Food Sci. Technol. Res., **18** (6), 795 – 804, 2012

The Function of Lactic Acid Bacteria on Biogenic Amines Production by Food-Borne Pathogens in Arginine Decarboxylase Broth

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Received March 29, 2012; Accepted July 3, 2012

The function of lactic acid bacteria (LAB) on biogenic amines (BAs) accumulation by gram negative and positive food-borne pathogens (FBP) was investigated in arginine decarboxylase broth (ADB). Main amines produced by gram negative and positive bacteria were putrescine (PUT), spermidine, agmatine (AGM) and 2-phenylethylamine in ADB. There were not significant differences in BAs production between gram negative and gram positive bacteria ($p > 0.05$), except for histamine and trimethylamine. The function of LAB strains on ammonia and BAs production by gram negative and gram positive FBP was strain dependent. The all used bacteria appeared to convert arginine into PUT through AGM. LAB strains generally seemed to stimulate amine that generated by FBP. The highest stimulation effect on PUT production was found for *Pseudomonas aeruginosa* in the presence of *Lb. plantarum* (about 8 fold higher). Consequently, the function of LAB strains on ammonia (AMN) and BAs production by gram negative and positive FBP varied depending on strains although LAB used with some pathogens had stimulation effect on AMN and BAs.

Keywords: agmatine, putrescine, lactic acid bacteria, food-borne pathogens

Introduction

The functions of biogenic amines in relationship to diversity in bacteria, cell growth, amine metabolism, and their presence in food as well as methodological aspects have been studied. Biogenic amines (BAs) in foods are mainly generated by decarboxylation of the corresponding amino acids by microorganisms (Moreno-Arribas *et al.*, 2003).

Histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), tryptamine (TRP), 2-phenylethylamine (PHEN), spermine (SPN), and spermidine (SPD) are the most important biogenic amines in foods (Shalaby, 1996). Biogenic amines are regarded as toxic when exceeding a certain level and are consequently accused of causing food poisoning (Yongjin *et al.*, 2007). BAs, especially HIS, TRP, PHEN and TYR are biologically active amines and they have physiological effects in human especially either psychoactive or vasoactive (Lovenberg, 1973). Furthermore, BAs like TYR as well as diamines such as PUT and CAD have been described as precursors of carcinogenic nitrosamines (Lange and Wittmann, 2002). Maximum permissible limits of BAs for fish and fish products is limited to 200 mg/kg of the food in Germany, whilst it is only 100 mg/kg in Canada, Finland and Switzerland (Paleologos *et al.*, 2003; Brink *et al.*, 1990).

Production of BAs depends on not only intrinsic and extrinsic parameters of food such as environmental temperature and pH, aerobiosis and anaerobiosis, availability of carbon sources, presence of growth factors, cell growth phase, but also availability of precursors (amino acids) (Bover-Cid *et al.*, 2008; Greif *et al.*, 2006; Bunkova *et al.*, 2010).

Biogenic amines have favourable or unfavourable function for human, food and bacteria. For instances, some biogenic amines can act as free radical scavengers such as putrescine, cadaverine, spermidine (Ogawa *et al.*, 1996). Putrescine (PUT) and agmatine (AGM) have been reported to potentiate the toxicity of histamine (HIS) to humans by depressing HIS oxidation (Taylor, 1986). PUT and AGM also considered to be potential precursors for carcinogenic nitrosamines in the presence of nitrites (Hotchkiss *et al.*, 1977; Arena and Manca de Nadra, 2001). The spermine is able to

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regenerate tocopherol from the tocopheroxyl radical through hydrogenic donor from amino group as well (Greif *et al.*, 1999).

AGM may present in microorganisms, plants, invertebrates, mammalian tissue and other organisms (Reis and Regunathan, 2000; Ineba *et al.*, 2004). Four L-arginine-degrading pathways are functional in *Pseudomonas putida* and *Pseudomonas aeruginosa*. The L-arginine succinyl transferase pathway, the L-arginine deiminase pathway and L-arginine oxidase/ dehydrogenase pathway contributes to L-arginine catabolism under aerobic and/or anaerobic conditions. Although the L-arginine decarboxylase pathway still is not well known, it may have its major function in the biosynthesis of the polyamines agmatine and putrescine (Lu, 2006; Schriek *et al.*, 2007). Recently, pathway (Fig. 1) for arginine catabolism has been well documented by Brauc *et al.* (2012). In organisms that has an intracellular arginine decarboxylase, AGM is produced directly from arginine and is converted to N-carbamoylputrescine by arginine decarboxylase enzymes (Griswold *et al.*, 2006). AGM can be produced by a broad range of microorganisms, including *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Lactobacillus hilgardii* (Sakakibara and Yangisava, 2003; Griswold *et al.*, 2006). PUT formation through AGM, as an intermediate amine, from arginine in *Lactobacillus hilgardii* X1B isolated from wine was also reported (Arena and Manca de Narda, 2001; Alberto *et al.*, 2007).

The BAs content of various foods have been widely studied and most of the studies have been focused on BAs formation of selected single bacteria isolates. LAB are the most important bacteria used in food fermentations. It is well known that common LAB strains produce substances such as hydrogen peroxide, weak organic acids, reuterin, diacetyl, bacteriocins and low-molecular-weight metabolites that inhibit pathogenic organisms in fermented foods (Brashears *et al.*, 2005; Olaoye and Ntuen, 2011). Studies on the interactions between mix bacterial populations (LAB and FBP) are very limited and no information exists regarding BA production mechanism of mixed culture. Recently, it was reported that environmental factors, such as temperature, influenced the interaction between bacterial strains in mixed cultures (Aguilar and Klotz, 2010). More research is required to distinguish amine production by lactic acid bacteria mixed with FBP. Therefore, the aim of the study was to investigate the function of LABs mixed with FBP on AGM and the other biogenic amine production in arginine decarboxylase broth (ADB).

