International Journal of Agricultural Technology 2017 Vol. 13(7.1): 1487-1504
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Detection and Identification of Bacterial Contamination in Meat by Matrix-Assisted Laser Desorption Ionization-Time of Flight - Mass Spectrometry

Aueangporn Somsri[1], Komkhae Pilasombut[2], Nualphan Ngamyeesoon[3] and Kittaporn Rumjuankiat[1*]

[1]Faculty of Biotechnology, College of Agriculture Innovation Biotechnology and Food, Rangsit University, Lak Hok, Mueang Pathum Thani District, Pathum Thani 12000, Thailand.; [2]Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand. [3]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Somsri, A., Pilasombut, K. and Rumjuankiat, K. (2017). Detection and Identification of Bacterial Contamination in Meat by Matrix-Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry. International Journal of Agricultural Technology 13(7.1): 1487-1504.

The detection and species confirmation of bacterial contaminated in chicken and pork were demonstrated by selective media, morphology and biochemical identification and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF MS). All meat samples both chicken and pork were randomly obtained from traditional market in eastern Bangkok, Thailand and 10 samples of each were taken for these studies. The selective media including xylose lysine deoxycholate agar (XLD) and salmonella shigella agar (SS) were used to detect *Salmonella* and *Shigella* species, while baird-parker agar (BP) and chromocult coliform agar (CC) were performed to analyse *Staphylococcus aureus* and coliform/*E. coli* bacteria. In addition, plate count agar (PCA) was also used for total bacteria count. Subsequently, bacterial randomly isolated from selective media were further identified by biochemical test. Meanwhile, bacterial contaminant from total plate count were studied using morphology test. Then, the species confirmation were performed by MALDI-TOF MS. There were 10, 12, 10 and 8 isolates, which were isolated from chicken and pork, collected from XLD and SS, CC, BP and PCA, respectively. All forty isolates were identified using MALDI-TOF MS as thirteen genera of *Proteus*, *Citrobacter*, *Staphylococcus*, *Salmonella*, *Serratia*, *Enterobacter*, *Escherichia*, *Lactococcus*, *Klebsiella*, *Aeromonas*, *Morganella*, *Macrococcus* and *Acinetobacter*. Considering to the isolate species, 8 isolates of *P. mirabilis*, *C. freundii*, *S. warneri*, *E. coli*, *K. pneumoniae*, *A. caviae*, *A. veronii* and *Salmonella* spp. were obtained from both chicken and pork. On the other hand, 4 isolates of *M. caseolyticus*, *M. morganii*, *S. aureus* and *A. baumannii* were only detected in pork, whereas only 5 isolates consisted of *S. pasteuri*, *S. epidermidis*, *S. fonticola*, *E. asburiae* and *L. lactis* were only detected in chicken.

Keywords: MALDI-TOF MS, bacterial contamination, meat

* **Coressponding Author**: Kittaporn Rumjuankiat; **E-mail address**: Kittaporn.r@rsu.ac.th

Introduction

Throughout the world, meat is the one of highly desirable food that is eaten routinely. Meat is an abundant source of nutrient comprised protein, vitamins and minerals causing highly susceptible to spoilage due to its high nutrient content. Therefore, public health have to concern about the safe steps in food handling, cooking, and storage for consumer (Angkititrakul *et al.*, 2013). Normally, a category of sources conduct to microbial contamination during slaughtering, dressing, chilling and cutting processes (Koutsoumanis and Sofos, 2004). Although these processes are a good hygienic practices, bacterial contamination still occur (Olajuyigbe *et al*., 2006). Meat animal carcasses and meat cuts are easily contaminated by spoilage bacteria including of *Pseudomonas*, *Micrococcus* spp., *Staphylococcus* spp., *Acinetobacter*, *Moraxella* and Enterobacteriaceae (Koutsoumanis and Sofos, 2004). While *Salmonella* spp., *Eschericia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* are the pathogenic bacteria which were isolated from fresh meat (Harris *et al.*, 2003; Ali *et al.*, 2010). However, the source of microbial contamination on meat comes from two major stages, contamination between live animals and post-slaughter contamination. Especially post-slaughter bacteria may come in contact with the meat carcass during processing and, if not properly handled, processed and preserved, they may support growth and serve as sources of various spoilage and pathogenic microorganisms. A variety of sources contribute microbial contamination during slaughtering, dressing, chilling and cutting processes when the muscles of animals are exposed to the environment. Sources of contamination include air, water, soil, faeces, processing, equipment, utensils and humans (Huffman, 2002; Koutsoumanis and Sofos, 2004).

Thailand is located in tropical regious. The markets in Thailand are classified in two categories; traditional open market under limited temperature, hygiene control and modern super market under better condition for food hygiene. These differences may cause the difference of the microbial quality of meat sold in each kind of the markets (Ananchaipattana *et al.*, 2016). Meat especially, pork, beef and chicken is the major diet for Thai people (Angkititrakul *et al.*, 2013). In the studies case of Thailand, 388 sample of raw meat (beef, chicken, pork and shrimp) purchased from open markets and supermarket exhibited statically ($P<0.05$) high contamination rate of *Salmonella* in 22 of 66 (36%) open market samples and in 12 of 75 (16%) sample from supermarkets (Minami *et al.*, 2010).

