Production of Biogenic Amines by Microflora Inoculated in Meats

Joong-seok Min, Sang-ok Lee, Aera Jang, Mooha Lee* and Yangha Kim[1]

School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, San 56-1 Sillim-dong, Gwanak-gu, Seoul, 151-742, Korea

ABSTRACT : The effects of microorganisms inoculated in beef, pork and chicken on the production of various biogenic amines (BA) were examined. *Acinetobacter haemolyticus, Aeromonas hydrophila subsp. hydrophila, Alcaligenes faecalis subsp. faecalis, Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Lactobacillus alimentarius, Lactobacillus curvatus, Leuconostoc mesenteroides subsp. Mesenteroides, Proteus mirabilis, Proteus vulgaris, Pseudomonas aerugina, Salmonella enteritidis* and *Salmonella typhimurium* were inoculated into beef, pork and chicken and incubated for 24 h at optimum temperatures of each bacterium. In ground beef, total amount of amines (TAA) produced was highest in the sample inoculated with *Bacillus cereus*, followed by *Enterobacter cloacae*. In ground pork, TAA was highest in the sample inoculated with *Alcaligenes faecalis*, followed by *Enterobacter cloacae*, *Proteus vulgaris* and *Bacillus cereus*. TAA of chicken breast was highest in the sample inoculated with *Alcaligenes faecalis*, followed by *Bacillus cereus* and *Lactobacillus alimentarius* while in chicken leg was the sample inoculated with *Proteus vulgaris*, followed by *Enterobacter aerogenes*, *Enterobacter cloacae* and *Alcaligenes faecalis*. Among biogenic amines produced, cadaverine (CAD) was detected at the highest level, followed by putrescine (PUT) and tyramine (TYM), their order being reversed by the kind of microorganism in beef and pork. In chicken breast and leg, CAD level was still the highest but PUT, TYM or PHM was the second highest, depending upon the kind of microorganism inoculated. In total, *Alcaligenes faecalis*, *Enterobacter cloacae* and *Bacillus cereus* were ones that produced a larger amount of BAs regardless of meat sources from different species. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1472-1478)***

Key Words : Biogenic Amines, Chicken, Beef, Pork, Bacteria, Total Amines

INTRODUCTION

Microbial spoilage of meat has always been of critical concern in the meat industry. It is generally accepted that the microorganisms on fresh carcass originate from two main sources: those derived from slaughterhouse environment and those from the intestinal tract. According to Nottingham (1992), the predominant organisms on the surface of freshly prepared carcass meat are Gram-negative bacteria such as *Acinetobacter*, *Aeromonas*, *Pseudomonas* and *Moraxella*, while other genera, including *Enterobacter* and *Escherichia*, are also found.

Biogenic amines (BA) are formed in foods as a result of amino acid decarboxylation catalyzed by bacterial enzymes. When they are consumed in sufficient quantities, BAs will cause headache, hypertension, fever and heart failure (Luthy and Schlatter, 1983; Nadon et al., 2001). A potential health risk will be elevated, especially when BAs are coupled with monoamine oxidase inhibitors, alcohol, and gastrointestinal diseases (Stratton et al., 1991). Many kinds of bacteria can decarboxylate amino acids in meat and poultry to the amines. Amino acid decarboxylases are found in certain *Enterobacteriacea*, *Clostridium*, *Lactobacillus*, *Streptococcus*, *Micrococcus*, *Pediococcus* and *Pseudomonas* species associated with meat (Shalaby, 1996). Santos (1998) reported that *Enterobacteriaceae* had higher amino acid decarboxylase activity than lactic acid bacteria (LAB) and Gram positive cocci. Durlu-Özkaya et al. (2001) suggested that the major BAs produced by *Enterobacteriaceae* were putrescine (PUT), cadaverine (CAD), tyramine (TYM) and histamine (HIM) in culture medium and meat products. LAB that are capable of decarboxylation of amino acids in various meat and meat products include *Lactobacillus buchneri*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus hilgardii*, *Carnobacterium piscicola*, and *Carnobacterium divergens* (Edwards et al., 1987; Tschabrun et al., 1990; Maijala and Eerola, 1993; Maijala et al., 1993; Butturini et al., 1995).

Lopez-Caballero et al. (2001) reported that PUT and HIM production was lowest under the 40% CO_2/60% O_2 gas mixture in *Shewanella putrefaciens* which is a microorganism specific to the spoilage of temperate-water marine fish species stored in ice (Gram et al., 1987). Gardini et al. (2001) investigated the combined effects of temperature, pH and NaCl concentration on BAs of *Enterococcus faecalis*. *Carnobacterium divergens* inoculated in meat-fat mixture was able to produce TYM (26-121 μg/g) (Masson et al., 1999). Leuschner et al. (1998) suggested that *Micrococcus varians* could oxidize TYM and decrease TYM in end products of fermented sausages. *Stenotrophomonas maltophilia* strains, the

* Corresponding Author: Mooha Lee. Tel: +82-2-880-4804, Fax: +82-2-873-4804, E-mail: moohalee@snu.ac.kr

[1] College of Human Ecology, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-gu, Seoul, 120-750, Korea.

