Genotypic and Phenotypic Characterization of Lactic Acid Bacteria Associated with Silage Fermentation of Pineapple Residue

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Abstract: The natural populations of lactic acid bacteria (LAB) and silage quality of pineapple residue silage were investigated in this study. A total of 34 LAB strains originally isolated from pineapple residue silage were characterized and identified by phenotypic and genotypic methods. These LAB strains were Gram-positive and catalase-negative bacteria, which were divided into four groups: Lactiplantibacillus plantarum (52.9%), Leivilactobacillus brevis (14.7%), Lacticaseibacillus paracasei (17.6%) and Leuconostoc citreum (14.7%). Lactiplantibacillus plantarum was the dominant species. Homofermentative strains accounted for 70.5%. After 30 days of ensiling, the pineapple residue silage could be well preserved with low pH value (3.65) and high content of lactic acid (75.57 g/kg of DM). In this study, LAB populations of pineapple residue silage fermentation were investigated, which indicated that pineapple residue silage was a potential good animal feed source. In addition, this result will be valuable for screening-appropriate inoculants aimed at improving the quality.

Keywords: fermentation characteristics; lactic acid bacteria; pineapple residue silage; 16S rRNA

1. Introduction

Pineapple (Ananas comosus L.) residues mainly refer to the skin and residual pulp of pineapple after being made into juice, jam, canned fruit, preserved fruit or wine, and usually contain abundant minerals and fiber, accounting for about 50% to 60% of the total amount of fresh fruit [1]. However, most of them are deposed onto fields or used as compost, which is a waste of resources and their spoilage causes unpleasant odors and attracts insects, rodents, and other vermin. In recent years, these residues have received great attention, with the aim to develop value-added alternatives.

Pineapple residues can partly or completely replace the roughage portion in the diet and partly replace the cereals in the diet of livestock [2]. One of the challenges in utilizing these residues is their stabilization during storage, because they have a high level of moisture (80–95%) making them difficult to store, as they can easily perish. Currently, dry storage of fresh pineapple residue is the primary method to prevent decomposition. However, dry storage has several inherent disadvantages including costs associated with drying and climate and regional limitations [3].

Ensiling is a common method to preserve moist forages for livestock. It depends upon the production of sufficient organic acid to inhibit the activity of undesirable microorganisms under anaerobic conditions [4–8]. During the fermentation process, lactic acid bacteria (LAB) utilize water-soluble carbohydrate (WSC) to produce lactic acid, the primary acid responsible for decreasing the pH in silage. It is well known that LAB play a major role in the natural ensiling process, so it is necessary to investigate the diversity of LAB in silage. Pang et al. [8,9] found that most of the characterized LAB in forage silages belonged to the
genus *Weissella*. However, to the best of our knowledge, there is little research regarding the LAB composition associated with pineapple residue silage. Yang et al. found that strains of LAB isolated from fruit residues such as banana leaf and stem, pineapple peel and papaya peel belonged to the genera *Lactiplantibacillus*, *Lactococcus*, *Weissella* and *Leuconostoc* [2]. Other studies on pineapple residue silage had showed that pineapple residue silage had the best nutritional value after 4 weeks (28 d) of fermentation [10]. Gowda, et al. reported that the fungal count on the 15th day of ensiling was minimal (<3–4 colony forming units) and contained 6–7% lactic acid (DM basis) [1]. Acaín et al. found that using pineapple residue silage instead of elephant grass to feed lambs would not affect the growth of lambs [11].

Therefore, the objective of the present study was to screen, isolate and identify the LAB in pineapple residue silage. Isolates were identified at the molecular level using 16S rRNA sequence analysis and *recA* gene amplification product. In order to evaluate the relationship between natural populations of LAB and silage quality, the fermentation characteristics and chemical composition of silage samples were also studied.