The application of suitable detection and identification techniques may also provide valuable information on the bacterial contamination, assisting the role of microorganisms in meat. The traditional methods of bacterial isolation and biochemical testing are laborious and number of techniques have been developed to allow more rapid detection and identification (Muhamadali *et al.*, 2016). The choice of bacterial identification methods is matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The beneficial of method are require minimal sample preparation, identification of wide range of bacteria and determine the genus, species and even subspecies of bacterial isolates (Nacef *et al.*, 2017). This method requires the biopolymer molecule normally present in the condensed phase be converted into intact, isolate ionized molecules in the gas phase. Then, ions are separated according to their molecules weight after migration in an electric field. Each molecule detected is characterized by: the molecular mass (m), the charge (z), the ratio mass/charge (m/z), and the relative intensity of the signal. The application of mass spectrometry are very large, comprising highly accurate analysis of peptide and determination of peptide sequences to identify and characterize the state of protein in biological sample (Carbonnelle *et al.*, 2011; Lasch *et al.*, 2016). Nicolaou *et al.*, (2012) have been studies about the detection and quantification of bacterial spoilage in milk and pork meat using MALDI-TOF MS and Multivariate Analysis. The result shown in this study that MALDI-TOF-MS is a sensitive technique in regard to detecting microbiological spoilage in milk and meat, and it is also very fast.

Therefore, the purpose of this study was to detect and biochemical identify of bacterial contamination in pork and chicken meat, then confirmed species by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Materials and methods

Meat samples preparation

Twenty fresh meat samples comprised 10 pieces of chicken breast and 10 pieces of pork loin were randomly purchased from 20 shops in open market, Ladkrabang, Bangkok, Thailand during April to May 2017. Briefly, twenty-five grams of each sample was placed in stomacher bag and mixed with 225 ml of normal saline (0.85% NaCl; Scharlau, France). The sample was homogenized in a stomacher (400 VW, Bag Mixer, France) in order to perform serial dilution. However, only the sample for *Salmonella* isolation was pre-enriched

in 225 ml of tryptic soy broth (TSB; Merck, Germany) before homogenization and then incubated for overnight at 37℃.

Morphology analysis

All bacterial colonies, which were obtained from coliform and total microbial count, were randomly collected for morphological analysis. Each bacterial with different colony phenotype was streaked on nutrient agar (NA; Merck, Germany) for single colony and then propagated in nutrient broth medium (NB) under aerobic conditions by shaking at 200 rpm at 37°C overnight. Their morphologies were determined using an optical microscope (Primo Star, USA).

Detection of bacterial contamination in meat samples

***Salmonella* spp.**

One millilitre of TSB cultured pre-enrichment was inoculated into 10 ml of tetrathionate broth base (TTB; Merck, Germany) and selenite cystine broth (SCB; Merck, Germany). Subsequently, all samples were incubated at 37℃ for 18-24 h. After enrichment, each sample was streaked onto xylose lysine desoxycholate (XLD; Merck, Germany) agar and salmonella shigella (SS; Merck, Germany) agar and observed after 24 h. Colorless colonies with black center on SS agar and slightly transparent red colonies with black center on XLD agar were suspected as *Salmonella*. Afterward, the presumed positive isolates from XLD or SS agar were confirmed by biochemical test (FDA-BAM, 2007).

S. aureus

The volume of 0.1 ml from serial dilution sample was spread onto baird-parker (BP; Merck, Germany) agar plate and incubated at 37℃ for 18-24 h. Characteristic appearance of jet black colonies and shiny with narrow white margins and surrounded by clear zone were considered to be presumptive of *S. aureus* and then, tested with biochemical test (FDA-BAM, 2016).

Coliform*/E. coli*

For coliform*/E. coli* species isolation, 0.1 ml of each dilution was spreaded onto chromocult coliform (CC; Merck, Germany) agar medium and incubated at 37℃ for 24 h. After incubation, colonial morphology of coliform and *E. coli* were pink and dark blue colonies, respectively. Later, only the colonies of *E. coli* were test with biochemical test as the second steps (AOAC, 2006).

Total plate count

Each 0.1 ml of serial dilution (10^{-1}-10^{-5}) was spread on plate count agar (PCA; Merck, Germany) and incubated at 37℃ for overnight (18-24 h). The colonies were sampling for determination (AOAC, 2006).

Biochemical tests of Salmonella* spp., *S. aureus and E. coli

Selective media were used for colonies screening as primary procedure. Afterwards, the suspected colonies of *Salmonella* spp., *S. aureus and E. coli* were tested by biochemical test. For the presumptive colonies of *Salmonella* spp. were transferred to both triple sugar iron agar (TSI; Merck, Germany) and motility indole-lysine (MIL; Merck, Germany) medium for overnight incubation at 37℃. Positive test of TSI was observed from red slant, yellow butt and H_2S produced (black butt) while, MIL characters change were investigated by 3 procedures as (i) stab line was a positive test for motility, (ii) a purple bottom was a positive test for lysine-decarboxylase and (iii) a red to pink reaction indicated the presence of indole and persistence of the bright yellow layer indicated a negative test. While, the pure cultures of *S. aureus* were confirmed by slide coagulase tests using rabbit plasma. Coagulase positive was investigated from clumping in coagulated plasma drop. For the suspected of *E. coli*, colonies were cultured in 5 ml of tryptophan broth (TB; Merck, Germany) and incubated at 37℃ overnight (18-24 h). The broth cultures were tested for indole production by 1-2 drop of Kovac's indole reagent, indole positive test of *E. coli* were detected by a red ring forms around the top surface of the TB. Biochemical identification of isolated pure culture was carried out by classical method according to Bergey's Manual of Determinative Bacteriology.