Received February 19, 2004; Accepted July 2, 2004

Table 1. Microorganisms used for production of biogenic amines

KCCM No.	Bacteria	Medium (broth)	Optimal temp. (°C)
40205	*Acinetobacter haemolyticus*	Nutrient	26
32586	*Aeromonas hydrophila subsp. hydrophila*	Nutrient	26
40078	*Alcaligenes faecalis subsp. faecalis*	Trypticase soy	30
11204	*Bacillus cereus*	Nutrient	30
11314	*Bacillus subtilis*	Nutrient	30
11783	*Enterobacter aerogenes*	Trypticase soy	37
11909	*Enterobacter cloacae*	Nutrient	30
11234	*Escherichia coli*	Trypticase soy	37
40979	*Lactobacillus alimentarius*	Lactobacillus MRS	30
40715	*Lactobacillus curvatus*	Lactobacillus MRS	37
11324	*Leuconostoc mesenteroides subsp. mesenteroides*	Lactobacillus MRS	30
11798	*Proteus mirabilis*	Nutrient	37
11758	*Proteus vulgaris*	Nutrient	37
11328	*Pseudomonas aerugina*	Nutrient	37
12021	*Salmonella enteritidis*	Nutrient	37
11862	*Salmonella typhimurium*	Nutrient	37

psychotropic or mesophilic bacteria, were a routine screening of HIM forming bacteria in albacore tuna and showed a strong lysine decarboxylating activity (Ben-Gigirey et al., 2000). Lakshmanan et al. (2002) observed that *Micrococcus*, *Alcaligenes*, *Flavobacterium*, *Acinetobacter*, *Shewanella* and *Pseudomonas*, were the predominant amine-forming bacteria during the ice storage of fish and shrimp. Some bacteria (for example, *Lactobacillus sakei*) are able to degrade BAs by means of amino oxidases (Dapkevicius et al., 2000).

Chen et al. (2002) reported that seven biogenic amines and two polyamines concentrations for all treatments were lower than those of other fermented meat products when raw cured meat was processed with various treatments such as citric acid, sodium hypophosphite, *monascus anka* mash, plum paste, lactic acid bacteria or organic acid spray. Therefore, in this study was carried out to examine which microorganisms produce specific biogenic amines most in various non-fermented meat sources so that the effective way to control the production of biogenic amines by bacteria in meat can be sought.

MATERIALS AND METHODS

Samples

Beef and pork loins were purchased from a slaughterhouse, and chicken legs and breasts were obtained from a local market, one day after the slaughter. All samples were put in an icebox for transport to the laboratory. Only lean flesh was taken from the samples.

To destroy the microorganisms contaminated on the surface of meat, the meat samples were treated by ultraviolet radiation (254nm, 40W UV ramp) for 15 min in the clean bench and ground aseptically. Ten grams of samples were weighed into a sterilized, 50 ml polypropylene conical tube (Beckton Dickinson & Co., Franklin Lakes, USA) for inoculation of bacteria.

Reagents

Nutrient broth, bacto agar, trypticase soy broth, lactobacillus MRS broth and bacto peptone were purchased from Becton Dickinson and Co. (Sparks, USA).

Amine standards (β-phenylethylamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, serotonin creatinine sulfate, tyramine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride and 1,7-diaminoheptane), sodium bicarbonate, sodium hydroxide, ammonium acetate and dansyl chloride were purchased from Sigma Chemical Co. (St. Louis, USA). Ammonia and perchloric acid (70%) were purchased from Showa Chemical Co. (Tokyo, Japan) and acetonitrile and acetone (HPLC grade) from TEDIA (Cincinnati, USA).

Cultivation and Inoculation of Microorganisms

For microbial production of amines, 16 species of microorganisms that may be associated with meat and poultry were obtained from Korean Culture Center of Microorganisms (Table 1). Fourteen species of bacteria were in the freeze-dried state and 2 species (*Lactobacillus curvatus* and *Proteus mirabilis*) in the slant media. Each bacterium was inoculated into a test tube (15×250 mm) containing 30 ml of the proper medium and incubated in the shaking incubator (220 rpm) for 20 h at its optimal temperature (Table 1). One milliliter of the incubated broth was taken and procedures were repeated for 3 times for each bacterium. The enriched bacterial broth (approximately $>10^8$ CFU/ml) were inoculated into the prepared sample. The inoculated sample was incubated at each bacterium's optimal temperature for 24 h and then subjected to the determination of the amounts of amines

Table 2. Production of biogenic amines[1] in ground beef by different microbial species(unit: μg/g)