2. Materials and Methods

2.1. Isolation and Characterization of LAB from Pineapple Residue Silage

The pineapple residue (*Ananas comosus* L.) was sampled in a fruit-processing factory in Henan Province, China in May 2017. Silages were prepared using a small-scale system, and approximately 200 g pineapple residue was chopped into pieces of about 20 mm length and packed into plastic bags (N-9, Asahi Kasei Co., Ltd., Tokyo, Japan), each group had 15 replicates. The bags were sealed with a Sharp Vacuum Seal/Package (SQ-202, Sharp Co., Ltd., Tokyo, Japan), and the plastic bags were stored at room temperature. Five bags for each the silage samples were collected at the 3rd, 7th and 30th day of ensiling process. Samples (10 g) were then blended with 90 mL sterilized water, and serially diluted from $10^{-1}$ to $10^{-5}$ in sterilized water. The number of LAB were measured by plate count on lactobacilli de Man, Rogosa and Sharpe (MRS) [12] agar incubated at 30 $^\circ$C for 48 h under anaerobic conditions (DG 250/min MACS; Don Whitley Science; London, England). Each LAB colony was isolated and purified twice by streaking on MRS agar plates. Pure cultures were grown on MRS agar at 30 $^\circ$C for 24 h, and then the purified strains were stored at $-80^\circ$C in nutrient broth (Difco). Coliform bacteria were counted on blue light broth agar (Nissui-Seiyaku Co., Ltd., Tokyo, Japan), incubated at 30 $^\circ$C for 48 h. Molds and yeasts were counted on potato dextrose agar (Nissui), incubated at 30 $^\circ$C for 24 h, and yeasts were distinguished from molds and other bacteria by colony appearance and the observation of cell morphology. Coliform bacteria and aerobic bacteria were counted on nutrient agar (Nissui), incubated at 30 $^\circ$C for 24 h under aerobic conditions. Colonies were counted as viable numbers of microorganisms in colony-forming unit CFU/g of fresh matter (FM).

2.2. Morphological, Physiological and Biochemical Tests of LAB

Morphology and Gram-staining response was examined after 24 h of incubation on MRS agar. Catalase activity and gas production from glucose were determined via the Cai’s methods [5]. Growth at different temperature was observed in MRS broth after incubation at 5 and 10 $^\circ$C for 14 d, and at 45 and 50 $^\circ$C for 7 d. Growth at pH 3.0, 3.5, 4.0, 5.0 and 8.0 was observed in MRS broth after incubation at 30 $^\circ$C for 7 d. Salt tolerance of LAB was tested in MRS broth containing 3.0 and 6.5% NaCl at 30 $^\circ$C for 48 h. Carbohydrate assimilation and fermentation of 49 compounds with 1 control were identified on API 50 CH strips (bioMerieux, Tokyo, Japan). API 50 CH is used in conjunction with API 50 CHL Medium (OT-50410) for the identification of LAB and related genera. The bacterial characteristics of 4 groups of strains were based on the carbohydrate fermentation map of the API 50 CHL database [13]. *Lactococcus lactis* strains were examined for their ability to produce c-aminobutyric acid and bile salt tolerance, as reported by Kim et al. [14] and Nomura et al. [15].
2.3. 16S rRNA Gene Sequencing and RecA Gene Polymerase Chain Reaction (PCR) Amplification

The strains grown at 30 °C for 24 h in MRS agar were used for 16S rRNA gene sequence. The coding region of 16S rRNA gene sequence was amplified by PCR thermal cycle. The sequences of the PCR products were determined directly with a sequencing kit using the prokaryotic 16S rRNA universal primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Sequence similarity searches were performed using the DNA Database of Japan (DDBJ) and the Basic Local Alignment Search Tool (BLAST). The sequence information was then imported into the Clustal X 1.81 software program (Hitachi Software Engineering Co., Tokyo, Japan) for assembly and alignment. The 16S rRNA sequences of strains were compared with the sequences from type LAB strains held in the DDBJ. Nucleotide substitution rates (Kunc values) were calculated and phylogenetic trees were constructed using the neighbor-joining method. Bacillus subtilis NCDO 1769 T was used as an outgroup organism. The topologies of trees were evaluated using bootstrap analysis of the sequence data with Molecular Evolutionary Genetic Analysis (MEGA) 7 software, based on 1000 random resampling. These sequences were aligned with the typical published sequences from DDBJ, GenBank and the European Molecular Biology Laboratory (EMBL).

