SMALL MOLECULAR PROTEIN CONTENTS AND THEIR ANTIOXIDANT ABILITIES OF PORCINE SI-RAW

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***Abstract*—In this study, the genus *Pediococcus acidilactici* was screened and identified by polymerase chain reaction (PCR) using 16S rDNA gene of lactic acid bacteria. *P. acidilactici* was used as a starter culture and inoculated into porcine Si-raw. During fermentation, samples were incubated at 25℃ for 7 days and determined for their growth rates and proteolytic activities. The results showed *P. acidilactici* grew very well and remained between 5-7 log cfu/g. The result showed that *P. acidilactici* had acid production activity. The increase in acidity was found when the samples were fermented for 3 days and remained above 19.28% at a later stage. No remarkable differences were detected between the inoculated sample and control sample on the SDS-PAGE electrophoregram. During fermentation, the ratio of soluble protein decreased with curing time. However, in the presence of Si-raw, proteins were decomposed to small molecules (M. W. <10,000) such as peptide and free amino acids. It was found that the ABTS and DPPH scavenging abilities increased with the fermentation time, and the values were about 7 and 20 times after 7 days. Furthermore, higher ABTS radical scavenging abilities were observed from the *P. acidilactici* inoculated samples than the control samples after fermented for 5 days. These findings suggested that bio-active compounds from the small molecule proteins might be potential resources for the development of antioxidant functions.**

Index Terms—bio-antioxidants, lactic bacteria, pork, proteolysis

I. INTRODUCTION

Si-raw is an aboriginal fermented meat product produced in Taiwan which is without adding starters during manufacturing (Chen et al., 2002). Mostly, fermentation of Si-raw is conducted by the action of natural lactic bacteria at ambient temperature, and pH value of products was below 4.27. Lactic bacteria are widely used in fermented meat products as starter cultures (Talon, et al., 2007; Hansen, 2002). They play an important role in the connection with flavor and color development (Leroy et al., 2005) and may contributing to a preservative effect against *Clostridium botulinum* in meat products (Aymerich et al., 1998).

Lactic bacteri, generally includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, are widely exist in nature. Recently researches indicated that Lactic bacteria not only improve sensory characteristcs of food, but also supply some physiological functions such as decrease in hypertension (Arihara, 2001 and 2006), cholesterol (Wang et al., 2009) as well as increase in gastrointestinal probiotics (Klaenhammer and Kullen, 1999), immunity (Villamil, 2002), and presenting some antioxidant ability (Kullisar et al., 2002; Stecchini et al., 2001;). It was noticed that biologically active peptides have now been studied and designing as functional ingredients. As demonstrated by Arihara (2006), carnosine, anserine, l-carnitine and conjugated linoleic acid were an considerable bioactive compounds in meat. Since previous works have been done on the muscle protein hydrolysis of traditional meat products in Taiwan (Chen and Guo, 1992; Chen et al., 2002, Chen et al., 2004), the objective of this study was to evaluate the role of screened stain (*P. acidilactici*) which was used as a starter in Si-raw, the growth of this organisms in Si-raw and their protein breakdown as well as antioxidant ability.

II. MATERIALS AND METHODS

A. Isolation and identification of lactic bacteria

Soil samples were collected using sterile bags from bamboo farm of Kaohsiung. After diluted and incubated at 37℃ for 48 hrs, the colonies grown on the agar plates (MRS agar, Merck) were isolated. Bacterial DNA were prepared after digested with a restriction DNA endonuclease *Eco*RI. PCR amplification methods were then used to classify and identify these isolates. The 16S rDNA sequences determined in this study were obtained from the database of the NCBI BLAST search program.

B. Si-raw preparation

Pork ham was cut into 1×2×2 cm cubes and cured with NaCl (3%) at 4℃ for 2 days. After draining of drip, the cubes were inoculated with *Pediococcus acidilactici* (10^7 CFU/per gram meat) starter. All si-raw samples were made of 70% cubes and 30% cooked rice and then stuffed into plastic bottle (50 ml volume). The samples were incubated at 25℃ for 7 days.