Bacterial identification by MALDI-TOF mass spectrometry

Colonies were picked up from selective media of each culture. These colonies were streaked on NA and incubated at 37℃ for 18-24 h. Single colony was then sequenced by Betagro Science Center Co., Ltd., Pathum Thani, Thailand. Briefly, all culture samples were confirmed using MALDI-TOF MS. Initially, single colony was smeared onto 96 well MALDI-TOF target plate. Subsequently, 1 µl of ready to use matrix solution which was a saturated solution of α-cyano-4-hydroxycinnamic acid was added. Finally, spots were dried at room temperature and then the samples were automatically analyzed using a MALDI-TOF mass spectrometer (Microflex, Bruker, Germany). For species identification, the Biotyper database 3.0 was determined. An identification (ID) score exceeding 2.3 (green colour) is predicted a highly

probable species identification; score 2.0-2.29 (green colour) implies that secure genus and probable species identification; score 1.70-1.99 (yellow colour) suggests that only probable genus identification and score values below 1.70 (red colour) means no significant similarity in any database.

Results

Morphological analysis

Bacterial contaminated isolations in raw meat were isolated by methods for *Salmonella* spp., *S. aureus*, coliform/*E. coli* and total microbial count. Forty isolates, which were isolated from 20 pieces of chicken and pork, were observed for their morphological and biochemical test. For morphological characteristic, bacteria were classified to 3 groups including, (i) Gram-negative bacteria in rod shape, (ii) Gram-positive bacteria in coccus shape, (iii) Gram-positive bacteria in spherical shape. Two groups of Gram-positive bacteria in coccus shape and Gram-positive bacteria in spherical shape were only found in chicken samples, whereas a group of Gram-negative bacteria in rod shape was found in both pork and chicken samples (Table 1).

Table 1. Coliform and total microbial count morphological characteristic using selective media

Samples	**Media**	**Morphology**		
		Colony color	Gram strain	Shape
Coliform				
Chicken				
KC-3-4	CC	pink	-	rod
KC-1-5	CC	pink	-	rod
Pork				
KP-5-7	CC	pink	-	rod
KP-6-1	CC	pink	-	rod
Total microbial count				
Chicken				
TC-2-3	PCA	white	-	rod
TC-6-7	PCA	white	+	coccus
TC-4-1	PCA	white	-	rod
TC-2-7	PCA	white	+	spherical
Pork				
TP-5-2	PCA	white	-	rod
TP-1-2	PCA	white	-	rod
TP-1-1	PCA	white	-	rod
TP-2-3	PCA	white	-	rod

Legend: + Positive, - Negative

Presumptive bacterial contaminants and confirmation test

The presumptive colonies consisted of 10 *Staphylococcus* colonies and 8 *E. coli* colonies were selected by selective media as the primary step. These colonies were chosen from black and shiny with narrow white margins and surrounded by clear zone colonies on BP agar for *S. aureus* detection, while dark blue colonies were collected from CC agar for *E. coli* investigation. The percentage of coagulase-positive *S. aureus* and indole-positive of *E. coli* were 30 and 75, respectively. Hence, the analysis of coagulase-negative *S. aureus* and indole-negative of *E. coli* supposed that they were the other species as shown in Table 2.

Table 2. Biochemical analysis of *S. aureus* and *E. coli* using coagulase and indole test

Meat samples	**Biochemical methods**	**Positive**		**Negative**	
		Isolates	Detection (%)	Isolates	Detection (%)
Pork	Coagulase test (*S. aureus*)	SP4-3, SP6-2	20	SP3-1, SP5-1, SP7-1	30
	Indole test (*E. coli*)	EP1-4, EP2-6, EP4-1	37.5	EP6-2	12.5
Chicken	Coagulase test (*S. aureus*)	SC4-2	10	SC1-4, SC1-5, SC2-5, SC3-5	40
	Indole test (*E. coli*)	EC-1-6, EC-2-1, EC-6-2	37.5	EC-4-6	12.5

The presumptive colonies of 10 *Salmonella* spp. were collected by selective media (XLD and SS agar) and then chosen from only black colonies. The result showed 2 positive tests of TSI and MIL in both chicken and pork as shown in Table 3**.**

Table 3. Biochemical analysis for the detection of *Salmonella* species.

Samples	TSI				MIL			
	Slant	Butt	H_2S	Gas	Top	Bottom	Indole	Motility
Pork								
P3-SS-1	Red	Yellow	+	-	Purple	Yellow	-	+
P1-TS-1	Red	Yellow	+	-	Purple	Purple	-	+
P1-SX-1	Red	Yellow	+	-	Purple	Yellow	-	+
P3-SX-1	Red	Yellow	+	-	Purple	Purple	-	+
P5-TS-2	Red	-	+	-	Purple	Purple	-	+
Chicken								
C3-TS-2	Yellow	Yellow	+	+	Purple	Yellow	-	+
C7-TX-2	Red	Yellow	+	-	Purple	Purple	-	+
C5-SS-4	Red	Yellow	+	-	Purple	Yellow	-	+
C5-SX-1	Red	Yellow	+	-	Purple	Purple	-	+
C7-SS-1	Red	Yellow	+	-	Purple	Yellow	-	+

