

The Impact of the Cell-Free Solution of Lactic Acid Bacteria on Cadaverine Production by *Listeria monocytogenes* and *Staphylococcus aureus* in Lysine-Decarboxylase Broth

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Abstract—The influences of cell-free solutions (CFSs) of lactic acid bacteria (LAB) on cadaverine and other biogenic amines production by *Listeria monocytogenes* and *Staphylococcus aureus* were investigated in lysine decarboxylase broth (LDB) using HPLC. Cell free solutions were prepared from *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Pediococcus acidilactici* and *Streptococcus thermophiles*. Two different concentrations that were 50% and 25% CFS and the control without CFSs were prepared. Significant variations on biogenic amine production were observed in the presence of *L. monocytogenes* and *S. aureus* ($P < 0.05$). The function of CFS on biogenic amine production by foodborne pathogens varied depending on strains and specific amine. Cadaverine formation by *L. monocytogenes* and *S. aureus* in control were 500.9 and 948.1 mg/L, respectively while the CFSs of LAB induced 4-fold lower cadaverine production by *L. monocytogenes* and 7-fold lower cadaverine production by *S. aureus*. The CFSs resulted in strong decreases in cadaverine and putrescine production by *L. monocytogenes* and *S. aureus*, although remarkable increases were observed for histamine, spermidine, spermine, serotonin, dopamine, tyramine and agmatine in the presence of LAB in lysine decarboxylase broth.

Keywords—Cell-free solution, lactic acid bacteria, cadaverine, food borne-pathogen.

I. INTRODUCTION

BIogenic amines (BAs) are produced by microbial decarboxylation of amino acids and are present in a wide range of food products, including fish, fish products, meat products, eggs, cheeses, fermented vegetable, fruits, soybean products, beers, wines, nuts, and chocolate [1]-[6]. In fact, microorganisms naturally present in food can convert free amino acids to biogenic amines by decarboxylation which can take place by two biochemical pathways, either that involving the endogenous decarboxylase enzyme naturally forming in the tissue considered, or due to the action of exogenous

enzymes released by various microorganisms associated with the environment, the storage and the processing procedure [7].

BAs are biologically active molecules that can be aliphatic (putrescine (PUT), cadaverine (CAD), spermine (SPN), spermidine (SPD), aromatic (tyramine (TYR), phenylethylamine (PHEN) or heterocyclic (histamine (HIS), tryptamine (TRPT) structures [8]. Polyamines amines are found in a wide range of food products, particularly protein-rich foods of both animal and plant origin [9], as well as in fermented products [10], [11]. The most significant biogenic amines occurring in foods are HIS, PUT, CAD, TYR, TRPT, PHEN, SPN, SPD and agmatine (AGM). The distribution of the various amines differs according to the food type, with meat being high in SPN, while foods of plant origins contain mostly PUT and SPD [12].

The consumption of food containing BAs causes several types of food borne diseases such as histamine poisoning and tyramine toxicity. Histamine is the most toxic amine detected in foods such as fish, cheese, wine and meat products [6]. The toxicological effect depends on histamine intake level, presence of other different amines, amino-oxidase activity and the intestinal physiology of the individual [13]. The other biogenic amines, such as putrescine and cadaverine, have been reported to enhance the toxicity of histamine [14]. The importance of estimating the concentration of biogenic amines in food and food products are related to their impact on human health and food quality.

Determination of polyamines present in food is an important analytical task for toxicological reasons since the high levels of BAs can be toxic for certain consumers. Moreover, quantification of polyamines is also essential from the quality point of view of meat and meat products [15], [16]. Good hygiene practices and proper handling are necessary to prevent food poisoning associated with consumption of food, which contains high level of biogenic amines.

There are numerous different bacterial species that have been reported to possess amino acid decarboxylase activity and to be responsible for biogenic amine production in food and food products. Decarboxylases are found in *Enterobacteriaceae*, *Pseudomonas* spp., *Enterococci*, some lactic acid bacteria, *Clostridium*, and *Lactobacillus* species [17], [1], [13]. Microorganisms with a decarboxylase activity can be spoiling or starter microbes [18], [6] Among specific spoilage organisms, *Enterobacteriaceae* have shown great

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capability to produce polyamines, in particular PUT and CAD, by decarboxylation of amino acids ornithine and lysine, respectively [19]-[24]. There are certain factors, which might affect biogenic amine-decarboxylase activity as well as the formation of BAs in food. Availability of substrate, temperature, pH, salt concentration has limiting effects on the biogenic amines-decarboxylase activity [25]-[28] and it also depends on variables such as the growth kinetics of the microorganisms and their proteolytic and decarboxylase activities [24].

