The isolation and identification of Bacillus velezensis ZN-S10 from vanilla (V. planifolia), and the microbial distribution after the curing process

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Abstract

The market value of vanilla beans (*Vanilla planifolia*) is constantly increasing due to their natural aroma and flavor properties that improve after a curing process, where bacteria colonization plays a critical role. However, a few publications suggest that bacteria play a role in the curing process. Hence, this study aimed to isolate *Bacillus* sp. that could be used for fermenting *V. planifolia* while analyzing their role in the curing process. *Bacillus velezensis* ZN-S10 identified with 16S rRNA sequencing was isolated from conventionally cured *V. planifolia* beans. The isolate (1 mL^{-1} of 10^{-7} CFU mL^{-1}) fermented and colonized non-cured vanilla pods. PCA results revealed distinguished bacterial communities of fermented vanilla and the control group, suggesting colonization of vanilla. Phylogenetic analysis showed that ZN-S10 was the dominant *Bacillus* genus member and narrowly correlated to *B. velezensis* EM-1 and *B. velezensis* PMC206-1, with 78 and 73% similarity, respectively. The bacterial taxonomic profiling of cured *V. planifolia* had a significant relative abundance of *Firmicutes, Proteobacteria, Cyanobacteria, Planctomycetes*, and *Bacteroidetes* phyla according to the predominance. *Firmicutes* accounted for 55% of the total bacterial sequences, suggesting their colonization and effective fermentation roles in curing vanilla.

Introduction

Vanilla (*Vanilla planifolia*) is a tropical orchid (Orchidaceae family) known worldwide for its aroma or flavor properties [1,2]. In *V. planifolia*, more than 200 compounds contribute to its distinctive flavor and aroma, with vanillin being the most abundant component [1,3]. Hence commercially, vanillin is required in vast quantities. Unfortunately, vanilla plants cannot supply adequate vanillin for the global market [4]. Flavoring agents such as vanillin (3-methoxy, 4-hydroxy-benzaldehyde), anise alcohol, guaiacol, and *p*-hydroxybenzaldehyde, found in natural cured vanilla are widely used in food or baking products, cosmetics, aromatherapy, and pharmaceuticals for various purposes [5,6]. Thus, vanilla beans can provide natural vanillin but are limited and costly [7,8]. To obtain a natural vanilla extract, *V. planifolia* must undergo a laborious and protracted curing procedure that also improves the vanilla's aroma [9,10]. In an agricultural farm or a greenhouse environment, matured green vanilla beans lack the distinctive vanilla flavor and are harvested odorless [11,12]. Hereafter, at post-harvest, a curing process is performed to initiate the formation of vanilla beans' distinctive aroma, flavor, and color properties. The conventional curing technique involves blanching or killing, fermentation (sweating), slow-drying, and conditioning the vanilla beans [13–15]. According to Peña-Barrientos, et al. [16], the curing process alters the physicochemical, and structural properties of vanilla beans, increasing the aromatic compounds of the bean, while the flavor attribute is also developed. Notably, fermented vanilla beans and their products have been reported to have an added distinctive aromatic value [17]. Other researchers have used the *Bacillus*-curing process to form better aromatic and flavor attributes caused by the hydrolysis of glucovanillin and the formation of vanillin compounds [18]. Hence, these studies have exhibited that *V. planifolia* can be colonized by *Bacillus* isolates inoculated on the vanilla bean pods. The glucosidase produced by these isolates influences

flavor production by hydrolyzing glucovanillin. Thus, the colonization of *Bacillus* in vanilla is tangled in flavor and aroma development during fermentation or the “sweating” stage in the curing process [19].

Fermentation in *V. planifolia* is anticipated as a strategy or as a valorization technique to obtain natural vanillin from vanilla beans, wherein glucovanillin is converted to vanillin. Hence, *Bacillus* sp., lactic acid bacteria (LAB) and other studied bacteria are added to green-uncured vanilla beans to improve their volatile profile [17,20]. It should also be noted that during fermentation, glucovanillin is hydrolyzed by glucosidase produced by *Bacillus* colonies on vanilla bean pods [21]. Buitimea-Cantua, et al. [22] reported that during the curing process of *V. planifolia*, the key precursors of vanillin (β-D-glucovanillin and vanillyl alcohol) are augmented during the sweating owing to the enzyme β-D-glucosidase activity that is influenced by pressure and moisture during this treatment. Similarly, vanillin is increased hence improving the flavor or aroma development and enzymatic activity [23]. Vanilla's glucovanillin content increases with the browning of the vanilla pods; after fermentation, the beans tend to have a higher glucose and vanillin content, subsequently an increased market value is attained [24–26]. The colonization of microorganisms on vanilla bean (*V. planifolia*) plays an essential role in flavor and aroma formation [18,21]. Gu, et al. [19] mentioned that the number of microorganisms such as bacteria communities that colonize *V. planifolia* during plant growth, at harvest, and after the curing process is numerous.

Statistical analysis or chemo-metrics can be employed after the curing process to observe the vanilla microbiome. A multivariate statistical approach; principal component analysis (PCA) can be used to minimize the dimensionality of a set of observations made up of a lot of interconnected variables [27,28]. Moreover, De Vrieze, et al. [29] mentioned that 16S ribosomal RNA-based analysis could be established as an accepted technique for profiling bacterial or fungi communities. Studies have shown that a 16S rRNA gene sequencing method analyzes bacteria and other microorganisms in a specific environmental sample to study the composition of the microbial population, and deduce the diversity, and abundance while exploring the relationship between microorganisms and the treatment [30–32], hence it has been also observed by Roling, et al. [33] in a vanilla flavor development study. Next-generation sequencing (NGS) technologies based on 16S rRNA gene sequencing are evolving rapidly, and it has become possible to sequence the entire genomes or transcriptomes of agricultural produce efficiently and economically [34–36]. Notably, few reports exhibited the involvement of bacteria communities while presenting the taxonomy and related profiling of these microorganisms. Therefore, this study isolated *Bacillus velezensis* ZN-S10 strain from vanilla beans (*V. planifolia*), to objectively employ bacterial fermentation of the obtained isolate (ZN-S10) in non-cured vanilla pods to explore the vanilla microbiome that may be associated with the curing process during the fermentation stage of *V. planifolia*. The phylogeny and taxonomy of the vanilla microbiome focused on bacteria communities involved when *V. planifolia* pods were fermented with *B. velezensis* ZN-S10 compared with traditionally cured vanilla beans was established.

