Diversity and the potency of indigenous bacteria in dengen fruit (Dillenia serrata), passion fruit (Passiflora edulis), and pineapple fruit (Ananas sp.) of South Sulawesi, Indonesia

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Abstract. Fruit plays an important role in plant conservation, public health, and welfare. The fruit is used by society as foodstuff, drinks, and condiments. The objectives of this study were to analyze the nutritional content in some fruits originated in South Sulawesi (dengen fruit, passion fruit, and pineapple fruit), to observe the metagenomic diversity and the correlation among nutritional content and alpha diversity, the potency of indigenous bacteria contains in the fruits. These fruits have historical and commercial value. Furthermore, dengen and pineapple are endemic fruits and almost extinct. Ripe fruit samples were obtained from public plantations in Luwu Raya, South Sulawesi Province, Indonesia. The indigenous bacteria in some fruits were observed by prepare each fruit juice. Each fruit juice was filtered using filter paper followed by nitrocellulose membranes pore of 0.45 and 0.20 µm respectively. Each fruit juice measured for its acidity degree using a pH meter and the nutritional contents using the titration methods. The chromosomal DNA of bacterial cells had extracted by FastDNA Spin Kit (MPBIO), and partial of 16S rDNA amplified with 341F-806R primers, and it analyzed by Illumina platform. The sequence of 16S rDNA was analyzed by MUSCLE v.3.8.31, QIIME v.1.7.0, R v.2.15.3, and SPSS v.20 software. The results showed that pineapple fruit has the highest sugar, reducing sugar, starch, and amylose content, while dengen fruit has the highest vitamin C content. The combination of sugars and vitamin C content may influence the dominant microbial genera. Degen fruit was dominated by Phylum Proteobacteria and it dominated by Genus of Acetobacter, Gluconobacter, and Komagataeibacter. Passion fruit and pineapple fruit were dominated by Phylum Firmicutes and Genus of Weissella. Genus of Acetobacter, Gluconobacter, and Komagataeibacter able to produce acetic acid, while Weissella is known as a lactic acid producer.

1. Introduction
Microbes are ubiquitous that can be found anywhere [1]. The presence of microbes in nature allows the flow of nutrients and energy, it has related to ecosystem stability [2]. These microbes can be
independent or associated with plants, animals, and humans [1]. Bacteria can be associated with plants in the rhizosphere, the surface (epiphytes), and in-plant tissues (endophytes) [3].

The presence of bacteria can benefit or detrimental to the plants as well as for animals and humans [1], [3], [4], [5], [6]. The increasing frequency of current outbreaks, thought to originate from the presence of enteric pathogenic bacteria in plants, is a challenge for microbiologists [3]. On the other hand, the interaction of bacteria with plants and their feasibility as biological control, providers of nutrients and growth regulators, production of plant and microbial metabolites, and the role of bacteria increasing tolerance in environmental stress are important in microbiological research [6], [7], [8]. The association among bacteria and fruit also allows the use of indigenous bacteria as a starter culture in the production of fermented food [9], [10], [11] and as a producer of active bacterial metabolites [12] in food, chemical, and pharmaceutical industries [11]. Therefore, studies on the association of bacteria with fruits is needed for technological development as an effort to preserve, improve the quality and productivity of fruit, related to food safety, commercial potency as a starter culture to produce fermented food and bioactive compounds. However, these challenges are difficult to answer because of a lack of knowledge about bacterial behavior in plants [2], [3], [7]. Furthermore, nutrient content and secondary metabolite of the fruit also affect the community structure of epiphytic and endophyte microbes.

The development of high-throughput sequencing (HTS) or next-generation sequencing (NGS) technology in molecular identification has become an alternative method for studying microbial communities directly from the environment without being cultured [13], [14], [15], [16], [17], [18]. This method allows a comprehensive description of the bacterial communities in the environment [4] and real-time [3]. Besides, this method becomes a solution for studying the un-culturable microbes [18] and microbes that live in extra-ordinary environments [17], [18].

This study examines the bacterial community using the NGS method and the potency of dominant bacteria in three origin fruits of South Sulawesi, namely dengen, passion fruit, and pineapple. These fruits are often used as groceries, drinks, and condiments. Dengan (*Dillenia serrata*) is one of Sulawesi’s endemic plants [19], produces sweetish-acid fruits, and it used for medicinal [20], [21]. Pineapple (*Ananas* sp.) was grown on big rocks without soil elements. This pineapple has a historical value for the people of South Sulawesi because it is the origin of the name of Wasuponda origin, where Wasu means stone and ponda means pineapple [22]. Both of these fruits are already rare and endangered. Unlike the dengen and pineapple fruits, passion fruit (*Passiflora edulis*) does not originate from Sulawesi. However, passion fruit has an important commercial value in South Sulawesi [8], [23] and passion fruit juice products are typical souvenirs of this province.

Research on microbial community structure using the NGS method for those fruits is limited in Brazilian passion fruit [24]. Whereas in South Sulawesi, it is still limited to rhizospheric Actinomycetes of passion fruit [8]. Therefore, the aims of this study are to examine: (a) the nutritional contents of each sample, diversity of bacteria, and nutritional correlation to bacterial alpha diversity; (b) bacterial community structure and the opportunities for utilization of indigenous bacteria in dengen fruit, passion fruit, and pineapple fruit for industrial development.