The recA multiplex-PCR assay was performed to distinguish the closely related species and subspecies of the Lactobacillus plantarum group [16].

2.4. Nucleotide Sequence Accession Number

The nucleotide sequences for the 16S rRNA gene described in this report were deposited with GenBank under accession number. AB969778, AB969779, AB969780 and AB969781 for the representative strains P15, P12, P22 and P24, respectively.

2.5. Chemical Composition and Fermentation Characteristics Analysis of Pineapple Residue Silage

After 30 days of ensiling, the silages were opened for the analysis of chemical composition and fermentation characteristics. The samples were dried in a forced-air drying oven at 65 °C for 48 h and ground to pass a 1 mm screen with a Wiley mill (ZM200, Retsch GmbH, Haan, Germany). Dry matter (DM), crude protein (CP), ether extract (EE) and ash were analyzed according to AOAC Methods 934.01, 976.05, 920.39 and 942.05, respectively [17,18]. Wet silage (10 g) was homogenized with 90 mL sterilized distilled water. Then, the pH was measured with a glass electrode pH meter (pH 213; HANNA; Italy). The ammonia-N was determined by steam distillation of the filtrates [19]. The concentration of organic acid including lactic acid, acetic acid, propionic acid and butyric acid were determined by high-performance liquid chromatography (HPLC) (column: Shodex RS Pak KC-811; Showa Denko K.K., Kawasaki, Japan; detector: DAD, 210 nm, SPD-20A; Shimadzu Co., Ltd., Kyoto, Japan; eluent: 3 mmol L⁻¹ HClO₄, 10 mL min⁻¹; temperature: 50 °C) [4].

2.6. Statistical Analysis

All the data obtained in the present study were analyzed using One-Way Analysis of Variance (ANOVA) to determine the fermentation quality of pineapple residues and pineapple residue silage, and Tukey’s honestly significant difference test was conducted for post hoc analysis via IBM SPSS 24.0 software (IBM Co., Armonk, NY, USA). Statistically significant difference was set at p < 0.05.

3. Results

3.1. The Microbial Composition, Chemical Component and Fermentation Characteristics of Pineapple Residue

The microbial composition, chemical components and fermentation characteristics of pineapple residue is shown in Table 1. In the fresh material, 10³ CFU/g of FM LAB, 10⁶ CFU/g of FM coliform bacteria, 10⁷ CFU/g of FM yeasts and 10³ CFU/g of FM molds were detected; the contents of organic matter (OM), CP and EE were 95.95%, 5.91% and 1.32%
on DM basis, respectively; the lactic and acetic acids in fresh pineapple residue were 3.78 and 1.22 g/kg of DM, respectively.

Table 1. The microbial composition, chemical component and quality of pineapple residue raw material and silage.