Apart from hygienic quality of raw materials, the addition of an amine-negative starter culture to carry out a controlled fermentation is suggested to prevent excessive amine accumulation. Starter cultures usually consist of one or several strains of lactic acid bacteria, micrococci and coagulase-negative staphylococci. Mixed starter culture (*Lactobacillus sakei* plus, *Staphylococcus carnosus* or *Staphylococcus xylosus*) drastically reduced the accumulation of TYR, CAD, and PUT in dry fermented sausages [29]. Likewise, significant reduction of CAD, PUT, HIS and TYR contents was observed in Thai fermented sausage (Nham) using *Lactobacillus plantarum* [30]. However, several studies failed to demonstrate the efficiency of the starter cultures to reduce amine production during sausage fermentation [31], [32].

It was reported that *L. plantarum* ZY40 plus *S. cerevisiae* JM19 significantly reduced the accumulation of PUT and CAD by more than 37% and 76%, respectively [33]. Similar results were obtained from researchers who reported that the use of starter cultures resulted in lower levels of PUT and CAD than those in naturally-fermented sausages [29], [34], [35].

The roles of some food-borne pathogens in the formation of biogenic amines in different types of foods have been studied. *Salmonella* strains have the ability to produce CAD, HIS, PHEN, PUT and TYR [36], whereas *Klebsiella pneumoniae* forms CAD and TYR [37]. The LAB strains can produce substances such as hydrogen peroxide, weak organic acids, reuterin, diacetyl, bacteriocins, and low-molecular-weight metabolites that inhibit pathogenic organisms [44] and also BA production [38].

Biogenic amine production has been most extensively studied with respect to HIS and TYR, probably two the most important BA of bacterial origin in food, due to their toxicological effects. The diamines like PUT and CAD were also investigated since they may potentiate the toxicity of the histamine and tyramine, and they even might serve as indicators of poor hygienic quality in some food substrates [39]. So far, most of the studies have been focused on BA formation of selected food or single bacteria isolates. There are no data regarding to stimulating or inhibiting effects of CFSs from specific LAB on cadaverine production by common food-borne pathogenic bacteria in vitro conditions. Thus, the impact of the cell-free solution of lactic acid bacteria on cadaverine and other biogenic amines production by *Listeria monocytogenes* and *Staphylococcus aureus* was investigated in lysine-decarboxylase broth.

II. MATERIALS AND METHODS

A. Bacterial Strains

Lactic acid bacteria strains which are *Lactococcus lactis* subsp. *lactis* IL 1403 (Lc. Lac. subs. *lactis*), *Leuconostoc mesenteroides* subsp. *cremoris* DSMZ 20346 (*Leu. mes. subs. cremoris*), *Pediococcus acidilactici* ATCC 25741 (*P. acidilactici*), and *Streptococcus thermophilus* NCFB 2392 (*S. thermophilus*) were obtained from Sutcu Imam University, (Kahramanmaraş, Turkey) in BGML stock culture.

The selected 2 FBPs were *Staphylococcus aureus* ATCC 29213 (*S. aureus*), *Listeria monocytogenes* ATCC 7677 (*L. monocytogenes*), which were purchased from American Type Culture Collection (Rockville, MD, USA).

B. Preparation of CFS from Lactic Acid Bacteria

The cells of the lactic acid bacteria strains were pre-grown in 10 ml MRS (De Man, Rogosa and Sharpe, Merck) medium (1%, v/v) at overnight incubation at 37°C, and then 7.5 ml of the culture was added aseptically to 750 ml MRS medium in a 1000 ml serum bottle. Samples were incubated for 16 h at 37°C without shaking. A cell-free solution was obtained by centrifuging at 9000 rpm for 20 min under refrigeration (at 4°C) from bacterial suspension. The cell-free solution was sterilized by membrane filtration (0,22µm, Sartorius).

C. Culture Media and Bacterial Extraction

Cadaverine production from all FBP strains in this work was monitored using lysine decarboxylase broth (LDB). The composition of the broth is 2g peptone, 1g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 5g NaCl (Merck 1.06404.1000, Darmstadt, Germany), 8.02g L-lysine (Sigma L5626, Steinheim, Germany) and 5 mg pyridoxal HCl (Sigma P9130, Steinheim, Germany) in per litre of water. The pH was adjusted according to their optimum growth pH with 1M KOH (Riedel-de Haen 06005, Seelze, Germany) or 6% trichloroacetic acid (TCA) (Riedel-de Haen 27242, Seelze, Germany). The LDB was pipetted in 10, 7.5 and 5 ml bottles and then autoclaved at 121°C in 15 min prior to use. Two different concentrations which were 50% (5ml CFS+5ml LDB) and 25% (2.5 ml CFS+7.5ml LDB) of cell-free solutions (CFS) were prepared and the control was only LDB without CFS.