2. Methods

2.1. Sample preparation

The dengen fruit was obtained from a community plantation in Battang, Wara Barat Subdistrict, and passion fruit obtained from Binturu, Wara Selatan Subdistrict, Palopo City, while pineapple fruit was obtained from Wasuponda Subdistrict, East Luwu Regency, South Sulawesi, Indonesia. Ripe fruits were taken to the Microbiology Laboratory of Mathematics and Natural Sciences Faculty Brawijaya University by using sterile plastic bags for further analysis. Each fruit sample was weighed and surface sterilized using NaOCl solution for 5 minutes then it rinsed 3 times in sterile distilled water. Each fruit is mashed using a blender and it added sterile distilled water at a ratio of 1: 1 (w/v). The fruit juice was stored at 4°C.
2.2. Measuring of acidity degree and nutritional contents

The fruit juices were analyzed for the acidity degree using a pH meter. While the total sugar, reducing sugar, starch, amylose, and vitamin C content have been analyzed according to Sudarmaji et al. [25] at the Food Laboratory of the Faculty of Agricultural Technology, Brawijaya University. The data statistically analyzed using the SPSS v20 software.

2.3. Chromosomal DNA extraction

Fruit juice filtered using a vacuum pump and nitrocellulose filter paper, 0.45 and 0.20 μm, respectively. The 0.20 μm porous nitrocellulose membrane contains bacterial cells trapped on its surface. The 0.20 μm membrane was cut into small pieces to set in the Lysing Matrix E Tube and the bacterial chromosomal DNA was extracted based on the modified FastDNA Spin Kit (MPBIO) protocol. Modification of DNA extraction procedures in the FastDNA Spin Kit (MPBIO) protocol carried out by homogenized the suspension in the Lysing Matrix E Tube for 10 seconds, just after sodium phosphate buffer was added. Another modification extended the incubation time of suspension up to 10 minutes after mixed with the protein precipitate solution. The chromosomal DNA was stored at -20°C for further analysis.

2.4. Analysis of chromosomal DNA by the NGS method

Qualitative analysis of chromosomal DNA was observed using 1% agarose gel while concentration and purity of it were determined using NanoDrop Spectrophotometer. Chromosomal DNA was diluted with sterile water up to 1 ng/µL concentration. The PCR reaction was carried out with the Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and used specific primers 341f (CCTAYGGGRBGCASCAG) and 806r (GGACTACNNGGGTATCTAA) of the V3-V4 region of 16S rDNA. The quality of the 16S rDNA amplicon was analyzed using agarose gel 2%. The 400-450bp size of the band was purified by the Qiagen Gel Extraction Kit (Qiagen, Germany) and is analyzed by the Illumina Platform. All stages of Next Generating Sequencing (NGS) were carried out in Novogen, Singapore.

Paired-end reads separated from the barcode and primer sequence, then merged using FLASH v1.2.7 software. Chimera was removed to get effective tags. All effective tags were analyzed by Uparse v7.0.1001 software to obtain the Operational Taxonomic Unit (OTU). Species annotated with the Mothur program against the SSURNA database of Silva Database. Phylogenetic relationships of all OTUs obtained using the MUSCLE v3.8.31 software. Alpha and beta diversity analyzed using the QIIME v1.7.0 and R v2.15.3 software. The correlation among acidity degree, nutritional content, and alpha diversity had analyzed by Pearson correlation using SPSS v20.0.

Alpha diversity describes the diversity and richness of the bacterial community in each sample [13]. Alpha diversity is measured through 3 matrices were richness, observed species, and diversity [17]. The bacterial diversity was determined by the Shannon diversity index and Simpson diversity index, while richness was calculated by Chao1 richness index and ACE richness index. The Shannon diversity index calculates diversity based more on richness than evenness, and vice versa on the Simpson diversity index. The Chao1 richness index focuses on species with low abundance and rare species considered to represent missing species, while the ACE richness index requires the right frequency of rare species to estimate the number of species lost [26].

According to taxonomic annotation, abundance information, and phylogenetic relationship of OTUs, the top rank phyla and genera of each sample selected to form the distribution histogram of relative abundance, ternary plots, and heatmap in phylum and genus level. The distribution histogram of relative abundance had drawn according to the top 10 genera of each sample. The ternary plot was according to the top 10 phyla and genera of all samples, while the heatmap based on the top 35 genera of all samples. Specific species of top 10 genera in high relative abundance selected to make the taxonomy tree by R & D software.
3. Results and discussion

3.1. Acidity degree and nutritional contents of fruit juices

The acidity degree (pH) and nutrient content of the three fruits were presented in Table 1. Juice acidity (pH) of dengen and passion fruits was 3.9, while pineapple juice has a higher pH (4.5). Pineapple juice has the highest total sugar and reducing sugar 14.46 % and 9.07% respectively, while dengen juice has the lowest total sugar and reducing sugar 0.23 and 0.09% respectively (p<0.05). Pineapple juice also has the highest starch and amylose content 38.67 % and 9.43% respectively (p<0.05), while passion fruit juice has the lowest concentration of both substances were 0.10 % and 0.01% respectively. Dengan fruit has the highest (56.84 mg/100g) while passion fruit has the lowest vitamin C content among three fruits (p>0.05).