Inocula	PHM	PUT	CAD	HIM	SER	TYM	SPD	SPM	TAA
Control	0.0^{e}±0.0	7.6^{i}±1.5	8.4^{m}±0.8	7.6^{bc}±0.2	1.6^{fg}±0.3	4.0^{g}±0.3	10.6^{d}±0.2	42.7^{abc}±0.0	82.4^{k}±3.3
Acinetobacter haemolyticus	2.4^{cd}±2.4	279.2^{c}±0.3	495.4^{c}±0.8	3.0^{d}±0.1	23.6^{bc}±2.3	123.8^{c}±0.8	3.1^{fg}±0.1	46.8^{a}±0.2	977.1^{c}±2.0
Aeromonas hydrophila	0.0^{e}±0.0	164.9^{f}±2.1	359.4^{e}±1.0	2.9^{d}±0.3	17.7^{cde}±3.8	81.2^{def}±0.7	3.2^{fg}±0.4	45.2^{ab}±0.6	674.4^{e}±6.3
Alcaligenes faecalis	2.5^{e}±0.2	5.0^{i}±0.3	1.8^{m}±0.2	2.7^{d}±0.0	27.8^{ab}±4.8	106.8^{cd}±0.3	2.0^{gh}±0.0	31.7^{c}±0.3	180.3^{j}±4.1
Bacillus cereus	6.7^{b}±0.6	337.0^{b}±1.9	711.9^{a}±1.6	3.1^{d}±0.1	33.9^{a}±1.0	326.8^{a}±0.8	4.1^{ef}±0.1	36.5^{abc}±1.0	1,460.0^{a}±5.1
Bacillus subtilis	1.4^{de}±0.1	267.3^{cd}±1.2	295.3±0.7^{gh}	2.6^{d}±0.2	16.6^{cde}±2.7	86.6^{de}±0.3	2.7^{gh}±0.0	35.8^{abc}±0.1	708.2^{e}±3.9
Enterobacter aerogenes	0.0^{e}±0.0	195.1^{e}±3.7	311.5^{f}±6.9	8.8^{bc}±0.1	4.0^{fg}±0.7	77.0^{def}±0.2	15.0^{c}±0.3	42.4^{abc}±0.6	653.8^{e}±9.3
Enterobacter cloacae	2.8^{cd}±0.4	501.6^{a}±1.5	544.7^{b}±0.7	3.4^{d}±0.3	30.0^{ab}±5.1	200.2^{b}±0.6	5.2^{e}±0.2	33.1^{bc}±0.6	1,321.0^{b}±5.6
Escherichia coli	0.0^{e}±0.0	202.0^{e}±10.3	432.6^{d}±3.8	9.9^{ab}±0.1	4.6^{fg}±0.8	105.6^{cd}±2.8	18.7^{b}±0.8	46.8^{a}±0.7	820.0^{d}±19.3
Lactobacillus alimentarius	1.0^{de}±0.2	120.9^{g}±0.2	299.8^{g}±0.9	2.8^{d}±0.2	19.0^{cd}±4.8	78.7^{def}±0.5	1.9^{gh}±0.1	40.2^{abc}±0.4	564.3^{f}±5.8
Lactobacillus curvatus	0.0^{e}±0.0	30.2^{h}±0.6	114.4^{l}±0.3	0.7^{d}±0.4	9.2^{efg}±4.6	87.4^{de}±43.7	1.59^{h}±0.8	30.7^{c}±15.3	274.2^{i}±64.7
Leuconostoc mesenteroides	4.3^{c}±0.3	4.5^{i}±0.7	3.4^{m}±2.9	6.5^{c}±3.6	17.1^{fg}±2.8	106.2^{cd}±3.4	1.2^{h}±0.6	39.6^{abc}±1.2	182.8^{j}±6.0
Proteus mirabilis	0.0^{e}±0.0	123.8^{g}±3.5	136.2^{k}±4.6	8.3^{bc}±0.1	0.0^{g}±0.0	50.6^{f}±0.5	19.2^{b}±0.3	45.1^{ab}±0.3	383.1^{h}±1.0
Proteus vulgaris	0.0^{e}±0.0	169.8^{f}±0.9	279.7^{i}±8.9	7.2^{bc}±0.0	4.8^{fg}±0.9	63.0^{ef}±0.1	15.6^{c}±0.3	38.5^{abc}±0.3	578.6^{f}±10.6
Pseudomonas aerugina	19.4±0.8^{a}	156.2±0.6^{f}	220.7±1.2^{j}	0.7±0.4^{d}	5.2±3.0^{fg}	52.9±2.2^{ef}	43.0±0.2^{a}	7.0±0.7^{d}	505.0^{g}±4.2
Salmonella enteritidis	0.0^{e}±0.0	195.0^{e}±3.2	435.8^{d}±0.9	12.1^{a}±0.1	11.0^{def}±1.6	105.5^{cd}±3.7	15.0^{c}±1.1	38.4^{abc}±0.2	812.8^{d}±1.1
Salmonella typhimurium	0.0^{e}±0.0	261.7^{d}±18.8	286.3^{hi}±1.4	7.1^{bc}±0.3	3.3^{fg}±0.8	51.8^{ef}±4.3	15.0^{c}±0.3	37.0^{abc}±0.4	662.2^{e}±23.5

$^{a, b, c, d, e, f, g, h, i, j, k, l, m}$ Means±SE with the different superscript in the same column were significantly different ($p<0.001$).

[1] PHM (β-phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), SER (serotonin), TYM (tyramine), SPD (spermidine), SPM (spermine), TAA (total amount of amines)

produced. Control samples were prepared without the inoculation.

Determination of biogenic amines

The method of Eerola et al. (1993) was modified for the determination of biogenic amines. Two grams of the sample were weighed into a 50 ml polypropylene conical tube (Beckton Dickinson & Co., Franklin Lakes, USA) and homogenized (Ultra-Turrax 25, IKA-Labortechnik, Staufen, Germany) in 10 ml of 0.4 M perchloric acid. The homogenized sample was centrifuged for 10 min at 3,000 rpm (Union 5KR, Hanil Co., Incheon, Korea) and the supernatant was filtered through filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, England). Ten milliliter of 0.4 M perchloric acid was added to the remnant and mixed thoroughly in a vortex mixer (Vortex-Genie2, Scientific Industries, Inc., Bohemia, USA). This mixture was centrifuged for 10 min at 3,000 rpm and the supernatant was filtered again through the same type of the filter paper. Finally, the volume of filtrate collected from both steps was adjusted to 25 ml with 0.4 M perchloric acid.

One milliliter of a sample extract was taken into a 15 ml polypropylene conical tube (Beckton Dickinson & Co., Franklin Lakes, USA) and 50 μl internal standard (1,000 ppm 1, 7-diaminoheptane) was added. Two hundreds microliters of 2 N sodium hydroxide, 300 μl of saturated sodium bicarbonate and 2 ml of dansyl chloride solution (10 mg dansyl chloride dissolved in 1 ml acetone) were added to sample extract before the incubation for 45 min at 40°C in a water bath. After the incubation, 100 μl ammonia was added to the reaction mixture for the removal of residual dansyl chloride. After 30 min at the ambient temperature, the volume of the reaction mixture was adjusted to 5 ml with acetonitrile. This reaction mixture was centrifuged for 5 min at 2,500 rpm. The supernatant was filtered with a 0.45 μm syringe filter with PVDF Membrane (Acrodisc® LC13 PVDF minispike, Pall Co., Ann Arbor, USA).

