerari et al., 2015). It has been identified as a subdominant genus in cheese (Casalta et al., 2009), Miang (Sukontasing et al., 2007), and Pu-erh tea fermentation (Zhao et al., 2015). In addition, *Enterococcus* species reportedly play a significant role in flavor development, probably through proteolysis, lipolysis, citrate breakdown, and bio-protection (Foulquie Moreno et al., 2006).

Lactococcus and *Bacillus* were identified using O2PLS analysis as the core functional genera during the Fu brick tea manufacturing process. *Lactococcus* is well known in the production of fermented milk products, where pertinent species convert chemical components and synthesize aroma substances. *Lactococcus* species also have antifungal activity, which may help to maintain the stability of the bacterial community (Leong et al., 2014). *Bacillus* species produce numerous volatile compounds, including pyrazines, aldehydes, ketones, and alcohols, and play an important role in the formation of aroma compounds in alkaline-fermented foods, condiments, and Chinese liquor (Azokpota et al., 2010). Thus, *Klebsiella*, *Lactococcus* and *Bacillus* might group together to form a core microbiome that contributes to the production of certain key metabolites during the manufacture of Fu brick tea.

5. Conclusions

The bacterial community found in the Fu brick tea ecosystem plays an important role during the manufacturing process. Our results indicate that this bacterial community, especially the core functional genera, can produce different substrates that provide a series of ecological habitats, which, in turn, modulate specialized populations. The core functional genera group together, forming a core microbiome. These genera are dominant across the different stages of the manufacturing process and contribute to the development of the special characteristics of Fu brick tea. These findings advance our understanding of the changes in the bacterial community during Fu brick tea manufacture and help elucidate the potential functions of specific genera in the development of the special characteristics of Fu brick tea during the manufacturing process.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.001.

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