Legend: + Positive, - Negative

Identification of bacterial contaminants by MALDI-TOF MS analysis

During study period, a total of 40 isolates were identified by conventional method using selective media and biochemical test. Therefore, the species confirmation of all bacteria were identified by MALDI-TOF MS. The results of MALDI-TOF for 40 isolates showed a valid score (score x ≥ 2.3; an accurate probable identification at the species level) approximately 45% (18 isolates), whereas the intermediate score (2.0 ≤ score x < 2.29; an accurate probable identification between the genus and the species level) exhibited about 55% (22 isolates) and no reliable identification (score x< 1.7; no significant similarity) as shown in Table 4. Among 40 isolates tested, an accurate result isolated by MALDI-TOF identification to the species level was corrected for 45% (18/40) of pathogenic bacteria including *Salmonella* spp., *E. coli, S. aureus, K. pneumoniae*, whereas 55% (22/40) of spoilage bacteria such as *P. mirabilis, C. freundii, S. epidermidis, S. pasteuri, S. warneri, M. caseolyticus, A. baumannii, A. caviae, M. morganii, E. asburiae, S. fonticola, L. lactis, A. veronii* was correctly. As a result, there were thirteen genera of *Proteus*, *Citrobacter*, *Staphylococcus*, *Salmonella*, *Serratia*, *Enterobacter*, *Escherichia*, *Lactococcus*, *Klebsiella*, *Aeromonas*, *Morganella*, *Macrococcus* and *Acinetobacter*. However, all 8 isolates consisted of *P. mirabilis*, *C. Freundii*, *S. warneri*, *E. coli*, *K. pneumoniae*, *A. caviae*, *A. veronii* and *Salmonella* spp. were detected from both chicken and pork, while 4 isolates of *M. caseolyticus*, *M. morganii*, *S. aureus* and *A. baumannii* were mearly detected in pork. There were 5 isolates

comprised *S. pasteuri, S. epidermidis, S. fonticola, E. asburiae* and *L. lactis* could be found only in chicken as shown in Table 4.

Table 4. Identification of bacterial contaminants isolated from chicken and pork using MALDI-TOF MS analysis

Samples	Number of strain		Identification Score				Bacterial identification
	Chicken	Pork	<1.7	1.7-2.0	2.0-2.29	>2.3	
Pathogenic bacteria							
C5-SS-4, C5-SS-1, C7-SS-1, P1-SX1, P3-SX-1	3	2	-	-	4	1	*Salmonella* spp.
TP1-2, EC4-5, EC2-1, EC6-2, EC1-6, EP6-2, EP2-6, EP4-1, EP6-1	4	5	-	-	-	9	*E. coli*
SP6-2, SP4-3	-	2	-	-	2	-	*S. aureus*
TP5-2, KC3-4	1	1	-	-	-	2	*K. pneumoniae*
Spoilage bacteria			-	-			
C7-TX-2, P1-TS-1	1	1	-	-	-	2	*P. mirabilis*
C3-TS-2, P3-SS-1, EP-1-4	1	2	-	-	2	1	*C. freundii*
SC4-2	1	-	-	-	1	-	*S. epidermidis*
SC1-5, SC2-5, TC6-7	3	-	-	-	3	-	*S. pasteuri*
SC1-4, SC3-5, SP7-1	2	1	-	-	3	-	*S. warneri*
SP3-1, SP5-1	-	2	-	-	2	-	*M. caseolyticus*
TP1-1	-	1	-	-	-	1	*A. baumannii*
TP2-3	-	1	-	-	1	-	*A. caviae*
P5-TS-2	-	1	-	-	-	1	*M. morganii*
TC2-3	1	-	-	-	1	-	*E. asburiae*
TC4-1	1	-	-	-	1	-	*S. fonticola*
TC2-7	1	-	-	-	1	-	*L. lactis*
KC1-5, KC5-7	1	1	-	-	1	1	*A. veronii*
Total	20	20	-	-	22	18	

Table 5. Comparison between conventional method and high-throughput identification analyzed by MALDI-TOF MS of *Salmonella* spp., *S. aureus* and *E. coli*

Samples	Bacterial identification	
	Biochemical test	MALDI-TOF MS
Salmonella spp.		
P1-TS-1	*Salmonella* spp.	*Salmonella* spp.
P3-SX-1	*Salmonella* spp.	*Salmonella* spp.
C5-SX-1	*Salmonella* spp.	*Salmonella* spp.
C7-TX-2	*Salmonella* spp.	*Salmonella* spp.
C7-SS-1	Unknown	*Salmonella* spp.
P3-SS-1	Unknown	*C. freundii*
C3-TS-2	Unknown	*C. freundii*
P5-TS-2	Unknown	*M. morganii*
C5-SS-4	Unknown	*P. mirabilis*
P1-SX-1	Unknown	*P. mirabilis*
% Detection of *Salmonella* spp.	40	*50*
S. aureus		
SP4-3	*S. aureus*	*S. aureus*
SP6-2	*S. aureus*	*S. aureus*
SC4-2	Unknown	*S. epidermidis*
SP3-1	Unknown	*M. caseolyticus*
SP5-1	Unknown	*M. caseolyticus*
SP7-1	Unknown	*M. caseolyticus*
SC1-4	Unknown	*S. warneri*
SC1-5	Unknown	*S. pasturi*
SC2-5	Unknown	*S. pasturi*
SC3-5	Unknown	*S. pasturi*
% Detection *of S. aureus*	20	20
E. coli		
EC2-1	*E. coli*	*E. coli*
EP2-6	*E. coli*	*E. coli*
EP4-1	*E. coli*	*E. coli*
EC1-6	*E. coli*	*E. coli*
EC6-2	*E. coli*	*E. coli*
EP6-2	Unknown	*E. coli*
EP4-6	Unknown	*E. coli*
EP1-4	Unknown	*C. freundii*
% Detection *of E. coli*	62.4	87.5