Nutrient broth (Merck 1.05443.0500, Darmstadt, Germany) was used for propagation of FBP strains and they were incubated according to their optimum growth temperature for 2 or 3 days. Production of biogenic amines was tested by 0.5 ml inoculating each food-borne pathogen strain in LDB for each CFS concentration. All of them were incubated at their optimum growth temperature for 72 hours. After that, 5 ml of the broth culture containing FBP strains were removed to separate bottles and then, 2 ml trichloroacetic acid was added. They were centrifuged at 3000 xg for 10 min and then filtered through a filter paper (Milipore). Finally, a 4 ml bacterial supernatant from centrifugation was taken for derivatization stage.

D. Derivatisation of Bacterial Supernatant

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance reaction with amines. For derivatisation of standard amine solutions, 100 µl was taken (4 ml bacterial supernatant) from each free base standard solution (10 mg/ml). Sodium hydroxide (2 M) was added, followed by 1 ml of 2% benzoyl chloride (dissolved in acetonitrile) and the solution was 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 was 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. Finally, the residue was solutioned in 1 mL of acetonitrile and 10 µl aliquots were injected into the HPLC.

E. Analytical Method and HPLC Apparatus

Biogenic amines analysis was carried out using the method of [40] and measured in milligram amines per litre broth. The confirmation of BAs production was accomplished with a rapid HPLC method with a reversed phase column by using a gradient elution program. The same analytical method was used for ammonia and trimethylamine separation.

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. For the BA analyses, the column was ODS Hypersil, 5µ, 250×4.6 mm (Phenomenex, Macclesfield, Cheshire, UK).

F. Chromatographic Conditions and HPLC Separation

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 µl and detection was monitored at 254 nm.

A standard curve for ammonia and each of the 12 amines in the range of 0 to 50 mg/mL was prepared. Correlation coefficient (r) of peak area against amine standard concentrations for each compound was calculated after injecting 5 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 therefore the gradient elution program used in this study was satisfactory.

G. Statistical Analysis

The data gathered from CFS of LAB strains were processed using one way analysis of variance (ANOVA), SPSS 15.0 for Windows (SPSS Inc., Chicago, IL. USA). The level of

significance was set at $P < 0.05$ and means were compared by Duncan t- tests.

III. RESULTS

Cadaverine-forming bacteria produced not only CAD but also other amines (PUT, CAD, SPD, TRPT, PHEN, SPN, HIS, AGM, SER, DOP) and ammonia (AMN) in LDB. Some other BAs were also produced in small amounts. Although the other amino acids (histidine, ornithine, etc.) were not added into LDB, the broth does contain peptone and beef extract that contain the other amino acids. The amounts of glutamic acid, arginine, lysine and tryptophan in bacteriological peptone (Oxoid LP0037; Oxoid Limited, Basingstoke, Hampshire, UK) and Lab Lemco (Oxoid CM0017; Oxoid Limited) were calculated as 304, 170, 132, 14 and 16 mg/L, respectively. The observed CAD, SPM, SPD, dopamine (DOP) and other BAs in LDB are, thus, likely to have been produced from the amino acids in peptone and beef extract. Table I shows effects of the CFS on CAD and other biogenic amines formation by *L. monocytogenes* and *S. aureus*. There were remarkable variations in BAs production by *L. monocytogenes* and *S. aureus* which produced considerable amount of CAD ($P < 0.05$).

The *L. monocytogenes* produced more than 300 mg/L of PUT, CAD, AGM (308.4, 500.9, 849.8 mg/L, respectively). During the growth in LDB, AGM reached a higher concentration compared to PUT and CAD. Considerable amount of amines were inhibited by CFS of LAB strains ($P < 0.05$). All CFS of LAB strains principally 25% CFS of *Lc. Lac. subsp. lactis* and 50% CFS of *P. acidilactici* decreased PUT production (64%). The concentrations of CAD decreased during the incubation of CFSs in LDB. Mainly 25% CFS of *S. thermophilus* and *P. acidilactici* inhibited CAD production (90%). AGM accumulation was decreased by all of CFS especially, the CFS of *Lc. lac. subs. lactis* (93%). DOP production significantly increased by all CFS of LAB strains in particular 25% CFS of *Lc. Lac. subs. lactis* and *S. thermophilus* comparing to the control. DOP production was higher than SPN, SPD, PHEN formation by *L. monocytogenes*. However *L. monocytogenes* produced lower than 10 mg/L TRPT, HIS, TYR, TMA except for SER. The CFSs of *S. thermophilus* induced 21-fold higher SER production by *L. monocytogenes* and 2-fold higher SER production by *S. aureus* (Tables I and II).