Materials and methods

Sample preparation

Green, matured vanilla (*V. planifolia*) bean samples were collected from a local farm in Pingtung, Taiwan (22°25'41.8"N 120°32'29.0"E). A 1 kg of vanilla bean pod pack was washed with sterilized water and then blanched in hot water (~ 80°C) for 2–3 min. The blanched vanilla pods were instantly placed in cooling boxes with paper tissues to let the pods to dry out. According to Xu et al. (2020), this “killing” stage inhibits the vegetative growth of the beans while allowing enzyme activation and initiating color change (from yellow/green to dark brown) and the flavor or aroma development of the final cured vanilla bean.

Curing of vanilla bean pods

The blanched vanilla beans were allowed to naturally ferment in a cool freezer; wherein the samples were stored in plastic boxes and wrapped in tissue paper and stored at 10 ℃. It should be noted that the tissue paper was changed every 2 days to avoid mold formation. Occasionally, cool-air drying and humidification were also done once a week with a lamina flow and a humidifier, respectively. Additionally, as the beans dried and showed yellow to brown color changes owing to phenol browning and lipid oxidation that occur simultaneously with the enzymatic hydrolysis of vanillin precursors [22]. Thus, brown to dark brown vanilla bean pods without molds were recognized as the best-quality cured samples and were used for experimentation.

Bacteria isolation and identification

A batch of the brown-dry cured vanilla bean pods were cut into 0.3–0.5 cm pieces and grounded into 0.5 g powder. Sterile distilled water was then added in 15 mL tubes with the vanilla powder, then agitated in ultrasound for 20 min, and stored at 20 ℃ for overnight, following the method of Chen, et al. [31]. A de Man, Rogosa, and Sharpe (MRS) agar medium [37] were modified and prepared on Petri dishes, maintained at pH 5.7, and autoclaved for 15 min with the composition presented in Table 1. About 500 µL of the supernatant was pipetted on MRS agar and then spread on the Petri dishes for oven incubation at 37 ℃ for 16 h. After the incubation, the bacteria were selected after three consecutive plates according to shape, color, and size to obtain pure isolates. The isolates were selected for identification and to be stored in 20% glycerol at -80 ℃ for further experiments.

Table 1
A modified MRS medium used in this study for bacterial isolation

Component	Composition (in g/L without agar)
Dextrose (D-glucose C□H□□O□)	15
Yeast extract	5
Sodium acetate ($C_2H_3NaO_2$)	5
Potassium phosphate (K_3PO_4)	2
Magnesium sulfate (MgSO□. 7 H_2O)	0.1
Tween 80 (1.00 mL)	–

A 16S rRNA gene sequencing technique [38] was used for the identification and classification of the pure bacteria isolates. The 16S rRNA molecular sequencing identification was performed by Mission Biotech Company (Taiwan), and arranged with ABI Big Dye Terminator v3.1. PCR mixtures that were amplified by initially holding at 94°C for 5 min. Besides, 40 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 30 s, and extensions at 72°C for 2 min 20 s. Then the reaction ended with a final extension at 72°C for 5 min and a hold at 4°C. In this study, F8 and R1510 primers were used for the amplification of 16S rRNA nucleotide sequences, obtainable on the National Center for Biotechnological Information (NCBI) website where the Basic Local Alignment Search Tool (BLAST) was used for phylogenetic analysis.

Hemolysis test

The blood agar test was conducted in the pure collected bacterial isolates to observe the safety of using these microbes for the food industry and animal use. In 1 L of distilled water, 40 g of a commercial agar-based medium (Infusion agar) (Himedia Laboratories Co., India), was suspended and then heated to boil at 121 ℃ at 15 lbs pressure. The agar medium was then allowed to cool and 5% *v/v* of sterile sheep-defibrinated blood was added and mixed well before pouring into Petri plates. The pure isolate bacteria were then streaked on these blood agar plates and then incubated at 37 ℃ for 16 h to observe the alpha, beta, and gamma properties of the bacteria. According to studies; *Staphylococcus* sp., *Streptococcus* sp., and *Enterococcus* sp. can be differentiated by the degree of hemolysis caused by their hemolysis [39,40]. Hence, the bacteria with beta (β) hemolysis [41] had been classified as complete hemolysis seen with the formation of a clear-transparent zone, and the plate with those properties was discarded in this study. Partial hemolysis was recognized with opaque-green zone formation on the blood agar plates, thus was also discarded in this study. The agar plates with notable growth on the blood agar medium characterized as gamma (γ) hemolysis were the targeted bacteria colonies and were used for identification and subsequent experiments of interest in this research. However, the bacteria with alpha (α) hemolysis were observed with partial hemolysis showing rupture or “digestion” with a lightened (yellow) and transparent area on the blood agar medium were discarded. Hence, this study performed a visual assessment of the hemolysis (α, β, γ) based on above-mentioned references.

Phylogenetic tree analysis based on 16S rRNA gene sequences

The evolutionary data of the phylogenetic tree was analyzed using the Neighbor-Joining method [42,43]. With branch lengths in the equal units as those of the evolutionary distances used to deduce the phylogenetic tree, the ideal tree was drawn to scale (2.00). The evolutionary distances are expressed in base substitutions per site and were calculated using the Maximum Composite Likelihood approach [44]. Beside each internal node in the tree is the percentage of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 96 positions in the final dataset. Evolutionary analyses were conducted and extracted NCBI 16S rDNA sequences from the aforementioned genome sequences and aligned them using MEGA11 [45]. The sequences were subjected prior to the NCBI database (https://www.ncbi.nlm.nih.gov/) for a FASTA search as well as obtaining the accession numbers according to the sequences.

Fermentation of V. planifolia

A new batch of green matured vanilla bean pods (non-blanched) was sorted according to sizes of approximately 15 cm considered as the best quality. The pods (1 kg) were then blanched for 2–3 min in hot water (~ 80 °C) and then allowed to dry for 3 h. After drying, the 1 kg bean pods were sprayed (1 mL of 10^{-7} CFU mL^{-1}) with *Bacillus velezensis* ZN-S10, then shaken in a plastic box to allow the bacteria to colonize and ferment the vanilla. The UV-Vis spectrometer was used to measure the OD value of the liquid to obtain the desired concentrate for bacterial fermentation. These bacteria-fermented samples were considered as the treatment group, while the vanilla beans that were uncured and allowed to ferment naturally i.e. without the addition of any isolated bacteria; were categorized as the control group.

Taxonomic profiling and Venn diagram construction

The microbial communities for each unique sample group were characterized according to their taxonomic profile using the relative microbial abundance. Only the bacterial OTUs of the phylum and genus levels were used for analysis in this research. Hence, to compare the genus and phylum levels between the bacterial communities for non-cured vanilla beans and bacteria-fermented (cured) samples were presented in a Venn diagram (in OTUs).

