

Research Paper

## The influences of fish infusion broth on the biogenic amines formation by lactic acid bacteria

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### Abstract

The influences of fish infusion decarboxylase broth (IDB) on biogenic amines (BA) formation by lactic acid bacteria (LAB) were investigated. BA productions by single LAB strains were tested in five different fish (anchovy, mackerel, white shark, sardine and gilthead seabream) IDB. The result of the study showed that significant differences in ammonia (AMN) and BA production were observed among the LAB strains in fish IDB ( $p < 0.05$ ). The highest AMN and TMA production by LAB strains were observed for white shark IDB. The all tested bacteria had decarboxylation activity in fish IDB. The uppermost accumulated amines by LAB strains were tyramine (TYM), dopamine, serotonin and spermidine. The maximum histamine production was observed in sardine (101.69 mg/L) and mackerel (100.84 mg/L) IDB by *Leuconostoc mesenteroides* subsp. *cremoris* and *Pediococcus acidophilus*, respectively. *Lactobacillus delbrueckii* subsp. *lactis* and *Pediococcus acidophilus* had a high TYM producing capability (2943 mg/L and 1157 mg/L) in sardine IDB.

**Key words:** biogenic amines, lactic acid bacteria, starter cultures, fish infusion broth.

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### Introduction

Biogenic amines (BA) production in seafood are one of the considerable public concern since histamine and possibly other biogenic amines such as cadaverine and putrescine are responsible for histamine fish poisoning (Taylor 1986; Jorgensen *et al.*, 2000) and serve as chemical indicators of fish spoilage (Alberto *et al.*, 2002). The toxicity of histamine appears to be enhanced by cadaverine (CAD) and putrescine (PUT) since they inhibit the histamine-detoxifying enzymes, which are diamine oxidase and histamine N-methyltransferase (Stratton *et al.*, 1991; Yongswatdigul *et al.*, 2004). Biogenic amines such as PUT, CAD, spermidine (SPD), spermine (SPN), histamine (HIM), tyramine (TYM) and tryptamine (TRP) are widely distributed in proteinaceous foods (Krizek *et al.*, 2004). Most studies have been focused on BA formation in fish and meat products (Ruiz-Cappillas and Jimenez-Colmenero, 2004) due to their proteinaceous nature and propensity to form BA from free amino acids (Magwamba 2010).

Accumulation of BA in foods requires (I) the availability of precursors (*i.e.* amino acids), (II) the presence of microorganisms with amino acid decarboxylases enzyme, which either derived from environmental contamination or from an added starter culture and (III) favourable conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity (Bodmer *et al.*, 1999; Karovicova and Kohajdova, 2005; Stadnik and Dolatowski, 2010). Although amino acid decarboxylases are not widely distributed among bacteria, species of many genera such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, *Photobacterium* and the lactic acid bacteria (*Lactobacillus*, *Pediococcus*, and *Streptococcus*) are capable of decarboxylating one or more amino acids (Halasz *et al.*, 1994; Silla-Santos 1996; Karovicova and Kohajdova, 2005; Özogul and Özogul, 2005).

BA production by bio-preservative features of lactic acid bacteria (LAB) have been reported (Connil *et al.*, 2002). Some strains of LAB synthesize histamine because

of their ability to possess the histidine decarboxylase gene (Landete *et al.*, 2005; Lucas *et al.*, 2005). The presence of BA in fermented foods is due to the decarboxylase activity of the LAB used as starter culture, and the action of some spoilage bacteria (Marcobal *et al.*, 2006). High BA contents have been reported in some fish products such as fish sauce, fish paste, fish salads, cold-smoked fish (Leuschner and Hammes, 1999; Petaja *et al.*, 2000; Yongsawatdigul *et al.*, 2004; Jorgensen *et al.*, 2000; Gonzalez-Rodriguez *et al.*, 2002; Thapa *et al.*, 2006; Udomsil *et al.*, 2010; Zaman *et al.*, 2010; Zhong-Yi *et al.*, 2010). The production of BA by LAB to be selected as starter cultures is not a desirable feature for food industry (Buchenuskes 1993). Thus some safety aspects of the LAB isolates of food products must be investigated before considering their use for the bio-preservation (Matamoros *et al.*, 2009). Before the use of the LAB in the fish product, the quantitative analysis of BA is very essential to confirm the bacterial production. A great amount of research has been focused on BA production in fish and fish products. However no study has been found on BA production by single LAB strains which is used in fish infusion broth. Therefore, the aim of the study was to investigate the function of some commercially important LAB strains on biogenic amine production in different fish infusion decarboxylase broth (IDB).

## 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). They were obtained from Sutcu Imam University, Kahramanmaras, Turkey in BGML stock culture. *Leuconostoc mesenteroides* subsp. *cremoris* (DSMZ 20346), *Lactobacillus acidophilus* (ATCC 11975), *Pediococcus acidophilus* (ATCC 25741) and *Lactobacillus delbrueckii* subsp. *lactis* (ATCC 10697) were purchased from Institute of Refik Saydam Hifzisiyhha (Ankara, Turkey).

### Fish species

In the present study, fish decarboxylase infusion broth was prepared using five different fish species which were gilthead seabream (*Sparus aurata*), sardine (*Sardinella aurita*) anchovy (*Engraulis encrasicolus*), white shark (*Carcharodon carcharias*), and mackerel (*Scomber scombrus*).

### Biogenic amine analysis

#### Culture media and bacterial extraction

Fish infusion broth was prepared according to method of Okuzumi *et al.* (1982) with minor modifications. Two hundred fifty grams of fish flesh was homogenised with 2 volumes of water (w/v), steamed at 100 °C for 1 hour and

filtered. The filtrate was enriched with 1% glucose and 0.5% NaCl. In order to decarboxylate amino acid by bacteria, 3 mg pyridoxal HCl addition was made in each infusion broth before autoclaving process.

MRS and M17 broth were used for propagation of LAB cultures. Lactic acid bacterial strains were incubated at 37 °C for 24 hour which after 0.5 mL of these bacterial cultures was removed and put into the fish IDB to decarboxylate amino acid.

For extraction of LAB cultures, 5 mL of the fish IDB containing LAB strains were removed to separate bottles and then 2 mL trichloroacetic acid was added. They were centrifuged at 3000xg for 10 min and then filtered through a Whatman filter paper (Whatman GmbH, Dassel, Germany). After that, 4 mL of bacterial supernatant was taken for derivatisation from each of LAB bacterial strains.

### Chemical reagents

All BA standards were purchased from Sigma-Aldrich (Munich, Germany). The mobile phase consisted of acetonitrile and HPLC grade water for amine analyses.