Table II shows BAs and AMN production by *S. aureus* in LDB with CFSs of LAB. Control samples had relatively high contents of PUT CAD and AMN although low contents of HIS, TYR, TRPT and TMA, showing considerable decreases in the overall contents of biogenic amines by *S. aureus* throughout 72 h incubating in only LDB. AMN production in control (1044.9 mg/L) was higher than BAs by *S. aureus*. All of CFS of LAB strains significantly showed inhibitor effect whereas both CFSs of 50 % of *Lc. Lac. subsp. lactis*, and *Leu. mes. subsp. cremoris* showed stimulator effects.

TABLE I
IMPACT OF CFS FROM *P. ACIDILACTICI* AND *S. THERMOPHILUS* ON CADAVERINE AND OTHER BIOGENIC AMINES PRODUCTION BY *L. MONOCYTOGENES* IN LDB (MG/L)

Biogenic Amines (mg/L)	Control in LDB	Treatment Groups			
		<i>Pediococcus acidilactici</i>		<i>Streptococcus thermophilus</i>	
		25%	50%	25%	50%
AMN	1324,60 ^{ax} (33,76) ^y	590,86 ^c (12,22)	727,15 ^d (32,29)	817,79 ^c (14,02)	921,29 ^b (21,04)
PUT	308,42 ^a (20,94)	203,27 ^b (2,80)	144,64 ^c (13,88)	212,42 ^b (8,48)	209,40 ^b (14,01)
CAD	500,98 ^a (21,83)	54,35 ^d (3,53)	106,80 ^c (14,06)	70,36 ^{de} (0,89)	118,74 ^b (6,89)
SPD	42,69 ^c (6,48)	37,48 ^d (4,18)	134,81 ^a (13,77)	105,42 ^b (9,70)	105,65 ^b (8,91)
TRPT	0,25 ^d (0,35)	7,89 ^b (1,25)	9,45 ^a (0,98)	0,00 (0,00)	6,05 ^c (0,06)
PHEN	51,67 ^d (6,80)	88,25 ^c (3,37)	85,88 ^c (7,39)	106,19 ^a (9,66)	97,79 ^{ab} (9,93)
SPN	26,15 ^e (0,11)	74,74 ^b (9,43)	92,09 ^a (1,98)	44,83 ^c (5,38)	41,96 ^d (0,04)
HIS	2,67 ^d (0,10)	15,92 ^b (0,00)	15,49 ^b (0,62)	14,52 ^{bc} (3,65)	21,43 ^a (3,38)
SER	8,28 ^f (2,46)	101,19 ^{cd} (15,08)	108,69 ^{bc} (6,38)	97,03 ^{de} (5,14)	181,61 ^a (14,27)
TYR	3,45 ^c (0,64)	65,98 ^b (8,87)	110,69 ^a (4,35)	65,04 ^b (7,68)	94,21 ^a (11,71)
TMA	7,48 ^c (3,43)	6,36 ^c (0,80)	12,54 ^b (0,45)	1,36 ^d (0,08)	14,60 ^a (0,44)
DOP	97,17 ^e (11,23)	161,19 ^{cd} (12,77)	213,25 ^b (13,44)	256,33 ^a (14,82)	149,80 ^{cd} (15,13)
AGM	849,88 ^a (26,78)	118,98 ^c (8,20)	103,57 ^{cd} (9,09)	180,25 ^b (10,13)	121,82 ^c (10,27)

HIS, histamine AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. Control: LDB, Concentration: 50% (5ml CFS+5ml medium) and 25% (2.5 ml CFS+7.5ml medium). Among groups ^x Mean value (n = 4), ^yStandard deviation, ^{a-c} indicate significant differences (P < 0.05) in a row.

TABLE II
IMPACT OF CFS FROM *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* AND *LEUCONOSTOC MESENEROIDES* SUBSP. *CREMORIS* ON CADAVERINE AND OTHER BIOGENIC AMINES PRODUCTION BY *L. MONOCYTOGENES* IN LDB (MG/L).