Ten microliters of filtered sample was injected in HPLC with a diode array detector (Agilent 1100, Agilent Techology Inc., Wilmington, USA) equipped with Spherisorb ODS_2 column (4.6×150 mm i.d., 5 μm, Waters, Milford, USA). Gradient elution program was used with the mixture of 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. Both solvents were vacuum-filtered by membrane filter (47 mm PTFE 0.45 μm, Pall Co., Ann Arbor, USA) and degassed with ultrasonicator (5210, Branson Ultrasonic Co., Danbury, USA). The flow rate was 1 ml/min. The gradient began at 50% (solvent A) and 50% (solvent B) and ended at 10% (solvent A) and 90% (solvent B) in 19 min. Ten minutes of waiting time before next analysis was necessary for equilibrium. The column temperature was 40°C. The amount of the dansyl derivatives of the biogenic amines were quantified by measurement of UV-absorption at 254 nm.

Statistical analysis

Statistical analysis was performed with the SAS program for windows V8 (SAS, 2000). One-way ANOVA was used to calculate the means and standard error while one-way ANOVA and Duncan's multiple range tests were carried out to analyze the significant differences in the

Table 3. Production of biogenic amines[1] in ground pork by different microbial species (unit: μg/g)

Inocula	PHM	PUT	CAD	HIM	SER	TYM	SPD	SPM	TAA
Control	0.0^{e}±0.0	4.4^{h}±0.2	13.2l±0.8	0.0^{d}±0.0	0.0^{f}±0.0	0.2^{k}±0.0	10.6^{e}±0.0	46.2^{de}±0.1	74.5^{l}±1.2
Acinetobacter haemolyticus	0.0^{e}±0.0	85.9^{f}±0.3	221.8^{h}±1.1	0.0^{d}±0.0	16.0^{b}±0.3	85.1^{i}±0.5	3.4^{h}±0.1	51.8^{b}±0.4	463.9^{h}±1.3
Aeromonas hydrophila	0.0^{e}±0.0	118.7^{e}±0.9	372.5^{e}±11.5	0.9^{cd}±0.5	15.3^{b}±1.0	106.3^{gh}±22.1	3.4^{h}±0.2	46.1^{de}±0.2	663.3^{ef}±29.8
Alcaligenes faecalis	191.0^{a}±2.8	762.7^{a}±5.9	1181.8^{a}±3.8	17.4^{a}±1.7	44.7^{a}±0.5	234.8^{a}±0.7	22.4^{b}±0.1	83.6^{a}±0.7	2,538.4^{a}±14.3
Bacillus cereus	2.8^{d}±0.2	195.8^{c}±1.1	467.0^{c}±4.9	1.2^{cd}±0.3	13.3^{bc}±1.7	162.1^{cd}±0.5	1.7^{ij}±0.0	41.2^{fgh}±0.6	885.1^{c}±7.3
Bacillus subtilis	83.5^{b}±1.3	44.5^{g}±0.8	302.8^{f}±2.6	0.0^{d}±0.0	10.4^{c}±1.09	173.4^{c}±1.0	2.1^{ij}±0.0	37.5^{i}±0.6	654.2^{ef}±4.3
Enterobacter aerogenes	0.0^{e}±0.0	150.1^{d}±17.9	363.5^{e}±2.1	3.5^{c}±2.0	0.4^{f}±0.1	126.1^{ef}±0.1	12.2^{d}±0.1	43.5^{ef}±0.7	699.3^{d}±13.1
Enterobacter cloacae	3.3^{d}±0.1	316.7^{b}±1.5	485.6^{b}±7.6	1.7^{cd}±0.7	12.1^{c}±0.5	201.0^{b}±0.8	2.8^{hi}±0.0	40.1^{ghi}±0.1	1,063.2^{b}±9.0
Escherichia coli	0.0^{e}±0.0	97.5^{ef}±11.6	384.9^{d}±0.5	9.2^{b}±1.1	6.7^{d}±3.0	119.3^{fg}±4.8	12.1^{d}±1.1	42.0^{fg}±2.4	671.6^{de}±14.1
Lactobacillus alimentarius	77.8^{c}±1.0	21.8^{h}±0.1	266.7^{g}±1.1	0.4^{d}±0.2	10.9^{c}±0.6	138.6^{e}±3.7	2.2^{ij}±0.2	39.0^{hi}±0.8	557.3^{g}±7.1
Lactobacillus curvatus	0.0^{e}±0.0	14.7^{h}±0.4	191.4^{i}±0.6	0.0^{d}±0.0	11.9^{c}±0.3	102.6^{gh}±0.9	2.2^{ij}±0.1	49.6^{bc}±0.6	372.4^{i}±0.9
Leuconostoc mesenteroides	2.6^{de}±0.2	16.6^{h}±0.8	9.0^{l}±1.0	1.2^{cd}±0.2	15.8^{b}±1.7	109.6f^{gh}±0.6	2.3^{ij}±0.0	45.0^{de}±1.9	202.1^{k}±2.6
Proteus mirabilis	0.0^{e}±0.0	82.7^{f}±11.5	373.9^{de}±4.0	3.8^{c}±2.2	2.4^{ef}±0.3	120.0f^{g}±0.3	9.4^{fg}±0.1	38.7^{hi}±0.6	630.8^{f}±6.1
Proteus vulgaris	0.0^{e}±0.0	180.6^{c}±12.0	491.4^{b}±1.0	7.9^{b}±0.2	0.5^{f}±0.2	154.9^{d}±0.6	20.7^{c}±0.1	47.7^{cd}±0.2	903.6^{c}±11.8
Pseudomonas aerugina	2.5^{de}±0.3	99.9^{ef}±0.9	107.6^{k}±0.2	0.3^{d}±0.2	4.0^{de}±0.0	43.5^{j}±0.1	33.0^{a}±0.1	16.8^{j}±0.3	307.6^{j}±1.7
Salmonella enteritidis	0.0^{e}±0.0	102.7^{ef}±10.5	371.1^{e}±3.2	6.6^{b}±0.2	1.3^{ef}±0.2	96.2^{hi}±0.4	9.9^{ef}±0.1	49.7^{bc}±0.2	637.6^{df}±8.1
Salmonella typhimurium	0.0^{e}±0.0	44.2^{g}±5.6	152.5^{j}±0.2	7.8^{b}±0.2	0.0^{f}±0.0	56.7^{i}±2.1	8.8^{g}±0.4	12.6^{k}±0.4	282.5^{j}±8.0

$^{a, b, c, d, e, f, g, h, i, j, k, l, m}$ Means±SE with the different superscript in the same column were significantly different ($p<0.001$).