| Item                          | Pineapple Residue | Silage              |
|-------------------------------|-------------------|---------------------|
| Microbial composition (CFU/g of FM): |                   |                     |
| Lactic acid bacteria (LAB)    | $4.5 \times 10^3$ | $1.0 \times 10^7$   |
| Coliform bacteria             | $3.0 \times 10^6$ | ND                  |
| Yeasts                        | $2.0 \times 10^7$ | $5.0 \times 10^4$   |
| Molds                         | $1.5 \times 10^3$ | ND                  |
| Chemical composition:         |                   |                     |
| Dry matter, % of FM           | 14.60$^a$         | 11.50$^b$           |
| Organic matter, % of DM       | 95.95$^a$         | 92.60$^b$           |
| Crude protein, % of DM        | 5.91              | 6.01                |
| Ether extract, % of DM        | 1.32              | 1.80                |
| Ammonia-N, g/kg of DM         | ND                | 0.17                |
| Fermentation characteristics: |                   |                     |
| pH                            | 5.56$^a$          | 3.65$^b$            |
| Lactic acid, g/kg of DM       | 3.78$^a$          | 75.57$^b$           |
| Acetic acid, g/kg of DM       | 1.22$^a$          | 20.12$^b$           |
| Propionic acid, g/kg of DM    | ND                | ND                  |
| Butyric acid, g/kg of DM      | ND                | ND                  |

Means within columns with different superscript letters differ significantly from each other ($p < 0.05$); CFU—colony-forming unit; FM—fresh matter; DM—dry matter; ND—not detected.

After 30 days of ensiling, LAB increased to $10^7$ CFU/g of FM while yeasts decreased to $10^4$ CFU/g of FM; coliform bacteria and molds were not detected. Compared to the fresh matter, pH value, DM and OM were lower ($p < 0.05$), and lactic and acetic acids were higher on the 30th day of ensiling.

3.2. The Morphological and Physiological Properties of Representative Strains Isolated from Pineapple Residue Silage

The morphological and physiological properties of representative strains isolated from pineapple residue silage are shown in Table 2. The 34 LAB strains were divided into 4 groups (A–D) according to growth temperature, salt tolerance, growth pH and carbohydrate fermentation method, and the number of strains in each group was group A (6 strains), group B (18 strains), group C (5 strains) and group D (5 strains). This analysis resulted in the delineation of four groups of isolates, each of which displayed a distinct carbohydrate fermentation pattern. All the isolates were Gram-positive and catalase-negative bacteria, which are unable to grow between 5 and 50 °C and able to grow between pH 4.0 to 8.0. The Groups A, B and C strains included rods that did not produce gas from glucose and could grow at pH 3.5 except for group C. The cocci-shaped strains in Group D were heterofermentative and unable to grow at pH 3.5. Strains A, B, C and D can grow in 3.0% NaCl. For the 6.5% NaCl, A and B can survive, while C and D cannot. Results of API 50 CH fermentation patterns showed that strains in group A and group C can use L-Arabinose, α-Methyl-D-glucoside, Melibiose, D-Raffinose, D-Turanose, Ribose, N-acetyl D-glucosamine, Salicine, Galactose and Trehalose, but cannot use Mannitol, Amygdaline, Esculine, Arbutine, Lactose, D-Tagatose and Gluconate, but in group C use Ribose, N-acetyl D-glucosamine, Salicine, Arbutine and Lactose, showing weak positivity; strains in group B can use Ribose, N-acetyl D-glucosamine, Salicine, Galactose, Trehalose, Mannitol, Amygdaline, Esculine, Arbutine and Lactose, but cannot use L-Arabinose, α-Methyl-D-glucoside, Melibiose, D-Raffinose, D-Turanose and D-Tagatose; strains in group D can use L-Arabinose, Ribose, Amygdaline and D-Tagatose.
Table 2. The characteristics of isolated strains from pineapple residue silage.