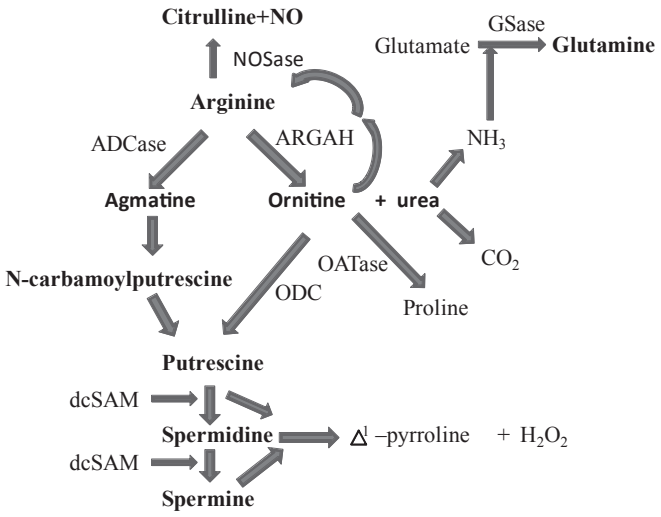

Fig. 1. Catabolic pathways of L-arginine. Arginine catabolism occurs via arginase (ARGAH), arginine decarboxylase (ADCase) and nitric oxide synthase (NOSase); the reaction products may be further metabolised to other amino acids and polyamines. GSase, glutamine synthase; dcSAM, decarboxylated Sadenosylmethionine; OATase, ornithine-d-aminotransferase.

Materials and Methods

Bacterial strains The used LAB species were *Lactococcus lactis* subsp. *cremoris* (MG 1363), *Lactococcus lactis* subsp. *lactis* (IL 1403), *Lactobacillus plantarum* (FI8595) and *Streptococcus thermophilus* (NCFB2392) were obtained from Department of the Biotechnology in Sutcu Imam University, Turkey in BGML stock culture.

The selected 8 food-borne pathogens were *Staphylococcus aureus* (ATCC29213), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Enterococus faecalis* (ATCC29212), *Pseudomonas aeruginosa* (ATCC27853), *Listeria monocytogenes* (ATCC7677) which were purchased from American Type Culture Collection (Rockville, MD, USA) and *Aeromonas hydrophila* (NCIMB1135) and *Salmonella paratyphi* A (NCTC13) which were obtained from National Collections of Industrial Food and Marine Bacteria (Aberdeen, UK) and National Collection of Type Cultures (London, UK), respectively.

Culture media and bacterial extraction The production of agmatine by all LAB and FBP strains used in this work was monitored using arginine decarboxylase broth (ADB). ADB was prepared using 1 g peptone, 0.5 g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 2.5 g NaCl (Merck 1.06404.1000, Darmstadt, Germany), 4.01 g L-arginine (Sigma, Steinheim, Germany) and 2.5 mg pyridoxal HCl (Sigma P9130, Steinheim, Germany) in 500 mL distilled water. The pH (5.5 − 6.8) were adjusted according to their optimum growth pH with 1M KOH (Riedel-deHaen 06005, Seelze, Germany) or 6% TCA (Riedel-deHaen 27242, Seelze, Germany). After that ADB was pipetted in 10 ml bottles and then autoclaved at 121°C in 15 min. prior to use.

MRS broth (Fluka 69966, Buchs, Switzerland) and Nutri-

ent broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of LAB and FBP cultures, respectively. Lactic acid and pathogen bacterial strains were incubated at 30 and 37°C for 2 or 3 days which after 0.5 mL of these bacterial cultures was removed and put into the ADB to allow these bacteria produce biogenic amine.

To remove the bacterial strains present in the growth broth (ADB), 5 mL of the ADB containing LAB and FBP strains were removed to separate bottles and then 2 mL sulphosalicylic acid was added. They were then centrifuged at 3000 *g* for 10 min and then filtered through a filter paper (125 mm, Schleicher & Schuell, Dassel, Germany). After that, 4 mL of bacterial supernatant were taken for derivatisation in order to analyse in HPLC.

Chemical reagents L-arginine and all BA standards were purchased from Sigma-Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and HPLC grade water for amine analyses. The used BA standards were histamine dihydrochloride, tyramine hydrochloride, typtamine hydrochloride, putrescine dihydrochloride, 2-phenylethylamine hydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, 5-hydroxytryptamine (serotonin), 3-hydroxytyramine hydrochloride (dopamine), agmatine sulphate, trimethylamine hydrochloride and ammonium chloride.

Derivatisation of bacterial extraction A stock solution was prepared by dissolving 2% benzoyl chloride (Sigma-Aldrich 25.995-0, Steinheim, Germany) in acetonitrile (Merck 1.00030.2500, Steinheim, Germany) to enhance the reaction with amines. For derivatisation of standard amine solutions, 100 mL was taken (4 mL for extracted bacterial cultures) from each free base standard solution (10 mg/mL). Sodium hydroxide (1 mL of 2 M) (Merck 1.06462.1000, Darmstadt, Germany) was added, followed by 40 µL of benzoyl chloride and the solution mixed on a vortex mixer for 1 min. The reaction mixture was left at room temperature for 20 min. After that, the benzoylation was stopped by adding 2 mL of saturated sodium chloride (Merck 1.06404.1000, Darmstadt, Germany) solution and the solution extracted twice with 2 mL of diethyl ether. The upper organic layer was transferred into a clean tube after mixing. Afterwards, the organic layer was evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 mL of acetonitrile and 10 µL aliquots were injected into the HPLC.

Analytical method Biogenic amines analysis was done using the method of Özogul (2004) and measured in mg amines per litre broth. The confirmation of biogenic amines production was accomplished using a rapid HPLC method with a reversed phase column by using a gradient elution program. Chromatographic separation was carried out using continuous gradient elution with acetonitrile (eluant A) and HPLC grade water (eluant B). The gradient started at 40 % acetonitrile and was then increased to 60% in 20 min. The total separation time was less than 20 min and the gradient was run for 25 min to ensure full separation. The injection volume was 10 mL and detection was monitored at 254 nm (wavelength range 220 − 400 nm). Same analytic method was used for ammonia and trimethylamine separation.

HPLC separation A standard curve for ammonia and each of the twelve amines in the range of 0 − 50 mg/mL was prepared. Correlation coefficient (r) of peak area against amine standard concentrations for each compound was calculated after injecting five replicates of each standard solution of amine. The correlation coefficient (r^2) in the curve was > 0.99 for each benzoylated amine and ammonia, which showed a linear relationship between amine concentration and detector response.

HPLC apparatus and column A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector and two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC), and a communication bus module (CBM-20A) with valve unit FCV-11AL was used. The column, purchased from Phenomenex (Macclesfield, Cheshire, UK), was Columbus C18 (150 × 4.6 mm).

Statistical analysis The mean value and standard deviation were calculated using the data obtained from the three samples for each treatment. One way ANOVA was used to determine the significance of differences at $P < 0.05$. All statistics were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL. USA).