[1] PHM (β-phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), SER (serotonin), TYM (tyramine), SPD (spermidine), SPM (spermine), TAA (total amount of amines).

counts of microorganisms in beef, pork and chicken, respectively.

RESULTS AND DISCUSSION

Table 2 shows that the amine concentrations in ground beef were different depending on the inocula. Spermidine (SPD) and spermine (SPM) were found relatively in large quantity in the control. They are naturally occurring polyamines in fresh pork and beef (Hernandez-Jover et al., 1996). The highest β-phenylethylamine (PHM) production was found in *Pseudomonas aerugina* followed by *Bacillus cereus*. Durlu-Özkaya et al. (2001) reported that *Citrobacter freundii*, *E. coli*, *Enterobacter taylorae*, *Hafnia alvei* and *Morganella morganii* produced PHM. *Bacillus cereus* and *Enterobacter cloacae* generated more PUT, CAD, serotonin (SER) and TYM than the other bacteria did. Heavy contamination by *Enterobacteriaceae* has resulted in large amount of CAD in beef (Slermr, 1981). The highest PUD concentration was detected in *Enterobacter* spp. (Durlu-Özkaya et al., 2001). TYM content is influenced by aerobic and lactic acid bacteria counts (Smith et al., 1993). The content of HIM was below 10 μg/g with the exception of that produced by *Salmonella enteritidis*. According to Durlu-Özkaya et al. (2001), the highest HIM forming bacteria was some *E. coli* strains. However, in this study, *E. coli* was the second to *Salmonella enteritidis*. TAA was highest in samples inoculated with *Bacillus cereus*, followed by *Enterobacter cloacae*.

As shown in Table 3, ground pork samples inoculated with diverse bacteria had various amines at different levels. The tendency in control pork samples was similar to that of ground beef except no detection of HIM and SER in ground pork. All biogenic amines were detected at the highest level in samples inoculated with *Alcaligenes faecalis* while *Bacillus cereus* was the one that produced the highest amount of all biogenic amines except HIM. More than 300 μg/g of PUT was found in sample inoculated with *Enterobacter cloacae* as well as *Alcaligenes faecalis*. *Bacillus cereus*, *Proteus vulgaris*, *Enterobacter cloacae* and *Alcaligenes faecalis* produced high amount of CAD (>400 μg/g). In samples inoculated with all bacteria except for *Alcaligenes faecalis*, HIM was detected below 10 μg/g. TYM that was 0.17 μg/g in control sample, was relatively low in samples inoculated with *Pseudomonas aerugina* and *Salmonella typhimurium*. It was found that HIM, TYM, PUT and CAD formation occurred during the storage of pork (Hernandez-Jover et al., 1996). Total amine level was highest in ground pork sample inoculated with *Alcaligenes faecalis* followed by *Enterobacter cloacae*.

In ground chicken breast and leg, amines were produced by the inoculation of various bacteria (Tables 4 and 5). The major biogenic amines in the control sample were CAD and HIM, similar to that in beef and pork. PHM was not found in the control of both parts while PUT, SER and TYM were found only in the breast sample. Silva et al. (2002) reported that PHM was not found in chicken products and that SPM was predominant polyamine, followed by SPD. PHM produced by *Lactobacillus alimentarius* showed the highest level followed by *Leuconostoc mesenteroides*. *Bacillus cereus*, *Leuconostoc mesenteroides*, *Enterobacter cloacae* and *Alcaligenes faecalis* produced larger amounts of PUT, particularly *Alcaligenes faecalis* produced 1,707.3 μg/g. Durlu-Özkaya et al. (2001) reported that the highest PUT concentration

Table 4. Production of biogenic amines[1] in ground chicken breast by different microbial species (unit: μg/g)

