ISOLATION AND IDENTIFICATION OF MICROORGANISMS INVOLVED IN BAMBANGAN FERMENTATION



BIOTECHNOLOGY RESEARCH INSTITUTE UNIVERSITI MALAYSIA SABAH 2012

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CHIN SENG YEE



BIOTECHNOLOGY RESEARCH INSTITUTE UNIVERSITI MALAYSIA SABAH 2012

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NAME	:	CHIN SENG YEE
MATRIC NO	:	PS05-013-004
TITLE	:	ISOLATION AND IDENTIFICATION OF MICROORGANISMS INVOLVED IN BAMBANGAN FERMENTATION
DEGREE	:	MASTER OF SCIENCE
VIVA DATE	:	27 MARCH 2012

DECLARED BY

 SUPERVISOR Associate Prof. Dr. Chye Fook Yee
 Signature
 CO-SUPERVISOR Prof. Datin Dr. Ann Anton

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Chin Seng Yee 27 October 2011



ABSTRACT

ISOLATION AND IDENTIFICATION OF MICROORGANISMS INVOLVED IN BAMBANGAN FERMENTATION

The objective of this study was to isolate and identify the microorganisms involved in *bambangan* fermentation, a well accepted indigenous fermented fruit of Sabah. Freshly prepared samples obtained from supplier were kept in ceramic jar (S1) and glass bottles (S2) respectively similar to the conditions of the supplier throughout the fermentation process. Microbiological and chemical analyses were carried out at each interval times. Moisture, ash and protein contents for both samples S1 and S2 increased significantly (p<0.05) during bambangan fermentation. Lactic acid increased notably during the process from 5.0 ± 1.5 mg/g to 108.7 ± 4.8 mg/g (S1) and 5.8±1.8 mg/g to 93.4±2.77 mg/g (S2), which present as the basis of pH reduction in the samples. Amount of glucose, fructose and sucrose were found as 1.35±0.60 mg/g (S1) and 2.5±0.97 mg/g (S2); 39.6±2.12 mg/g (S1) and 36.8±1.18 mg/g (S2); 2.92±1.12 mg/g (S1) and 5.1±1.08 mg/g (S2) at the end of fermentation. Alcohol content in samples S1 and S2 were $1.78\pm0.20\%$ and $1.91\pm$ 0.15% respectively after 10 days of fermentation. A total of 161 microbial isolates were identified throughout the *bambangan* fermentation. Microbial succession was observed during the *bambangan* fermentation with the coexist of lactic acid bacteria and yeast, which were believed to stay in a symbiosis correlation in the substrate during fermentation. The imperative microorganisms involved in bambangan fermentation were Lactobacillus plantarum, Pediococcus pentosaceus, Issatchenkia orientalis and Hanseniaspora guillermondii which play important roles in shaping the end product. The variation of microflora during the *bambangan* fermentation allied with the changes of chemical component in the samples. Among the 32 tested strains, Lactobacillus plantarum (LP1 and LP2); Lactobacillus paracasei spp paracasei (LPSP1) and Issatchenkia orientalis (IO1) were desired as potential probiotic strains among the entire tested strains ever since that they're capable to withstand at pH3, 0.3% bile salt concentration and performed antimicrobial properties as well. Most of the lactic acid bacteria strains and Issatchenkia orientallis showed significant glycodicidase activity. The occurrence of a wide diversity of LAB and yeast in fermented bambangan could in part be responsible for the desirable quality attributes associated with the product. Additional study using 16 rDNA sequencing could be carried out in the future to validate the unknown isolates (8%) and facilitate the development of potential starter cultures to upgrade the fermentation process with controllable parameters.