Principal component analysis (PCA)

The PCA was used in this study as a technique for analyzing the bacterial microbial communities. This technique is an ordination of reduced variables that are projected at the initial set of variables into new axes (PCs). Hence, the first-hand axis (PC1) is calculated on another axis (PC2) as an eigenvalue of the correlation (or covariance) matrix which is attained from the multivariate matrix of the data set, so a

restricted number of axes extracts the highest possible variance, and eigenvectors linked with eigenvalues that decide each PC direction [46,47].

Taxonomic abundance profiling of the bacterial communities

According to the abundance of information on the bacterial communities at the taxonomic level, a histogram at the phylum level and a heat map analysis at the genus level based on the Bray-Curtis distance were constructed to calculate the frequency of related communities of non-treated vanilla samples and bacteria fermented group. The grouping of the related bacterial communities that may influence the curing process during the fermentation of *V. planifolia* was exhibited in a bubble plot and a circos presentation at phylum and genus levels, respectively.

Results and discussion

Strain identification

The bacteria used in this study was isolated from vanilla beans (*Vanilla planifolia*). The isolation was done from previously naturally fermented and cured batch of vanilla bean pods in the laboratory. A 16S rRNA gene sequencing was conducted based on Johnson, et al. [48] method. The isolated bacteria strain was *Bacillus velezensis* ZN-S10 acquiring a complete genome. The *Bacillus* genus is classified as a gram-positive bacterium, from the *Bacillaceae* family. It is rod-shaped, and aerobic with the formation of endospores; hence shows whitish, round, and medium-sized colonies [49–51].

Isolated pure colonies and hemolysis test findings

This study found that *Bacillus velezensis* ZN-S10 colonies were effectively isolated from vanilla bean pods (*V. planifolia*) when grown with an overnight culture in MRS agar plates and incubated at 37 ℃ for 16 h. As observed in **Fig. 1**, the colonies were fairly spread with off-white colonies on the surface of MRS agar Petri dishes after three consecutive plates (MRS subculture), hence the single pure colonies (Fig. 1A) were found. The hemolysis test characterized by Savardi, et al. [52] as alpha, beta, and hemolysis was conducted with blood agar. The isolated bacteria (*B. velezensis* ZN-S10) exhibited gamma hemolysis (Fig. 1B) exhibiting no hemolysis since the bacteria did not absorb blood agar. The absence of hemolysis showed that the pure isolate was safe for agricultural use in both handling and application.

Figure 1. *Bacillus velezensis* ZN-S10 colonies. The ZN-S10 pure isolate appearance showing the single colonies on the fourth streak region (circled) grown on MRS agar Petri-plate, incubated at 37 ℃ for 16 h (A); and the gamma hemolysis of the ZN-S10 strain (B) grown on blood agar.

The phylogenetic tree of the evolutionary relationship of taxa

In this study, the phylogenetic tree was constructed based on the 16S rRNA gene sequences, where the accession numbers of the isolated strain and related sequences were obtained from the NCBI web database (https://www.ncbi.nlm.nih.gov/). Bacterial phylogenetic analyses are frequently carried out to investigate the evolutionary relationships among distinct bacterial species and genera [53]. Hence, we performed the comparison of the *Bacillus velezensi*s strains acquired from non-cured vanilla beans employing the Neighbor-Joining method to show the phylogenetic relationships of the strains (Fig. 2). The *B. velezensi*s strain ZN-S10 used for fermentation was distinct from other members of the *Bacillus* group. Hence, based on the constructed phylogenetic tree (Fig. 2), *B. velezensis* ZN-S10 were the major bacteria that colonized the vanilla bean pods, and the strain was used for fermentation at the curing process for a new batch of non-cured vanilla bean pods. The strain ZN-S10 accounted for the bootstrap value of 79%, with lesser differences of similar isolate sequences as neighbor strains of *B. velezensis* EM-1, PMC206-1, and C5 strains with bootstrap values of 73%.

Comparison and variation of bacterial communities in naturally fermented and treated vanilla

A Venn diagram (Fig. 3) was constructed for the number of common (uncured vanilla beans) and unique species (bacteria-fermented cured vanilla) in each group. All the sequences of each sample were compared with the NCBI RefSeq 16S rRNA database using EPI2ME v3.0 (Oxford Nanopore Technologies, UK). Hence, the number of variants was very similar to the mean expression of species in the mean groups; showing 230 OTUs amongst the naturally fermented vanilla beans and a very similar population of 233 OTUs in the treatment group. Between the two groups (control and treated vanilla), 230 OTUs were shared by both, while the non-treated pods had the majority of 253 OTUs in the bacterial communities. The shared OTUs between the naturally fermented vanilla (control) and *B. velezensis* ZN-S10 fermented samples suggested the successful colonization with microorganisms; hence effective fermented and cured vanilla beans. Similarly, Chen., et al. [21] reported that *Bacillus* sp. colonizes vanilla beans and is classified as β-D-glucosidase-producing bacteria. The bacterial community population was analyzed with 16s rRNA gene sequencing presented in operational taxonomic units (OTUs) as shown in Fig. 3 **(right).** The results reported that the bacteria-fermented vanilla beans had a higher population (~ 390 OTUs) than the control as expected; hence showing the success of curing the beans with *B. velezensis* ZN-S10 used as a fermentation tool. Studies have also proved that bacteria communities are involved during the curing process of vanilla beans [18]. Hence, the occurrence of the ZN-S10 showed their role in the effective fermentation of *V. planifolia* during the curing process.

Principal component analysis of the bacterial communities

The microbial communities were profiled with principal component analysis (PCA) to visualize the differences between microorganisms obtained from naturally fermented vanilla and the beans that were treated with 10^{-7} CFU mL^{-1} of *B. velezensis* ZN-S10. As presented in Fig. 4, the microbial population accounted for 95.83% of PC 1 of the total variance in treated samples, while control samples showed that PC 2 accounted for 4.17% in the PCA score plot.

Findings on the microbiome of the vanilla before and after fermentation with B. velezensis