Inocula	PHM	PUT	CAD	HIM	SER	TYM	SPD	SPM	TAA
Control	0.0^{i}±0.0	5.0^{m}±0.1	15.0^{l}±0.1	7.2^{e}±0.2	1.3^{d}±0.4	0.5^{o}±0.3	22.3^{d}±0.3	82.8^{de}±0.0	134.1^{m}±0.6
Acinetobacter haemolyticus	103.9^{e}±1.9	36.1^{l}±0.6	889.0^{f}±44.5	2.4^{f}±1.2	1,50.2^{b}±20.3	103.4^{f}±1.6	11.0^{def}±1.6	87.7^{c}±2.3	1,383.7^{f}±58.8
Aeromonas hydrophila	38.9^{h}±2.4	66.6^{j}±3.2	663.8^{hi}±8.4	2.0^{f}±1.2	2,10.3^{a}±20.6	57.5^{k}±1.4	7.4^{ef}±3.7	106.5^{a}±5.2	1,153.0^{h}±17.7
Alcaligenes faecalis	2,51.1^{c}±2.5	1,707.3^{a}±8.5	2,001.2^{b}±14.1	37.9^{b}±1.6	62.4^{c}±3.9	134.9^{e}±0.9	6.9^{ef}±0.3	57.7^{j}±0.3	4,259.4^{a}±28.3
Bacillus cereus	2,23.4^{d}±1.3	313.0^{d}±0.8	2,588.2^{a}±13.1	11.2^{e}±1.6	16.9^{d}±1.5	201.0^{c}±1.0	3.4^{f}±0.1	63.0^{i}±1.0	3,420.2^{b}±18.4
Bacillus subtilis	81.6^{f}±0.5	208.2^{e}±1.9	686.2^{h}±3.1	2.3^{f}±0.6	61.2^{c}±3.9	89.3^{h}±0.2	13.2^{def}±0.1	68.8^{h}±0.2	1,210.8^{h}±7.4
Enterobacter aerogenes	0.0^{i}±0.0	51.6^{k}±4.0	975.4^{e}±6.8	10.8^{e}±0.1	11.1^{d}±0.4	99.6^{g}±0.4	42.0^{bc}±0.3	86.7^{cd}±0.3	1,277.2^{g}±2.1
Enterobacter cloacae	50.3^{g}±0.2	412.0^{c}±2.5	1,199.0^{a}±15.7	16.8^{d}±0.6	48.0^{c}±1.0	265.2^{b}±1.1	6.8^{ef}±0.1	73.1^{g}±0.4	2,071.3^{e}±20.1
Escherichia coli	0.0^{i}±0.0	80.4^{ij}±8.2	628.4^{i}±5.3	8.4^{e}±0.1	7.7^{d}±0.9	1.9^{o}±0.2	49.7^{ab}±0.1	92.9^{b}±0.0	869.2^{j}±4.1
Lactobacillus alimentarius	432.3^{a}±3.7	213.3^{e}±2.7	1,756.2^{c}±10.1	25.4^{c}±3.9	22.0^{d}±5.3	339.4^{a}±1.8	17.4^{de}±14.2	63.3^{i}±1.3	2,869.3^{c}±15.1
Lactobacillus curvatus	14.0^{i}±0.3	99.2^{h}±0.2	534.9^{i}±0.6	0.5^{f}±0.3	6.2^{d}±0.2	38.0^{l}±0.3	11.6^{def}±0.1	79.3^{ef}±0.1	783.7^{k}±0.9
Leuconostoc mesenteroides	409.1^{b}±2.9	548.5^{b}±2.9	948.6^{e}±16.4	60.6^{a}±3.3	48.1^{c}±3.3	143.4^{d}±0.8	5.1^{f}±0.0	64.1^{i}±0.6	2,227.6^{d}±26.5
Proteus mirabilis	0.0^{i}±0.0	153.4^{g} ±6.4	249.1^{l}±4.3	7.6^{e}±0.2	4.2^{d}±0.7	4.7^{n}±0.1	33.1^{c}±0.1	90.8^{bc}±0.7	542.9^{l} ±3.9
Proteus vulgaris	0.0^{i}±0.0	83.6^{i}±6.7	688.4^{h}±0.7	15.4^{d}±0.1	9.9^{d}±0.4	83.8^{i}±0.4	35.5^{c}±0.2	74.8^{g}±0.2	991.4^{i}±6.9
Pseudomonas aerugina	2.9^{j}±0.7	172.9^{f}±5.0	829.0^{g}±8.2	8.0^{e}±0.2	11.7^{d}±1.1	70.6^{j}±0.1	58.9^{a}±0.0	27.0^{j}±0.2	1,180.9^{h}±4.0
Salmonella enteritidis	0.0^{i}±0.0	105.0^{h}±8.7	681.7^{h}±0.2	8.6^{e}±0.0	4.1^{d}±0.9	17.2^{m}±0.3	42.9^{b}±0.1	79.5^{ef}±0.2	938.9^{j}±9.2
Salmonella typhimurium	0.0^{i}±0.0	79.5^{ij}±6.6	687.9^{h}±2.5	16.7^{d}±0.4	8.3^{d}±0.8	83.9^{i}±0.5	33.9^{c}±0.4	76.2^{fg}±0.1	986.5^{i}±6.3

$^{a, b, c, d, e, f, g, h, i, j, k, l, m}$ Means±SE with the different superscript in the same column were significantly different ($p<0.001$).

[1] PHM (β-phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), SER (serotonin), TYM (tyramine), SPD (spermidine), SPM (spermine), TAA (total amount of amines).

Table 5. Production of biogenic amines[1] in ground chicken leg by different microbial species (unit: μg/g)