### Preparation of standard amine solution

HIM dihydrochloride (165.7 mg), TYM hydrochloride (126.7 mg), TRP hydrochloride (122.8 mg), PUT dihydrochloride (182.9 mg), 2-phenylethylamine (PHEN) hydrochloride (130.1 mg), CAD dihydrochloride (171.4 mg), SPD trihydrochloride (175.3 mg), SPN tetrahydrochloride (172.0 mg), 5-hydroxytryptamine (Serotonin, SER) (133.9 mg), 3-hydroxytyramine hydrochloride (Dopamine, DOP) (123.8 mg), agmatine (AGM) sulphate (175.4 mg), trimethylamine (TMA) hydrochloride (161.7 mg) and ammonium chloride (296.9 mg) were dissolved in 10 mL HPLC grade water. The final concentration of free base for each amine was 10 mg mL<sup>-1</sup> solution.

### Derivatisation of extract from bacterial broth culture

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For derivatisation of standard amine solutions, 100 µL was taken (4 mL for extracted bacterial cultures) from each free base standard solution (10 mg mL<sup>-1</sup>). 1 mL of sodium hydroxide (2 M) was added, followed by 1 mL of 2% benzoyl chloride (dissolved in acetonitrile) and the solution mixed on a vortex mixer for 1 min. The reaction mixture was left at room temperature for 5 min and then centrifuged for 10 min. After that, the benzylation was stopped by adding 2 mL of saturated sodium chloride 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

BA analysis was done using the method of Özogul (2004) and measured in mg amines per litre broth. The confirmation of BA production was accomplished using a rapid HPLC method with a reversed phase column by using a gradient elution program. Same analytic method was used for ammonia and trimethylamine (TMA) separation.

## 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 was a reverse-phase, ODS Hypersil 5, 250x4.6 mm (Phenomenex, Macclesfield, Cheshire, UK) for the BA analyses.

## Statistical analysis

The mean value and standard deviation were calculated from the data obtained from the four 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

Ammonia (AMN) and BA production by LAB strains in five different fish IDB were given in Tables 1 to 5. Significant differences in AMN were found among the LAB strains and fish IDB ( $p < 0.05$ ). The all used bacteria produced more than 200 mg/L of AMN. *Lc. lactis* subsp. *cremoris* produced the highest amount of AMN (591.72 mg/L) in sardine IDB, ( $p < 0.05$ ). The lowest AMN production was observed by *Strep. thermophilus* in sardine infusion broth (Table 2), whereas LAB strains had the highest ability to produce AMN in white shark infusion broth (Table 5).

There were also significant differences in BA production among the LAB strains ( $p < 0.05$ ) for all fish IDB. The strains produced all amine in fish IDB (Tables 1 to 5) apart from spermine (SPN) and 2-phenylethylamine (PHEN). Putrescine (PUT) production by LAB strains was ranged from 1.85 (for *Strep. thermophilus* in white shark IDB) to 139.98 mg/L (for *Ped. acidophilus* in anchovy IDB).

Significant differences in histamine (HIM) production was observed among the LAB strains ( $p < 0.05$ ). *Lc. lactis* subsp. *cremoris*, *Leuc. mesenteroides* subsp. *cremoris* and *Lc.* produced cadaverine (CAD) more than 100 mg/L in sardine IDB, whereas CAD production by LAB strains in the other mediums was less than 93 mg/L. In mackerel IDB, the lowest spermidine (SPD) production was found for *Lactococcus lactis* subsp. *cremoris*

**Table 1** - Biogenic amines production (mg/L) by lactic acid bacteria in anchovy (*Engraulis encrasicolus*) infusion decarboxylase broth.

	<i>Lb.delb.</i> subsp. <i>lactis</i> * <i>Leu.mex.</i> subsp. <i>crem.</i>	<i>Lb.acidophilus</i>	<i>Ped.acidophilus</i>	<i>Lc.cremoris</i>	<i>Lc.lactis</i>	<i>Lb.plantarum</i>	<i>Strep.thermophilus</i>
AMN	530.36 <sup>a</sup> ± 29.71 <sup>abcd</sup>	572.68 ± 7.95 <sup>bc</sup>	360.68 ± 21.35 <sup>a</sup>	670.95 ± 36.79 <sup>a</sup>	281.96 ± 22.08 <sup>f</sup>	407.76 ± 16.65 <sup>e</sup>	597.09 ± 35.21 <sup>b</sup>
PUT	39.61 ± 2.51 <sup>cd</sup>	44.16 ± 2.15 <sup>c</sup>	37.85 ± 0.37 <sup>cd</sup>	139.98 ± 10.11 <sup>a</sup>	14.49 ± 2.05 <sup>e</sup>	32.09 ± 1.20 <sup>d</sup>	38.56 ± 1.48 <sup>cd</sup>
CAD	39.06 ± 1.01 <sup>c</sup>	41.47 ± 2.01 <sup>c</sup>	33.64 ± 2.22 <sup>d</sup>	110.51 ± 3.17 <sup>a</sup>	17.80 ± 1.42 <sup>e</sup>	32.46 ± 2.66 <sup>d</sup>	33.40 ± 1.99 <sup>d</sup>
SPD	43.84 ± 3.50 <sup>e</sup>	100.72 ± 10.06 <sup>c</sup>	58.53 ± 2.24 <sup>d</sup>	196.83 ± 4.06 <sup>a</sup>	24.55 ± 2.00 <sup>f</sup>	55.32 ± 1.14 <sup>de</sup>	64.75 ± 3.24 <sup>d</sup>
TRPT	17.76 ± 0.37 <sup>b</sup>	14.26 ± 1.34 <sup>de</sup>	6.11 ± 0.38 <sup>e</sup>	1.64 ± 0.12 <sup>f</sup>	12.45 ± 1.10 <sup>d</sup>	15.61 ± 0.52 <sup>bc</sup>	6.08 ± 0.11 <sup>e</sup>
PHEN	39.72 ± 2.99 <sup>d</sup>	38.12 ± 2.36 <sup>de</sup>	21.21 ± 2.30 <sup>f</sup>	16.44 ± 0.63 <sup>f</sup>	99.28 ± 10.30 <sup>b</sup>	116.27 ± 7.54 <sup>a</sup>	27.78 ± 0.81 <sup>ef</sup>
SPN	63.02 ± 5.80 <sup>b</sup>	63.96 ± 4.09 <sup>b</sup>	36.74 ± 2.19 <sup>cd</sup>	17.14 ± 0.20 <sup>e</sup>	0.89 ± 0.01 <sup>f</sup>	40.10 ± 2.82 <sup>c</sup>	29.77 ± 2.81 <sup>d</sup>
HIM	47.10 ± 1.42 <sup>a</sup>	22.88 ± 1.40 <sup>b</sup>	19.960/49 <sup>c</sup>	2.69 ± 0.16 <sup>c</sup>	4.77 ± 0.19 <sup>e</sup>	11.77 ± 0.15 <sup>d</sup>	22.39 ± 0.47 <sup>b</sup>
SER	589.27 ± 25.24 <sup>a</sup>	307.15 ± 6.70 <sup>c</sup>	80.18 ± 4.41 <sup>f</sup>	135.09 ± 6.86 <sup>c</sup>	301.84 ± 15.43 <sup>c</sup>	477.28 ± 17.08 <sup>b</sup>	469.59 ± 2.83 <sup>b</sup>
TYM	75.33 ± 4.16 <sup>e</sup>	56.29 ± 4.13 <sup>f</sup>	69.74 ± 3.87 <sup>ef</sup>	224.42 ± 12.50 <sup>b</sup>	153.64 ± 8.18 <sup>d</sup>	61.02 ± 2.38 <sup>ef</sup>	170.30 ± 6.79 <sup>c</sup>
TMA	414.60 ± 17.91 <sup>a</sup>	0.07 ± 0.00 <sup>d</sup>	1.34 ± 0.11 <sup>d</sup>	7.17 ± 0.13 <sup>d</sup>	97.91 ± 2.82 <sup>c</sup>	247.56 ± 8.21 <sup>b</sup>	5.60 ± 0.35 <sup>d</sup>
DOP	11.39 ± 0.60 <sup>g</sup>	128.50 ± 7.88 <sup>d</sup>	103.89 ± 9.77 <sup>e</sup>	200.71 ± 5.97 <sup>b</sup>	554.30 ± 17.18 <sup>a</sup>	48.53 ± 3.14 <sup>f</sup>	201.43 ± 7.29 <sup>b</sup>
AGM	34.26 ± 2.99 <sup>d</sup>	20.27 ± 2.01 <sup>e</sup>	42.51 ± 2.92 <sup>c</sup>	40.87 ± 2.19 <sup>cd</sup>	55.12 ± 1.49 <sup>b</sup>	47.37 ± 1.56 <sup>c</sup>	47.21 ± 3.43 <sup>c</sup>