C. Bacterial count and acidity determenation

Ten grams of Si-raw sample were homogenized, diluted, and inoculated on MRS agar plates and then incubated at 37℃ for 48 hrs. Colony was expressed as cell forming units per gram (CFU/g) of sample. For pH values, blended samples (10：90 by a ratio of sample and distilled water) were measured by using a pH meter (SP 701, Suntex, Taiwan). The acidity was determined by the method of titratable acidity and calculated as percentage of lactic acid.

D. Protein components analysis

The soluble protein was extracted by the method of Sanz et al., (1999) and determined according to the method of Lowry. SDS-PAGE gel which was as described by Laemmli (1970) was used to measure the sarcoplasmic protein pattern. Low molecular components (M. W. < 10,000) were extracted from soluble protein potions and centrifuged using Amicon filters. After centrifuged at 5000×g (4℃, 20 min) and reacted with o-phthaldialdehyde reagent, absorbance of the filtrates was measured at 340 nm and calculated depending on the Gly-Leu standard curve. The low molecular components (LMC) were also kept frozen (-18℃) until for the further radical scavenging activity analysis.

E. Radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) and ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt) were used as free radicals to evaluate the antioxidant activity of LMC. DPPH• free radical scavenging activity of LMC was estimated according to the procedure described by Yamaguchi et al., (1998) with slight modifications. A 96 well plate was used to read the decrease in absorbance at 517 nm by an ELISA reader at the end of the reaction time of 16 min. The percentage of remaining DPPH• was calculated by the formula:

DPPH scavenging effect % = [1- absorption of LMC/ absorption of a blank sample] ×100%.

ABTS free radical scavenging ability was determined according to the method modified by Re et al. (1999). The decrease in absorbance (734 nm), reflecting the ABTS radical scavenging capacity of LMC, was obtained and calculated as ABTS scavenging effects % :

ABTS scavenging effect % = [1- absorption of LCM/ absorption of a blank sample] ×100%.

III. RESULTS AND DISCUSSION

The 16S rDNA sequences of strains which were isolated using MRS agar were determined and confirmed by PCR-amplified gene fragments (Fig. 1). The identification obtained from PCR assays showed 98 % (compare base 1494, accession number DQ294960, source GB) agreed with *Pediococcus acidilactici* 16S rDNA sequencing identification. When Si-raw samples fermented at 25℃ for 7 days, lactic acid bacteria were reached and maintained above 6-8 log cfu/g. As shown in Fig. 2, both natural lactic acid bacteria and inoculated *Pediococcus acidilactici* could be adapted at this ferment condition. However, as shown in Fig. 3, the increase in acidity of Si-raw samples with or without inoculated with *Pediococcus acidilactici* had a smilar pattern during the fermentation stage. This implies parts of original bacteria may be related to acid production. Similar patterns of the decrease in pH values which might further influence the growth of lactic acid bacteria were also observed for the samples with or without inoculated with *Pediococcus acidilactici* .

Changes in the sarcoplasmic protein between the samples with/without inoculation were without remarkably different Disappearnce of the components with higher molecular weight were detected after 2 days of fermentation (Fig. 4). Diversity results showed that the low molecular components (LMC) appeared. This implies that the protein mignht be hydrolyzed by endogenetic autolysis and also due to bacteria proteolytic activity. The antioxidant properties of LMC have been reported recently (Kullisar et al., 2002; Stecchini et al., 2001). Based on the reaction of OPA with free

primary amino groups, a remarkable increase of extractable LMC contents was observed after 4 days of fermentation (Fig. 5). This results implies proteolysis of sarcoplasmic was beneficial in the small molecular production.

LMC was extracted at different stages and the scavenging activities for DPPH & ABTS radical scavenging were measured to evaluate the antioxidant efficacy. Results showed that the radical scavenging abilitis of LMC was fementation time related. The Sir-raw fermented at 25℃ for 7days had increasing DPPH & ABTS radical scavenging abilitis, while the samples inoculated with *Pediococcus acidilactici* appeared to have a better ABTS scavenging ability than the control samples. Using the DPPH & ABTS assay, many studies have been demonstrated that peptide is critical to lipid oxidative inhibition (Kawashima et al., 1979; Saito et al., 2003). From the results of this study, Si-raw could be used to provide some biological components after fermentation.

IV. CONCLUSION

In conclusion, the antioxidant activities of the small molecular protein extracts from Si-raw which was previously inoculated with *Pediococcus acidilactici* as a starter or natural flora fermentation. Furthermore studies are needed to focusing on the production, identification, and utilization of these functional extracts.

