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Characteristics of lactic acid bacteria isolates and their effect on silage fermentation of fruit residues

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ABSTRACT

The natural lactic acid bacteria (LAB) population, chemical composition, and silage fermentation of fruit residues were studied. Eighty-two strains of LAB were isolated from fruit residues such as banana leaf and stem, pineapple peel, and papaya peel. All strains were gram-positive and catalase-negative bacteria, and they were divided into 7 groups (A–G) according to morphological and biochemical characters. Strains in groups A to F were rods, and group G was cocci. Group F produced gas from glucose; other groups did not. Groups A to C and F formed DL-lactic acid, whereas groups D, E, and G formed L-lactic acid. Based on the 16S rRNA gene sequence and DNA-DNA hybridization analysis, groups A to G strains were identified as *Lactobacillus plantarum* (54.9% of the total isolates), *Lactobacillus paraplantarum* (3.6%), *Lactobacillus nagelii* (8.5%), *Lactobacillus perolens* (4.9%), *Lactobacillus casei* (11.0%), *Lactobacillus fermentum* (9.8%), and *Enterococcus gallinarum* (7.3%), respectively. *Lactobacillus plantarum* and *Lactobacillus casei* are the most frequently isolated from fruit residues as a dominant species, and they could grow at a lower pH conditions and produce more lactic acid than other isolates. Pineapple and papaya peels contained higher crude protein (11.5–13.8%) and water-soluble carbohydrate (16.8–22.4%), but lower acid detergent fiber contents (21.2 to 26.4%) than banana stems and leaves (8.2% crude protein, 42.8% acid detergent fiber, and 5.1% water-soluble carbohydrate). Compared with banana stem and leaf silages, the pineapple and papaya peel silages were well preserved with a lower pH and higher lactate content. The study suggests that the fruit residues contain excellent LAB species and abundant feed nutrients, and that they can be preserved as silage to be potential food resources for livestock.

Key words: fruit residue, lactic acid bacteria, silage fermentation

INTRODUCTION

Banana, pineapple, and papaya are popular tropical fruits in the world, their production per year worldwide was 81.2 million, 18.8 million, and 0.3 million t, respectively (Liu and Zhang, 2013). Hainan Island, located in the South China Sea, has a humid subtropical climate with warmer temperatures and tropical monsoon climate (Tang et al., 2008). Commercial processing of tropical fruits produces large quantities of residues every year in many countries, including China, but some residues are burned and dumped into landfills whereas others are used as compost, which leads to wasted resources and possible environmental problems because of unsuitable disposal (Ouyang, 2010). Demand for efficient use of food byproducts has increased due to economic and environmental concerns. Fruit residues from bananas, pineapples, and papayas are high in nutrients such as vitamins, minerals, and fiber (Council for Science and Technology, 2005) and can be potential feed resources for livestock. However, these fruit residues are highly perishable because of their high moisture content. Technology to provide long-term storage for the resulting silage as high quality animal feed needs to be developed.

Silage is now a common preserved fresh forage as feed in the world, and this fermentation technology is also considered to be available for the preservation and preparation of fruit residue. Generally, silage preservation depends on the production of sufficient acid to inhibit activity of undesirable microorganisms under anaerobic conditions (Cai et al., 1998). Naturally occurring lactic acid bacteria (**LAB**) are responsible for silage fermentation and can also influence silage quality (Lin et al., 1992). During silage fermentation, LAB convert sugar into lactic acid (Muck, 1989). As a result, the pH is reduced and the forage is preserved (Cai, 1999). However, from a silage fermentation point of view, to

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our knowledge, very little information is available on the silage preparation with fruit residues.

In the present experiment, 82 strains of LAB were isolated from fruit residues of banana, pineapple, and papaya. Their taxonomic status was studied through 16S rDNA sequence and DNA-DNA hybridization analysis. To develop the silage fermentation technique for tropical fruits residues, their natural LAB population, chemical composition, and silage fermentation were also studied.