| Characteristics                      | Group A | Group B | Group C | Group D |
|--------------------------------------|---------|---------|---------|---------|
|                                      | P 12    | P 15    | P 22    | P 24    |
| No. of isolates                      | 6       | 18      | 5       | 5       |
| Shape                                | Rod     | Rod     | Rod     | Cocci   |
| Gram stain                           | +       | +       | +       | +       |
| Gas from glucose                     | +       | +       | −       | −       |
| Catalase                             | −       | −       | −       | −       |
| Fermentation type                    | Homo    | Homo    | Homo    | Hetero  |
| Growth at temperature:              |         |         |         |         |
| 5 °C                                 | −       | −       | −       | −       |
| 10 °C                                | +       | +       | −       | −       |
| 45 °C                                | +       | +       | +       | +       |
| 50 °C                                | −       | −       | −       | −       |
| Growth at pH:                        |         |         |         |         |
| 3.0                                  | -       | w       | −       | −       |
| 3.5                                  | +       | +       | −       | −       |
| 4.0                                  | +       | +       | +       | +       |
| 5.0                                  | +       | +       | +       | +       |
| 8.0                                  | +       | +       | +       | +       |
| Growth in NaCl (%):                  |         |         |         |         |
| 3.0                                  | +       | +       | +       | +       |
| 6.5                                  | +       | +       | −       | −       |
| Carbohydrate fermentation:           |         |         |         |         |
| Maltose                              | +       | +       | +       | +       |
| D-Fructose                           | +       | +       | +       | +       |
| D-Glucose                            | +       | +       | +       | +       |
| L-Arabinose                          | +       | −       | +       | +       |
| Ribose                               | +       | +       | w       | +       |
| D-Xylose                             | +       | +       | +       | +       |
| Galactose                            | +       | +       | −       | −       |
| Mannitol                             | −       | +       | −       | −       |
| α-Methyl-D-glucoside                 | +       | −       | +       | −       |
| N-acetyl glucosamine                 | +       | +       | w       | w       |
| Amygdaline                           | +       | +       | −       | −       |
| Arbutine                             | +       | +       | w       | w       |
| Esculine                             | −       | +       | −       | −       |
| Salicine                             | +       | +       | w       | w       |
| Lactose                              | −       | +       | w       | −       |
| Melibiose                            | +       | −       | +       | −       |
| Saccharose                           | +       | +       | +       | +       |
| Trehalose                            | +       | +       | +       | −       |
| D-Raffinose                          | +       | −       | +       | −       |
| Starch                               | w       | w       | −       | −       |
| β-Gentiobiose                        | w       | +       | w       | −       |
| D-Turanose                           | +       | −       | +       | −       |
| D-Tagatose                           | −       | −       | −       | +       |
| Gluconate                            | −       | w       | −       | w       |

+, positive; w, weakly positive; −, negative; Homo, homofermentative; Hetero, heterofermentative; The rest of the carbohydrates were not fermented by the isolated strains and are not listed.

3.3. 16S rRNA Gene Sequence Analysis

Phylogenetic trees were shown in Figures 1 and 2. According to the phylogenetic analysis, the strain P12 from Group A was categorized in the Lactocaseibacillus paracasei cluster in a 100% bootstrap cluster. A representative strain in Group B, namely P15, clearly belonged to the genus Lactiplantibacillus (L.), because it was clustered in the L. plantarum branch, which included L. pentosus, L. plantarum, L. argentoratensis, L. paraplantarum and L. fabifermentans, on the phylogenetic tree (Figure 1). The strain P22 from Group C was closely related to the Levilactobacillus brevis with a bootstrap value of 100%. The strain P24
from Group D was categorized in the *Leuconostoc (Ln.)* cluster because it was grouped with *Ln. citreum* on the phylogenetic tree, and this grouping was supported with a bootstrap value of 98% (Figure 2).

**Figure 1.** Phylogenetic tree showing the relative positions of rod-shaped LAB strains isolated from pineapple residue silage by the neighbor-joining method of complete 16S rRNA sequences. Bootstrap values for 1000 replicates are shown at the nodes of the tree. *Bacillus subtilis* is used as an outgroup organism. The bar indicates 1% sequence divergence. Knuc = nucleotide substitution rate.
Lacticaseibacillus paracasei 1, 2, 3, 4 and 5 are from pineapple residue silage by the neighbor-joining method of complete 16S rRNA sequences. Bootstrap values for 1000 replicates are shown at the nodes of the tree. 