\**Lactococcus lactis* subsp. *cremoris* MG 1363. *Lactococcus lactis* subsp. *lactis* IL 1403. *Lactobacillus plantarum* F18595. *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346. *Lactobacillus acidophilus* ATCC 11975. *Pediococcus acidophilus* ATCC 25741. *Lactobacillus delbrueckii* subsp. *lactis* ATCC 10697 and *Streptococcus thermophilus*, AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIM, histamine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYM, tyramine; TMA, trimethylamine; DOP, Dopamine, AGM, agmatine. <sup>a</sup>Mean value. <sup>b</sup>Standard deviation (n = 4). Different lowercase letters (a-g) in a row indicate significant differences ( $p < 0.05$ ) among bacteria.

**Table 2** - Biogenic amines production (mg/L) by lactic acid bacteria in sardine (*Sardinella aurita*) infusion decarboxylase broth.

	<i>Lb.delb.</i> subsp. <i>lactis</i> * <i>Leu.mes.</i> subsp. <i>crem</i>	<i>Lb.acidophilus</i>	<i>Ped.acidophilus</i>	<i>Lc.cremoris</i>	<i>Lc.lactis</i>	<i>Lb.plantarum</i>	<i>Strep.thermophilus</i>
AMN	399.04 <sup>a</sup> ± 16.44 <sup>ac</sup>	335.78 ± 7.60 <sup>d</sup>	410.71 ± 6.32 <sup>bc</sup>	591.72 ± 15.38 <sup>a</sup>	262.90 ± 4.10 <sup>e</sup>	428.42 ± 18.46 <sup>b</sup>	206.28 ± 8.81 <sup>f</sup>
PUT	58.93 ± 2.71 <sup>b</sup>	43.22 ± 2.48 <sup>e</sup>	26.67 ± 2.16 <sup>d</sup>	75.85 ± 6.82 <sup>a</sup>	18.12 ± 1.58 <sup>e</sup>	33.22 ± 2.08 <sup>d</sup>	29.62 ± 2.29 <sup>d</sup>
CAD	92.14 ± 2.58 <sup>b</sup>	125.04 ± 10.28 <sup>e</sup>	75.69 ± 7.40 <sup>bc</sup>	108.89 ± 8.79 <sup>a</sup>	60.20 ± 3.11 <sup>e</sup>	83.29 ± 5.31 <sup>b</sup>	41.08 ± 3.36 <sup>d</sup>
SPD	101.51 ± 9.19 <sup>c</sup>	146.41 ± 11.97 <sup>a</sup>	43.11 ± 2.36 <sup>e</sup>	124.20 ± 4.54 <sup>b</sup>	66.22 ± 3.14 <sup>d</sup>	66.27 ± 5.48 <sup>d</sup>	28.06 ± 1.49 <sup>f</sup>
TRPT	15.33 ± 1.20 <sup>a</sup>	3.09 ± 0.12 <sup>bc</sup>	3.02 ± 0.23 <sup>bc</sup>	8.12 ± 0.43 <sup>b</sup>	2.42 ± 0.15 <sup>e</sup>	6.17 ± 0.52 <sup>e</sup>	8.86 ± 0.20 <sup>b</sup>
PHEN	0.00 ± 0.00 <sup>f</sup>	32.45 ± 2.05 <sup>c</sup>	10.45 ± 0.63 <sup>e</sup>	81.35 ± 1.72 <sup>a</sup>	0.00 ± 0.00 <sup>f</sup>	15.27 ± 0.38 <sup>d</sup>	0.00 ± 0.00 <sup>f</sup>
SPN	104.02 ± 8.22 <sup>a</sup>	22.15 ± 1.63 <sup>f</sup>	21.38 ± 0.46 <sup>f</sup>	82.34 ± 3.47 <sup>b</sup>	70.42 ± 4.83 <sup>e</sup>	35.33 ± 2.84 <sup>e</sup>	14.48 ± 0.74 <sup>f</sup>
HIM	21.00 ± 0.09 <sup>e</sup>	101.69 ± 6.99 <sup>a</sup>	67.42 ± 4.93 <sup>b</sup>	24.77 ± 2.07 <sup>e</sup>	59.47 ± 3.63 <sup>e</sup>	20.03 ± 1.66 <sup>e</sup>	35.73 ± 1.22 <sup>d</sup>
SER	110.02 ± 6.30 <sup>ef</sup>	160.32 ± 11.42 <sup>d</sup>	95.31 ± 6.01 <sup>f</sup>	219.31 ± 7.33 <sup>c</sup>	419.25 ± 22.31 <sup>a</sup>	117.06 ± 5.92 <sup>ef</sup>	310.71 ± 6.71 <sup>b</sup>
TYM	2943.51 ± 113.54 <sup>a</sup>	374.25 ± 22.89 <sup>d</sup>	658.86 ± 35.00 <sup>e</sup>	345.74 ± 18.16 <sup>d</sup>	154.39 ± 9.54 <sup>e</sup>	700.41 ± 42.77 <sup>c</sup>	163.78 ± 11.87 <sup>e</sup>
TMA	0.38 ± 0.03 <sup>d</sup>	1.99 ± 0.08 <sup>c</sup>	2.61 ± 0.25 <sup>b</sup>	0.43 ± 0.02 <sup>d</sup>	4.29 ± 0.03 <sup>a</sup>	2.47 ± 0.16 <sup>b</sup>	0.21 ± 0.01 <sup>d</sup>
DOP	324.66 ± 16.95 <sup>b</sup>	145.79 ± 5.31 <sup>d</sup>	166.21 ± 8.61 <sup>d</sup>	276.74 ± 14.14 <sup>b</sup>	349.46 ± 23.14 <sup>a</sup>	91.08 ± 6.16 <sup>e</sup>	88.42 ± 4.36 <sup>e</sup>
AGM	33.35 ± 1.72 <sup>cd</sup>	56.52 ± 3.25 <sup>a</sup>	28.93 ± 1.19 <sup>d</sup>	39.64 ± 2.08 <sup>b</sup>	31.38 ± 1.32 <sup>e</sup>	22.41 ± 1.42 <sup>e</sup>	21.19 ± 0.72 <sup>e</sup>