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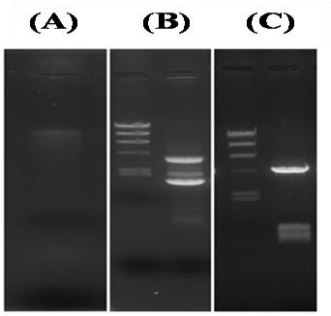

Fig.1 Agarose gel electrophoresis of amplified 16S rDNA of *Pediococcus acidilactici* isolated from the Bamboo shoots garden soil.(A) original DNA (B) undigested 16s rDNA (C) *Eco*RI-digested 16S rDNA of *Pediococcus acidilactici* Uga146-3 at Similarity (Compare Base) of 98% (1494).

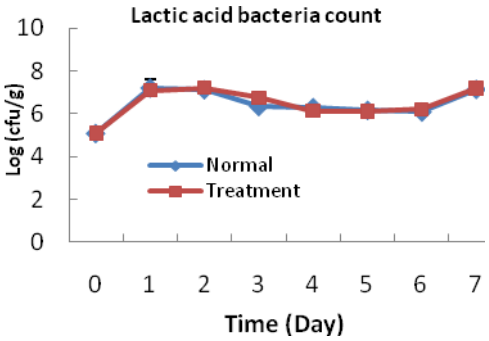

Fig. 2 Changes in bacterial count of during Si-raw fermentation.

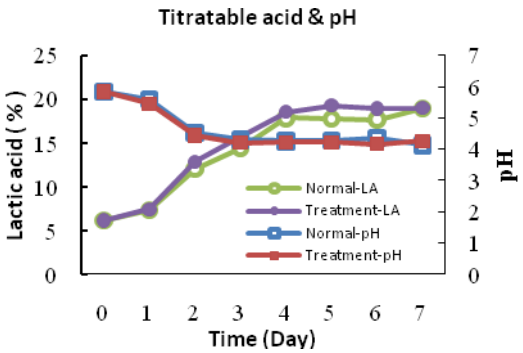

Fig. 3 Changes in titrable acid and pH value during Si-raw fermentation.

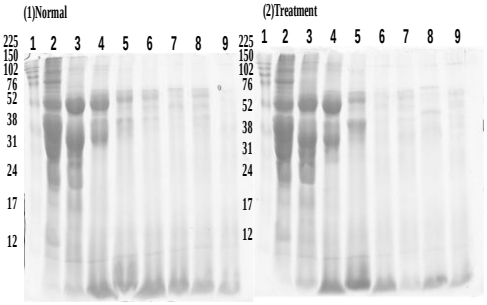

Fig. 4 Electrophoretic patterns of Si-raw sarcoplasmic protein obtained by SDS-PAGE. (lane 1 MW markers, lanes 2-9 correspond to 0-7 days of fermentation)

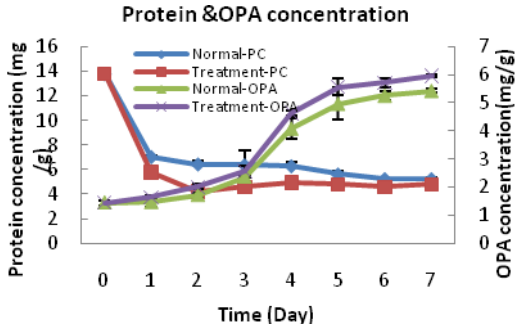

Fig. 5 Changes in soluble protein and peptide concentration during Si-raw fermentation.

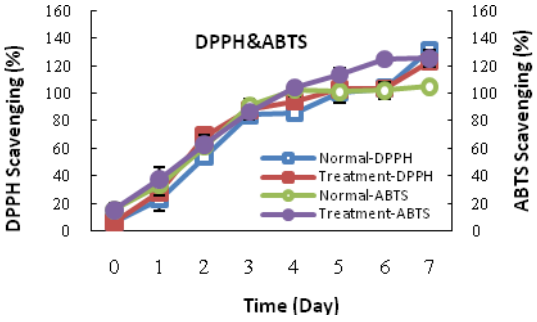

Fig. 6 Evolution of DPPH and ABTS radical scavenging ability of LMC during different Si-raw extracted stage.