Results and Discussion

Biogenic amine production by single LAB cultures

BAs and ammonia (AMN) production by LAB (10^{11} cfu/mL) in arginine decarboxylase broth (ADB) were given in Table 1. LAB strains produced high amount of AMN. Although AMN production by *Lc. lactis subsp. cremoris* and *Lc. lactis subsp. lactis* were similar, significant differences in AMN production were observed among the LAB strains. Arena *et al.* (1999) reported that *Lb. plantarum* N8 and N4 strains isolated from orange degraded L-arginine to citrulline, ornithine and ammonia. AMN production by LAB strains in ADB was very high (> 1070 mg/L), indicating arginine degradation into AMN through arginine. L-arginine content of broth was 8020 mg/L. Although the other amino acids (lysine, ornithine, tyrosine etc.) were not added into arginine decarboxylase broth, the broth does contain peptone and beef extract which contains the other amino acids. The amounts of glutamic acid, arginine, lysine, tyrosine and tryptophan

Table 1. Biogenic amines and ammonia production (mg/L) by lactic acid bacteria in arginine decarboxylase broth (ADB).

Microorganism	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	HIS	SER	TYR	TMA	DOP	AGM
Lactococcus lactis subsp. cremoris	2554.86^{xa}	172.76^{b}	1.63^{c}	6.63^{a}	0.00^{b}	15.77^{a}	0.00^{a}	0.84^{c}	0.71^{c}	0.22^{a}	0.07^{b}	3.36^{c}	1.35^{c}
	202.68^{y}	11.76	0.15	0.58	0.00	1.99	0.00	0.04	0.08	0.01	0.01	0.22	0.06
Lactococcus lactis subsp. lactis	2349.01^{a}	107.43^{c}	2.51^{b}	6.73^{a}	0.00^{b}	18.83^{a}	0.00^{a}	1.45^{b}	0.70^{c}	0.11^{b}	0.34^{b}	3.63^{c}	10.22^{a}
	148.50	2.56	0.03	0.56	0.00	1.38	0.00	0.03	0.05	0.01	0.01	0.21	1.65
Lactobacillus plantarum	1872.60^{b}	33.98^{d}	5.68^{a}	0.38^{c}	0.00^{b}	9.22^{b}	0.00^{a}	2.25^{a}	0.91^{b}	0.24^{a}	0.09^{b}	5.35^{b}	8.62^{a}
	88.73	1.59	0.33	0.03	0.00	0.45	0.00	0.12	0.07	0.01	0.00	0.43	0.19
Streptoccocus thermophilus	1079.15^{c}	228.59^{a}	1.81^{c}	5.46^{b}	19.58^{a}	8.67^{b}	0.06^{a}	0.33^{d}	2.70^{a}	0.00^{c}	13.71^{a}	6.39^{a}	5.98^{b}
	106.17	10.94	0.07	0.07	0.71	0.28	0.00	0.02	0.06	0.00	1.08	0.02	0.41

AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIS, histamine; SPD, spermidine; TRP, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. ^{x} Mean value, ^{y} Standard deviation (n = 4) ND: not detected. Different lowercase letters ($^{a-c}$) in a row indicate significant differences ($P < 0.05$) among bacteria species.

in peptone and beef extract are calculated as 304 mg/L, 170 mg/L, 132 mg/L, 14 mg/L and 16 mg/L, respectively (Oxoid L37; Oxoid L29) .

BAs production by LAB were statistically significant ($P < 0.05$). LAB strains produced not only AGM but also other amines such as PUT, cadaverine (CAD), 2-phenylethylamine (PHEN) and dopamine (DOP) in ADB. The observed BAs in ADB are likely to have been produced from the amino acids in peptone and beef extract. However, none of the strains was able to produce tryptamine (TRP) and spermine (SPN) except for *Strep. thermophilus*. Straub *et al.* (1995) reported that *Lactococcus spp.*, *Pediococcus spp.*, *Streptococcus spp.* and *Leuconostoc spp.* did not have amino acid decarboxylase activity.

AGM is reported to be produced from arginine by the reaction of arginine decarboxylase that had been secreted from lactic acid bacteria and nitric acid-reducing bacteria (Umezu *et al.* 1977; Ineba *et al.* 2004). In the present study, AGM production by LAB strains were ranged from 0 to 11 mg/L. Arena and Nadra (2001) reported that *Lactobacillus hilgardii* X1B was able to degrade arginine by two pathways which are arginine deiminase and arginine decarboxylase. The isolate was able to produce putrescine from ornithine and from agmatine. PUT was the most produced amine in ADB. The used LAB strains were seemed to convert AGM into PUT. PHEN production by LAB strains was generally higher than that of AGM and other amines (except for PUT). HIS and TYR production by LAB were low (> 2.3 vs. 0.3 mg/L).

The Effect of LAB Strains on BAs Production by Gram Negative FBP AMN and BAs production by LAB (10^{11} cfu/mL) mixed with gram negative bacteria (10^{11} cfu/mL) were given in Tables 2 − 3. The function of LAB strains on AMN and BAs production by gram negative FBP was strain dependent, which was in agreement with results of previous study (Özogul, 2011). *Lb. plantarum* and *Strep. thermophilus* inhibited significantly ($p < 0.05$) the AMN accumulation by *A. hydrophila* and *P. aeruginosa*, although *Lc. lactis* subsp. *lactis* and *Lb. plantarum* resulted in higher AMN production by *E. coli* and *S. paratyphi* A. Heterofermentative lactic acid bacteria may degrade arginine, leading to the formation of ammonia and citrulline, among other substances (Orduña *et al.* 2001). Schneider, Kiupakis, and Reitzer (1998) reported ammonia-producing arginine succinyltransferase pathway in *E. coli*. In the current study, *E. coli* produced 2209.87 mg/L of ammonia in ADB. *Lc. lactis subsp. cremoris* (for *P. aeruginosa* and *S. parathypi* A) and *Strep. thermophilus* (for *E. coli* and *S. paratyphi* A) showed no effect on AMN accumulation in ADB. In the previous study (Özogul, 2011), it was found that all of the mix cultures (LAB with FBP) inhibited the AMN accumulation significantly ($p < 0.05$) except for *E. coli* with *Lc. lactis* subsp. *lactis* and *Lb. plantarum* in histidine decarboxylase broth.