\**Lactococcus lactis* subsp. *cremoris* MG 1363. *Lactococcus lactis* subsp. *lactis* IL 1403. *Lactobacillus plantarum* F18595. *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346. *Lactobacillus acidophilus* ATCC 11975. *Pediococcus acidophilus* ATCC 25741. *Lactobacillus delbrueckii* subsp. *lactis* ATCC 10697 and *Streptococcus thermophilus*. AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIM, histamine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYM, tyramine; TMA, trimethylamine; DOP, Dopamine, AGM, agmatine. <sup>a</sup>Mean value. <sup>b</sup>Standard deviation (n = 4). Different lowercase letters <sup>(a-e)</sup> in a row indicate significant differences (p < 0.05) among bacteria.

**Table 3** - Biogenic amines production (mg/L) by lactic acid bacteria in gilthead seabream (*Sparus aurata*) infusion decarboxylase broth.

	<i>Lb.delb.</i> subsp. <i>lactis</i> * <i>Leu.mes.</i> subsp. <i>crem</i>	<i>Lb.acidophilus</i>	<i>Ped.acidophilus</i>	<i>Lc.cremoris</i>	<i>Lc.lactis</i>	<i>Lb.plantarum</i>	<i>Strep.thermophilus</i>
AMN	446.71 <sup>x</sup> ± 18.96 <sup>ye</sup>	494.40 ± 6.46 <sup>d</sup>	501.95 ± 32.13 <sup>d</sup>	523.20 ± 22.26 <sup>d</sup>	675.14 ± 25.37 <sup>a</sup>	625.78 ± 14.43 <sup>bc</sup>	589.46 ± 7.92 <sup>e</sup>
PUT	32.32 ± 1.23 <sup>d</sup>	27.49 ± 1.15 <sup>e</sup>	22.48 ± 0.54 <sup>f</sup>	44.21 ± 1.82 <sup>e</sup>	76.98 ± 1.86 <sup>a</sup>	53.91 ± 1.00 <sup>b</sup>	41.05 ± 2.06 <sup>c</sup>
CAD	33.08 ± 2.29 <sup>e</sup>	36.01 ± 2.79 <sup>e</sup>	44.02 ± 1.92 <sup>d</sup>	63.28 ± 4.09 <sup>b</sup>	73.31 ± 2.36 <sup>a</sup>	52.52 ± 3.48 <sup>c</sup>	42.80 ± 2.11 <sup>d</sup>
SPD	42.81 ± 1.57 <sup>e</sup>	60.45 ± 4.10 <sup>d</sup>	33.10 ± 3.11 <sup>f</sup>	87.08 ± 2.43 <sup>e</sup>	96.30 ± 4.16 <sup>b</sup>	116.68 ± 4.35 <sup>a</sup>	69.14 ± 3.55 <sup>d</sup>
TRPT	3.86 ± 0.24 <sup>e</sup>	2.70 ± 0.15 <sup>f</sup>	0.84 ± 0.02 <sup>g</sup>	8.43 ± 0.62 <sup>c</sup>	15.04 ± 0.30 <sup>a</sup>	10.79 ± 0.45 <sup>b</sup>	5.88 ± 0.20 <sup>d</sup>
PHEN	15.43 ± 1.31 <sup>e</sup>	14.37 ± 0.86 <sup>e</sup>	14.87 ± 0.26 <sup>e</sup>	35.76 ± 0.76 <sup>d</sup>	209.52 ± 3.81 <sup>a</sup>	52.15 ± 1.08 <sup>c</sup>	71.88 ± 0.97 <sup>b</sup>
SPN	19.61 ± 1.58 <sup>d</sup>	28.35 ± 0.13 <sup>c</sup>	28.25 ± 1.09 <sup>c</sup>	63.45 ± 1.56 <sup>b</sup>	85.01 ± 2.96 <sup>a</sup>	67.74 ± 3.24 <sup>b</sup>	62.32 ± 2.81 <sup>b</sup>
HIM	5.62 ± 0.48 <sup>e</sup>	13.54 ± 0.29 <sup>d</sup>	14.18 ± 1.38 <sup>d</sup>	30.74 ± 1.25 <sup>e</sup>	73.70 ± 3.05 <sup>a</sup>	41.06 ± 1.42 <sup>b</sup>	29.09 ± 1.69 <sup>c</sup>
SER	36.67 ± 1.38 <sup>d</sup>	228.07 ± 5.62 <sup>a</sup>	234.28 ± 16.92 <sup>a</sup>	123.09 ± 10.93 <sup>c</sup>	191.91 ± 7.57 <sup>b</sup>	133.97 ± 10.84 <sup>c</sup>	127.97 ± 9.12 <sup>c</sup>
TYM	23.22 ± 1.23 <sup>e</sup>	83.65 ± 3.01 <sup>d</sup>	94.84 ± 2.59 <sup>d</sup>	145.12 ± 2.81 <sup>c</sup>	375.17 ± 20.28 <sup>a</sup>	177.01 ± 6.26 <sup>b</sup>	181.62 ± 6.19 <sup>b</sup>
TMA	0.12 ± 0.01 <sup>e</sup>	3.59 ± 0.21 <sup>c</sup>	4.09 ± 0.09 <sup>b</sup>	5.86 ± 0.45 <sup>a</sup>	6.02 ± 0.09 <sup>a</sup>	0.51 ± 0.04 <sup>de</sup>	0.62 ± 0.05 <sup>d</sup>
DOP	172.37 ± 8.15 <sup>c</sup>	323.49 ± 25.14 <sup>a</sup>	91.33 ± 8.71 <sup>d</sup>	45.48 ± 3.66 <sup>e</sup>	225.01 ± 9.17 <sup>b</sup>	100.26 ± 7.61 <sup>d</sup>	77.23 ± 6.63 <sup>d</sup>
AGM	25.93 ± 1.10 <sup>e</sup>	40.39 ± 3.29 <sup>bc</sup>	51.47 ± 3.08 <sup>a</sup>	42.17 ± 3.57 <sup>b</sup>	49.70 ± 0.05 <sup>a</sup>	31.82 ± 2.17 <sup>d</sup>	31.78 ± 0.76 <sup>d</sup>