Biogenic Amines (mg/L)	Control in LDB	Treatment Group			
		<i>Lactococcus lactis</i> subsp. <i>lactis</i>		<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	
		25%	50%	25%	50%
AMN	1324,60 ^{ax} (33,76) ^y	546,45 ^c (13,97)	771,26 ^c (7,77)	691,23 ^{cd} (12,61)	910,82 ^b (23,98)
PUT	308,42 ^a (20,94)	170,40 ^{cd} (10,95)	206,33 ^b (13,27)	227,18 ^b (11,09)	197,38 ^{bc} (15,33)
CAD	500,98 ^a (21,83)	134,18 ^{bc} (11,43)	111,41 ^c (11,34)	131,52 ^{bc} (10,02)	137,61 ^b (11,72)
SPD	42,69 ^d (6,48)	24,76 ^e (5,81)	115,00 ^{bc} (11,67)	123,23 ^{ab} (12,59)	132,31 ^a (13,12)
TRPT	0,25 ^d (0,35)	3,09 ^c (0,13)	9,53 ^a (0,08)	8,95 ^{ab} (0,07)	0,00 (0,00)
PHEN	51,67 ^d (6,80)	72,66 ^c (5,78)	78,45 ^c (5,05)	123,55 ^a (13,63)	115,72 ^{ab} (6,90)
SPN	26,15 ^e (0,11)	69,30 ^d (8,25)	85,79 ^{bc} (6,89)	93,46 ^{ab} (5,08)	106,56 ^a (9,46)
HIS	2,67 ^d (0,10)	9,31 ^b (0,73)	10,66 ^b (0,05)	8,95 ^{bc} (0,07)	18,23 ^a (2,57)
SER	8,28 ^e (2,46)	76,12 ^d (8,48)	82,58 ^c (3,06)	129,13 ^a (13,15)	118,72 ^{ab} (7,35)
TYR	3,45 ^c (0,64)	43,38 ^c (8,14)	57,66 ^b (5,75)	98,39 ^a (7,10)	27,17 ^d (1,85)
TMA	7,48 ^b (3,43)	5,33 ^c (0,02)	7,73 ^b (1,20)	8,77 ^b (0,31)	12,51 ^a (2,41)
DOP	97,17 ^e (11,23)	249,76 ^a (14,22)	140,72 ^d (11,66)	213,88 ^b (17,42)	174,32 ^c (11,35)
AGM	849,88 ^a (26,78)	70,36 ^d (7,33)	62,55 ^c (9,81)	99,08 ^b (8,34)	89,20 ^c (4,12)

HIS, histamine AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. Control: LDB, Concentration: 50% (5ml CFS+5ml medium) and 25% (2.5 ml CFS+7.5ml medium). Among groups ^x Mean value (n = 4), ^yStandard deviation, ^{a-c} indicate significant differences (P < 0.05) in a row

PUT production was found as 607.97 mg. L⁻¹. Particularly, PUT content progressively decreased from initially 607.97 mg/L to 121.64 mg/L by 25% CFS of *P. acidilactici* during incubation. Compared with the control, CAD accumulation significant decreased from initially 948.08 mg/L to 51.64 mg/L by 25% CFS of *P. acidilactici* follow 50% CFS of *P. acidilactici*, 25% CFS of *Lc. Lac. subsp. lactis*.

IV. DISCUSSION

Quantitatively the most important biogenic amines in the present experiment were CAD, PUT, AGM and ammonia that produced by *S. aureus* and *L. monocytogenes* in LDB. Diamines, PUT and CAD, are usually common amines often related to the activity of *Enterobacteria*. It was reported that the biogenic amine-producer strains were included in a wide range of *Enterobacterial* species, including *Kluyvera intermedia*, *Enterobacter aerogenes*, *Yersinia kristensenii*, *Serratia grimesii*, *Serratia ficaria*, *Yersinia rodheii*, *Providencia vermicola* and *Obesumbacterium proteus* [17]. Among *Enterobacteria*, PUT was the amine more frequently

produced (87%), followed by CAD (85%). It was studied that the distribution of the decarboxylases of lysine, arginine, and ornithine among the species of *Enterobacteria* [41]. In present study, although PUT, CAD production by *L. monocytogenes* was 308.4, and 500.9 mg/L, production of PUT and CAD by *S. aureus* was 607.9 948.1 mg/L, respectively. Similarly, it was investigated potentially production of biogenic amines for 56 coagulase-negative *Staphylococci* isolated from industrial Spanish dry-cured ham processes [42]. The only *Staphylococci* with aminogenic capacity were a histamine-producing *Staphylococcus capitis* strain, and a *Staphylococcus lugdunensis* strain that simultaneously produced PUT and CAD. As they reported that the sequence of several *Staphylococcal* lysine decarboxylases is available, this implies that CAD production seems to be a frequent biochemical property among *Staphylococcal* species. Prolific or nonprolific HIS-forming bacterial strains produced not only HIS but also other amines (CAD, PUT, SPM etc.) in specific decarboxylase broths.