For *Salmonella* spp. detection, both method of biochemical test and MALDI-TOF MS identified such as P1-TS-1, P3-SX-1, C5-SX-1 and C7-TX-2, while the isolates of P3-SS-1, C5-SS-4, C7-SS-1, P1-SX-1, P5-TS-2 and C3-TS-2 could not identify by biochemical test but it could identify by MALDI-TOF MS and there were 4 species comprised *C. freundii*, *P. mirabilis, Salmonella* spp., *M. morganii* as shown in Table 5. *Salmonella* spp. detection percentage showed a value of 40 and 50 when using biochemical test and MALDI-TOF MS, respectively. Although, the isolates of SP4-3 and SP6-2 were identified as *S. aureus* using both method for *S. aureus* isolation, 8 isolates including SC4-2, SP3-1, SP5-1, SP7-1, SC1-4, SC1-5, SC2-5 and SC3-5 could not identify by biochemical method. Nevertheless, these isolates were identified as 4 species (*S. epidermidis*, *S. warneri*, *S. pasturi and M. caseolyticus*) and the percentage of *S. aureus* detection was only 20 by MALDI-TOF MS methods.

Among 8 isolates of *E. coli* identification, biochemical test could identify as *E. coli* approximately 62.5% (5/8), whereas 87.5% (7/8) of *E. coli* could identify by MALDI-TOF MS. Only 1 isolate (EP1-4) was identified as *C. freundii* (Table 5).

Discussion

The cause of bacterial contamination in meat consisting the spread of bacteria during slaughter and processing, temperature and distribution (Nychas *et al.*, 2008). Some species of microbial contaminants originated from gastrointestinal tract of animal which had contacted during slaughtering, dressing, chilling and cutting (Koutsoumanis and Sofos, 2004). Furthermore, raw meat usually contaminated by various microorganism in open-air local retail shops (Ali *et al.*, 2010). In Thailand, Thai people usually buy raw materials from open market for cooking in their home for the day. Some raw material such as pork, chicken and beef sold in open market is supplied daily (Indrawattana *et al.*, 2011). The personal vendors normally unwrap raw meat at room temperature, resulting in these raw meat are highly contaminated with microorganisms, rodents, insects, sewage, water or human (Ananchaipattana *et al.*, 2012).

In our study, forty bacterial contaminants were isolated from fresh meat which collected from open market in Ladkrabang, Bangkok, Thailand. The samples (12 isolates) of coliform and total microbial count was studied by morphological analysis. It could be separated to three groups. The main group was Gram-negative bacteria in rod shape and could be found in both of chicken and pork samples. Based on biochemical and MALDI-TOF methods, thirteen

genus of bacterial contaminants were successfully identified as pathogenic and spoilage bacteria. The most important pathogens associated with fresh meat included *E. coli, K. pneumoniae, Salmonella* spp. and *S. aureus.*

Salmonella spp., *E. coli* and *Klebsiella* are members of the Enterobacteriaceae group, (Gwida *et al*., 2014) especially *Salmonella* spp. and *E. coli* are the species, which colonize in the gastrointestinal tracts of a variety of animals particularly animals raised for human consumption (Resta-Lenert *et al.*, 2003; Dunkley *et al.*, 2009). Additionally, these species can contact or transmit by farm animals, pets or person-to-person (Zhao *et al.*, 2001). Ordinarily, *Salmonella* spp. and *E. coli* are an indicator of food safety and sanitation (Keeratipibul *et al*., 2010). They can cause severe foodborne disease such as diarrhea and gastroenteritis (Hanson *et al.*, 2003). The previous studies revealed that *E. coli* and *Salmonella* spp. were detected in daily product, raw and undercook poultry, ground beef and pork (Zhao *et al.*, 2001; Gómez-Aldapa *et al.*, 2012). In Thailand, pathogenic *E. coli* were detected approximately 25% and 10% in chicken and pork, respectively (Sukkua *et al*., 2017). Moreover, the other bacteria can be found in meat also.

Angkititrakul *et al.*, (2013) studied about *Salmonella* species from local retail market in Thailand, the prevalence of pork and chicken showed the percentage about 65% and 75%, respectively. In our studies, the isolation of *Salmonella* spp. and *E. coli* were detected from both chicken and pork using MALDI-TOF MS analysis. Therefore, both *Salmonella* spp. and *E. coli* usually occurred in open market or retail meat (Sukkua *et al.*, 2017). *Klebsiella* is commonly an opportunistic and a hospital-acquired pathogen, causing various clinical syndromes (Frazee *et al.*, 2009). Generally, *Klebsiella* infections in humans include (i) community-acquired pneumonia, (ii) nosocomial infection (Yinnon *et al.*, 1996), (iii) urinary tract infection or UTI (López-Sastre *et al.*, 2005), (iv) rhinoscleroma and ozena, (v) Chronic pulmonary disease (Sinha *et al.*, 2003), and (vi) colonization. Normally, *K. pneumoniae* is colonizer in the gastrointestinal tracts of human and domesticated animal such as turkey, chicken and pork (Kim *et al.*, 2005; Davis *et al.*, 2015). *K. pneumoniae* is a better indicator for meat sanitation and hygiene at the retail market (Stiles and Ng, 1981). In Thailand, *K. pneumonia*e was detected in pork from central, eastern, northern, southern Bangkok about 95.65%, 80.30%, 93.75% and 79.55%, respectively (Namkratok and Nuanualsuwan, 2017). Moreover, the species of *P. mirabilis, M. morganii, E. asburiae, C. freundii* and *S. fonticola* are the member of Enterobacteriaceae as well as *Salmonella* spp., *E. coli* and *Klebsiella*. They can detect in food (Lindberg *et al.*, 1998), chicken and meat beef (Gwida *et al*., 2014).