MATERIALS AND METHODS

Fruit Residue Collection and Microbiological Analysis

Samples of banana (*Musa sapientum* L.) stems and leaves were collected from the Research Base of Tropical Crops Genetic Resources Institute of Tropical Agricultural Sciences Academy of China (Danzhou, Hainan province, China). As shown in Table 1, fruit residue samples of pineapple [*Ananas comosus* (L.) Merr.] peels and papaya [*Chaenomeles speciosa* (Sweet) Nakai] peels were collected from a local fruit processing factory (Kangle Foods factory, Danzhou, Hainan province, China).

The number of microorganisms in the fruit residue was measured by the plate count method. Fruit residue samples (10 g) were blended with 90 mL of sterilized water, and were serially diluted 10^{-1} to 10^{-5} in sterilized water. The number of LAB was measured by the plate count method on lactobacilli de Man, Rogosa, Sharpe (**MRS**) agar (Difco Laboratories, Detroit, MI) incubated at 30°C for 48 h under anaerobic conditions (Anaerobic box; TE-HER Hard Anaerobox, ANX-1; Hirosawa Ltd., Tokyo, Japan). For isolation of LAB, 10 to 20 strains on MRS agar medium were picked randomly from each silage sample, and a total of 86 isolates were collected, of which 82 isolates were considered to be LAB, as determined by the Gram stain appearance, catalase test, and lactic acid productivity, and their physiological properties were then determined by the isolation and identification methods for LAB (Kozaki et al., 1992). Coliform bacteria were counted on blue light agar (Nissui-seiyaku, Tokyo, Japan) incubated at 30°C for 48 h; mold and yeast were counted on potato dextrose agar (Nissui-seiyaku) incubated for 24 h at 30°C. Yeast was distinguished from mold and bacteria by colony appearance and observation of cell morphology. Bacilli and aerobic bacteria were distinguished by the colony shape and counted on nutrient agar (Nissui-seiyaku) incubated for 24 h at 30°C under aerobic conditions. Colonies were counted as viable numbers of microorganisms in colony forming units per gram of fresh matter (**FM**). Each LAB colony was purified twice by streaking on a MRS agar plate. The pure cultures were grown on MRS agar at 30°C for 24 h, resuspended in a solution of nutrient broth (Difco) and dimethyl sulfoxide at a ratio of 9:1, and stored as stock cultures in a deep freezer (Sanyo, Tokyo, Japan) at −80°C until further examination.

Morphological, Physiological, and Biochemical Test

Gram stain of LAB and morphological characteristics were determined after 24 h of incubation on MRS agar. Catalase activity and gas production from glucose were determined as described by Kozaki et al. (1992). Growth at different temperatures was detected in MRS broth after incubation at 5 and 10°C for 10 d, and at 45 and 50°C for 7 d. Growth at pH 2.5, 3.0, 3.5, 4.0, and 7.0 was observed in MRS broth after incubation at 30°C for 7 d. Salt tolerance of LAB was tested in MRS broth containing 3.0 and 6.5% NaCl. Carbohydrate assimilation and fermentation of 49 different compounds with one control were identified on API 50 CH strips (bioMerieux, Tokyo, Japan). These strains were divided into 7 groups (A–G) according to morphological and biochemical characters, and the representative strains of each group were selected by their different fermentation patterns of API 50 CH.

Table 1. Lactic acid bacteria strains used in this study[1]

Sample	Collection site	Representative strain
Banana stem and leaf	The Research Base of Tropical Crops Genetic Resources Institute of Tropical Agricultural Sciences Academy of China, Danzhou, Hainan province, China	HN3, HN4, HN7, HN8, HN9, HN10, HN11, HN12, HN15, HN16, HN17, HN18
Pineapple peel	Fruit processing factory, Kangle Foods factory, Danzhou, Hainan province, China	HN2, HN6
Papaya peel	Fruit processing factory, Kangle Foods factory, Danzhou, Hainan province, China	HN1, HN5, HN13, HN14

[1]HN strains were determined by the Gram stain appearance, catalase test, and lactic acid productivity. The representative strains were selected by their different morphological and biochemical characters.