**Figure 2.** Phylogenetic tree showing the relative positions of cocci-shaped LAB strains isolated from pineapple residue silage by the neighbor-joining method of complete 16S rRNA sequences. Bootstrap values for 1000 replicates are shown at the nodes of the tree. *Bacillus subtilis* is used as an outgroup organism. The bar indicates 1% sequence divergence. Knuc = nucleotide substitution rate.

### 3.4. Amplification Products Obtained from the *recA* Gene Multiplex Assay

The amplification products obtained from the *recA* gene are shown in Figure 3. Based on Bergey’s manual of Systematic Bacteriology, the amplification products shown in lanes 1, 2, 3, 4 and 5 are from *Lacticaseibacillus paracasei* JCM 16167<sup>T</sup> (negative control), *L. paraplantarum* JCM 12533<sup>T</sup>, *L. pentosus* JCM 1558<sup>T</sup>, *L. plantarum* JCM 1149<sup>T</sup> and *L. argentoratensis* JCM 16169<sup>T</sup>, respectively, and Lane 6 shows the PCR amplification product from strain P15. P15 and JCM 1149<sup>T</sup> had the same amplification product. Therefore, P15 was clearly identified as *L. plantarum*.

**Figure 3.** Amplification products obtained from the *recA* multiplex assay. Lane M contained a 600 bp PLUS DNA ladder (Tiangen Biotech Co, Ltd., Beijing, China). Lanes 1, 2, 3, 4 and 5 contain PCR amplification products from *Lacticaseibacillus paracasei* JCM 16167<sup>T</sup> (negative control), *L. paraplantarum* JCM 12533<sup>T</sup>, *L. pentosus* JCM 1558<sup>T</sup>, *L. plantarum* JCM 1149<sup>T</sup> and *L. argentoratensis* JCM 16169<sup>T</sup>, respectively; Lane 6, PCR amplification product from P15.
4. Discussion

It is well established that LAB play a major role and can out-compete undesirable microorganisms, such as coliform bacteria in natural silage fermentation. Many studies have been conducted to investigate the natural population of LAB in forage and grass-based silages, and they have found that the quantity and species of LAB become a significant factor in predicting the adequacy of silage fermentation and the necessity to apply bacterial inoculants to silage materials [20]. Cai found that the number of LAB was generally low (less than $10^3$) in forages and vegetables [5]. It has been reported that the residues of banana, pineapple and papaya presented relatively high numbers—$10^3$ to $10^5$ (cfu/g of FM)—of LAB, and found that the main microbial groups on fruit residue were *Lacticaseibacillus plantarum* and *Lacticaseibacillus casei*, which played an important role in silage fermentation [2].

In this investigation, 40 strains isolated from pineapple residue silage were screened, of which 34 were considered as LAB determined by culture on MRS agar, Gram stain appearance, catalase test and lactic acid production from glucose. All the presumptive LAB were further characterized by sugar fermentation assays using API50 CH strips. This led to four groups of isolates, each displaying an obvious carbohydrate fermentation pattern. The various groups presumably represented three genera: *Lactiplantibacillus*, *Levilactobacillus* and *Leuconostoc*, which were the dominant counts of the LAB population in pineapple residue silage.

Sequencing of 16S rRNA has been successfully applied for the identification of bacteria at the species level [21]. The representative strains of groups A (P12), C (P22) and D (P24) were identified as *Lacticaseibacillus paracasei*, *Levilactobacillus brevis* and *Ln. citreum*. *L. plantarum* cluster members had similar 16S rRNA gene sequences with a difference of only 2 bp [21]. Because of the fundamental role of the recA gene, its gene product is considered to be a phylogenetic marker for distantly related species [22]. PCR analysis of recA gene products showed that the four strains were significantly different to group B strains. Strain P15 was a group B strain and had the same product (318 bp) as *L. plantarum* subsp. *plantarum*. Therefore, strain P15 can be identified as *L. plantarum* subsp. *plantarum*.