| Parameters       | Degen (D) | Passion Fruit (M) | Pineapple (N) |
|------------------|-----------|-------------------|---------------|
| pH               | 3.9       | 3.9               | 4.5           |
| Total sugar (%)  | 0.23\(^a\) | 2.38\(^b\)        | 14.46\(^c\)   |
| Reducing sugar (%)| 0.09\(^a\) | 2.30\(^b\)        | 9.07\(^c\)    |
| Starch (%)       | 0.19\(^a\) | 0.10\(^a\)        | 38.67\(^b\)   |
| Amylose (%)      | 0.01\(^a\) | >0.01\(^a\)       | 9.43\(^b\)    |
| Vitamin C (mg/100 g) | 56.84\(^c\) | 6.59\(^a\)        | 29.33\(^b\)   |

The letters that following numbers indicate Tukey’s difference test at the significance level \(\alpha = 0.05\)

Correlation test between pH and nutrient content showed that pH significantly correlated with starch and amylose content (Table 3). The starch and amylose content will increase with increasing pH and vice versa. The positive correlation significant is also exists between starch and amylose content. Amylose is one of the polymer components in starch [27], [28], [29], so that if the amylose content increases, the starch content will also increase.

3.2. Alpha diversity of bacterial communities of the fruits

Based NGS analysis showed that dengen hast's most total tags were 127,079 tags while the lowest 122,945 tags in passion fruit. Dengan fruit has most taxon tags (125,390 tags) while passion fruit has the lowest 118,450 tags. Passion fruit has the highest unique tags (4,442 tags) while dengen fruit has lowest was 1,671 unique tags (Table 2). Passion fruit, pineapple, and dengen fruit have OTUs number were 444 OTUs, 233 OTUs, and 99 OTUs respectively.

The correlation between tags and the number of OTUs (Table 3) showed that the total tags, taxon tags, and unique tags were significantly positive correlation. This positive correlation due to all effective tags (total tags) is annotated and grouped into annotated tags (taxon tags), unique tags (only appear once and in only in one sample), and un-notated tags (unclassified tags). Total sugars, reducing sugars, starches, and amylose content had a positive correlation but not significant (p>0.05) with total tags and taxon tags of the fruits. Vitamin C content also has a positive correlation but not significant (p>0.05) with total tags and taxon tags but it has a negative correlation with unique tags and OTUs numbers. Sugar and vitamins are nutrients needed by bacteria, but the needs vary for each species [30]. Carbohydrates are generally needed by almost all bacteria as a carbon source [31], while vitamin C can stimulate [32], [33], inhibit, [34], [35] or no effect [36] to the growth of certain bacteria. The amount of sugar, starches, and amylose in sufficient quantities allows the growth of various types of bacteria and allows many unique species to grow.
Table 2. Tags, OTUs number, bacterial diversity, and bacterial richness.

| Parameters          | Fruit                   |
|---------------------|-------------------------|
|                     | Degen (D) | Passion Fruit (M) | Pineapple (N) |
| Total Tags          | 127,079   | 122,945            | 126,788       |
| Taxon Tags          | 125,390   | 118,450            | 125,013       |
| Unique Tags         | 1,671     | 4,442              | 1,775         |
| OTUs Number         | 99        | 444                | 233           |
| Shannon diversity index | 2.370    | 1.675              | 1.039         |
| Simpson diversity index | 0.759    | 0.326              | 0.249         |
| Chao1 richness index | 97.615    | 447.000            | 234.814       |
| ACE richness index  | 112.227   | 452.159            | 237.772       |

Total tags are the number of effective tags. Taxon tags are annotated tags. Unique tags are the tags that are only found once and in one sample. OTU numbers are the number of tags that have been grouped based on the ≥97% similarity of sequences and shows the number of genera or species.

Table 2 shows that dengen fruit has the highest diversity of bacterial communities, followed by passion fruit, and the lowest in pineapple fruit. The diversity of bacterial communities was not a significant correlation with pH and nutrient content of the fruits (Table 3). However, the pH, total sugar, reducing sugar, starch, and amylose contents had a negative correlation with the diversity index, while vitamin C has a positive correlation with the diversity index. A positive correlation between diversity index and vitamin C was also reported by Kato et al. [33] and suggested that vitamin C can stimulate the growth of certain bacteria. Table 3 also shows that diversity was not a significant correlation with total tags, taxon tags, unique tags, but the Simpson diversity index has a non-significant negative correlation with OTUs numbers. This is due to the Simpson diversity index also indicated the dominance of certain species in a community [26].

The passion fruit has the highest bacterial richness while dengen has the lowest bacterial richness (Table 2 and Figure 1). The bacterial richness index does not correlate significantly with pH and nutrient content of the fruits (Table 3). Nevertheless, vitamin C content has a negative correlation with bacterial richness index. It means that if the vitamin C content in the fruits increases, the richness index will decrease. Vitamin C’s effect on bacterial growth is different for each species. Vitamin C is mostly known as an antibacterial that arrests bacterial growth [34], [35], but some need vitamin C to grow [32], [33]. Thus, only certain species can survive in fruit with high vitamin C. Therefore, species richness may decrease due to high vitamin C content. Table 3 also shows that the richness index has a significant positive correlation with OTUs numbers. The OTU grouping based on sequences that have ≥97% similarity and indicated as a species. This means that if the number of species increases, the richness index will increase.
Figure 1. Rarefaction curve of fruits.

The rarefaction curve (Figure 1) is a curve made based on the number of random sequences and the number of OTUs that represent it. The rarefaction curve indirectly depicts the richness of microbial community in the samples [26]. The flat part of the curve illustrates that the number of randomly drawn sequences can already represent the microbial community and only rare species remain. Figure 1 shows that the number of 80,000 sequences taken at random can represent microbial communities in all fruit samples.