The vanilla microbiome after fermentation with *B. velezensis* ZN-S10 was evaluated with high-throughput amplicon sequencing of the bacterial 16S rRNA gene showed a predominance of *Firmicutes, Proteobacteria, Cyanobacteria, Planctomycetes*, and *Bacteroidetes*, respectively (Fig. 5**).** These genera made up ~95% of the total data set wherein at phylum level, a high dominance (≈ 80%) of *Firmicutes* with the subdominant of *Proteobacteria*, and a small fold of *Cyanobacteria* was observed (Fig. 5A) with the vanilla beans that were blanched and allowed to ferment naturally. Nevertheless, the vanilla samples fermented with isolated bacteria from previously naturally fermented vanilla batches also showed a high dominance of *Firmicutes*, (≈ 60%) which was slightly lower in abundance with control samples. The microbiome of treated samples was followed by *Proteobacteria* and *Cyanobacteria* which were a two-fold increase as compared to control samples (Fig. 5A). Researchers have also reported that *Proteobacteria* and *Cyanobacteria* were the common families found in the core of the vanilla microbiome [30]. Also, according to Xiong, et al. [54], the monoculture practice of growing vanilla beans contributes to the high dominance of *Firmicutes* due to low bacterial and fungal communities that occur during the continuous cropping of the vanilla beans. Furthermore, the relative abundances of *Firmicutes, Actinobacteria, Bacteroidetes*, and *Basidiomycota* are common phyla in vanilla plants especially when grown in a monoculture system. Hence, in this study, the results indicated that at a phylum level *Firmicutes, Proteobacteria*, and *Cyanobacteria* were the bacterial groups (Fig. 5A **& B**) with key influence in the curing of vanilla beans. The treated vanilla bean pods showed a higher relative abundance of *Cyanobacteria* and *Proteobacteria* as compared to the non-treated samples (Fig. 5B). Moreover, it should be noted *Planctomycetes*, and *Actinobacteria* were presented in the bacteria-fermented vanilla although the communities in this group were in small abundance, rather than in the control samples (Fig. 5B). Notably, the discussed findings of the bacteria communities were found on the cured *V. planifolia* beans for both treated samples (ZN-S10 fermented) and non-treated pods.

According to the heat map analysis (Fig. 6), the vanilla microbiome at the genus level presented significant differences in the relative abundance across the bacteria-fermented samples (C3-1-9) and the control groups. Hence, an increase of *Firmicutes, Proteobacteria*, and *Cyanobacteria* was observed while *Bacteroidetes*, *Verrucomicrobia*, and *Planctomycetes* were reduced with a significant decrease of *Actinobacteria* on the microbiome of bacteria-treated vanilla samples. Similarly, *Firmicutes* were remarkably increased in the control group, and *Proteobacteria and Cyanobacteria* were reduced while significant decreases were observed in *Bacteroidetes* and *Verrucomicrobia*, with a slight increase in *Planctomycetes* and *Actinobacteria* in contrast with the microbiome of the treated vanilla.

Figure 7 shows the bubble plot had four bacterial classes that include; *Firmicutes, Cyanobacteria, Planctomycetes*, and *Proteobacteria* among others were the most abundant in the microbiome of naturally fermented vanilla and samples fermented with *B. velezensis*. Hence, at the genus level, *Priesta* had a higher relative abundance of 0.5 for *Firmicutes* phylum on both control and treated vanilla bean samples.

The distinct bacterial communities were presented in a circos plot at genus and phylum levels (Fig. 8) that was detected in naturally fermented vanilla (control) and the vanilla treated with *B. velezensis* for fermentation purposes during the curing process. We identified 5 different genera in both the control and treated vanilla samples. Hence, at the genus level, the treated vanilla samples had dominant *Priesta* (45%), while the naturally fermented vanilla had 55% dominance. Interestingly, at this level larger fractions of *Pseudoxanthomonas* (96%), *Microcoleus* (90%), *Loriellopsis* (95%), and *Paenibacillus* (97%), as compared to the control group (Fig. 8A). Nevertheless, the control vanilla microbiota was slightly supplemented with *Microcoleus* (10%), and *Loriellopsis* (5%); also showing a lower abundance of *Paenibacillus* (3%), Psedoxanthomans (4%), which was contrary to the microbiota of the treated vanilla. Therefore, the *Priesta* or *Bacillus* were presented as the predominant bacterial community at the genus level. It should also be noted that the *Firmicutes* were the most dominant phylum (Fig. 8B) as observed with higher relative abundance at the phylum level.

Conclusions

In this research, *Bacillus velezensis* ZN-S10 was successfully isolated from *Vanilla planifolia* and was used for bacterial fermentation in green-matured non-cured vanilla beans. The bacteria communities were also analyzed with the NGS technique, where 16s rRNA gene sequencing to explore the genus and phylum level of the bacteria population involved. The annotations provided by the sequence data, multiple aspects of the development and biochemistry of vanilla beans could be understood to be involved in the colonization of non-cured vanilla beans, while microorganisms are important in fermentation for the curing process in *V. planifolia*. The 16S rRNA gene sequencing in this study was shown to be very crucial for the understanding of the bacterial ecology, abundance, and relationship taxonomy to improve the procedures done for the curing process to achieve high-quality cured vanilla beans. The findings of the bacterial communities suggested that at the genus level *Priesta* were dominant; hence were involved in the colonization or fermentation of *V. planifolia*. Moreover, at the phylum level, the Bray-Curtis distance revealed a higher relative abundance of *Firmicutes*, hence these bacteria had an important role in the curing process of the vanilla beans. Further research on the cured vanilla beans cannot only be used to extract the bacteria responsible for fermentation but for natural vanilla extract as well as for the preparation of other vanilla products. The isolated bacteria from the vanilla, *B. velezensis* ZN-S10 could also be explored for other properties, such as plant-growth-promoting attributes, possible biocontrol qualities or pathogen suppression, and other biotechnological applications.

Declarations

Ethical statement

The authors would like to declare that vanilla bean pods (*Vanilla planifollia*) were harvested, green and matured with permission from Pingtung Lin Tsai Bian Association Farm, Taiwan (22°25'41.8"N, 120°32'29.0"E). The website link of the farm is as follows https://www.agriharvest.tw/archives/73970

confirming that the harvest of the vanilla was grown under the appropriate shade and cultivation media before it was harvested and used in this study. The farm also confirmed that the vanilla was following the national regulations of the Ministry of Agriculture in Taiwan (https://eng.moa.gov.tw/); hence vanilla bean pods were grown following international guidelines. Authors do not have any conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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Figures