In organisms having an intracellular arginine decarboxylase such as *P. aeruginosa*, AGM is produced directly from arginine and is converted to N-carbamoylputrescine by arginine decarboxylase (Nakada *et al.*, 2001). The result obtained from previous study showed that none of the bacteria including *K. pneumoniae*, *Ent. faecalis*, and *Pseudomonas spp.* did not decarboxylate arginine except *Psychrobacter immobilis*, although some of these strains accumulated PUT, CAD, SPN and even HIS (Özogul and Özogul, 2007). Bunkova *et al.* (2010) reported that AGM production in decarboxylation broth containing lysine, histidine, tyrosine and arginine was only found for *Enterobacteriaceae* family, namely in three strains of *E. coli* and one strain of *Proteus vulgaris*. Production of AGM by one strains of *E. coli* was low (7.20 mg/L), the other three strains produced medium amounts of AGM (22.85 − 32.29 mg/L). In the current study, all used gram negative bacteria as well as *Enterobacteriaceae* strains had an ability to convert arginine to agmatine. *Enterobacteriace-*

Table 2. Biogenic amines and ammonia production (mg/L) by mix cultures (lactic acid bacteria and gram negative food-borne pathogens) in arginine decarboxylase broth (ADB).

Microorganism	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	HIS	SER	TYR	TMA	DOP	AGM
A. hydrophila (AH)	2349.69^{xb}	89.59^{c}	9.28^{d}	15.92^{b}	0.00^{b}	14.03^{c}	0.00^{c}	0.51^{e}	1.60^{c}	0.37^{d}	0.09^{c}	2.04^{e}	6.35^{d}
	119.59^{y}	(7.85)	0.56	0.85	0.00	1.19	0.00	0.04	0.12	0.03	0.01	0.06	0.27
AH × CREM	2699.62^{a}	255.67^{a}	74.31^{b}	18.14^{a}	1.63^{a}	36.53^{a}	1.55^{c}	1.08^{c}	18.99^{a}	11.01^{b}	24.62^{a}	68.27^{a}	16.40^{b}
	212.08	38.34	6.93	0.59	0.04	1.93	0.06	0.03	1.47	0.75	1.93	6.69	1.34
AH × LAC	1068.87^{d}	24.97^{d}	33.13^{cd}	0.00^{e}	0.00^{b}	4.88^{d}	0.00^{c}	0.64^{d}	2.56^{c}	0.62^{d}	0.05^{c}	11.89^{d}	25.48^{a}
	42.43	1.41	1.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.13	2.12
AH × PLAN	1405.00^{c}	145.97^{b}	418.88^{a}	3.09^{d}	1.53^{a}	0.00^{e}	164.82^{a}	1.33^{b}	8.80^{b}	14.77^{a}	1.29^{c}	29.21^{c}	13.55^{bc}
	103.36	2.46	22.69	0.14	0.11	0.00	5.25	0.00	0.59	1.47	0.10	1.27	1.34
AH × STREP	1431.07^{c}	94.21^{c}	55.55^{bc}	7.61^{c}	0.00^{b}	19.56^{b}	19.79^{b}	1.66^{a}	6.84^{b}	5.64^{c}	6.52^{b}	51.21^{b}	12.17^{c}
	59.73	8.92	2.87	0.20	0.00	0.86	0.88	0.03	0.65	0.37	0.38	3.13	0.43
E.coli (EC)	2209.87^{b}	101.36^{c}	4.37^{b}	36.16^{a}	0.00^{d}	14.20^{c}	0.00^{c}	0.42^{d}	0.45^{e}	0.00^{d}	0.04^{d}	1.61^{c}	6.09^{c}
	79.39	8.73	0.35	1.50	0.00	1.27	0.00	0.01	0.02	0.00	0.01	0.16	0.32
EC × CREM	2910.67^{a}	191.99^{b}	19.19^{b}	12.85^{c}	9.08^{b}	64.74^{a}	3.82^{c}	3.64^{b}	74.94^{a}	20.71^{b}	71.03^{b}	108.39^{b}	14.73^{a}
	196.36	13.19	0.58	0.90	0.68	3.33	0.32	0.27	1.07	1.32	4.86	6.71	0.86
EC × LAC	3107.55^{a}	166.74^{b}	46.56^{b}	3.37^{e}	5.22^{c}	35.81^{b}	38.25^{b}	1.40^{c}	59.55^{b}	7.35^{c}	77.87^{a}	162.36^{a}	10.37^{b}
	162.53	10.38	1.34	0.17	0.30	2.63	2.47	0.08	1.90	0.54	1.33	14.85	0.89
EC × PLAN	2207.91^{a}	127.99^{c}	9.32^{b}	6.81^{d}	1.00^{d}	32.62^{b}	0.00^{b}	1.41^{c}	15.67^{d}	9.09^{c}	6.42^{c}	112.51^{b}	14.19^{a}
	153.53	4.84	0.72	0.55	0.01	0.98	0.00	0.08	0.57	0.10	0.55	6.90	0.79
EC × STREP	1961.85^{b}	353.80^{a}	650.67^{a}	23.65^{b}	11.68^{a}	36.77^{b}	239.32^{a}	8.50^{a}	25.34^{c}	32.98^{a}	1.52^{cd}	91.31^{b}	12.94^{a}
	127.64	16.81	35.58	1.39	0.52	1.01	13.18	0.50	1.27	2.37	0.07	6.98	1.08
K. pneumoniae (KP)	2172.84^{b}	73.61^{c}	7.60^{d}	22.51^{a}	0.00^{d}	11.08^{d}	0.00^{d}	0.57^{b}	0.70^{d}	0.33^{d}	0.24^{d}	3.65^{d}	4.63^{d}
	101.51	3.04	0.01	1.56	0..00	0.46	0.00	0.02	0.06	0.03	0.02	0.14	0.16
KP × CREM	1474.91^{d}	49.60^{c}	2.94^{e}	4.22^{d}	0.76^{c}	19.44^{c}	2.96^{c}	0.71^{b}	3.66^{cd}	5.58^{c}	5.67^{d}	7.91^{d}	4.23^{d}
	145.43	3.47	0.86	0.31	0.06	1.70	0.09	0.01	0.33	0.13	0.35	0.58	0.16
KP × LAC	2222.31^{b}	227.01^{b}	27.50^{c}	8.84^{c}	2.46^{b}	33.12^{b}	24.82^{a}	0.70^{b}	59.68^{a}	14.36^{b}	70.96^{a}	101.54^{a}	12.34^{b}
	122.35	16.30	1.17	0.04	0.22	1.63	1.16	0.01	4.08	0.94	1.12	9.90	1.04
KP × PLAN	2931.24^{a}	232.86^{ab}	43.86^{a}	20.05^{b}	0.00^{d}	50.82^{a}	12.62^{b}	23.90^{a}	17.01^{b}	17.87^{a}	42.71^{c}	73.55^{b}	18.67^{a}
	228.13	16.08	1.25	0.35	0.00	1.28	0.74	0.71	0.86	1.27	3.61	6.81	1.27
KP × STREP	1819.99^{cd}	269.96^{a}	32.61^{b}	9.32^{c}	5.37^{a}	36.32^{b}	2.95^{c}	1.39^{b}	6.81^{c}	19.12^{a}	57.84^{b}	46.60^{c}	9.60^{c}
	103.84	22.92	1.63	0.60	0.06	2.91	0.01	0.12	0.01	1.34	3.92	3.24	0.35