Table 3. Pearson Correlation coefficients among pH, nutritional contents, tags, OTUs number, richness, and diversity of bacteria.

| Parameters code | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12  | 13  | 14  |
|-----------------|----|----|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| 1 pH            | 0.990 | 0.972 | 1.000** | 1.000** | -0.055 | 0.444 | 0.458 | -0.471 | -0.128 | -0.853 | -0.616 | -0.123 | -0.149 |
| 2 Total sugar   | 0.995 | 0.990 | 0.990 | -0.194 | 0.314 | 0.328 | -0.343 | 0.012 | -0.918 | -0.721 | 0.017 | -0.009 |
| 3 Reducing sugar| 0.971 | 0.971 | -0.289 | 0.220 | 0.235 | -0.249 | 0.110 | -0.952 | -0.785 | 0.115 | 0.089 |
| 4 Starch        | 1.000** | -0.053 | 0.446 | 0.459 | -0.473 | -0.130 | -0.852 | -0.615 | -0.125 | -0.151 |
| 5 Amylose       | -0.054 | 0.445 | 0.458 | -0.472 | -0.129 | -0.852 | -0.616 | -0.124 | -0.150 |
| 6 Vitamin C     | 0.870 | 0.863 | -0.855 | -0.983 | 0.568 | 0.820 | -0.984 | -0.979 |
| 7 Taxon tags    | 1.000** | -1.000* | -0.945 | -0.089 | 0.432 | -0.944 | -0.952 |
| 8 Unique tags   | -1.000** | -0.940 | 0.074 | 0.418 | -0.939 | -0.947 |
| 9 OTUs number   | 0.935 | 0.959 | -0.404 | 0.933 | -0.943 |
| 10 Shannon      | -0.409 | -0.702 | 1.000** | 1.000* |
| 11 Simpson      | 0.937 | -0.413 | -0.389 |
| 12 Chao1        | -0.706 | -0.687 |
| 13 ACE          | 1.000* |

Parameters code: 1 = pH, 2 = total sugar, 3 = reducing sugar, 4 = starch, 5 = amylase, 6 = vitamin C, 7 = total tags, 8 = taxon tags, 9 = unique tags, 10 = OTUs number, 11 = Shannon diversity index, 12 = Simpson diversity index, 13 = Chao1 richness index, 14 = ACE diversity index.

* Indicates correlate significance at α = 0.05.
** Indicates the significance at α = 0.01.

The numbers indicate the R-value (the strength value of the correlation between variables, 0 is represented by no correlation/neutral, and 1 is represented as a very strong correlation). The coefficient (+) and (-) indicates the direction of correlation between variables, (+) indicate positive, and (-) indicate negative.
3.3. Beta diversity of bacterial communities of the fruits
Beta diversity is a community analysis comparing the difference between microbial communities among samples [13]. Figure 2a shows that dengen and pineapple fruit has a beta diversity coefficient of 0.764 while passion fruit and dengen fruit has a beta diversity coefficient of 0.750 of bacterial communities. Passion fruit and pineapple fruit has the lowest 0.051 coefficients of bacterial community difference.

![Figure 2a](image1)
![Figure 2b](image2)
![Figure 2c](image3)

**Figure 2.** Beta diversity analysis of samples based on weight unifrac distance: (a) beta diversity heatmap, (b) PCoA analysis, (c) UPGMA cluster tree.
Sample code of D means dengen fruit, M means passion fruit, and N means pineapple fruit. Each grid of beta diversity heatmap represents the dissimilarity coefficient between pairwise fruit samples. The percentage of each axis on PCoA Graph indicates the contribution value to discrepancy among samples. UPGMA Tree plotted in the left and relative abundance in phylum level in the right. Samples with the closest distance are grouped in a node. The length of the line between the sample/branching point to the next branching point is the average distance between two samples/nodes. The line color on the UPGMA tree represents the line of each sample. The color on the relative abundance graph represents each phylum.
The relationship of the bacterial community among three fruit was visualized by Principal Coordinates Analysis (PCoA) based on weighted unifrac distance (Figure 2b). It was shown that the bacterial community of passion fruit and pineapple fruit clustered in the left of the graph along PC1 (99.68% of the total variations) and separated from dengen fruit. However, those three fruits separated from each other based on PC2, which accounted for 0.32% of explained variations. The UPGMA cluster tree based on weighted unifrac distance (Figure 2c) showed that passion fruit and pineapple fruit have a short distance (0.03) and cluster to form a node. Dengen fruit has a long-distance 0.35 with passion fruit-pineapple fruit’s node. The visualization of PCoA and UPGMA cluster trees indicates that passion fruit and pineapple fruit have a similar bacterial community due to both fruits dominated by Phylum Firmicutes. Dengen fruit was different from other fruits, it was dominated by Phylum Proteobacteria.

3.4. Bacterial community structure of the fruits
The Venn diagram of the three fruit samples (Figure 3) showed that there was 165 common OTUs consist of 31 OTUs found in dengen fruit and passion fruit, 96 OTUs in passion fruit and pineapple fruit, 6 OTUs in dengen fruit and pineapple fruit, and 32 OTUs found in all fruits. The most specific OTUs found in passion fruit (285 OTUs). Pineapple fruit had 90 specific OTUs and only 18 specific OTUs found in dengen fruit.