ABSTRAK

Obiektif kajian ini adalah untuk mengenalpasti mikroflora yang terlibat dalam fermentasi bambangan, sejenis buah fermentasi tempatan yang terkenal di Sabah. Sampel yang baru disediakan oleh pengusaha disimpan dalam tajau (S1) dan botol kaca (S2) masing-masing sepanjang tempoh fermentasi sepertimana yang dilakukan oleh pengusaha bambangan fermentasi. Analisis mikrobiologi dan kimia dijalankan pada selang masa tertentu, kandungan kelembapan, abu dan protein bagi kedua-dua sampel S1 dan S2 meningkat dengan signifikan (p<0.05) pada akhir proses fermentasi. laktik asid meningkat dari 5.0±1.5 mg/g to 108.7±4.8 mq/q (S1) dan 5.8±1.8 mq/q to 93.4±2.77 mq/q (S2), dimana peningkatannya merupakan perdorongan bagi penurunan pH dalam sampel. Kandungan glukose, fruktose dan sukrose didapati sebanyak 1.35±0.60 mg/g (S1) dan 2.5±0.97 mg/g (S2); 39.6±2.12 mg/g (S1) dan 36.8±1.18 mg/g (S2); 2.92±1.12 mg/g (S1) dan 5.1±1.08 mg/g (S2) pada hujung proses fermentasi. Kandungan alkohol dalam sampel S1 and S2 merupakan $1.78 \pm 0.20\%$ dan $1.91 \pm 0.15\%$ masing-masing selepas 10 hari fermentasi. Sebanyak 161 mikroorganisma diisolat sepanjang proses fermentasi bambangan. Penggantian jenis-jenis mikroflora berlaku dengan muncul sesama antara bacteria laktik asid dan yis, dengan hubungan simbiosis dalam subtrak. Microflora utama dalam proses fermentasi terdiri daripada Lactobacillus plantarum, Pediococcus pentosaceus, Issatchenkia orientalis dan Hanseniaspora guillermondii dengan memainkan peranan yang penting dalam membentukan produk akhir. Di antara 32 strains mikroorganisma yang diuji, Lactobacillus plantarum (LP1 and LP2); Lactobacillus paracasei spp paracasei (LPSP1) dan Issatchenkia orientalis (IO1) dipilih sebagai strain yang mempunyai potensi probiotik memandangkan stain-strain ini berkebolehan untuk terus hidup dalam keadaan pH3, 0.3% garam bile dan menunjukkan kecekapan dalam menyekat pertumbuhan mikroorganisma yang patogenik. Kebanyakan bacteria asid dan Issatchenkia orientallis menunjukkan aktiviti glikosidasi yang laktik merangsangkan. Perubahan jenis mikroflora semasa fermentasi bambangan serentak dengan perubahan komponen kimia dalam sampel. Pemunculan sekumpulan LAB dan yis dalam fermentasi bambangan menyumbangkan pembentukan mutu produk yang memuaskan. Kajian lanjutan dengan menggunakan 16 rDNA boleh digunakan untuk mendapat identiti mikrofloramikroflora yang kurang pasti (8%) dimana ia dapat menyumbangkan dalam pembangunan kultur pemula yang berpotensi bagi meningkatkan mutu proses fermentasi dengan parameter yang terkawal.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
CFU/g	Colony forming unit per gram
g	Gram
Μ	Molar
ml	Mililitre
°C	Degree Celsius
mg/g	Milligram per gram
LAB	Lactic acid bacteria
h	Hours
min	Minutes
w/v 599-1	Weight per volume
cm	Centimeter
kg 🖉	Kilogram
ppm	Part per million
AOAC	Association of Official Analytical
Ν	Normality
μm	Micrometer
μΙ	Microlitre
rpm	Revolution per minute
nm	nanometer
KH_2PO_4	Potassium dihydrogen phosphate
uv	Ultraviolet
≥	More or equal than

- Degree
- < Less than



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ASSOC. PROF. DR. CHYE FOOK YEE

(PENYALIA UTAMA)

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CHAPTER 1

INTRODUCTION

Modern food biotechnology has moved a long way since ancient times of empirical food fermentations, which is one of the oldest technologies used for food preservation. This process had been evolved, refined and diversified over the centuries. Nowadays, a variety of food products is derived from this technology in households, small-scale food industries as well as in large enterprise within developing countries. Although advances in food science and technology have given rise to a wide range of new food technologies, fermentation remained an important preservation technique throughout the history of mankind, which is also an affordable technology and accessible to all populations (Okonko *et al.*, 2006).

As stated by FAO (2004), fermented foods find wide consumers acceptance in the developing world and contribute substantially to food security in a number of developing countries. They are generally appreciated for their unique attributes on pleasant flavour, aroma, and improved texture. Moreover, it also provides excellent protein source by improving the balance of essential amino acids and their availability. Similarly, fermentations increase the content and availability of vitamins such as thiamine, riboflavin, niacin and folic acid which have profound effects on human health (Afoakwa *et al.*, 2007).

Traditional fermentation processes are developed largely as an art, rather than through scientific principles. Its traditional applications have been based on experiences gained through trial and error by consecutive generations of food producers and households who have used the technology for the domestic preparation and preservation of foods. Despite progress made in this field, the technology is still often empirically applied without a comprehensive understanding of the underlying principles of the fermentation process and the requirements for ensuring quality and safety of food. Such approach presents a major pitfall, as it may lead to unsafe products depending on the process, environmental setting, and the condition of the raw materials (Steinkraus, 2009).