\**Lactococcus lactis* subsp. *cremoris* MG 1363. *Lactococcus lactis* subsp. *lactis* IL 1403. *Lactobacillus plantarum* F18595. *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346. *Lactobacillus acidophilus* ATCC 11975. *Pediococcus acidophilus* ATCC 25741. *Lactobacillus delbrueckii* subsp. *lactis* ATCC 10697 and *Streptococcus thermophilus*. AMN, ammonia; PUT, putrescine; CAD, cadaverine; HIM, histamine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYM, tyramine; TMA, trimethylamine; DOP, Dopamine, AGM, agmatine. <sup>a</sup>Mean value. <sup>b</sup>Standard deviation (n = 4). Different lowercase letters <sup>(a-e)</sup> in a row indicate significant differences (p < 0.05) among bacteria.

**Table 4** - Biogenic amines production (mg/L) by lactic acid bacteria in mackerel (*Scomber scombrus*) infusion decarboxylase broth.

	<i>Lb.delb.</i> subsp. <i>lactis</i> * <i>Leu.mes.</i> subsp. <i>crem</i>	<i>Lb.acidophilus</i>	<i>Ped.acidophilus</i>	<i>Lc.cremoris</i>	<i>Lc.lactis</i>	<i>Lb.plantarum</i>	<i>Strept.thermophilus</i>
AMN	298.15 <sup>a</sup> ± 21.95 <sup>e</sup>	349.57 ± 24.08 <sup>de</sup>	439.08 ± 16.68 <sup>bc</sup>	423.11 ± 11.79 <sup>bc</sup>	357.18 ± 35.19 <sup>de</sup>	506.60 ± 20.90 <sup>a</sup>	455.19 ± 43.94 <sup>bb</sup>
PUT	8.17 ± 0.48 <sup>c</sup>	8.57 ± 0.81 <sup>c</sup>	16.24 ± 1.23 <sup>b</sup>	29.10 ± 1.21 <sup>a</sup>	5.77 ± 0.32 <sup>e</sup>	17.70 ± 0.97 <sup>b</sup>	15.30 ± 1.37 <sup>b</sup>
CAD	6.78 ± 0.67 <sup>c</sup>	6.34 ± 0.39 <sup>c</sup>	42.92 ± 2.49 <sup>a</sup>	18.68 ± 0.97 <sup>b</sup>	3.53 ± 0.17 <sup>d</sup>	8.00 ± 0.64 <sup>e</sup>	8.69 ± 0.38 <sup>e</sup>
SPD	59.72 ± 4.83 <sup>e</sup>	40.14 ± 2.37 <sup>d</sup>	122.93 ± 6.28 <sup>b</sup>	148.55 ± 9.47 <sup>a</sup>	11.54 ± 0.53 <sup>e</sup>	61.48 ± 4.23 <sup>c</sup>	36.96 ± 2.95 <sup>d</sup>
TRPT	3.96 ± 0.33 <sup>e</sup>	3.24 ± 0.22 <sup>ef</sup>	8.25 ± 0.56 <sup>b</sup>	10.45 ± 0.48 <sup>a</sup>	0.56 ± 0.05 <sup>e</sup>	7.04 ± 0.36 <sup>e</sup>	2.56 ± 0.16 <sup>f</sup>
PHEN	12.65 ± 0.37 <sup>e</sup>	9.87 ± 0.73 <sup>cd</sup>	8.64 ± 0.63 <sup>d</sup>	29.11 ± 1.57 <sup>b</sup>	70.50 ± 2.61 <sup>a</sup>	0.00 ± 0.00 <sup>e</sup>	10.42 ± 0.36 <sup>cd</sup>
SPN	15.52 ± 1.16 <sup>d</sup>	21.61 ± 1.48 <sup>e</sup>	51.30 ± 2.99 <sup>a</sup>	28.56 ± 0.79 <sup>b</sup>	8.62 ± 0.54 <sup>c</sup>	2.61 ± 0.22 <sup>f</sup>	15.43 ± 0.79 <sup>d</sup>
HIM	50.72 ± 1.03 <sup>e</sup>	35.00 ± 2.58 <sup>d</sup>	74.89 ± 4.96 <sup>b</sup>	100.84 ± 6.43 <sup>a</sup>	50.30 ± 3.50 <sup>c</sup>	43.43 ± 2.12 <sup>c</sup>	33.34 ± 2.20 <sup>d</sup>
SER	72.90 ± 1.27 <sup>f</sup>	103.53 ± 7.27 <sup>de</sup>	126.78 ± 3.22 <sup>d</sup>	231.32 ± 17.67 <sup>b</sup>	96.01 ± 9.46 <sup>de</sup>	594.13 ± 41.66 <sup>a</sup>	134.03 ± 6.65 <sup>d</sup>
TYM	156.21 ± 8.58 <sup>c</sup>	312.48 ± 24.85 <sup>a</sup>	120.45 ± 8.99 <sup>d</sup>	228.29 ± 17.52 <sup>b</sup>	217.95 ± 13.07 <sup>b</sup>	158.39 ± 12.15 <sup>c</sup>	171.73 ± 11.42 <sup>c</sup>
TMA	2.88 ± 0.09 <sup>b</sup>	1.75 ± 0.14 <sup>b</sup>	5.23 ± 0.49 <sup>b</sup>	4.84 ± 0.24 <sup>b</sup>	2.57 ± 0.10 <sup>b</sup>	132.71 ± 10.63 <sup>a</sup>	2.05 ± 0.11 <sup>b</sup>
DOP	85.14 ± 3.20 <sup>d</sup>	89.53 ± 4.25 <sup>d</sup>	182.73 ± 6.77 <sup>b</sup>	224.74 ± 9.85 <sup>a</sup>	91.63 ± 2.88 <sup>d</sup>	32.96 ± 2.88 <sup>c</sup>	97.85 ± 1.29 <sup>d</sup>
AGM	44.071.79 <sup>b</sup>	47.471.93 <sup>b</sup>	38.61 ± 1.40 <sup>c</sup>	75.12 ± 4.12 <sup>a</sup>	25.10 ± 1.03 <sup>c</sup>	26.72 ± 1.17 <sup>de</sup>	18.34 ± 1.18 <sup>f</sup>

\**Lactococcus lactis* subsp. *cremoris* MG 1363. *Lactococcus lactis* subsp. *lactis* IL 1403. *Lactobacillus plantarum* F18595. *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346. *Lactobacillus acidophilus* ATCC 11975. *Pediococcus acidophilus* ATCC 25741. *Lactobacillus delbrueckii* subsp. *lactis* ATCC 10697 and *Streptococcus thermophilus*, AMN, ammoniac; PUT, putrescine; CAD, cadaverine; HIM, histamine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYM, tyramine; TMA, trimethylamine; DOP; Dopamine, AGM, agmatine. <sup>a</sup>Mean value. <sup>b</sup>Standard deviation (n = 4). Different lowercase letters (a-e) in a row indicate significant differences (p < 0.05) among bacteria.