![Venn diagram of fruits](image)

**Figure 3.** Venn diagram of fruits.
Each circle represents one sample/group. The value represents the OTUs number (number of species), values in overlapping parts represent common OTUs and the others represent specific OTUs in each fruit sample. The color represents the sample and overlapping parts among fruit samples.

Figure 4a showed that dengen fruit dominated by Proteobacteria (100%), whereas passion fruit and pineapple fruit dominated by Firmicutes (95% and 98%, respectively) and Proteobacteria (3% and 1.5%, respectively). The dominance of the two phyla was also illustrated by ternary plot (Figure 4b), where the abundance of Phylum Proteobacteria in dengen fruit, passion fruit, and pineapple fruit were 95.74%, 2.77%, and 1.48% respectively. While the abundance of Phylum Firmicutes about 0.20%, 47.77%, and 52.02%, respectively. The difference in the dominant bacteria among fruits thought to be
influenced by the nutrient content in it. Table 1 shows that the carbohydrate content in dengen is the lowest but highest in vitamin C.

At the genus level (Figure 5a), dengen fruit was mainly composed of Genus *Acetobacter* (46%), *Gluconobacter* (31%), and *Komagataeibacter* (18%). Passion fruit mainly composed of Genus *Weissella* (82%), *Lactobacillus* (6%), *Pediococcus* (3%), *Romboutsia* (1%), *Acinetobacter* (1%), and *Escherichia-Shigella* (1%). Pineapple fruit composes of Genus *Weissella* (86%), *Lactobacillus* (6%), *Pediococcus* (3%), *Acinetobacter* (1%), and *Leuconostoc* (1%). Top three genera that dominated in dengen, passion fruit, and pineapple fruit were (shown by the ordinate circle in the ternary plot, Figure 5b), were *Weissella* (0.05%, 48.64%, 51.32%), *Acetobacter* (99.48%, 0.52%, 0.00%), and *Komagataeibacter* (99.36%, 0.64%, 0%). The cluster of the top 35 genera based on the abundance in fruit samples, showed that 21 genera members of Phylum Firmicutes, 9 genera of Phylum Proteobacteria, 3 genera of Phylum Bacteriodetes, and 1 genus of Phylum Spirochaetes (Figure 5c.).

The top 10 dominant genera in all samples consist of Genus *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella*, *Romboutsia*, *Acetobacter*, *Gluconobacter*, *Komagataeibacter*, *Escherichia-Shigella*, and *Acinetobacter* (Figure 6). The Genus *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella*, *Romboutsia*, *Escherichia-Shigella*, and *Acinetobacter* found in passion fruit and pineapple fruit. Genus *Romboutsia* was just found in passion fruit, while Genus *Acetobacter*, *Gluconobacter*, and *Komagataeibacter* were found abundantly in dengen fruit. Genus *Lactobacillus* and *Acinetobacter* consist of 8 species and 4 species respectively, while each Genus of *Pediococcus*, *Leuconostoc*, *Weissella*, *Acetobacter*, *Gluconobacter*, *Komagataeibacter*, and *Escherichia-Shigella* has 1 species. In this study, the bacterial community in passion fruit was different from the results of Cruz et al. [24] who reported that the passion fruit peel from wild areas in Brazil dominated by *Streptomyces mashuensis* as an antibacterial producer. The difference of those results due to the characteristics of the sample, environmental factors, the nature of the microorganism itself, and pesticide application [4].

![Image](72x371 to 301x578)

**Figure 4.** Microbial Community structure in phylum level of fruits: (a) abundance relative of phyla; (b) ternary plot in phylum level.

“Others” in abundance relative of phylum represents a total relative abundance of the rest phyla besides the top 10 phyla. Three vertexes represent three fruits sample or group. Each circle represents the dominant phylum and the size of circles is proportional to the relative abundance. The ordination of each cycle shows a dominance percentage of a certain phylum in each fruit sample.
Figure 5. Microbial Community structure in genus level: (a) relative abundance of genera; (b) ternary plots of genera; (c) taxonomic abundance cluster genera heatmap.

"Others" in abundance relative of genera represents a total relative abundance of the rest genera besides the top 10 genera. Three vertexes represent three fruit samples or group, each circle represent dominant genus and the size of circles are proportional to the genus relative abundance, and the ordinate of each cycle proportional a dominance percentage of the certain genus in each fruit sample. The tree proportional to the taxonomic cluster, the X-axis represents fruit sample name, the Y-axis represents the genus, the value of ‘z’ represents the distance between the raw score and the mean of standard deviation, (-) means the raw score is below the mean and (+) means the raw score is above the mean.

Bacterial species that identified members of top 10 genera in three fruits consist of E. coli [4], Lb. brevis, Lb. fermentum, Lb. pentosus, P. pentosaceus, W. confusa [30], [37], L. citreum [38], were found in plants. Besides those species, Genus Weissella, Leuconostoc [4], Acinetobacter [39], Acetobacter, Gluconobacter, Komagataeibacter [40], Romboutsia [41] also found in plants. Romboutsia sp., Lb. murinus, Lb. intestinalis, Lb. johnsonii, Lb. reuteri, and Lb. Salivarius were specific bacterium species in passion fruit. Lactobacillus can grow in all habitats that provide sufficient carbohydrates [30]. Likewise, the acetic acid bacteria such as Acetobacter, Gluconobacter, and Komagataeibacter have generally inhabited a sugar-rich niche [40]. Table 1 showed that
pineapple has the highest while dengen fruit has the lowest sugar content. Table 1 also shows that dengen fruit contains the highest vitamin C. Vitamin C is reported as a trigger of acetic acid bacterial growth [32]. Also, *Gluconobacter* is reported as a vitamin C producer [40], [42].