Following biochemical and phylogenetic analysis, the isolates of pineapple residue silage fell within well-recognized groups of LAB, and it was found that most of the characterized LAB belonged to *Lactiplantibacillus* and *Leuconostoc*. The species diversity was observed because four species were also identified: *Lacticaseibacillus paracasei* (17.6% of the total number of LAB isolates in this study), *L. plantarum* (52.9%), *Levilactobacillus brevis* (14.7%) and *Leuconostoc citreum* (14.7%). Furthermore, homofermentative species accounted for 70.6% of the total LAB community. *Lactiplantibacillus* and *Leuconostoc* were also found living in plant material and dairy products and several reports have declared that they dominate the microbial population in forage crops and grass silages, which is consistent with the results of our investigation, as well as those of other authors [23–26]. The most prevalent species in pineapple residue silage, *L. plantarum*, is reported to be the most dominant in the fermentation of forage crops and grass silages. As shown in Table 2, strains in group B (*L. plantarum*) had a relatively low pH (3.50) tolerance. For this reason, these strains could grow well in an anaerobic environment and produce more lactic acid even in pH values below 4.0, which was in accordance with the low pH value (3.65) of pineapple residue after 30 days of ensiling in present study. The heterofermentative *Leuconostoc* did not improve the fermentation characteristics and may cause organic matter loss [4]. In addition, the low frequencies (14.7%) of *Leuconostoc* found in the pineapple residue silage indicated that it did not play a key role in the ensiling process compared with *L. plantarum*. However, silage microbial screening is an effective way to obtain live lactic acid bacteria in silage based on MRS medium culture method [27,28]. Many microorganisms can survive on the surface of silage material, but cannot be cultured on selective medium, so there may be cognitive bias. With the development and application of high-throughput sequencing technology, it is possible to better understand the composition and dynamics of the microbial community during fermentation [29]. Therefore, the concept of using metagenomics or real-time high-throughput sequencing methods to explore the application effect of specific
strains in this study on pineapple residue silage and its impact on the overall microbial community of pineapple residue silage fermentation is worthy of further study.

At 30 d of ensiling, the pineapple residue silage can be well preserved with a high content of lactic acid (75.57 g/kg of DM) and low pH value (3.65). That is probably due to the homofermentative LAB which were dominant in the pineapple residue silage and had a strong survival ability under relatively low pH conditions (Table 2). As we know, homofermentative LAB, such as Lactiplantibacillus plantarum, can produce almost exclusively lactic acid from fermentative sugars and reduce pH value quickly [30,31]. The reduction of molds, coliform bacteria and yeasts in the ensiling process was mainly related to the acidic and anaerobic environment conditions not suitable for their growth [32]. They still survived at the level of 10^4 CFU/g of FM when the pH value of the pineapple residue silage was 3.65. Pieper et al. [33] reported that fermentative activity of microorganism depended on the water available in the substrate and increasing moisture content could enable fermentation activity of microorganism. The water content of pineapple residues silage was much higher than that of usual silage (55–70%), which indicated the possibility of a high level of water activity. This might be the main reason why the yeasts were not strongly inhibited even under the conditions of higher lactic acid concentration and lower pH value [34,35]. During the ensiling fermentation process, yeast converts six-carbon sugar into ethanol, which is then oxidized to acetic acid. The high moisture content (beyond 85%) might contribute to the growth of yeasts even with low pH in our study. Therefore, the increase in acetic acid content also means the possibility of frequent yeast metabolism. In addition, the reduction of DM and OM contents compared to raw matter was probably due to yeasts fermenting soluble carbohydrate in the ensiling process. Concentrations of butyric acid from all the samples were not detected, which was indicative of well-preserved silages.

5. Conclusions

The pineapple residue can be well preserved with a low pH value and a high content of lactic acid in the ensiling process. In addition, the identification results revealed the LAB composition inhabiting pineapple residues and enabling the future design of appropriate inoculants aimed at improving the fermentation quality of silage.

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