Staphylococci are ubiquitous Gram-positive bacteria that distributed in nature or environmental source (Marino *et al.*, 2011) including dust, water, soil, humans and animals (Rumjuankiat *et al*., 2016). Staphylococci are normally bacterial microflora of human and animal, especially the skin and mucosal surface (Simeoni *et al.*, 2008). Additionally, these species are frequently isolated from various foods such as poultry, milk and minced meat (Huber *et al.*, 2011; Simeoni *et al*., 2008). *Staphylococcus* species, consisting *S. aureus* and *S. epidermidis*, can cause disease in human. Especially, *S. aureus* is the most important foodborne pathogen and causing of disease outbreak related to food consumption such as urinary tract infections and gastrointestinal tract infection (Marino *et al.*, 2011). According to, the previous studies showed the presence of *S. aureus* in food might be a possible cross-contamination by food workers (Gundogan *et al.*, 2005). In our study, *S. aureus* was absent in chicken, resulting in this sample are safe. In contrast, *S. aureus* were detected only from pork. Furthermore, *S. epidermidis* are opportunistic pathogenic species and can cause severe infections (Irlinger, 2008). It is the most predominant and persistent staphylococci that often isolated from human skin, milk and dry fermented sausages (Morot-Bizot *et al.*, 2004). Nevertheless, there are some species of Staphylococci including *S. warneri*, *S. pasteuri* and *M. caseolyticus* are nonpathogenic species and frequently detected in food and environment, respectively (Martín *et al.*, 2006; Baba *et al.*, 2009). The most common *Staphylococcus* species including *S. aureus, S. epidermidis, S. saprophyticus, S. pasturi, S. sciuri, S. warneri* and *xylosus* were isolated from food and food environments (Marino *et al.*, 2011). Obviously, these species can contaminate in food and meat if not handled properly, considered to the various food and environment. Not only bacterial contaminants as mentioned above but the others genera including *L. lactis*, *A. baumannii*, *A. caviae* and *A. veronii* also detected in food such as poultry product, fruit, vegetable, meat, poultry meat and fermented sausage etc (Berlau *et al*., 1999; Melas *et al*., 1999; Barakat *et al*., 2000; Neyts *et al*., 2000; Noonpakdee *et al*., 2003; Fournier and Richet, 2006; Li *et al*., 2011).

In recent year, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has acquainted as a high-throughput method for the routine identification of bacterial isolates (Van Veen *et al*., 2010). Based on bacterial identification, MALDI-TOF MS has been displayed to be easier, more rapid, accurate and cost-efficient than conventional phenotypic techniques or molecular methods (Pavlovic *et al*., 2013). Several research studies have been report the identification of food associated bacteria such as *Salmonella* spp. (Kuhns *et al*., 2012), *Listeria* spp. (Barbuddhe *et al*., 2008) and *vibrio* spp. (Dieckmann *et al*., 2010), *Campylobacter* spp. (Bessede

et al., 2011) using MALDI-TOF MS analysis. However, there were a few reports of bacterial identification isolated from fresh meat and meat product by MALDI-TOF MS. Recently, a number of lactic acid bacteria (LAB), which were collected from pork meat and pork meat product, were identified using SDS-PAGE, 16S rRNA gene sequencing and MALDI-TOF MS. The result exhibited that MALDI-TOF MS has been proved as an appropriate LAB identification method in this study (Nicolaou *et al.*, 2012). Consequently, MALDI-TOF MS has established to be a technique with powerful capability in bacterial characterization when compare to other methods.

Conclusion

In summary MALDI-TOF MS is promising platform for fast, flexible, and reliable identification of meat microbial isolates. The method complies with a variety of requirements for meat microbial laboratories. Especially the simple protocol and shortened analysis time assists in the maintenance of high level of food safety.

Acknowledgement

The authors gratefully acknowledge funding for this research from Rangsit University and thank for technical support from Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

References

Ali, N. H., Farooqui, A., Khan, A., Khan, A. Y. and Kazmi, S. U. (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. The Journal of Infection in Developing Countries 4(6): 382-388.

Ananchaipattana, C., BARI, M. L. and Inatsu, Y. (2016). Bacterial contamination into ready-to-eat foods sold in middle Thailand. Biocontrol Science 21(4): 225-230.

Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongsawat, S., Isobe, S. and Inatsu, Y. (2012). Bacterial contamination in retail foods purchased in Thailand. Food Science and Technology Research 18(5): 705-712.

Angkititrakul, S., Polpakdee, A. and Chuanchuen, R. (2013). Prevalence of *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* in raw meat in Thai self-service style restaurants in KhonKaen municipality. Thai Journal of Veterinary Medicine 43(2): 265-268.

AOAC. (2006). AOAC Official Methods: Chapter 17. *In* Horwitz, W. and Latimer, G.W. Official methods of analysis of AOAC international. Maryland: AOAC international.

Baba, T., Kuwahara-Arai, K., Uchiyama, I., Takeuchi, F., Ito, T. and Hiramatsu, K. (2009). Complete genome sequence of *Macrococcus caseolyticus* strain JSCS5402, reflecting the ancestral genome of the human-pathogenic staphylococci. Journal of Bacteriology 191(4): 1180-1190.

Barakat, R. K., Griffiths, M. W. and Harris, L. J. (2000). Isolation and characterization of *Carnobacterium*, *Lactococcus*, and *Enterococcus* spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat. International Journal of Food Microbiology 62(1): 83-94.