TABLE III
IMPACT OF CFS FROM *P. ACIDILACTICI* AND *S. THERMOPHILES* ON CADAVERINE AND OTHER BIOGENIC AMINES PRODUCTION BY *STAPHYLOCOCCUS AUREUS* IN LDB (MG/L)

Biogenic Amines (mg/L)	Control in LDB	Treatment Group			
		<i>Pediococcus acidilactici</i> 25%	<i>Pediococcus acidilactici</i> 50%	<i>Streptococcus thermophiles</i> 25%	<i>Streptococcus thermophiles</i> 50%
AMN	1044,89 ^{ax} (44,67) ^y	436,68 ^{de} (13,84)	912,84 ^b (18,16)	491,12 ^d (15,71)	652,18 ^c (17,509)
PUT	607,97 ^a (19,57)	121,64 ^d (1,67)	157,82 ^b (10,21)	161,30 ^b (15,47)	144,66 ^c (7,68)
CAD	948,08 ^a (24,52)	51,64 ^{cd} (3,94)	66,74 ^c (9,36)	85,61 ^b (5,32)	88,78 ^b (9,64)
SPD	48,46 ^c (1,26)	31,53 ^d (0,79)	64,68 ^a (4,72)	48,93 ^c (0,79)	53,75 ^b (7,48)
TRPT	3,77 ^{cd} (0,24)	0,19 ^d (0,01)	5,55 ^b (1,24)	29,43 ^a (4,59)	0,00 (0,00)
PHEN	118,65 ^a (11,57)	36,12 ^c (9,50)	66,39 ^b (8,55)	61,23 ^b (5,58)	110,94 ^a (10,47)
SPN	35,49 ^c (3,47)	18,01 ^d (0,03)	90,21 ^a (10,38)	58,83 ^b (9,52)	85,05 ^{ab} (5,85)
HIS	5,24 ^{de} (0,09)	8,42 ^d (2,93)	12,44 ^{bc} (3,03)	14,51 ^b (0,50)	28,67 ^a (2,90)
SER	47,99 ^e (2,15)	79,46 ^{bc} (4,73)	62,22 ^{de} (28,12)	96,36 ^b (6,80)	120,91 ^a (6,96)
TYR	47,23 ^d (2,59)	42,24 ^d (14,36)	59,57 ^c (8,47)	94,40 ^{ab} (3,93)	96,66 ^a (6,47)
TMA	0,12 ^e (0,09)	5,76 ^d (0,15)	7,03 ^c (3,44)	10,07 ^b (0,80)	15,84 ^a (0,56)
DOP	277,96 ^{bc} (19,08)	371,86 ^a (28,16)	231,53 ^d (14,09)	337,47 ^{ab} (2,79)	232,64 ^d (8,65)
AGM	84,84 ^d (4,44)	118,66 ^{bc} (14,24)	132,90 ^b (14,00)	173,58 ^a (10,38)	181,32 ^a (23,19)

HIS, histamine; AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. Control: LDB, Concentration: 50% (5ml CFS+5ml medium) and 25% (2.5 ml CFS+7.5ml medium). Among groups ^x Mean value (n = 4), ^yStandard deviation, ^{a-c} indicate significant differences (P < 0.05) in a row.

The biogenic amine production was depended on the microbial flora, availability of precursors and physicochemical factors, such as temperature, pH, salt, oxygen and sugar concentration [43]. The production of BAs by decarboxylase-positive microorganisms is affected by several environmental factors, such as pH, temperature and NaCl concentration [25]-[28] and it also depends on variables such as the growth

kinetics of the microorganisms and their proteolytic and decarboxylase activities [22].

Formation of PUT, CAD, AGM by *L. monocytogenes* was 308.4, 500.9, 849.8 mg/L, respectively. During the growth in LDB, AGM reached a higher concentration compared to PUT and CAD. All CFS of LAB strains mainly 25% CFS of *Lc. Lac. subsp. lactis* and 50% CFS of *P. acidilactici* decreased PUT production up to 64% while treatment of 25% CFS of *S.*

thermophilus and *P. acidilactici* inhibited CAD production up to 90%.