CREM: *Lactococcus lactis* subsp. *cremoris*; LAC: *Lc. lactis subsp.* lactis; PLAN: *Lactobacillus plantarum*; THERM: *Streptococcus thermophilus*. AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIS, histamine; SPD, spermidine; TRP, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. ^{x} Mean value, ^{y} Standard deviation (n = 4) ND: not detected. Standard deviation (n = 4). Different lowercase letters ($^{a-d}$) in a row indicate significant differences ($P < 0.05$) among groups.

Table 3. Biogenic amines and ammonia production (mg/L) by mix cultures (lactic acid bacteria and gram negative food-borne pathogens) in arginine decarboxylase broth (ADB).

Microorganism	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	HIS	SER	TYR	TMA	DOP	AGM
P. aeruginosa (PA)	2716.88^{b}	57.12^{d}	2.40^{d}	2.56^{d}	0.00^{c}	19.64^{d}	0.00^{d}	0.42^{b}	1.77^{d}	0.21^{c}	0.17^{b}	3.67^{d}	10.98^{b}
	182.71	2.27	0.01	0.18	0.00	1.29	0.00	0.03	0.15	0.01	0.01	0.14	1.31
PA × CREM	2515.18^{bc}	177.32^{c}	17.72^{c}	13.18^{c}	0.89^{c}	60.24^{a}	1.85^{c}	1.91^{b}	3.42^{c}	30.74^{a}	4.97^{b}	42.27^{b}	9.43^{b}
	115.17	4.93	1.66	0.30	0.06	2.85	0.16	0.01	0.37	1.87	0.04	2.83	0.89
PA × LAC	4607.79^{a}	245.95^{b}	38.45^{a}	13.05^{c}	0.29^{c}	39.78^{b}	0.00^{d}	23.54^{a}	2.31^{cd}	4.41^{b}	0.07^{b}	10.27^{c}	7.18^{c}
	57.36	15.49	3.27	0.89	0.03	1.41	0.00	1.38	0.21	0.34	0.00	0.87	0.55
PA × PLAN	2369.10^{c}	443.07^{a}	33.40^{ab}	25.57^{b}	5.27^{b}	43.34^{b}	3.16^{b}	3.35^{b}	11.45^{a}	5.66^{b}	7.98^{b}	30.10^{b}	10.96^{b}
	30.59	11.39	2.10	0.91	0.08	2.85	0.04	0.12	1.04	0.36	0.38	1.77	0.05
PA × THERM	976.51^{d}	398.47^{b}	28.92^{b}	27.44^{a}	25.51^{a}	32.61^{c}	10.67^{a}	755.27^{a}	7.04^{b}	4.89^{b}	138.73^{a}	78.70^{a}	13.30^{a}
	75.32	5.35	1.43	0.87	1.35	1.17	1.03	33.85	0.05	0.04	12.07	1.67	0.53
S. paratyphi A (SP)	1531.88^{b}	220.81^{c}	1.29^{d}	13.20^{a}	0.00^{c}	10.18^{d}	0.00^{d}	0.21^{c}	0.56^{c}	0.17^{d}	0.15^{b}	0.98^{c}	3.67^{d}
	82.30	13.44	0.10	0.47	0.00	0.19	0.00	0.02	0.04	0.02	0.00	0.08	0.30
SP × CREM	1193.61^{b}	138.07^{d}	27.65^{c}	9.24^{b}	0.29^{c}	34.75^{b}	1.17^{c}	39.31^{a}	1.10^{c}	2.97^{c}	0.06^{b}	8.45^{c}	5.36^{c}
	91.48	7.86	0.57	0.33	0.01	1.24	0.12	2.18	0.05	0.23	0.00	0.70	0.20
SP × LAC	2188.95^{a}	378.88^{a}	80.90^{b}	10.38^{b}	7.80^{a}	54.81^{a}	6.98^{b}	3.26^{b}	50.53^{a}	8.07^{b}	43.58^{a}	94.83^{a}	8.59^{b}
	203.03	11.95	6.25	0.68	0.78	0.94	0.31	0.28	1.99	0.64	2.14	6.12	0.20
SP × PLAN	2377.71^{a}	154.26^{d}	26.51^{c}	5.27^{c}	0.00^{c}	29.07^{c}	0.00^{d}	3.96^{b}	6.73^{b}	8.70^{b}	0.33^{b}	35.07^{b}	11.23^{a}
	224.00	10.26	1.31	0.50	0.00	1.99	0.00	0.19	0.61	0.35	0.00	2.01	0.58
SP × THERM	1563.01^{b}	253.10^{b}	243.2^{a}	2.69^{d}	3.68^{b}	27.02^{c}	14.42^{a}	1.36^{bc}	2.88^{c}	29.82^{a}	40.48^{a}	96.31^{a}	10.25^{a}
	134.16	8.49	19.30	0.18	0.25	0.51	0.59	0.07	0.16	1.90	3.29	8.87	0.67

CREM: *Lc. lactis* subsp. *cremoris*; LAC: *Lc. lactis* subsp. *lactis*; PLAN: *Lactobacillus plantarum*; THERM: *Streptococcus thermophilus*. AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIS, histamine; SPD, spermidine; TRP, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. ^{x} Mean value, ^{y} Standard deviation (n = 4) ND: not detected. Standard deviation (n = 4). Different lowercase letters ($^{a-d}$) in a row indicate significant differences ($P < 0.05$) among groups.