**Table 5** - Biogenic amines production (mg/L) by lactic acid bacteria in white shark (*Carcharodon carcharias*) infusion decarboxylase broth.

	<i>Lb.delb.</i> subsp. <i>lactis</i> * <i>Leu.mes.</i> subsp. <i>crem</i>	<i>Lb.acidophilus</i>	<i>Ped.acidophilus</i>	<i>Lc.cremoris</i>	<i>Lc.lactis</i>	<i>Lb.plantarum</i>	<i>S.thermophilus</i>
AMN	3424.54 <sup>x</sup> ± 106.34 <sup>z</sup>	2712.24 ± 112.13 <sup>e</sup>	3526.19 ± 264.71 <sup>c</sup>	4348.38 ± 264.03 <sup>b</sup>	2769.90 ± 173.46 <sup>de</sup>	4991.38 ± 211.26 <sup>a</sup>	3193.06 ± 81.83 <sup>cd</sup>
PUT	6.62 ± 0.40 <sup>de</sup>	123.24 ± 9.56 <sup>a</sup>	16.96 ± 0.67 <sup>c</sup>	13.25 ± 0.99 <sup>cd</sup>	2.37 ± 0.14 <sup>c</sup>	6.13 ± 0.60 <sup>de</sup>	1.85 ± 0.08 <sup>e</sup>
CAD	7.51 ± 0.34 <sup>de</sup>	8.29 ± 0.22 <sup>d</sup>	27.82 ± 1.50 <sup>a</sup>	12.50 ± 0.35 <sup>c</sup>	2.59 ± 0.24 <sup>f</sup>	2.36 ± 0.10 <sup>f</sup>	6.74 ± 0.12 <sup>e</sup>
SPD	36.88 ± 3.06 <sup>b</sup>	33.79 ± 1.63 <sup>b</sup>	626.58 ± 58.19 <sup>a</sup>	57.16 ± 3.31 <sup>b</sup>	23.59 ± 1.15 <sup>b</sup>	15.12 ± 1.24 <sup>b</sup>	17.28 ± 1.30 <sup>b</sup>
TRPT	21.22 ± 1.12 <sup>c</sup>	39.84 ± 1.34 <sup>a</sup>	1.18 ± 0.10 <sup>f</sup>	14.83 ± 0.71 <sup>d</sup>	1.63 ± 0.06 <sup>f</sup>	8.28 ± 0.70 <sup>e</sup>	0.57 ± 0.02 <sup>f</sup>
PHEN	90.37 ± 3.23 <sup>a</sup>	87.33 ± 3.35 <sup>a</sup>	16.82 ± 1.56 <sup>cd</sup>	69.10 ± 2.26 <sup>b</sup>	20.36 ± 0.59 <sup>c</sup>	67.31 ± 2.87 <sup>b</sup>	14.06 ± 0.88 <sup>d</sup>
SPN	25.95 ± 2.76 <sup>d</sup>	0.00 ± 0.00 <sup>e</sup>	30.49 ± 2.10 <sup>c</sup>	22.87 ± 0.53 <sup>d</sup>	0.00 ± 0.00 <sup>e</sup>	0.00 ± 0.00 <sup>e</sup>	66.20 ± 1.69 <sup>b</sup>
HIM	38.21 ± 1.28 <sup>a</sup>	22.16 ± 1.25 <sup>c</sup>	28.26 ± 0.75 <sup>b</sup>	26.15 ± 1.78 <sup>b</sup>	16.95 ± 1.21 <sup>d</sup>	2.69 ± 0.13 <sup>f</sup>	9.24 ± 0.24 <sup>c</sup>
SER	183.18 ± 4.38 <sup>c</sup>	124.09 ± 5.40 <sup>d</sup>	223.11 ± 4.74 <sup>b</sup>	98.62 ± 1.83 <sup>e</sup>	239.63 ± 13.51 <sup>b</sup>	100.37 ± 5.40 <sup>e</sup>	222.07 ± 11.20 <sup>b</sup>
TYM	40.14 ± 3.15 <sup>e</sup>	42.08 ± 3.42 <sup>c</sup>	75.83 ± 5.30 <sup>a</sup>	28.38 ± 2.34 <sup>d</sup>	15.70 ± 0.92 <sup>e</sup>	8.86 ± 0.36 <sup>e</sup>	53.68 ± 3.44 <sup>b</sup>
TMA	4.61 ± 0.38 <sup>b</sup>	2.80 ± 0.14 <sup>b</sup>	8.31 ± 0.33 <sup>b</sup>	2.64 ± 0.09 <sup>b</sup>	1.45 ± 0.04 <sup>b</sup>	4.08 ± 0.19 <sup>b</sup>	786.75 ± 18.60 <sup>a</sup>
DOP	365.16 ± 13.58 <sup>a</sup>	125.53 ± 2.76 <sup>d</sup>	356.09 ± 22.35 <sup>a</sup>	205.81 ± 13.7 <sup>z</sup>	100.99 ± 5.32 <sup>d</sup>	223.40 ± 5.75 <sup>bc</sup>	251.70 ± 8.55 <sup>b</sup>
AGM	18.13 ± 0.71 <sup>cd</sup>	18.14 ± 0.67 <sup>cd</sup>	41.62 ± 0.32 <sup>a</sup>	17.52 ± 0.33 <sup>cd</sup>	14.40 ± 1.29 <sup>d</sup>	18.82 ± 1.07 <sup>cd</sup>	20.15 ± 1.48 <sup>c</sup>

\**Lactococcus lactis* subsp. *cremoris* MG 1363. *Lactococcus lactis* subsp. *lactis* IL 1403. *Lactobacillus plantarum* F18595. *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346. *Lactobacillus acidophilus* ATCC 11975. *Pediococcus acidophilus* ATCC 25741. *Lactobacillus delbrueckii* subsp. *lactis* ATCC 10697 and *Streptococcus thermophilus*, AMN, ammoniac; PUT, putrescine; CAD, cadaverine; HIM, histamine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYM, tyramine; TMA, trimethylamine; DOP; Dopamine, AGM, agmatine. <sup>a</sup>Mean value. <sup>b</sup>Standard deviation (n = 4). Different lowercase letters (a-e) in a row indicate significant differences (p < 0.05) among bacteria.