Barbuddhe, S. B., Maier, T., Schwarz, G., Kostrzewa, M., Hof, H., Domann, E., Chakreborty, T. and Hain, T. (2008). Rapid identification and typing of *listeria* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and Environmental Microbiology 74(17): 5402-5407.

Berlau, J., Aucken, H. M., Houang, E. and Pitt, T. L. (1999). Isolation of *Acinetobacter* spp. including *A. baumannii* from vegetables: implications for hospital-acquired infections. Journal of Hospital Infection 42(3): 201-204.

Bessede, E., Solecki, O., Sifre, E., Labadi, L. and Megraud, F. (2011). Identification of *Campylobacter* species and related organisms by matrix assisted laser desorption ionization–time of flight (MALDI□TOF) mass spectrometry. Clinical Microbiology and Infection 17(11): 1735-1739.

Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J. L., Ferron, A., Gutmann, L. and Nassif, X. (2011). MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clinical Biochemistry 44(1): 104-109.

Davis, G. S., Waits, K., Nordstrom, L., Weaver, B., Aziz, M., Gauld, L., Grande, H., Bigler, R., Horwinski, J., Porter, S. and Stegger, M. (2015). Intermingled *Klebsiella pneumoniae* populations between retail meats and human urinary tract infections. Clinical Infectious Diseases 61(6): 892-899.

Dieckmann, R., Strauch, E. and Alter, T. (2010). Rapid identification and characterization of *Vibrio* species using whole□cell MALDI□TOF mass spectrometry. Journal of Applied Microbiology 109(1): 199-211.

Dunkley, K. D., Callaway, T. R., Chalova, V. I., McReynolds, J. L., Hume, M. E., Dunkley, C. S., Kubena, K.L., Nisbet, D.J. and Ricke, S. C. (2009). Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe 15(1): 26-35.

FDA BAM. (2007). Bacteriological analytical manual, *Salmonella*. Chapter, pp: 5, December. 2007 edition.

FDA BAM. (2016). Bacteriological Analytical Manual, *Staphylococcus aureus*. Chapter 12, March 2016 edition.

Fournier, P. E., Richet, H. and Weinstein, R. A. (2006). The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clinical Infectious Diseases 42(5): 692-699.

Frazee, B. W., Hansen, S. and Lambert, L. (2009). Invasive infection with hypermucoviscous *Klebsiella pneumoniae*: multiple cases presenting to a single emergency department in the United States. Annals of Emergency Medicine 53(5): 639-642.

Gómez-Aldapa, C. A., del Refugio Torres-Vitela, M., Villarruel-López, A. and Castro-Rosas, J. (2012). The role of foods in *Salmonella* infections. In *Salmonella*-A dangerous foodborne pathogen. InTech : 21-46.

Gundogan, N., Citak, S., Yucel, N. and Devren, A. (2005). A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. Meat Science 69(4): 807-810.

Gwida, M., Hotzel, H., Geue, L. and Tomaso, H. (2014). Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. International Scholarly Research Notices 2014: 1-6.

Hanson, R., Kaneene, J. B., Padungtod, P., Hirokawa, K. and Zeno, C. (2003). Prevalence of *Salmonella* and *E. coli*, and their resistance to antimicrobial agents, in farming communities in northern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 33: 120-126.

Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H. and Busta, F. F. (2003). Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh□cut produce. Comprehensive Reviews in Food Science and Food Safety 2(s1): 78-141.

Huber, H., Ziegler, D., Pflüger, V., Vogel, G., Zweifel, C. and Stephan, R. (2011). Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Veterinary Research: 7(1): 6.

Huffman, R. D. (2002). Current and future technologies for the decontamination of carcasses and fresh meat. Meat Science 62(3): 285-294.

Indrawattana, N., Nibaddhasobon, T., Sookrung, N., Chongsa-nguan, M., Tungtrongchitr, A., Makino, S. I., Tungyong, W. and Chaicumpa, W. (2011). Prevalence of *Listeria monocytogenes* in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods. Journal of Health, Population, and Nutrition 29(1): 26.

Irlinger, F. (2008). Safety assessment of dairy microorganisms: coagulase-negative staphylococci. International Journal of Food Microbiology 126(3): 302-310.

Keeratipibul, S., Meethong, S., Techaruwichit, P. and Thephuttee, N. (2010). Prevalence of *Escherichia coli* and enterococci in a Thai frozen cooked chicken plant, and modeling of the cleaning and sanitizing procedure. Food Control 21(8): 1104-1112.

Kim, S. H., Wei, C. I., Tzou, Y. M. and An, H. (2005). Multidrug-resistant *Klebsiella pneumoniae* isolated from farm environments and retail products in Oklahoma. Journal of Food Protection 68(10): 2022-2029.

Koutsoumanis, K. and Sofos, J. N. (2004). Microbial contamination of carcasses and cuts. Encyclopedia of Meat Sciences: 727-737.

Kuhns, M., Zautner, A. E., Rabsch, W., Zimmermann, O., Weig, M., Bader, O. and Groß, U. (2012). Rapid discrimination of *Salmonella enterica* serovar Typhi from other serovars by MALDI-TOF mass spectrometry. PLoS One 7(6): e40004.

Lasch, P., Jacob, D., Grunow, R., Schwecke, T. and Doellinger, J. (2016). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) for the identification of highly pathogenic bacteria. TrAC Trends in Analytical Chemistry 85: 103-111.