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**Figure 6.** Taxonomic tree of TOP ten genus.

The column represents different taxonomic ranks. The size of the slice area represents the relative abundance of species.

The species in the top 10 genera are not yet known for their potency in plants. However, some species were known as a pathogen for animals and humans, such as *E. coli* [4], *Weissella confusa* [43], *Acinetobacter schindleri* and *Acinetobacter ursingii* [44]. The presence of enteric bacterial in plants generally occurs due to the use of animal manure in agricultural practices [3]. The species *E. coli* and *Acinetobacter ursingii* are found in passion fruit and pineapple fruit (Figure 6) that grow in residential areas.

Some bacteria of the fruits could use in the food and health industries. Lactic acid bacteria such as *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* were reported used as probiotics, lactic acid-producing, and antimicrobial [30], [45], [46], [47], [48], [49], [50]. *Acetobacter aceti*, *Gluconobacter frateurii*, and *Komagataeibacter saccharivorans* were known as acetic acid-producing bacteria. *Gluconobacter frateurii* was reported as a producer of vitamin C and *Komagataeibacter saccharivorans* as a producer of bacterial cellulose [40], [42], [51]. *Romboutsia* was tested as a fermenting bacterium that adapted in the human intestine [52], [53], while a strain of *Acinetobacter johnsonii* was reported in heavy metal remediation [54].
4. Conclusion

Among the three fruits, pineapple fruit has the highest carbohydrate content, while dengen fruit has the highest vitamin C content. The passion fruit has the highest while dengen fruit has the lowest number of bacterial OTU but it has highest bacterial diversity index and pineapple fruit. Among the nutrition contents and alpha diversity correlation, sugars and vitamin C content have an un-significantly effect to the diversity index. The dengen fruit was inhabited 100% by Phylum Proteobacteria dominantly consist of Genus Acetobacter, Gluconobacter, and Komagataeibacter, while passion fruit and pineapple dominantly composed of Phylum Firmicutes (95% and 98%) and Genus Weisella (82% and 86%) which known as probiotics. Species Romboutsia sp., Lb. murinus, Lb. intestinalis, Lb. johnsonii, Lb. reuteri, and Lb. Salivarius were specific bacteria of passion fruit. The top 8 genera are known as organic acid-producing bacteria, such as acetic acid and lactic acid. The potential of each dominant bacterium requires further testing.

References
[1] Stark L A 2010 Beneficial microorganisms: countering microbephobia Life Sciences Education, 9 387-389.
[2] Panizzon J P, Junior H L P, Knaak N, Ramos R C, Ziegler D R, and Fiuza L M 2015 Microbial diversity: relevance and relationship between environmental conservation and human health Brazilian Archives of Biology and Technology 58 1 137-145.
[3] Panigrahy A, Babu S, Vivekanandhan G, Subashkumar R, and Thayumanavan T 2011 Development of a metagenomic DNA extraction procedure and PCR detection of human enteric bacteria in vegetable salad tissues Research in Biotechnology 2 1 11-19.
[4] Telias A, White J R, Pahl D M, Ottesen A R, and Walsh C S 2011 Bacterial community diversity and variation in spray water sources and the tomato fruit surface BMC Microbiology 11 81 1-13.
[5] Ottesen A R, Pena A G, White J R, Pettengill J B, Li C, Allard S, Rideout S, Allard M, Hill T, Evans P, Strain E, Musser S, Knight R, and Brown E 2013 Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato) BMC Microbiology 13 144 1-12.
[6] Kafle A, Garcia K, Petta V, Yakha J, Soupir A, and Bücking H 2018 Beneficial plant microbe interactions and their effect on nutrient uptake, yield, and stress resistance of soybeans: soybean-biomass, yield and productivity. Minobu Kasai, IntechOpen, DOI: 10.5772/intechopen.81796. Available from: https://www.intechopen.com/books/soybean-biomass-yield-and-productivity/beneficial-plant-microbe-interactions-and-their-effect-on-nutrient-uptake-yield-and-stress-resistance.
[7] Berg G, Koberl M, Rybakova D, Muller H, Grosch R, and Smalla K 2017 Plant microbial diversity is suggested as the key to future biocontrol and health trends FEMS Microbiology Ecology 93 5 1-9.
[8] Ali A, Junda M, Rante H, and Nuramelia R 2018 Characterization of Actinomycetes antagonist Fusarium oxysporum f.sp.passiflora isolated from rhizosphere soil of purple passion fruit plants, South Sulawesi, Indonesia IOP Conf. Series: Journal of Physics: Conf. Series 1028 1-8.
[9] Bonatsou S, Tassou C C, Panagou E Z, and Nychas G E 2017 Table olive fermenting using starter cultures with multifunctional potential Microorganisms 5 2 30 1-16.
[10] Kavitake D, Kandasamy S, Devi P B, and Shetty P H 2018 Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – a review Food Bioscience 21 34-44.
[11] Lynch K M, Zannini E, Wilkinson S, Daenen L, and Arendt E K 2019 Physiology of acetic acid bacteria and their role in vinegar and fermented beverages Comprehensive Reviews in Food Science and Food Safety 18 588-625.
[12] Sriram K P, Mangrolia U, and Osborne W J 2020 Isolation and characterization of cultivable indigenous endophytic bacteria in the tender coconut Food Biotechnology 34 3 228-242.
[13] Lazupone C A, Hamady M, Kelley S T, and Knight R 2007 Quantitative and qualitative β
diversity measures lead to different insights into factors that structure microbial communities. *Applied and Environmental Microbiology* 73 5 1576-1585.