All of CFSs of LAB strains considerably inhibited CAD and PUT accumulation by *L. monocytogenes* and *S. aureus*. The LAB strains can produce substances such as hydrogen peroxide, weak organic acids, reuterin, diacetyl, bacteriocins, and low-molecular-weight metabolites that inhibit pathogenic organisms [44] and also BA production [38]. In previous work, the pH values of CFS of *S. thermophilus*, *Leu. mes. subsp. cremoris*, *Lc. Lac. subsp. lactis* and *P. acidilactici* were reported as 4.04, 4.03, 4.15 and 4.39, respectively [45]. The pH of a supernatant can be lowered by several factors which

are the types and concentrations of LAB and fermentable carbohydrates that are present, the rate of acid production and growth, the presence of any inhibitory factors as well as the initial pH and buffering capacity of the food [46]. The antimicrobial activity of LAB strains was determined against indicator strains using well diffusion assay. Results of the well diffusion test showed that all of the CFS of LAB strains revealed inhibitory activity against indicator bacteria. They reported that CFS from *P. acidilactici*, and *S. thermophilus* showed more inhibitor effect than *Lc. lac. subsp. lactis*, *Leu. mes. subsp. cremoris* on *L. monocytogenes* and *S. aureus* [45].

TABLE IV
IMPACT OF CFS FROM *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* AND *LEUCONOSTOC MESAENTEROIDES* SUBSP. *CREMORIS* ON CADAVERINE AND OTHER BIOGENIC AMINES PRODUCTION BY *STAPHYLOCOCCUS AUREUS* IN LDB (MG/L)

Biogenic Amines (mg/L)	Control in LDB	Treatment Group			
		<i>Lactococcus lactis</i> subsp.		<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	
		25%	50%	25%	50%
AMN	1044,89 ^{bx} (44,67) ^y	639,86 ^c (18,06)	1156,22 ^{ab} (20,79)	431,41 ^d (10,87)	1191,26 ^a (28,49)
PUT	607,97 ^a (19,57)	209,91 ^d (7,83)	234,36 ^b (28,34)	178,31 ^{cd} (9,79)	224,85 ^b (10,31)
CAD	948,08 ^a (24,52)	71,38 ^d (10,80)	141,46 ^b (15,04)	101,92 ^c (9,79)	162,73 ^b (3,97)
SPD	48,46 ^d (1,26)	107,83 ^c (9,91)	279,57 ^a (17,74)	101,14 ^c (11,07)	134,68 ^b (9,12)
TRPT	3,77 ^{cd} (0,24)	3,89 ^{cd} (0,01)	10,24 ^b (1,34)	0,00 (0,00)	9,76 ^b (1,61)
PHEN	118,65 ^a (11,57)	90,39 ^b (4,20)	115,30 ^a (10,30)	88,14 ^b (5,74)	116,59 ^a (6,98)
SPN	35,49 ^d (3,47)	82,76 ^c (10,02)	144,79 ^a (14,24)	97,27 ^c (11,18)	124,13 ^b (1,45)
HIS	5,24 ^e (80,09)	14,40 ^{cd} (2,96)	21,23 ^{bc} (5,63)	27,46 ^{ab} (0,11)	29,51 ^a (3,05)
SER	47,99 ^c (2,15)	81,91 ^{cd} (2,56)	159,42 ^a (14,29)	96,37 ^{bc} (10,03)	150,96 ^a (1,62)
TYR	47,23 ^d (2,59)	83,40 ^{bc} (11,35)	134,16 ^a (18,42)	73,79 ^{bc} (10,08)	132,84 ^a (10,81)
TMA	0,12 ^d (0,09)	7,64 ^c (1,83)	18,99 ^{ab} (2,47)	7,76 ^c (0,66)	20,58 ^a (2,78)
DOP	277,96 ^a (19,08)	207,19 ^b (14,13)	136,49 ^d (5,22)	164,78 ^c (14,21)	255,64 ^{ab} (11,35)
AGM	84,84 ^c (4,44)	213,69 ^a (14,50)	176,58 ^b (13,93)	95,94 ^{de} (10,65)	123,43 ^{cd} (8,98)

HIS, histamine AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRPT, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TYR, tyramine; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. Control: LDB, Concentration: 50% (5ml CFS+5ml medium) and 25% (2.5 ml CFS+7.5ml medium). Among groups ^a Mean value (n = 4), ^bStandard deviation, ^{a-c} indicate significant differences (P < 0.05) in a row.