Inocula	PHM	PUT	CAD	HIM	SER	TYM	SPD	SPM	TAA
Control	0.0^{g}±0.0	0.0^{j}±0.0	9.1^{l}±0.3	7.8^{ef}±0.1	0.0^{h}±0.0	0.0^{m}±0.0	23.4^{g}±0.4	63.0^{c}±0.0	103.2^{n}±0.1
Acinetobacter haemolyticus	146.9^{b}±3.1	275.2^{fg}±7.0	336.5^{k}±0.9	7.5^{ef}±0.9	71.4^{a}±11.3	87.0^{h}±1.5	16.2^{j}±0.3	79.6^{a}±2.8	1,020.1^{k}±4.8
Aeromonas hydrophila	29.6^{e}±0.7	239.9^{g}±1.0	443.7^{l}±1.3	4.4^{g}±0.2	58.8^{b}±4.1	30.7^{l}±1.8	21.5^{h}±0.4	75.7^{b}±0.2	904.1^{l}±4.4
Alcaligenes faecalis	388.6^{a}±3.7	813.4^{a}±6.2	947.0^{f}±7.7	7.5^{ef}±1.0	33.4^{d}±4.6	186.0^{c}±1.9	13.5^{i}±0.3	38.9^{i}±0.2	2,428.3^{c}±14.8
Bacillus cereus	7.9^{f}±0.4	572.8^{c}±2.7	745.7^{g}±4.3	4.3^{g}±0.2	29.2^{d}±1.6	64.5^{j}±0.4	16.6^{j}±0.1	48.0^{h}±0.3	1,488.7^{g}±9.3
Bacillus subtilis	28.2^{e}±1.0	182.1±1.5^{h}	336.4±1.2^{k}	6.1±1.4^{fg}	44.3±2.0^{c}	71.4±0.7^{i}	16.1±0.0^{j}	56.6±1.1^{e}	741.2±5.1^{m}
Enterobacter aerogenes	0.0±0.0^{g}	713.8^{b}±4.4	1,489.4^{b}±2.0	58.1^{a}±0.2	3.1^{gh}±1.2	268.5^{b}±2.8	26.2^{e}±0.0	50.3^{gh}±0.2	2,609.4^{b}±0.4
Enterobacter cloacae	2.0^{g}±0.3	837.7^{a}±6.7	1,359.5^{d}±7.5	3.6^{g}±0.1	53.2^{bc}±1.0	111.6^{f}±0.3	19.6^{i}±0.1	56.3^{e}±0.2	2,443.7^{c}±14.1
Escherichia coli	0.0^{g}±0.0	258.4^{g}±4.2	675.5^{h}±2.2	9.7^{e}±0.7	3.5^{gh}±0.2	52.6^{k}±1.4	36.7^{a}±0.8	60.1^{cd}±0.2	1,096.6^{j}±3.1
Lactobacillus alimentarius	0.0^{g}±0.0	173.5^{h}±1.6	1,134.6^{e}±4.5	4.2^{g}±0.4	14.9^{ef}±0.4	88.4^{h}±0.6	14.4^{k}±0.1	59.8^{d}±0.1	1,489.7^{g}±6.9
Lactobacillus curvatus	86.8^{c}±1.1	71.7^{i}±0.9	1,409.4^{c}±7.4	3.4^{g}±0.1	8.9^{efgh}±0.0	158.8^{e}±0.4	16.0^{j}±0.0	78.0^{ab}±0.1	1,832.9^{f}±7.7
Leuconostoc mesenteroides	0.7^{g}±0.3	3.3^{j}±0.5	5.8^{l}±1.0	0.8^{h}±0.0	16.1^{e}±0.4	35.5^{l}±0.7	2.1^{m}±0.0	30.2^{k}±0.3	94.3^{n}±0.7
Proteus mirabilis	53.7^{d}±3.0	313.9^{ef}±18.8	1,363.3^{d}±1.9	12.8^{d}±1.6	11.5^{efg}±2.0	92.7^{h}±0.1	28.3^{d}±0.1	35.8^{j}±0.2	1,912.1^{e}±20.3
Proteus vulgaris	0.0^{g}±0.0	797.1^{a}±12.76	1,797.0^{a}±17.1	16.8^{c}±2.2	4.9^{fgh}±0.6	393.9^{a}±1.6	33.7^{b}±0.7	52.2^{fg}±2.7	3,095.7^{a}±3.4
Pseudomonas aerugina	0.0^{g}±0.0	352.5^{e}±12.8	622.7^{i}±3.1	13.5^{d}±0.2	0.0^{h}±0.0	103.2^{g}±3.5	29.7^{c}±0.3	25.9^{l}±0.8	1,147.5^{i}±6.0
Salmonella enteritidis	0.0^{g}±0.0	313.0^{ef}±2.0	759.3^{g}±0.8	19.0^{c}±0.2	1.2^{gh}±0.2	35.6^{l}±0.2	24.5^{f}±0.1	54.1^{ef}±0.5	1,206.7^{h}±2.4
Salmonella typhimurium	0.0^{g}±0.0	401.9^{d}±54.0	1,373.3^{d}±0.0	35.5^{b}±0.9	0.9^{gh}±0.3	179.2^{d}±5.7	30.3^{c}±0.1	39.1^{i}±0.0	2,060.2^{d}±49.7

$^{a, b, c, d, e, f, g, h, i, j, k, l, m}$ Means±SE with the different superscript in the same column were significantly different ($p<0.001$).

[1] PHM (β-phenylethylamine), PUT (putrescine), CAD (cadaverine), HIM (histamine), SER (serotonin), TYM (tyramine), SPD (spermidine), SPM (spermine), TAA (total amount of amines).

was detected in *Enterobacter* spp. strains. The amine produced at the highest level was CAD formed by *Bacillus cereus*. The content of HIM in ground chicken breast was different from that of ground beef or pork. *Leuconostoc mesenteroides* formed 60.6 μg/g of HIM, *Alcaligenes faecalis* 37.9 μg/g and *Lactobacillus alimentarius* 25.4 μg/g. *Aeromonas hydrophila* and *Acinetobacter haemolyticus* produced 210.3 and 15.0 μg/g of SER, respectively. More TYM was produced in the order of *Lactobacillus alimentarius*, *Enterobacter cloacae* and *Bacillus cereus*. Bacteria that have revealed tyrosine decarboxylase activity in various foods are *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus bulgaricus*, *Escherichia coli* and *Pseudomonas* spp. (Santos, 1996). Total amine level was in the order of samples inoculated with *Alcaligenes faecalis*, *Bacillus cereus*, and *Lactobacillus alimentarius*.

In leg samples, *Alcaligenes faecalis*, *Acinetobacter haemolyticus* and *Lactobacillus curvatus* formed a large amount of PHM. *Enterobacter cloacae*, *Alcaligenes faecalis*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Bacillus cereus* produced PUT at the level above 500 μg/g and *Proteus vulgaris*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Salmonella typhimurium Enterobacter cloacae* and *Lactobacillus curvatus* produced above 1,000 μg CAD/g of chicken leg. The content of HIM in the sample inoculated with *Enterobacter aerogenes* was 58.1 μg/g and that inoculated with *Salmonella typhimurium* 35.5 μg/g. SER was abundantly detected in the samples inoculated with *Acinetobacter haemolyticus*, *Aeromonas*

hydrophila, *Enterobacter cloacae* and *Bacillus subtilis*. TYM was rich in the sample inoculated with *Proteus vulgaris*, *Enterobacter aerogenes* and *Alcaligenes faecalis*. Total amine level was highest in sample inoculated with *Proteus vulgaris*, followed by *Enterobacter aerogenes*, *Enterobacter cloacae* and *Alcaligenes faecalis*.