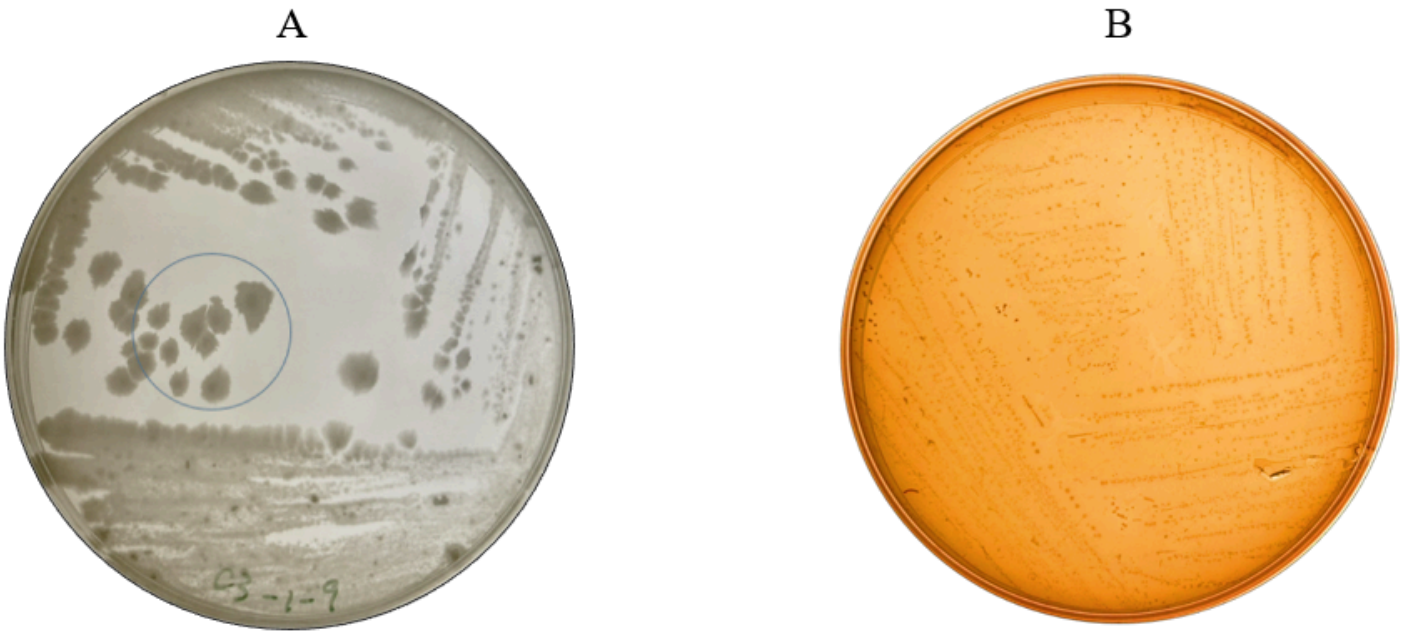

Figure 1

Bacillus velezensis ZN-S10 colonies. The ZN-S10 pure isolate appearance showing the single colonies on the fourth streak region (circled) grown on MRS agar Petri-plate, incubated at 37 ℃ for 16 h (A); and the gamma hemolysis of the ZN-S10 strain (B) grown on blood agar.

Figure 2

The phylogenic tree of 10 random sequences from the *Bacillus velezensis* strains, highlighting their position within the *Bacillus* genus. The tree was built with MEGA, using the neighbor-joining technique. The percentage numbers at nodes are of bootstrap values, calculated by 1000 repeats of the tree. This analysis involved 10 nucleotide sequences of *Bacillus velezensis* strains, with a total of 96 positions in the final dataset.

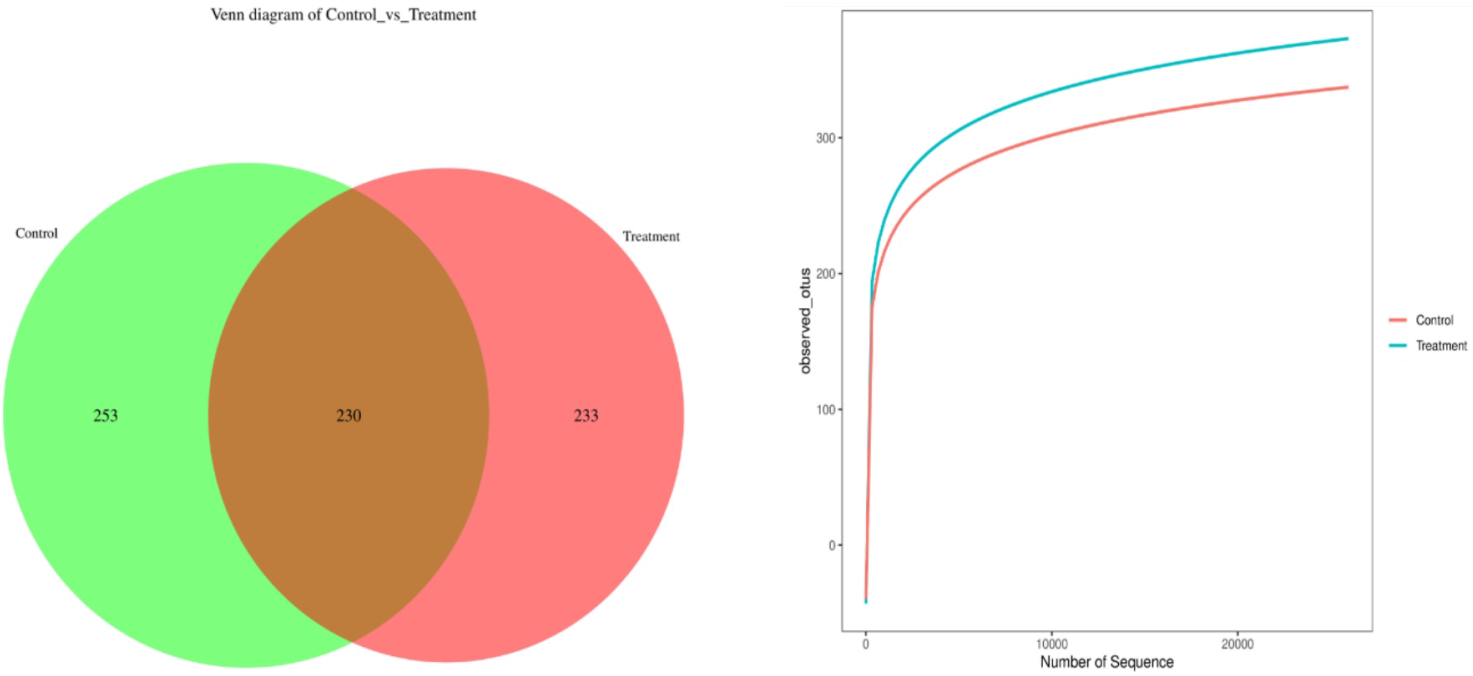

Figure 3

Venn diagram (left) at shared and the unique OTUS of the vanilla microbiome and schematic diagram (right) of dilution curve results of naturally fermented (control) vanilla beans and *Bacillus velezensis* ZN-S10-treated samples. representing the control vanilla beans and *Bacillus velezensis*ZN-S10-treated samples.