ae strains such as *E. coli*, *S. paratyphi* A and *K. pneumoniae* produced 6.09, 4.63 and 3.67 mg/L of AGM respectively. LAB strains had significant effect on increasing of AGM accumulation by gram negative FBP in ADB ($P < 0.05$), in spite of the fact that their effect was strain-dependent for *P. aeruginosa*. *Lc. lactis subsp. cremoris* and *Lb. plantarum* seemed to have no effect on AGM production by *P. aeruginosa.* In general, the strongest stimulation effect on AGM production by gram negative FBP was observed in the presence of *Lc. lactis subsp. lactis* and *Lb. plantarum.* LAB require essential amino acids for growth (Neviani *et al.*, 1995; Garault *et al.*, 2000) and to obtain essential amino acids, is able to degrade proteins into small peptides and amino acids which can be taken up from the environment by transporters (Christensen *et al.*, 1999; Pastink, 2009). The amino acid-decarboxylating activity was reported for some LAB strains (Coisson *et al.*, 2004; Landete *et al.*, 2008), whereas a number of lactic acid bacteria associated with food fermentations, exhibit the potential for degrading histamine and tyramine through the production of mono- and di-amino-oxidases (Leuschner *et al.*, 1998; Holzapfel, 2002). Studies on the interactions between mix bacterial populations (LAB and FBP) are very limited and no information exists regarding BA production mechanism of mixed culture. The increased production of BA in mixed culture is the result of presence of amine positive LAB strains. Competitive behaviour of these strains also may affect biogenic amine production of mixed culture.

The presence and accumulation of biogenic amines also depend on many factors, such as, availability of free amino acids and sugars, pH, water activity, temperature, bacterial density, bacterial competition for nutrients, and synergistic effect between micro-organisms (Stratton *et al.*, 1991; Gardini *et al.*, 2001; Suzzi and Gardini, 2003; Aguilar and Klotz, 2010).

Arginine is metabolised through agmatine deiminase, resulting in formation of PUT (Jorgensen *et al.*, 2000). PUT were the most accumulated amine by gram negative FBP, ranged from 57 mg/L for *P. aeruginosa* to 220 mg/L for *S. paratyphi* A. In histidine decarboxylase broth, lower PUT production by same FBP was found, ranging from 9.8 to 19.2 mg/L (Özogul, 2011). LAB strains significantly stimulated PUT production, apart from *Lc. lactis* subsp. *lactis* mixed with *A. hydrophila* and *Lc. lactis subsp. cremoris* mixed with *S. paratyhpi* A which resulted in significant decreases on amine production. *P. aeruginosa* was reported to be more able to produce putrescine from agmatine than the other bacteria (Landete *et al.*, 2008). In the present study, the highest stimulation effect was found for *P. aeruginosa* in the presence of *Lb. plantarum* (about 8 fold higher).

SPD and SPN are formed from PUT by successive donation of one or two aminopropyl groups, respectively, from decarboxylated S-adenosylmethionine in all organisms (Smith, 1981). LAB strains showed generally an inhibitory effect on SPD production by gram negative FBP, whereas the presence of LAB induced the production of high SPD production by *P. aeruginosa*. *Strep. thermophilus* and *Lc. lactis* subsp. *cremoris* was the most effective bacteria on stimulating SPD production by *A. hydrophila* and *P. aeruginosa*, respectively. SPN was generally seemed to be increased by presence of LAB strains, although some LAB strains showed no effect on amine production ($P > 0.05$). While *A. hydrophila* had not an ability to produce SPN alone, *Lb. plantarum* resulted in about 160 fold higher SPN production in ADB. *Strep. thermophilus* had also significant effect on SPN production by *E. coli*, *P. aeruginosa* and *S. parathypi* A.

Lopez-Sabater *et al.* (1996) found that the production of HIS by *K. pneumoniae* was 216 ppm in a culture broth assay. HIS production by *K. pneumoniae* was stimulated by *Lb. plantarum* ($p < 0.05$) while the other tested LAB did not have any effects on HIS formation in histidine decarboxylase broth (Özogul, 2011). In the present study, while HIS was accumulated a negligible quantity (< 0.57 mg/L) in ADB, some LAB strains resulted significant HIS production. Though the effect of LAB was strains dependent on biogenic amines by FBP strain, the strongest stimulation effect among the tested LAB strains on HIS production was observed for *Strep. thermophilus* mixed with *P. aeruginosa*. In this way, *P. aeruginosa* accumulated 750 fold higher HIS in ADB in the presence of *Strep. thermophilus*. *Lc. lactis* subsp. *cremoris* mixed with *S. paratyphi* A and *Lb. plantarum* mixed with *K. pneumoniae* also yielded medium HIS production (> 40 mg/L).

CAD production by gram negative FBP was between 1.29 mg/L for *S. paratyphi* A and 9.28 mg/L for *A. hydrophila*. The used LAB strains increased CAD production by FBP, whereas they had no significant effect on CAD production by *E. coli* ($p > 0.05$). The effect of *Strep. thermophilus* on CAD production by *E. coli* and *S. paratyphi* A as well as *Lb. plantarum* mixed with *A. hydrophila* was highest. The production of PHEN by gram negative FBP was above the 10 mg/L and generally increased with presence of LAB strains. Although none of the gram negative bacteria produced TRP, *Strep. thermophilus* stimulated significantly TRP production by *E. coli* and *P. aeruginosa*. Poor production of SER, DOP and TMA by gram negative bacteria were obtained. SER and DOP production by some FBP also increased with presence of LAB strains. TMA production by *P. aeruginosa* was 130 fold higher in the presence of *Strep. thermophilus*.

Gram negative FBP produced also low amount of TYR (< 0.4 mg/L). LAB strains also induced higher TYR accumulation in ADB. The highest TYR accumulation was found for

Strep. thermophilus mixed with *E. coli* and *S. paratyphi* A, and *Lc. lactis subsp. cremoris* mixed with *P. aeruginosa*.

The Effect of LAB Strains on BAs Production by Gram positive FBP Table 4 shows AMN and BAs production by gram positive FBP (10^{11} cfu/mL) in ADB. AMN production by gram positive FBP were above the 1600 mg/L. Significant differences in AMN production by gram positive FBP was observed in the presence of LAB strains ($P < 0.05$). There was also significant differences in AMN production between gram negative and gram positive FBP mixed with LAB ($P < 0.05$) (Tables 2 − 4). LAB strains significantly increased AMN production by *Ent. faecalis* and *Staph. aureus* (for *Lb. plantarum* and *Strep. thermophilus*). AMN production by *L. monocytogenes* did not affected in the presence of LAB strains except for *Lc. lactis* subsp. *lactis* which had inhibitory effect.