In summary, CAD was detected at the highest level, followed by PUT and TYM when beef, pork or chicken was spoiled. As for the microorganisms involved in biogenic amines production, *Alcaligenes faecalis*, *Enterobacter cloacae* and *Bacillus cereus* were ones that produced a larger amount of BAs regardless of meat sources from different species.

ACKNOWLEDGEMENT

This work was supported by Korea Research Foundation Grant (KRF-2001-042-G00008).

REFERENCES

Ben-Gigirey, B., J. M. V. B. de Sousa, T. G. Villa and J. Barros-Velazquez. 2000. Characterization of biogenic amine-producing Stenotrophomonas maltophilia strains isolated from white muscle of fresh and frozen albacore tuna. International Journal of Food Microbiology 57:19-31.

Butturini, A., P. Aloisi, R. Tagliazucchi and C. Cantoni. 1995. Production of biogenic amines by enterobacteria and lactic acid bacterial isolated from meat products. Industrie Alimentari 34:105-107.

Chen, M. -T, Y. -S. Lin, H. -T. Tsai and H. -L. Kuo. 2002. Efficiency of hurdle technology applied to raw cured meat (Si-raw) processing. Asian-Aust. J. Animal. Sci. 15 (11):1646-1652.

Dapkevicius, M. L. N. E., M. J. R. Nout, F. M. Rombouts, J. H. Houben and W. Wymenga. 2000. Biogenic amine formation and degradation by potential fish silage starter microorganisms. International Journal of Food Microbiology 57:107-114.

Durlu-Özkaya, F., K. Ayhan and N. Vural. 2001. Biogenic amines produced by Enterobacteri-aceae isolated from meat products. Meat Science 58:163-166.

Edwards, R. A., R. H. Dainty, C. M. Hibbard and S. V. Ramantanis. 1987. Amines in fresh beef of normal pH and the role of bacteria in changes in concentration observed during storage in vacuum packs at chill temperatures. Journal of Applied Bacteriology 63:427-434.

Eerola, S., R. Hinkkanen, E. Lindfors and T. Hirvi. 1993. Liquid chromatographic determination of biogenic amines in dry sausages. Journal of AOAC International 76(3):575-577.

Gardini, F., M. Martuscelli, M. C. Caruso, F. Galgano, M. A. Crudele, F. Favati, M. E. Guerzoni and G. Suzzi. 2001. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. International Journal of Food Microbiology 64:105-117.

Gram, L., G. Trolle and H. H. Huss. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Microbiology 4:65-72.

Hernandez-Jover, T., M. Izquierdo-Pulido, M. T. Veciana-Nogues and M. T. Vidal-Carou. 1996. Biogenic amines sources in cooked cured shoulder pork. J. Agric. Food Chem. 44:3097-3101.

Lakshmanan, R., R. J. Shakila and G. Jeyasekaran. 2002. Survival of amine-forming bacteria during the ice storage of fish and shrimp. Food Microbiology 19:617-625.

Leuschner, R. G. K., R. Kurihara and W. P. Hammes. 1998. Effect of enhanced proteolysis on formation of biogenic amines by lactobacilli during Gouda cheese ripening. International Journal of Food Microbiology 44:15-20.

Lopez-Caballero, M. E., J. A. Sanchez-Fernandez and A. Moral. 2001. Growth and metabolic activity of Shewanella putrefaciens maintained under different CO_2 and O_2 concentrations. International Journal of Food Microbiology 64:277-287.

Luthy, J. and C. Schlatter. 1983. Biogene amine in Lebensmitteht zür Wirkung von Histamine, Tyramin und B-phenylethylamine auf den Menschen. Z. Lebensm. Unters. Forsch. 177:439-443.

Maijala, R. and S. Eerola. 1993. Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat Science 35(3):387-395.

Maijala, R. L., S. H. Eerola, M. A. Aho and J. A. Hirn. 1993. The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. Journal of Food Protection 56:125-129.

Masson, F., G. Johansson and M. C. Montel. 1999. Tyramine production by a strain of Carnobacterium divergens inoculated in meat-fat mixture. Meat Science 52:65-69.

Nadon, C. A., M. A. Ismond and R. Holley. 2001. Biogenic amines in vacuum-packaged and carbon dioxide-controlled atmosphere-packaged fresh pork stored at -1.5°C. J. Food Prot. 64(2):220-227.

Nottingham, P. M. 1992. Microbiology of Carcass Meats. In: Meat Microbiology. (Ed. M. H. Brown). Applied Science Publishers Ltd, London, pp. 13-66.

Santos, M. H. S. 1998. Amino acid decarboxylase capability of microorganisms isolated in Spanish fermented meat products. International Journal of Food Microbiology 39:227-230.

Santos, M. H. S. 1996. Biogenic amines: their importance in foods. International Journal of Food Microbiology 29:213-231.

SAS Institute Inc. 2000. The SAS system for windows (Release 8.01). SAS Institute Inc., Cary, NC, USA.

Shalaby, A. R. 1996. Significance of biogenic amines to food safety and human health. Food Research International 29(7):675-690.

Silva, C. M. G. and M. B. A. Gloria. 2002. Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1°C and in chicken-based meat products. Food Chem. 78:241-248.

Slermr, J. 1981. Biogenic amine als potentieller chemoscher qualitatserdikator für flasch. Fleichwirtschaft 61:921-962.

Smith, J. S., P. B. Kenny, C. L. Kastner and M. M. Moore. 1993. Biogenic amine formation in fresh vacuum packaged beef during storage at 1°C for 120 days. J. Food Protect. 56:497-532.

Stratton, J. E., R. W. Hutkins and S. L. Taylor. 1991. Biogenic amines in cheese and other fermented foods: a review. J. Food Prot. 54:460-470.

Tschabrun, R., K. Sick, F. Bauer and P. Kranner. 1990. Bildung von Histamin in schnittfesten Rohwürsten. Fleischwirtschaft 70:448-45.