Microorganisms, by virtue of their metabolic activities contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf-life and safety. Thus, the essential role of bacteria, yeasts and moulds in the generation of fermented foods came to be understood and this ultimately resulted in more controlled and efficient fermentations (Giraffa, 2004). Studies on microbial dynamics, substrate related interactions and metabolic activities of different microbial groups, key enzymes and the role of technical and other process parameters have provided a firm basis for improvement of traditional processes (Holzapfel, 2002). Many studies on indigenous fermented foods particularly their microbiological and biochemical changes have been documented around the world (Oguntoyinbo, 2007; Anihouvi et al., 2006; Lucia et al., 2007; Susana, et al., 2005; Elena et al., 2007; Pablo et al., 2008). Besides, research on indigenous fermented fruits such as tempoyak (Adnan and Tan, 2007); Masau fruit (Loveness et al., 2007); table olive (Alejandro et al., 2007); cocoa (Nielsen et al., 2007); grape (Christine et al., 2006) and apple must (Emmanuel et al., 2006) were also reported due to lactic acid bacteria (LAB) and yeasts.

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The biological and microbiological bases of the fermentation processes of most of the indigenous fermented foods are partly understood. What little information is available often deals with the identification and perhaps preliminary characterization of the primary microflora in the finished product. There is undoubtedly a need to produce these foods in circumstances where quality and safety can be guaranteed. This in turn necessitates a more thorough understanding of the microorganisms involved, in terms of the types and their specific activities, so that the fermentation process can be more reliable and predictable. Thus it is likely that basic microbiological analyses in conjunction with the appropriate technological developments will be sufficient to achieve these objectives (Robert, 2006).

Bambangan (Mangifera pajang) belonging to family Anacardiaceae is an indigenous fruit with brownish skin and yellow colour flesh that grows wild in the forest of Borneo Island (Rukayah, 1999). This mango-like fruit can be eaten fresh or to be processed into fermented products that retain its high degree of sensory qualities with soft and tender texture, mango-like fragrance, pale yellow colour, salty and sour taste. It can be eaten with rice, as pickle or used as a condiment with fish and it's usually served by the locals as their traditional ethnic food and regularly promoted as indigenous food to the tourists. Fermented *bambangan* are made based on the empirical knowledge by mixing the fruit cubes and its grated seed together with salt and left to ferment at ambient temperature (28°C - 30°C) in a tightly closed container for 7-10 days.

The fermentation of *bambangan* remains as a cottage industry in Northern Borneo and its method of processing may vary among producers. Unlike other traditional fermented foods; fermented *bambangan* undergoes spontaneous fermentation that typically results from the competitive activities of a variety of unknown microorganisms. Those best adapted to the food substrate ultimately dominate the process. Yet, such fermentation process is neither predictable nor controllable and often applied without comprehensive understanding of the underlying principles of the fermentation process. This process had caused a major pitfall in production and lead to inconsistent product quality depending on the process. In addition, poor hygiene practices during production of bambangan usually results in spoilage of final product and poor keeping quality. Studies should be premeditated to examine the technological constraints of fermented *bambangan* so as to upgrade its fermentation process. Hence, it is important to assess the microbiological and biochemical changes during this traditional process to acquire information that could be used in improving the control of the fermentation process, product quality and acceptability. Therefore, this study was undertaken to isolate and identify the microorganisms involved in the spontaneous fermentation of *bambangan*. The specific objectives of this research were:

a. To isolate, identify and characterize the microorganisms involved in *bambangan* fermentation.

- b. To determine the proximate composition and chemical changes during *bambangan* fermentation
- c. To evaluate the beneficial and functional properties of the isolated strains.

Rationales of the study

Isolation and identification of microorganisms involved at each stage of bambangan fermentation is crucial to provide a comprehensive inspiration of the key microfloras that actually drive he fermentation process ever since spontaneous fermentation typically results from the competitive activities of a variety of microorganisms including contaminants. Those best adapted to the food substrate and to technical control parameters, eventually dominate the process. Other than microbiological, proximate and chemical changes in the substrate will come into view as well as an indicator if fermentation carried out is generally allied with the variation of microflora during the process. These parameters might offer a perception on the sensory attribute of the end product, which will have an effect on the consumer's acceptability. At present, beneficial or functional microorganisms are broadly applied in food industry for health promoting properties, enzymes production, starter culture and et cetera. Consequently, screening the isolates for beneficial and functional properties in this study may give a better understanding on their contribution in the fermentation process and facilitate the selection of potential starter culture for bambangan fermentation. Apart from that, these isolates may also be applied in other food industry based on their particular properties.