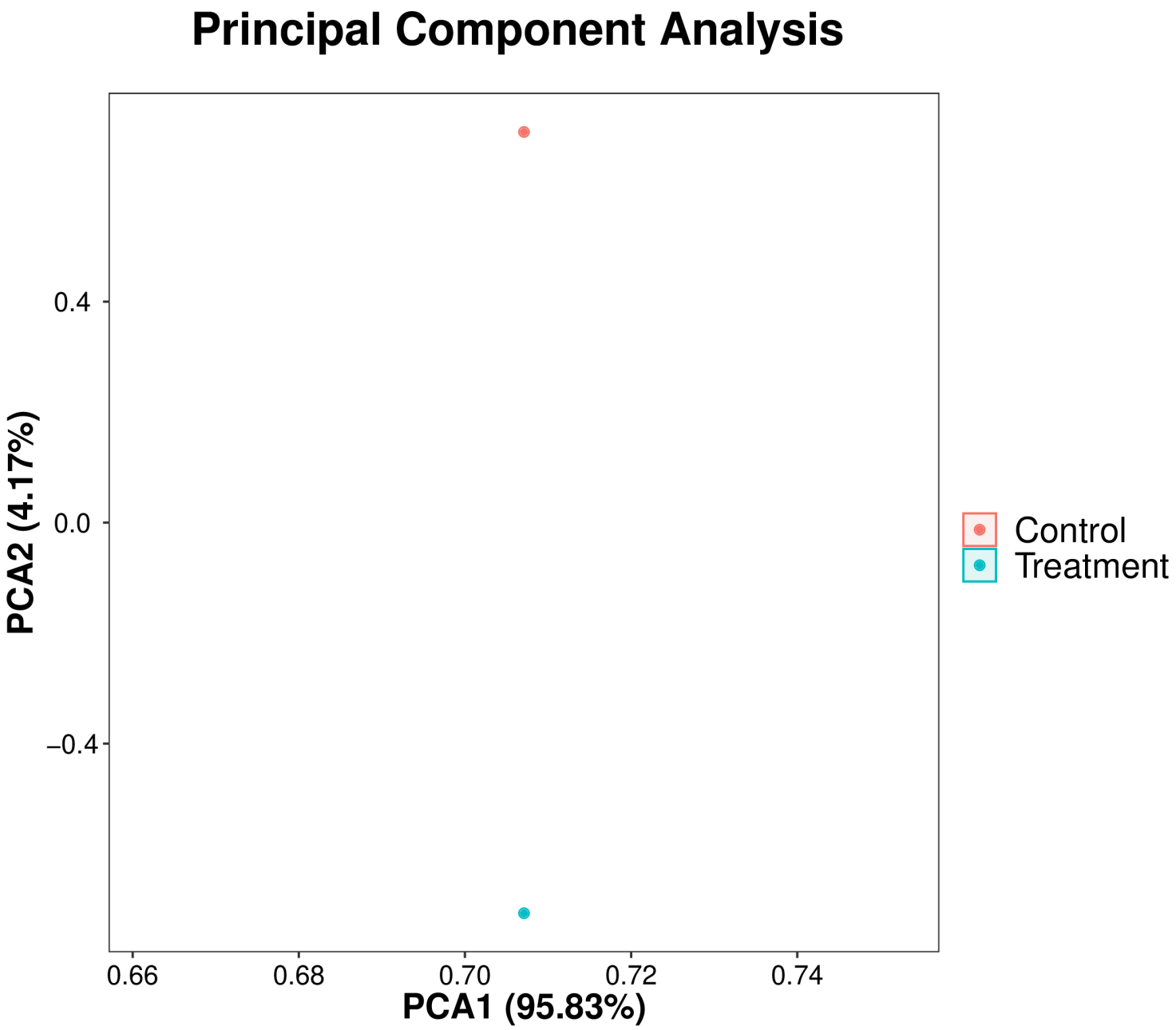

Figure 4

The principal component analysis (PCA) of the bacterial communities obtained from naturally fermented vanilla (control) and *B. velezensis* ZN-S10-treated vanilla beans.