Some of the researchers stated that the addition of negative amine-producer starter culture to carry out a controlled fermentation could be an advisable practice to reduce excessive amine accumulation [19], [47]-[50]. In the current study, all of CFSs of LAB strain displayed inhibitor effect on CAD accumulation. The effects of 25% CFSs of *P. acidilactici* and *S. thermophilus* on *L. monocytogenes* and *Lc. Lac. subsp. lactis*, *Leu. mes. subsp. cremoris* on *S. aureus* were 2-fold higher than 50% concentration of their CFSs. It was observed that some of the LAB strains belonging to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Enterococcus* genera can decarboxylate tyrosine and lysine [51]. In order to see their decarboxylase activity, an analytical test for HIS, TY, PUT and CAD production by starter cultures was suggested [52], [47].

SPD and SPM were naturally occurring BAs in food and their formation is not related to bacterial spoilage [53]. In this study, the CFSs had generally significant stimulator effect on SPD and SPN production since there is increasing differences between control and CFSs treatments. CFS of *Leu. mes. subsp. cremoris* and 50 % CFS of *Lc. Lac. subsp. lactis* increased SPD production by both pathogens comparing to control and the other treatments groups. LAB with most of the pathogen showed considerably stimulation effects on SPN, SPD, PHEN, HIS, TMA, DOP. It was reported that 13 strains (6 *Streptococcus*, 3 *Enterococcus*, 3 *Lactococcus* and 1 *Lactobacillus*) which were isolated from different sources showed strong abilities to produce BAs, especially TYR and SPM [54].

Statistically, considerable amount of AMN by FBP were inhibited by CFS of LAB strains (P<0.05). Treatment of 25%

CFS of all LAB strain had the highest effect on AMN production following 50% CFS of *S. thermophilus* and *P. acidilactici*. AMN production by *S. aureus* was increased by 50% CFS of *Leu. mes. subsp. cremoris* and *Lc. lac. subsp. lactis*. In LDB (the control), AMN production by *L. monocytogenes* was significantly higher (1324.6 mg/L) than *S. aureus* production. CFS of 25% of *Leu. mes. subsp. cremoris*, and *P. acidilactici* showed the highest inhibitor effect on AMN production (60%) by *S. aureus* following CFS of (25%) *S. thermophilus*. CFS of 25% of *P. acidilactici* resulted in great increases in DOP production by *S. aureus*. HIS production by both FBPs was less than 5 mg/L thus all CFSs showed stimulator effects regarding to histamine formation.

Amino acid decarboxylases are found in many microorganisms of food concern. They have been observed in *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella*, *Shigella* [3], [55]-[57], *Staphylococcus*, *Micrococcus* and *Kocuria* [58], [59]. It was reported that *S. aureus*, *K. pneumoniae* and *L. monocytogenes* produced higher than 300 mg/L PUT, and thus these bacteria can be classified as medium amine former [60]. In the present study PUT production was found as 607.97 mg/L. Particularly, PUT formation by 25% CFS of *P. acidilactici* progressively decreased from initially 607.97 mg/L to 121.64 mg/L. As well as other CFSs of LAB strains significantly decreased PUT production by *S. aureus*. Compared with the control CAD accumulation significant decreased from initially 948.08 mg/L to 51.64 mg/L by 25% CFS of *P. acidilactici* followed 50% CFS of *P. acidilactici* and 25% CFS of *Lc. Lac. subsp. lactis*. Inhibition of CAD production was observed in all of CFS of LAB strains. AMN production was the higher than BAs 1044.89 mg/L by *S. aureus*. All of CFS of LAB strains showed inhibitor effect, but only 50 % CFS of *Lc. Lac. subsp. lactis* increased AMN production that was almost 10 %.

LAB strains such as *Lc. Lac. subsp. lactis*, *Lc. Lac. subsp. cremoris*, *L. plantarum* and *S. thermophilus* revealed lower activity of SER and DOP (1 and 5.5 mg/L, respectively) in histidine decarboxylase broth [49]. It was stated that *S. thermophilus* and *L. plantarum* showed inhibition effect on CAD production by *L. monocytogenes* and *E. coli* [60]. CAD was the main amine detected in Egyptian salted fermented bouri fish samples during ripening and storage, followed by PUT [55]. Addition of amine-negative starter cultures has been proposed to prevent amine formation in dry sausages. Mixed starter cultures (*Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus*) considerably lessened (about 90%) the presence of PUT, CAD and TYR in Spanish sausages [29].

V. CONCLUSION

Inhibition of CAD production was observed all of CFS of LAB strains. Most of all CFS of LAB strains revealed statistically inhibitor effect because weak organic acids, particularly lactic acid slowed down microbial growth, reduced pH quickly and lessened the production of AMN and BAs. Accordingly, in order to avoid the formation of high content of biogenic amines in fermented food by bacteria, it is

advisable to use CFS for food and food products in the food industry.

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