Georgnaras *et al.* (1995) found that *Listeria* strains had not an ability to produce BAs. However, in ADB, main amines produced by gram positive bacteria were PUT, SPD, AGM and PHEN. There was not significant differences in BAs production between gram negative and gram positive bacteria ($P > 0.05$), except for HIS and TMA. Gram-positive bacteria are usually associated with histamine production in fermented foods (Silla Santos, 1996), whereas in raw fish and meat, gram-negative bacteria are known to produce histamine when exposed to inappropriate temperatures (Smit *et al.*, 2008). Two different kinds of histidine decarboxylase were found in gram-negative and gram-positive bacteria. Histidine decarboxylase of gram-negative bacteria use pyridoxal phosphate as a cofactor for activity, whereas histidine decarboxylase of gram-positive bacteria use a different catalytic mechanism based on a pyruvoyl group linked at the active site (Tanase *et al.*, 1985; van Poelje *et al.*, 1990; Lucas *et al.*, 2005). *Ent. faecalis* and *Staph. aureus* had not an ability to produce HIS in ADB. However, Chang *et al.* (2008) reported that *Staph. aureus* isolates produced HIS, ranging from 12.7 to 33.0 mg/kg in culture medium. The effect of LAB strains (except for *Lc. lactis subsp. cremoris*) on HIS production by *Ent. faecalis* was also poor. *Lc. lactis subsp. cremoris* mixed with *Ent. faecalis*, *Lc. lactis subsp. lactis* mixed with *L. monocytogenes* and *Strep. thermophilus* mixed mixed with *Staph. aureus* accumulated 3-6 folds higher HIS than that of

Table 4. Biogenic amines and ammonia production (mg/L) by mix cultures (lactic acid bacteria and gram positive food-borne pathogens) in arginine decarboxylase broth (ADB).

Microorganism	AMN	PUT	CAD	SPD	TRP	PHEN	SPN	HIS	SER	TYR	TMA	DOP	AGM
Ent.faecalis (EF)	1898.77^{c} 74.85	104.94^{c} 8.85	1.61^{d} 0.07	12.47^{b} 0.82	0.00^{d} 0.00	11.92^{c} 0.93	0.00^{b} 0.00	0.00^{b} 0.00	1.59^{c} 0.10	0.17^{d} 0.02	0.04^{d} 0.00	1.91^{d} 0.11	10.78^{b} 2.10
EF × CREM	2369.93^{b} 209.84	364.15^{a} 29.19	21.18^{c} 1.30	5.88^{c} 0.55	8.83^{a} 0.45	46.80^{a} 1.88	2.18^{b} 0.04	3.26^{a} 0.21	55.54^{b} 4.44	10.06^{c} 0.64	74.80^{b} 4.03	109.59^{b} 8.19	7.20^{c} 0.26
EF × LAC	3229.85^{a} 105.70	160.90^{b} 11.85	30.68^{b} 2.47	2.50^{d} 0.10	0.86^{c} 0.06	32.01^{b} 1.64	31.23^{a} 2.44	1.37^{b} 0.08	152.66^{a} 5.22	15.10^{b} 0.81	202.04^{a} 19.17	87.53^{c} 5.00	16.29^{a} 0.85
EF × PLAN	2311.55^{b} 211.41	323.04^{a} 10.79	18.75^{c} 0.82	16.34^{a} 1.02	0.00^{d} 0.00	33.65^{b} 3.11	1.59^{b} 0.09	1.51^{b} 0.09	7.86^{c} 0.71	21.42^{a} 1.50	2.44^{cd} 0.20	85.65^{c} 5.25	11.72^{b} 0.84
EF × THERM	2421.70^{b} 57.18	359.12^{a} 16.39	37.31^{a} 1.58	3.37^{d} 0.04	2.06^{b} 0.11	34.41^{b} 1.99	2.56^{b} 0.11	1.52^{b} 0.05	5.12^{c} 0.40	9.62^{c} 0.40	24.06^{c} 1.76	151.61^{a} 7.98	16.43^{a} 0.95
L. monocytogenes (LM)	1841.57^{ab} 83.94	109.64^{d} 5.74	3.78^{d} 0.11	12.33^{b} 0.45	0.00^{d} 0.00	14.06^{d} 1.15	0.00^{d} 0.00	0.29^{c} 0.02	1.22^{c} 0.05	0.74^{d} 0.03	0.05^{b} 0.00	1.77^{c} 0.18	0.28^{d} 0.03
LM × CREM	1645.68^{bc} 21.69	257.54^{c} 7.70	107.58^{b} 2.83	13.52^{b} 0.35	0.15^{c} 0.01	25.14^{c} 1.61	5.78^{a} 0.31	0.34^{c} 0.01	22.83^{a} 0.92	6.99^{b} 0.51	29.74^{a} 1.82	44.64^{a} 3.11	6.69^{c} 0.52
LM × LAC	1544.45^{c} 77.24	299.34^{b} 27.71	53.85^{c} 4.16	4.17^{c} 0.02	1.61^{a} 0.04	30.80^{b} 2.33	1.30^{c} 0.11	1.83^{a} 0.13	6.82^{b} 0.34	4.81^{c} 0.47	0.26^{b} 0.00	15.71^{b} 1.03	10.47^{b} 0.16
LM × PLAN	2002.77^{a} 82.63	493.65^{a} 15.29	126.20^{a} 8.23	23.05^{a} 2.22	0.00^{d} 0.00	39.07^{a} 1.81	0.00^{d} 0.00	1.15^{b} 0.12	1.56^{c} 0.16	6.75^{b} 0.12	0.05^{b} 0.00	13.44^{b} 1.15	9.26^{b} 0.39
LM × THERM	1944.77^{a} 131.22	234.10^{c} 9.78	63.12^{c} 3.15	13.68^{b} 1.13	0.40^{b} 0.04	42.65^{a} 2.28	1.92^{b} 0.07	1.64^{a} 0.03	23.43^{a} 1.41	8.60^{a} 0.73	0.48^{b} 0.02	16.82^{b} 1.01	13.28^{a} 0.82
Staph. aureus (SA)	2508.24^{c} 143.15	57.83^{c} 3.54	1.64^{e} 0.13	7.24^{c} 0.40	0.53^{b} 0.05	0.92^{d} 0.02	0.00^{b} 0.00	0.00^{d} 0.00	0.74^{d} 0.02	0.07^{c} 0.00	0.10^{d} 0.00	2.21^{d} 0.14	1.42^{d} 0.14
SA × CREM	2114.98^{d} 89.16	304.25^{a} 22.89	28.28^{b} 2.37	5.08^{d} 0.37	2.92^{a} 0.28	56.39^{a} 3.32	1.89^{b} 0.01	0.99^{c} 0.00	15.90^{b} 1.44	37.04^{a} 1.63	16.87^{c} 1.07	90.20^{a} 5.37	8.45^{a} 0.28
SA × LAC	1819.03^{e} 97.67	295.27^{a} 7.39	33.97^{a} 2.11	10.33^{b} 1.22	0.61^{b} 0.01	42.86^{b} 3.85	80.07^{a} 7.18	1.30^{b} 0.01	51.99^{a} 1.32	10.60^{b} 0.92	83.66^{a} 7.47	42.07^{b} 1.19	6.92^{b} 0.06
SA × PLAN	3362.33^{a} 119.29	337.59^{a} 27.78	23.28^{c} 1.63	13.01^{a} 0.94	0.54^{b} 0.01	53.78^{a} 2.20	3.07^{b} 0.17	0.95^{a} 0.06	10.84^{c} 0.94	12.69^{b} 0.77	1.19^{d} 0.09	26.41^{c} 1.69	6.27^{c} 0.04
SA × THERM	2930.21^{b} 53.63	188.58^{b} 15.46	17.21^{d} 1.01	2.98^{e} 0.25	0.71^{b} 0.03	28.04^{c} 0.91	2.31^{b} 0.15	3.37^{a} 0.10	1.52^{d} 0.05	10.89^{b} 0.95	68.83^{b} 5.96	46.96^{b} 3.92	7.88^{a} 0.42