[14] Gilbert J A, O’Dor R, King N, and Vogel T M 2011 The importance of metagenomic surveys to microbial ecology: or why Darwin would have been a metagenomic scientist *Microbial Informatics and Experimentation* 1 5 1-3.

[15] Hjort K, Presti I, Elvang A, Marinelli F, and Sjoling S 2014 Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. *Appl Microbiology Biotechnology* 98 2819-2828.

[16] Sebastien M, Margarita M, and Haissam J M 2015 Biological control in the microbiome era: challenges and opportunities. *Biological Control* 89 98–108.

[17] Zhang J, Sun Q, Zeng Z, Chen S, and Sun L 2015 Microbial diversity in the deep-sea sediments of Iheya North and Iheya Ridge, Okinawa Trough *Microbiological Research* 177 43-52.

[18] Saminathan T, Garcia M, Ghimire B, Lopez C, Bodunrin A, Nimmakayala P, Abburi V L, Levi A, Balagurusamy N, and Reddy U K 2018 Diverse watermelon cultivars reveal the role of fruit associated microbiome in carbohydrate metabolism and ripening of mature fruits. *Frontier in Plant Science* 9 4 1-13.

[19] Lim T K 2012 *Edible medicinal and non-medicinal plants volume 2, fruits* London, New York Springer.

[20] Jalil J, Sabandar C W, Ahmat N, Jamal J A, Jantan I, Aladdin N, Muhammad K, Buang F, Mohamad H F, and Sahidin I 2015 Inhibitory effect of triterpenoids from *Dillenia serrata* (Dilleniaceae) on prostaglandin E2 production and quantitative HPLC analysis of its koetjapic acid and betulinic acid contents *Molecules* 20 3206-3220.

[21] Sabandar C W, Jalil J, Ahmat N, and Aladdin N 2017 Medicinal uses, chemistry and pharmacology of *Dillenia* species (Dilleniaceae) *Phytochemistry* 134 6-25.

[22] Pongsibanne L K, Naping H, Hamdat S, and Ariffin A 2018 Social cultural transformation in attitude and behaviour of Padoe community (a case study of Padoe community in mining area of PT. Vale, Tbk. In Wasuponda, Luwu District, South Sulawesi Province) *International Journal of Sociology and Anthropology Research* 4 5 1-19.

[23] Nadja R A, Langkong J, Amullah A, Arsyad M, Jamil M H, Viantika N M, Tenriawaru A N, Rahmadanih, Akhsan, Sulili A, Nurlaela, and Ginting N M 2019 Development strategy of passion fruit agro-industry: evidence from South Sulawesi, Indonesia *IOP Conf. Series: Earth and Environmental Science* 343 1-9.

[24] Cruz A F, Barka G D, Blum L E B, Tanaka T, Ono N, Kanaya S, and Reineke A 2019 Evaluation of microbial communities in peels of Brazilian tropical fruits by amplicon sequence analysis *Brazilian Journal of Microbiology* 50 3 739-748.

[25] Sudarmaji S, Suhardi, and Haryono B 1984 *Prosedur analisa untuk bahan makanan dan pertanian* Yogyakarta Liberty.

[26] Kim B, Shin J, Guevarra R B, Lee J H, Kim D W, Seol K, Lee J, Kim H B, and Isaacson R E 2017 Deciphering diversity indices for a better understanding of microbial communities *Journal Microbiology Biotechnology* 27 12 2089-2093.

[27] Chung H, Liu Q, Lee L, and Wei D 2011 Relationship between the structure, physicochemical properties and *in vitro* digestibility of rice starches with the different amylose contents *Food Hydrocolloids* 25 968-975.

[28] Syahariza Z A, Sar S, Hasjimi J, Tizzotti M J, and Gilbert R G 2013 The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains *Food Chemistry* 136 742-749.

[29] Cai J, Man J, Huang J, Liu Q, and Wei W 2015 Relationship between structure and functional properties of normal rice starches with different amylose contents. *Carbohydrate Polymers* 125 35-44.

[30] De Vos P, Garrity G M, Jones D, Krieg N R, Ludwig W, Rainey F A, Schleifer K, and Whitman W B 2010 *Bergey’s Manual of Systematic Bacteriology Second Ed. Volume Three USA* Springer.

[31] Aidelberg, G, Towbin B D, Rothschild D, Dekel E, Bren A, and Alon U 2014 Hierarchy of
non-glucose sugars in *Escherichia coli* BMC Systems Biology 8 133 1-12.

[32] Keshk S M A S 2014 Vitamin C enhances bacterial cellulose production in *Glucanacetobacter xylinus* Carbohydrate Polymers 99 98-100.

[33] Kato I, Vasquez A, Moyerbrailean G, Land S, Djuric Z, Sun J, Lin H, and Ram J L 2016 Nutritional correlates of human oral microbiome *Journal of the American College of Nutrition* 0 0 1-11.

[34] Taneja N K, Dhintra S, Mittal A, Naresh M, and Tyagi J S 2010 *Mycobacterium tuberculosis* transcriptional adaptation, growth arrest and dormancy phenotype development is triggered by vitamin C *Plos One* 5 5 e10860.

