Pre-screening of biogenic amine production from isolated bacteriocin-producing lactic acid bacteria associated in traditional Thai fermented meat products

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Abstract – Bacteriocin-producing lactic acid bacteria (LAB) strains isolated from various traditional Thai fermented meat products were pre-screened for biogenic amine (BA) production using the improved decarboxylase broth and plate supplement with 0.005% pyridoxal-5-phosphate and 0.006% brom cresol purple under anaerobic condition at 37º C for 4 days. Tyrosine, histidine, lysine and ornithine are the amino acid base used for BA production in this study. The results informed that many strains could possible to produce BA only from tyrosine, only one *W. cibaria* (KMITL-QU 21) could possible to produce BA from all studied amino acids. *P. pentosaceus* TISTR 536 and *Lb. plantarum* KMITL-QU 54 are the strains that showed no BA production from all studied amino acids. Therefore, both of these strains are decided to be further used as starter cultures to improve the quality and safety of traditional Thai fermented meat products.

Key Words – Biogenic amines, bacteriocin-producing lactic acid bacteria, traditional Thai fermented meat products

I. INTRODUCTION

Biogenic amines (BA), are organic bases of low molecular weight, can be formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms, and are usually produced by the decarboxylation of free amino acids or by amination and transamination of aldehydes and ketones. Removal of the α-carboxyl group from a precursor amino acid leads to the corresponding BA (1). Histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, and spermidine are the most important BA in foods (2). Many reports informed that different BA levels in final products depending on the presence of decarboxylating microorganisms, either derived from environmental contamination or from starter cultures, and the conditions supporting their growth and activity (3). Since there are many reports informed the beneficial effect of using lactic acid bacterial (LAB) starter cultures in the production of fermented meat products especially the bacteriocin-producing strains (4, 5), this has been known that starter cultures especially with bacteriocin-producing strains are able to compete with nonstarter bacteria during the later phase of ripening and throughout storage can further avoid excessive biogenic amines production (4, 6). The inability of the culture to form BA but also its ability to grow well at the temperature intended for processing of the product and competitiveness in suppressing the growth of wild amine producing microflora should be taken into consideration in the selection of starter cultures (6). Our previous study had informed many LAB strains from Thai fermented meat products which known to produce various kinds of bacteriocins (5). These bacteriocin-producing LAB strains decide to be used as starter cultures for the good quality and safety improvement in our Thai fermented meat production. Thus, the preliminary investigation of BA production by these isolated bacteriocin-producing LAB was conducted and reported in this study.

II. MATERIALS AND METHODS

Bacteriocin-producing LAB strains

Pediococcus pentosaceus TISTR 536 (pediocin PA-1 producer), *P. pentosaceus* M13 (pediocin PA-1 producer),, *Lactococcus lactis* N100 (nisin Z producer), *Lb. plantalum* SS7 (plantaricin W producer), *Lb. plantalum* KMITL-QU54 (pediocin like bacteriocin producer) and *Weisella cibaria* KMITL-QU21 (produce unknow bacteriocin) isolated from traditional Thai fermented meat products (5) were used in the study.

Activation and subculturing of LAB strains

In order to promote the enzyme induction before the actual screening test, all LAB strains which kept in Freeze-dried form were activated by growing them in de Man-Rogosa-Sharpe (MRS) broth for 24 h. at 37ºC. All strains were subcultured 3 to 5 times in MRS broth containing 0.1% of each precursor amino acid comprising L-Tyrosine disodium salt hydrate (Tyr), L- Histidine monohydrochloride monohydrate (His), L-Lysine monohydrochloride (Lys), and L-Ornitine monohydrochloride (Orn).

Qualitative screening of biogenic amines formation of bacteriocin-producing LAB strains

10 µl of each subcultured strain was screened for Tyr, Hist, Lys, and Orn formation by growing each strain in the Improved decarboxylase medium (IDM) broth in addition to supplementation 0.005% of pyridoxal-5-phosphate and 0.006% of

Bromcersol purple as described by Bover-Cid and Holzapfel (7) compared to the IDM broth without amino acid (as control). Then, all broths were incubated under anaerobic condition for 4 days at 37°C. Meanwhile, one loop of each subcultured strain was confirmed by streaking in duplicate on IDM plates as described by Bover-Cid and Holzapfel (7) compared to the IDM plate without amino acid (as control). All plates were also incubated under anaerobic condition for 4 days at 37°C. Positive decarboxylase results to cause BA in both medium broths and plates were recorded when a purple color occurred in the IDM broth and around the colonies on IDM plate or Tyr precipitate disappeared around the colonies or in the IDM broth.

III. RESULTS AND DISCUSSION

The study of pre-screening for biogenic amine production of isolated bacteriocin-producing LAB from traditional Thai fermented meat products was informed in the IDM broth and on the IDM plates (Table 1). It was revealed that *P. pentosaceus* TISTR 536 and *Lb. plantarum* KMITL-QU 54 showed no BA formation with 4 amino acids used in this study. *P. pentosaceus* M13, *Lb. plantarum* SS7 and *Lc. lactis* N100 revealed to produce only BA from Tyr (no precipitate in the IDM broth and on IDM plate), while *W. cibaria* KMITL-QU21 revealed to produce BA from all amino acids (Tyr, Lys, His and Orn) that used in this study.

LAB especially the inability to form BA but also its ability to grow well at the temperature intended for processing of the product and competitiveness in suppressing the growth of wild amine producing microflora should be taken into consideration in the selection of starter cultures for meat fermentation (6). The formation by the bacteriocin-producing strains can increase their competitiveness especially with pathogenic bacteria (4, 5). A rapid pH decrease caused by amine negative starter cultures can largely prevent biogenic amines accumulation in fermented meat products. Moreover, starter cultures able to compete with nonstarter bacteria during the later phase of ripening and throughout storage can further avoid excessive BA production (5, 6). Therefore, *P. pentosaceus* TISTR 536 and *Lb. plantarum* KMITL-QU54 are the possible strains for using as starter cultures in order to gain the good and safety production for traditional Thai fermented meat products.

Table 1 Possibility of biogenic amine (BA) production results by Lactic Acid Bacteria on the improved decarboxylase medium plate

LAB strain	Tyrosine	Lysine	Histidine	Ornithine
P. pentosaceus (536)	-*	-	-	-
P. pentosaceus (M13)	+*	-	-	-
Lb. plantarum (SS7)	+*	-	-	-
Lb. plantarum (KMITL-QU 54)	-*	-	-	-
Lc. lactis (N100)	+*	-	-	-
W. cibaria (KMITL-QU 21)	+*	+	+	+

-* = precipitate not disappear, +* = precipitate disappear,
- = color not change to purple, + = color chage to purple

IV. CONCLUSION

The study of BA production of isolated bacteriocin-producing LAB strains from traditional Thai fermented meat products using the IDM in both broth and plate informed that *P. pentosaceus* TISTR 536 and *Lb. plantarum* KMITL-QU 54 are the strains that showed no BA production from all studied amino acids. Therefore, both of these non BA-producing strains are decided to be further used as starter cultures to improve the quality and safety of traditional Thai fermented meat products.

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