(11.54 mg/L). In anchovy IDB, SPD production by *Lactobacillus* spp. was 55 mg/L. *Lb. delbrueckii* subsp. *lactis*, *Lc. lactis* subsp. *lactis* and *Strep. thermophilus* in sardine IDB as well as *Lc. lactis* subsp. *lactis* in mackerel IDB had not an ability to produce PHEN. However, *Lc. lactis* subsp. *cremoris* was found as good PHEN producer in gilthead seabream IDB (209.52 mg/L). *Lactococcus* spp. had also good activity to produce PHEN in anchovy IDB (Table 1). In white shark IDB, significant PHEN production was observed for *Lb. delbrueckii* subsp. *lactis* and *Leu. mesenteroides* subsp. *cremoris*.

Most of LAB strains produced below 9 mg/L of TMA. However, *Lb. plantarum* and *Strep. thermophilus* accumulated significant amount of TMA (784.46 and 786.75 mg/L, respectively) in white shark IDB ( $p < 0.05$ ) (Table 5). LAB strains *i.e.* *Lb. delbrueckii* subsp. *lactis*, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* also had a high TMA formation in anchovy IDB (Table 1). *Lc. lactis* subsp. *lactis* was one of the LAB strains produced good amount of TMA in mackerel IDB. Tryptamine (TRP) was one of the lowest produced amine by LAB strains. Agmatine (AGM) production by LAB strains was between 14 and 101 mg/L.

## Discussion

*Lc. lactis* subsp. *lactis* (4991.38 mg/L) and *Ped. acidophilus* (4348.38 mg/L) were characterized as the highest AMN producer in shark IDB among the tested LAB strains. In anchovy IDB, *Ped. acidophilus* produced the highest amount of AMN (670.95 mg/L), whereas *Lc. lactis* subsp. *cremoris* accumulated the lowest level of that (281.96 mg/L). *Lc. lactis* subsp. *cremoris* and *Lb. plantarum* in gilthead seabream IDB were the highest ability in AMN production ( $p < 0.05$ ). In mackerel IDB, AMN production was ranged from 298 mg/L (*Lb. delbrueckii* subsp. *lactis*) to 507 mg/L (*Lc. lactis* subsp. *lactis*).

The highest accumulated amines by LAB strains were generally TYM, DOP, SER and SPD, although other amines produced at significant levels ( $p < 0.05$ ). Bunkova *et al.* (2009) reported that *Lc. lactis* subsp. *cremoris*, *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* produced TYM but did not produce other tested amines. Herring and tuna fish salads inoculated with *Lactobacillus curvatus* LTH 975 had PUT, CAD, HIM, TYM accumulation (Leuschner and Hammes, 1999). However, in cold-smoked fish fermented with LAB, the LAB used was unable to produce CAD, HIM or TYM (Leuschner and Hammes, 1999). Similar results were found by Thapa *et al.* (2006) who reported none of the strains of LAB including *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lb. plantarum*, *Leu. mesenteroides* and *Pediococcus pentosaceus* isolated from traditionally processed fish products produced BA.

Özogul and Özogul (2005) defined four categories in order to simplify the discussion of amine production by

bacterial strains, which are: prolific amine former ( $> 1000$  mg/L), good amine former (100-1000 mg/L), medium amine former (10-100 mg/L) and poor amine former ( $< 10$  mg/L). According to this category, LAB strains seemed to poor PUT producer in white shark and mackerel IDB (Tables 4 and 5). However, *Leuc. mesenteroides* subsp. *cremoris* in white shark IDB, and *Lc. lactis* subsp. *cremoris* in sardine and gilthead seabream IDB accumulated high amount of PUT ( $> 75$  mg/L). In mackerel IDB, PUT production was above the 5.7 mg/L and the highest production was found for *Lb. plantarum* (30.71 mg/L). *Lb. plantarum* N4 isolated from wine was able to produce putrescine from ornithine (Arena and Manca de Nadra, 2001). PUT was reported as main amine produced by *Leu. mesenteroides* and *Lactobacillus zeae* (Morrena-Arribas and Polo, 2008).

CAD was one of the most abundant amines in fish sauce with maximum reported value of 1429 ppm (Zaman *et al.*, 2010). In the present study, *Lc. lactis* subsp. *cremoris*, *Leuc. mesenteroides* subsp. *cremoris* and *Lc. acidophilus* were good CAD producer in sardine IDB ( $> 100$  mg/L) (Table 2). Similar production was also observed for *Ped. acidophilus* in anchovy IDB (Table 1). In the other mediums, CAD production by LAB strains was less than 93 mg/L. In gilthead seabream IDB, the highest CAD production was observed for *Lc. lactis* subsp. *cremoris*. In the recently study, Özogul (2011) reported that lower CAD production by *Lc. lactis* subsp. *cremoris* and *Strep. thermophilus* in histidine decarboxylase broth was found ( $< 17$  mg/L). In the current study, the lowest CAD production by LAB strains was generally observed for white shark and mackerel IDB. There were also not significant differences between white shark and mackerel IDB in terms of CAD production by LAB strains apart from *Ped. acidophilus* and *Lc. acidophilus*.

SPD was one of the most accumulated amine by LAB strains. *Leuc. mesenteroides* subsp. *cremoris*, *Ped. acidophilus* and *Strep. thermophilus* produced more than 100 mg/L of SPD in sardine, mackerel and anchovy IDB, respectively. However, SPD production by *Lc. acidophilus* was 6-fold higher in white shark, compared with those bacteria. However, SPD production by LAB strains did not differ statistically except for *Lb. acidophilus* in white shark IDB ( $p > 0.05$ ). The LAB strains produced medium amount of SPD in gilthead seabream IDB. Zaman *et al.* (2010) reported that SPD, TRP, PHEN and SPN were minor amines in fish sauce. Similarly, TRP was one of the lowest produced amine by LAB strains. The lowest TRP production was observed from *Lc. lactis* subsp. *cremoris* (mackerel IDB) and *Strep. thermophilus* (white shark IDB), whereas *Leuc. mesenteroides* subsp. *cremoris* and *Lb. plantarum* accumulated medium amount of TRP in white shark IDB.

Özogul (2011) reported that none of the LAB strains produced SPN in histidine decarboxylase broth. Similar results were obtained from this study associated with SPN

production by *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Leu. mesenteroides* subsp. *cremoris* in white shark IDB. *Lb. plantarum* was one of the highest amounts of SPN producer. Similarly, SPN production by *Lc. lactis* subsp. *cremoris* in anchovy IDB was negligible level, whereas *Strep. thermophilus* produced high amount of SPN (116.71 mg/L). No significant differences in SPN were observed between *Lc. lactis* and *Lb. plantarum* in anchovy IDB ( $p > 0.05$ ). There were also not significant differences ( $p > 0.05$ ) in SPN production among the *Ped. acidophilus*, *Lc. lactis* subsp. *lactis*, *Lb. plantarum* and *Strep. thermophilus* in gilthead seabream IDB (~64 mg/L). In mackerel IDB, *Lc. lactis* subsp. *cremoris* produced low amount of SPN (8.62 mg/L), while SPN production by *Lb. acidophilus* was 51.30 mg/L ( $p < 0.05$ ).