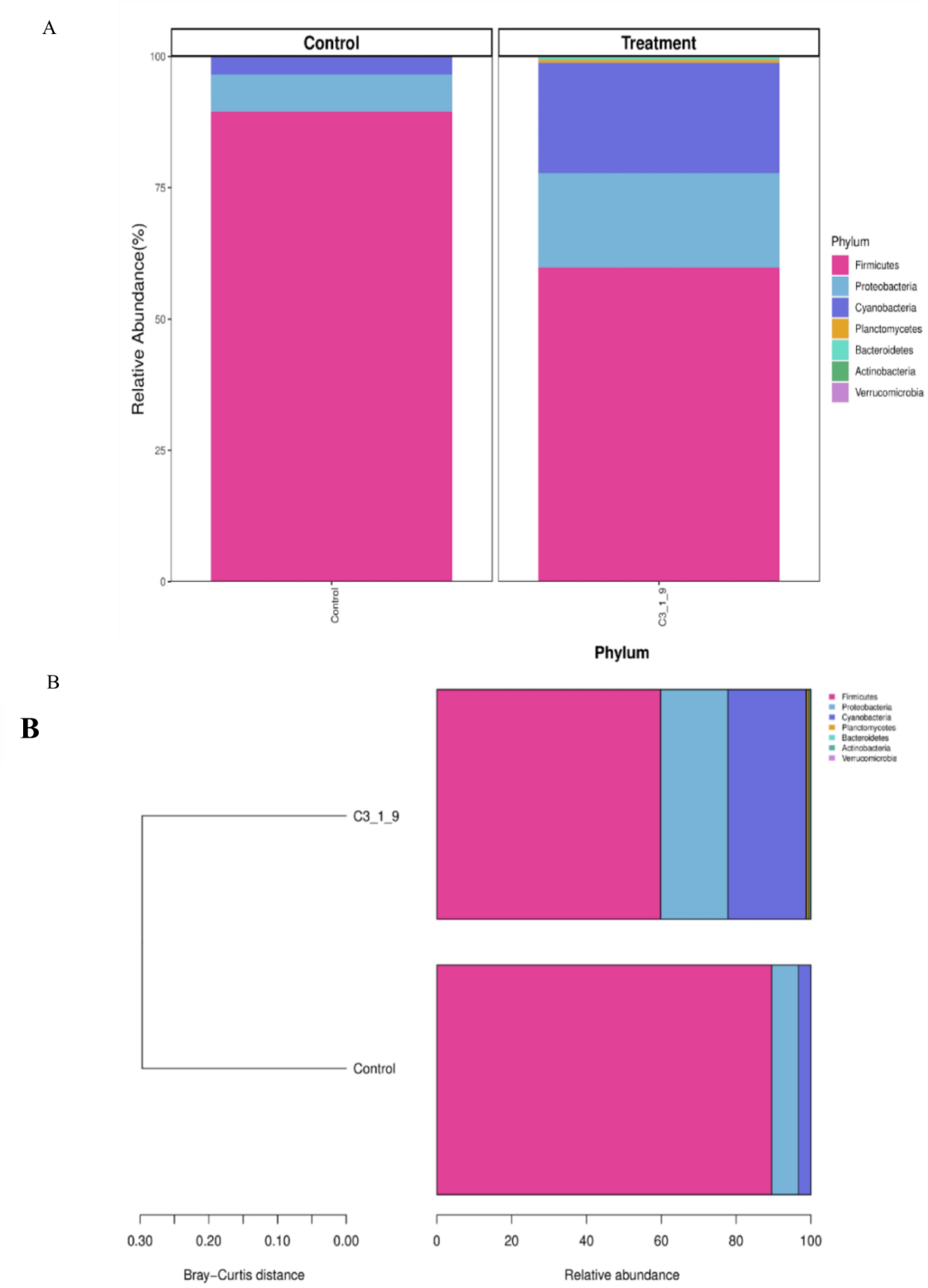

Figure 5

The bacterial abundance of the vanilla microbiome. (A) Histogram of the bacterial composition at its relative abundance (%) presented at the phylum level of the control group (naturally fermented vanilla pods) and *Bacillus velezensis* ZN-S10 fermented vanilla (C3-1-9). (B) The relative sample abundance at the phylum level was analyzed with the Bray-Curtis distance of non-treated vanilla samples (control) and *B. velezensis* ZN-S10 fermented (C3-1-9).

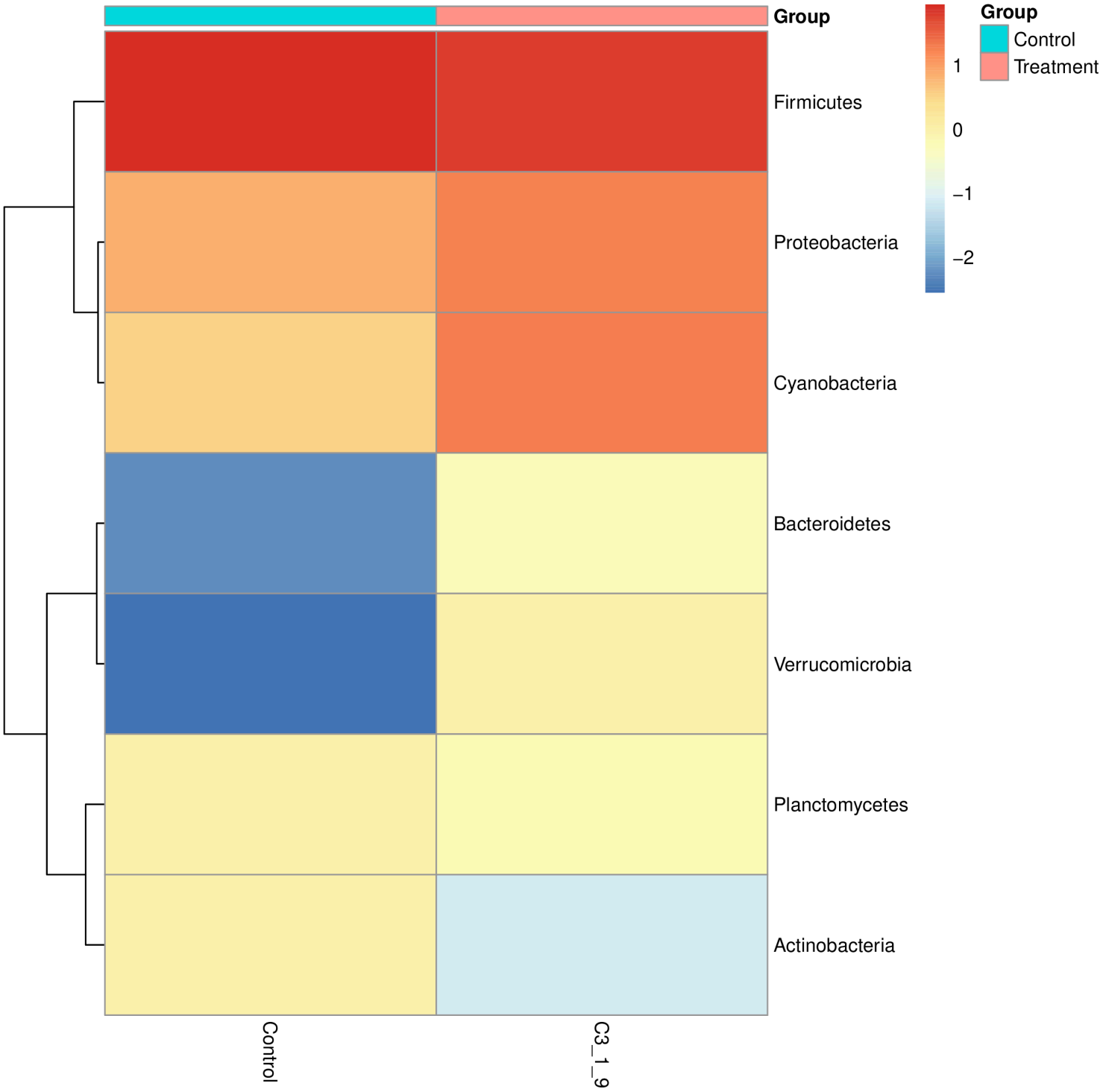

Figure 6

The heat map analysis of the vanilla microbiota composition at the genus level for Bacillus velezensis ZN-S10 fermented samples (C3-1-9) as compared with the control group.