CREM: *Lc. lactis* subsp. *cremoris*; LAC: *Lc. lactis* subsp. *lactis*; PLAN: *Lactobacillus plantarum*; THERM: *Streptococcus thermophilus* AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIS, histamine; SPD, spermidine; TRP, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. ^{x} Mean value, ^{y} Standard deviation (n = 4) Different lowercase letters ($^{a-d}$) in a row indicate significant differences ($P < 0.05$) among groups.

single gram positive bacteria.

None of gram positive bacteria produced SPN as gram negative FBP. *L. monocytogenes* and *Staph. aureus* among the FBP characterized as the lowest AGM producer in ADB ($P < 0.05$). AGM production by *Ent. faecalis* was 10.78 mg/L.

In terms of BAs, significant differences was found only for PUT, TMA and DOP between gram negative and gram positive FBP mixed with LAB ($P < 0.05$). The used LAB strains had a stimulation effect on AGM production, although the effect of them on the production by *Ent. faecalis* was strain dependent.

Gram positive FBP seemed to convert arginine into PUT rather than AGM, as gram negative bacteria. PUT production by single gram positive FBP was between 57 and 110 mg/L. *L. monocytogenes* and *Ent. faecalis* were one of the high amount of PUT producer ($P < 0.05$) and produced similar amount of PUT. All of LAB strains led to an increase in PUT production (3 − 5 folds higher). The highest effects were found for *Lb. plantarum* mixed with *Staph. aureus* ($P < 0.05$).

Klebsiella oxytoca was found as the highest cadaverine producer with value of 325 mg/L in lysine decarboxylase broth (Özogul and Özogul, 2005). CAD production by gram positive FBP was less than 4 mg/L and increased with presence of LAB strains ($P < 0.05$). The highest CAD accumulation was observed for *Lb. plantarum* mixed with *L. monocytogenes*. SPD production by FBP varies depending on LAB strains. SPD production by *Ent. faecalis* reduced by LAB strains except for *Lc. lactis subsp. lactis*. *Lc. lactis subsp. cremoris* and *Strep. thermophilus* had inhibitory effect on reducing SPD production by *Staph. aureus*. TRP was not produced by *Ent. faecalis* and *L. monocytogenes*. LAB strains apart from *Lc. lactis* subsp. *cremoris* seemed to no effect on TRP production. Although SPN production by single gram positive LAB and FBP were not observed in ADB, *Lc. lactis* subsp. *lactis* mixed with *Ent. faecalis* and *Staph. aureus* resulted in high SPN accumulation in broth. Similar effect was also found for SER. Engesser *et al.* (1990) also reported that *Ent. faecalis* formed significant concentrations of TYR under experimental conditions. Kim *et al.* (2009) found that *Enterobacter* strains isolated from fish species did not produce DOP. In the present study, TYR and DOP production by single gram positive FBP was low (> 2.5 mg/L). TYR production by *Ent. faecalis* and *Staph. aureus* was increased in the presence of *Lb. plantarum* and *Lc. lactis subsp. cremoris* (> 20 mg/L). Although the highest TYR production among the tested FBP was found for *L. monocytogenes* ($P < 0.05$), *L. monocytogenes* produced lower TYR presence of LAB strains compared to *Ent. faecalis* and *Staph. aureus* (Table 4). There was significant increase in the value of DOP produced by FBP in the presence of *Strep. thermophilus* and *Lc. lactis subsp. cremoris*.

Gram positive FBP produced negligible amounts of TMA. In the presence of LAB strains apart from *Lb. plantarum* on ADB, production of TMA by *Ent. faecalis* and *Staph. thermophilus* was high. However, there was not any effect of LAB strains (except for *Lc. lactis subsp. cremoris*) on TMA production by *L. monocytogenes*.

Conclusions

In conclusion, all bacterial cultures tested in this study had the ability to produce AGM (0.2 − 11 mg/L), PUT (33 − 228 mg/L), CAD (1 − 10 mg/L), PHEN (0.9 − 20 mg/L), and DOP (0.9 − 7 mg/L). Putrescine was the major amine indicating conversion of arginine into PUT through AGM pathway. The result obtained from this study showed that the function of LAB strains on AMN and BAs production by gram negative and positive FBP varies depending on strains, although LAB used with some selected pathogens had stimulation effect on AMN and BAs. *P. aeruginosa* accumulated 75 fold higher HIS in ADB in the presence of *Strep. thermophilus*.

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