Li, Y., Liu, Y., Zhou, Z., Huang, H., Ren, Y., Zhang, Y., Li, G., Zhou, Z. and Wang, L. (2011). Complete genome sequence of *Aeromonas veronii* strain B565. Journal of Bacteriology 193(13): 3389-3390.

Lindberg, A. M., Ljungh, A., Ahrne, S., Löfdahl, S. and Molin, G. (1998). Enterobacteriaceae found in high numbers in fish, minced meat and pasteurised milk or cream and the presence of toxin encoding genes. International Journal of Food Microbiology 39(1): 11-17.

López Sastre, J. B., Fernández Colomer, B., Coto Cotallo, G. D. and Ramos Aparicio, A. (2005). Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatrica 94(4): 451-457.

Marino, M., Frigo, F., Bartolomeoli, I. and Maifreni, M. (2011). Safety□related properties of staphylococci isolated from food and food environments. Journal of Applied Microbiology 110(2): 550-561.

Mart ń, B., Garriga, M., Hugas, M., Bover-Cid, S., Veciana-Nogu és, M. T. and Aymerich, T. (2006). Molecular, technological and safety characterization of Gram-positive catalase-positive cocci from slightly fermented sausages. International Journal of Food Microbiology 107(2): 148-158.

Melas, D. S., Papageorgiou, D. K. and Mantis, A. I. (1999). Enumeration and confirmation of *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sobria* isolated from raw milk and other milk products in Northern Greece. Journal of Food Protection 62(5): 463-466.

Minami, A., Chaicumpa, W., Chongsa-Nguan, M., Samosornsuk, S., Monden, S., Takeshi, K., Makino, S. and Kawamoto, K. (2010). Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control 21(3): 221-226.

Morot□Bizot, S. C., Talon, R. and Leroy, S. (2004). Development of a multiplex PCR for the identification of *Staphylococcus* genus and four staphylococcal species isolated from food. Journal of Applied Microbiology 97(5): 1087-1094.

Muhamadali, H., Weaver, D., Subaihi, A., AlMasoud, N., Trivedi, D. K., Ellis, D. I., Dennis, L. and Goodacre, R. (2016). Chicken, beams, and *Campylobacter*: rapid differentiation of foodborne bacteria via vibrational spectroscopy and MALDI-mass spectrometry. Analyst 141(1): 111-122.

Nacef, M., Chevalier, M., Chollet, S., Drider, D. and Flahaut, C. (2017). MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. International Journal of Food Microbiology 247: 2-8.

Namkratok, R. and Nuanualsuwan, S. (2017) Prevalence of *Klebsiella pneumoniae* contaminated pork in Bangkok Markets. Thai Journal of Veterinary Medicine 47: 89-90.

Neyts, K., Huys, G., Uyttendaele, M., Swings, J. and Debevere, J. (2000). Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. Letters in Applied Microbiology 31(5): 359-363.

Nicolaou, N., Xu, Y. and Goodacre, R. (2012). Detection and quantification of bacterial spoilage in milk and pork meat using MALDI-TOF-MS and multivariate analysis. Analytical Chemistry 84(14): 5951-5958.

Noonpakdee, W., Santivarangkna, C., Jumriangrit, P., Sonomoto, K. and Panyim, S. (2003). Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. International Journal of Food Microbiology 81(2): 137-145.

Nychas, G. J. E., Skandamis, P. N., Tassou, C. C. and Koutsoumanis, K. P. (2008). Meat spoilage during distribution. Meat Science 78(1): 77-89.

Olajuyigbe, O. O., Oluremi, B. B. and Umaru, D. G. (2006). Bacterial spoilage of fresh meat in some selected Lagos markets. Ife Journal of Science 8(2): 193-198.

Pavlovic, M., Huber, I., Konrad, R. and Busch, U. (2013). Application of MALDI-TOF MS for the identification of food borne bacteria. The Open Microbiology Journal 7: 135.

Resta-Lenert, S. and Barrett, K. E. (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 52(7): 988-9.

Rumjuankiat, K., Keawsompong, S. and Nitisinprasert, S. (2016). Bacterial contaminants from frozen puff pastry production process and their growth inhibition by antimicrobial substances from lactic acid bacteria. Food Science and Nutrition 5(3): 454-465.

Simeoni, D., Rizzotti, L., Cocconcelli, P., Gazzola, S., Dellaglio, F. and Torriani, S. (2008). Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities. Food Microbiology 25(1): 196-201.

Sinha, R., Panjabi, C., Varma, M., Vijayan, V. K. and Shah, A. (2003). Chronic *Klebsiella pneumonia* in an immunocompetent host. Journal-Association of Physicians of India 51: 306-308.

Stiles, M. E. and Ng, L. K. (1981). Enterobacteriaceae associated with meats and meat handling. Applied and Environmental Microbiology 41(4): 867-872.

Sukkua, K., Pomwised, R., Rattanachuay, P., Khianngam, S. and Sukhumungoon, P. (2017). Characterization of extraintestinal pathogenic *Escherichia coli* from meat in southern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health 48(1): 98-108.

Van Veen, S. Q., Claas, E. C. J. and Kuijper, E. J. (2010). High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. Journal of Clinical Microbiology 48(3): 900-907.

Yinnon, A. M., Butnaru, A., Raveh, D., Jerassy, Z. and Rudensky, B. (1996). *Klebsiella bacteraemia*: community versus nosocomial infection. QJM: An International Journal of Medicine 89(12): 933-942.

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. and Meng, j. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Applied and Environmental Microbiology 67(12): 5431-5436.

(Received: 30 October 2017; accepted: 25 November 2017)