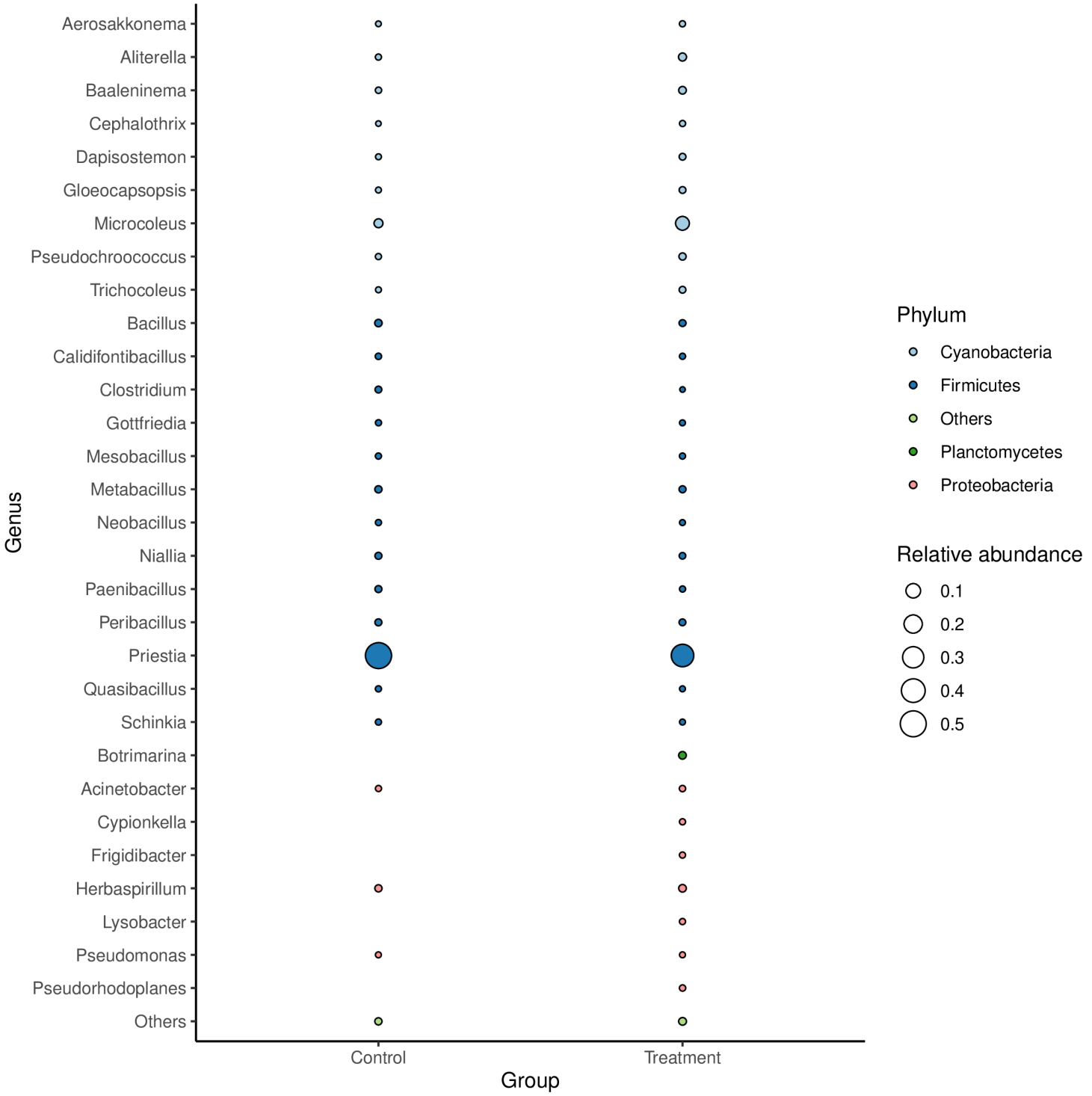

Figure 7

The taxonomic bubble plot of the bacteria in vanilla at the relative abundance at genus and phylum levels for the control and treatment groups. Circle sizes represent the relative abundance, and colors indicate the bacterial phylum groups.

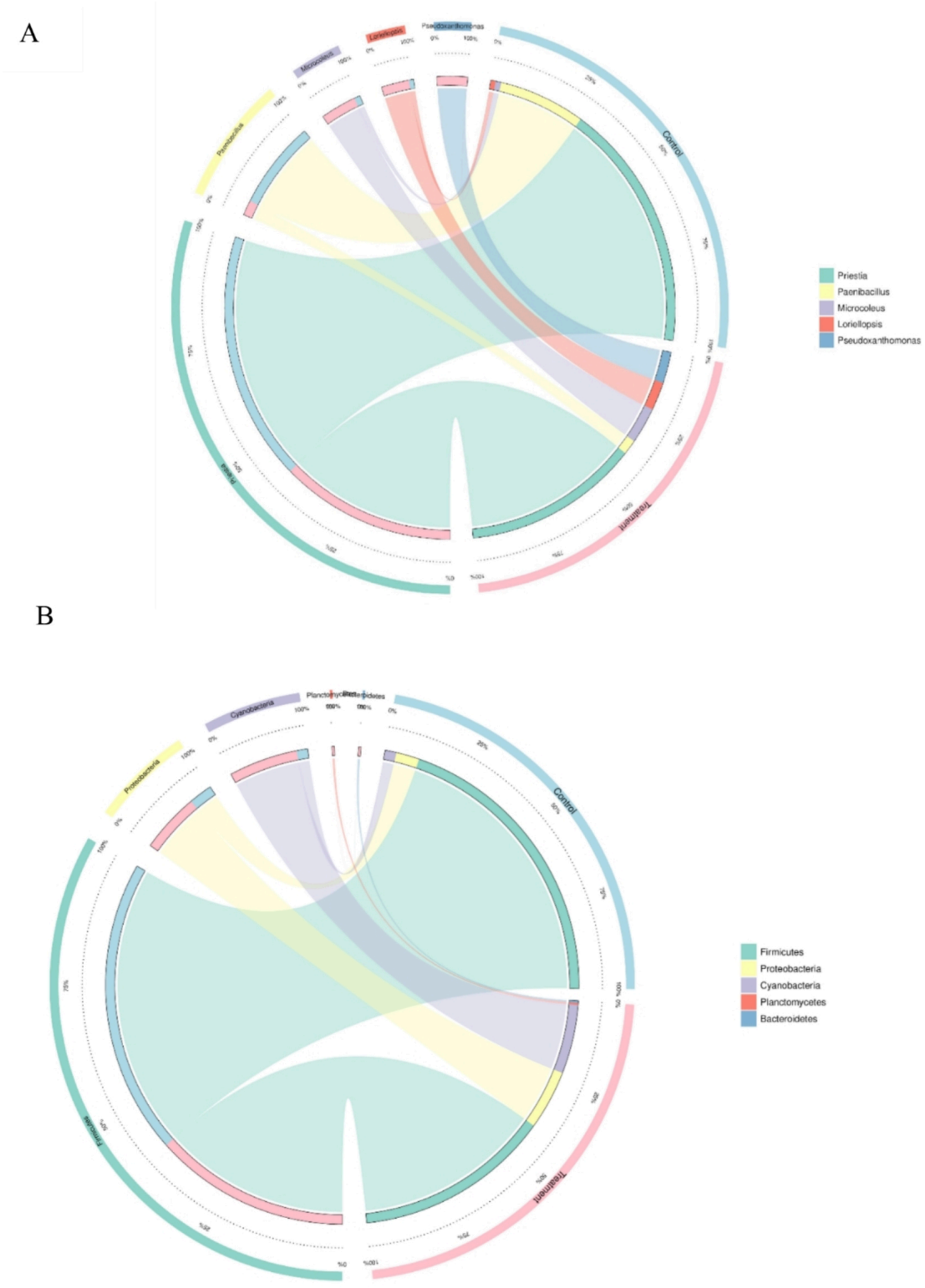

Figure 8

Circos representation of the most abundant bacterial genera between naturally fermented vanilla (control) and*B. velezensis* ZN-S10 fermented samples. (A) The circus plot of the relative abundance at the phylum level. (B) The circos plot at the genus level for the microbiota found in vanilla beans.

Supplementary Files

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