[35] Kallio J, Jaakkola M, Maki M, Kilpelainen P, and Virtanen V Vitamin C inhibits *Staphylococcus aureus* growth and enhances the inhibitory effect of quercetin on growth of *Escherichia coli* in vitro *Planta Med* 78 1824-1830.

[36] Pandit S, Ravikumar V, Abdel-Haleem A M, Derouiche A, Mokkapati V R S S, Sihlbom C, Mineta K, Gojobori T, Gao X, Westerlund F, and Mijakovic I 2017 Low concentrations of vitamin C reduce the synthesis of extracellular polymers and destabilizes bacterial biofilms *Frontiers in Microbiology* 2 5299.

[37] Filannino P, Cagno R D, and Gobbetti M 2018 Metabolic and functional paths of lactic acid bacteria in plant foods: get out of the labyrinth *Current Opinion in Biotechnology* 49 64-72.

[38] Laguerre S, Amari M, Vuillemin M, Robert H, Loux V, Klopp C, Morel S, Gabriel B, Remaud-Simeon M, Gabriel V, Moulis C, and Fontagne-Faucier C 2012 Genome sequences of three *Leuconostoc citreum* strains, LBAE C10, LBAE C11, and LBAE E16, isolated from wheat sourdoughs. *Journal of Bacteriology* 194 6 1610-1611.

[39] Romero F M, Marina M, and Pieckenstain F L 2014 The communities of tomato (*Solanum lycopersicum* L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing *FEMS Microbiology Letter* 351 187-194.

[40] Matsushita K, Toyama H, Tonouchi N, Okamoto A, and Kainuma 2016 Acetic acid bacteria: ecology and physiology *Japanese Springer*.

[41] Bao L, Cai W, Zhang X, Liu J, Chen H, Wei Y, Jia X, and Bai Z 2019 Distinct microbial community of phyllosphere associated with five tropical plants on Yongxing Island, South China Sea. *Microorganisms* 7 11 525.

[42] Brenner D J, Krieg N R, and Staley J T 2010 *Bergey’s Manual of Systematic Bacteriology* 2nd Ed. Volume Two Part C USA Springer.

[43] Lee M R, Huang Y T, Liao C H, Lai C C, Lee P I, and Hsueh P R 2011 Bacteremia caused by *Weissella confusa* at a university hospital in Taiwan, 1997-2007 *Clinical Microbiology and Infection* 17 8 1226-1231.

[44] Dortet L, Legrand P, Soussy C, and Cattoir V 2006 Bacterial identification, clinical significance and antimicrobial susceptibility of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *Journal of Clinical Microbiology* 44 12 4471-4478.

[45] Jongenurakkun B, Wang Q, Xu S H, Tada Y, Minamida K, Yasokawa D, Sugi M, Hara H, and Asano K 2008 *Pediococcus pentosaceus* NB-17 for probiotic use *Journal of Bioscience and Bioengineering* 106 1 69-73.

[46] Serna L C, Valencia L J H, and Campos R G 2011 Lactic acid bacteria with antimicrobial activity against pathogenic agent causing of bovine mastitis *Biotechnologia en el Sector Agropecuario y Agroindustrial* 9 1 97-104.

[47] Vidhyasagar V and Jeevaratnam K 2013 Evaluation of *Pediococcus pentosaceus* strains isolated from Idly batter for probiotic properties in vitro *Journal of Functional Foods* 5 235-243.

[48] Pujato S A, Quiberoni A L, Candidoti M C, Reinheimer J A, and Guglielmotti D M 2014 *Leuconostoc citreum* MB1 as biocontrol agent of *Listeria monocytogenes* in milk *Journal of Dairy Research* 81 137-145.

[49] Fusco V, Quero G M, Cho G, Kabisch J, Meske D, Neve H, Bockelmann W, and Franz C M A 2015 The Genus *Weissella*: taxonomic, ecology and biotechnological potential *Frontiers in Microbiology* 6 155 1-22.
[50] Ilavenil S, Vijayakumar M, Kimb D H, Arasu M V, Park H S, Ravikumar S, and Choi K C 2015 Assessment of probiotic, antifungal, cholesterol lowering properties of Pediococcus pentosaceus-KCC-23 isolated from Italian ryegrass Journal of the Science of Food and Agriculture 96 2 593-601.

[51] Abdelhady H M, Hassan E A, El-Salam S S A, and Abdullah S M 2015 Bacterial cellulose production as affected by bacterial strains and some fermentation conditions Nature and Science 13 3 30-40.

[52] Gerritsen J, Umanets A, Staneva I, Hornung B, Ritari J, Paulin L, Rijkers G T, de Vos W M, and Smidt H 2018 Romboutsia hominis sp. Nov., the first human gut-derived representative of the Genus Romboutsia, isolated from Ileostoma effluent International Journal of Systematic and Evolutionary Microbiology 68 3 3479-3486.

[53] Gerritsen J, Hornung B, Renckens B, van Hijum S A F T, dos Santos V A P M, Rijkers G T, Schaap P J, de Vos W M, and Smidt H 2017 Genomic and functional analysis of Roboutsia ilealis CRIB1 reveals adaptation to the small intestine PeerJ 1-28.

[54] Boswell D C, Hewitt C J, and Macaskie L E 1998 An application of bacterial flow cytometry: evaluation of the toxic effect of four heavy metals on Acinetobacter sp. With potential for bioremediation of contaminated wastewaters Biotechnology Letters 20 9 857-863.