HIM production varies depending on bacterial strains and IDB. Matamoros *et al.* (2009) reported that HIM and tyramine (TYM) production for *Leuconostoc gelidum*, *Lactococcus piscium*, *Lactobacillus fuchuensis* and *Carnobacterium alterfunditum* isolated from seafood products were below the 5 mg/L. HIM was the only major BA produced by various strains of lactic acid bacteria from fish sauce (Udomsil *et al.*, 2010). In the recent study, it was shown that HIM production in histidine decarboxylase broth was negligible by *Lc. lactis* subsp. *cremoris*, whereas *Lc. lactis* subsp. *lactis*, *Strep. thermophilus* and *Lb. plantarum* did not produce HIM (Özogul, 2011). However, in the present study, the all strains had an ability to produce HIM in fish IDB, ranging from 2 to 102 mg/L (Tables 1-5) indicating that fish muscle was more favourable than specific medium on HIM production. The lowest HIM accumulation was found for *Lc. lactis* subsp. *lactis* (2.69 mg/L) and *Ped. acidophilus* (2.69 mg/L) in white shark and anchovy IDB, respectively. In anchovy IDB, the highest HIM production was found for *Lb. delbrueckii* subsp. *lactis* (47.10 mg/L), whereas *Leu. mesenteroides* subsp. *cremoris*, *Lb. plantarum* and *Strep. thermophilus* produced similar amount of HIM ( $p > 0.05$ ). *Lactobacillus buchneri* LB14 and *Lactobacillus buchneri* ST2A produced 344 and 401 mg/L HIM in decarboxylase medium contained histidine, lysine, ornitine and tyrosine (Choudhury *et al.*, 1990). Although most of LAB strains seemed to be medium HIM producer in fish IDB, *Leu. mesenteroides* subsp. *cremoris* and *Ped. acidophilus* was good HIM producer (~100 mg/L) in sardine and mackerel IDB, respectively.

No significant differences in HIM production was observed among the *Lb. delbrueckii* subsp. *lactis*, *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* (~50 mg/L) in mackerel IDB. In gilthead seabream IDB, the lowest HIM production was found for *Lb. delbrueckii* subsp. *lactis* (5.62 mg/L) though there were no significant differences in HIM production between *Lc. lactis* subsp. *cremoris* (73.70 mg/L) and *Lc. lactis* subsp. *lactis* (74.37 mg/L) ( $p > 0.05$ ). Tuna and herring salad inoculated with *Lb.*

*buchneri* revealed 900 ppm and 670 ppm histamine, respectively (Leuschner and Hammes, 1999).

Fadda *et al.* (2001) found that *Lb. plantarum*, *Lb. casei* and *Pediococcus acidilactici* isolated from fermented sausages did not produce TYM in a medium supplemented with tyrosine at 20 mg/L. Udomsil *et al.* (2010) reported trace amounts of TYM production (1-5 mg/100 mL) by various LAB strains from fish sauce. In the current study, TYM was one of the main amines produced by LAB strains. Among the fish IDB, most of LAB strains showed high activity in producing TYM in sardine IDB (> 300 mg/L) (Table 2). The reported upper TYM limit of 100-800 mg kg<sup>-1</sup> to be toxic doses in foods (Brink *et al.*, 1990; Kim *et al.*, 2009), which were generally exceed by most of LAB strains. *Lb. delbrueckii* subsp. *lactis* (2943.51 mg/L) and *Ped. acidophilus* (1157.25 mg/L) had the highest ability to produce TYM in sardine IDB. In white shark, most of the LAB strains produced less than 55 mg/L. Marino *et al.* (2008) reported that *Strep. thermophilus* was a TYM-producer strain. In the present study, *Strep. thermophilus* (241.57 mg/L), *Ped. acidophilus* (224.42 mg/L), *Lb. plantarum* (170.30 mg/L) and *Lc. lactis* subsp. *cremoris* (153.64 mg/L) produced the highest amount of TYM in anchovy IDB. There were not significant differences in TYM production between *Leu. mesenteroides* subsp. *cremoris* and *Lb. acidophilus* (~90 mg/L), and also for *Lc. lactis* subsp. *lactis*, *Lb. plantarum* and *Strep. thermophilus* (~180 mg/L) in gilthead seabream IDB.

The LAB strains were generally good SER producer. Among the fish IDB, the highest SER production was found by *Lc. lactis* subsp. *lactis* in mackerel IDB and *Lb. delbrueckii* subsp. *lactis* in anchovy IDB, whereas *Lb. delbrueckii* subsp. *lactis* was produced the lowest amount of SER in gilthead seabream IDB ( $p < 0.05$ ). *Leu. mesenteroides* subsp. *cremoris* was the main LAB produced highest amount of SER in gilthead seabream IDB. LAB strains such as *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lb. plantarum* and *Strep. thermophilus* showed lower activity of SER and DOP (1 and 5.5 mg/L, respectively) in histidine decarboxylase broth (Özogul, 2011). There were significant differences in DOP production among the LAB strains in fish IDB ( $p < 0.05$ ). LAB strains produced significant amount of DOP, especially for sardine and white shark IDB. Although *Lb. delbrueckii* subsp. *lactis* was poor DOP producer in anchovy IDB, the highest DOP production by *Lc. lactis* subsp. *cremoris* was found in this medium ( $p < 0.05$ ). In mackerel IDB, DOP production was ranged from 32.96 mg/L for *Lc. lactis* subsp. *lactis* to 224.74 mg/L for *Ped. acidophilus*.

AGM is formed from arginine by the enzyme of arginine decarboxylase secreted from lactic acid and nitric acid-reducing bacteria during the fermentation process (Umezue *et al.*, 1977). The lowest AGM production by LAB strains was in white shark IDB (< 42 mg/L) while the highest AGM accumulation was found for *Strep. thermophilus*

(100.32 mg/L) and *Ped. acidophilus* (75.12 mg/L) in anchovy and mackerel IDB ( $p < 0.05$ ), respectively. In sardine IDB, AGM production by LAB strains was below the 57 mg/L.

## Conclusion

The study results showed that the tested LAB strains had an ability to produce high amount of BA in fish IDB, which were mainly TYM, HIM, DOP, SER and SPD production. Fish species also appeared to play an important role in BA production by LAB strains. Therefore both criteria have to be taken into consideration in order to prevent the bacterial BA production and food poisoning related to fish consumption. Consequently, the ability of LAB strains to produce biogenic amines in fish products can be considered as criteria for the selection of strains and fish species.

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