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Assessment of biogenic amine production by lactic acid bacteria isolated from Serbian traditionally fermented foods

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A B S T R A C T

The aim of this study was to monitor the production of biogenic amines by 156 selected lactic acid bacteria (LAB) strains isolated from Serbian traditionally fermented sausages and cheeses. The method for the determination of biogenic amines is liquid chromatography tandem mass spectrometry (LC-MS/MS). The measured concentrations of putrescine, cadaverine, tryptamine and spermidine (except in 13 LAB strains) were below the detection limit of the analytical method (0.1 mg/L), whereas those of histamine, tyramine and spermine were above the limit of detection, but still not significant. Tyramine was the only amine that had a measured concentration of 59.89 ± 0.06 mg L^{-1}. Most of the tested LAB strains produced tyramine in broth below 26 mg L^{-1} with the exception of *Enterococcus faecalis* strains (59.89 ± 6.66 mg L^{-1}) and *Enterococcus faecium* strains (47.33 ± 8.58 mg L^{-1}). The low concentrations of biogenic amines are considered non-significant from both a technological and safety point of view.

Keywords: biogenic amines, lactic acid bacteria, Serbian traditional fermented food, liquid chromatography tandem mass spectrometry.

И З В О Д

Циљ овог истраживања је био праћење производње биогених амина од стране 156 одабраних сојева бактерија млечне киселине (БМК), које су изоловане из српских традиционално ферментисаних кобасица и сирева. Метода за одређивање биогених амина је течна хроматографија са тандем масеном спектрометријом, ЛЦ-МС/МС. Утврђене концентрације путресцина, кадаверина, триптамина и спермидина (изузев код 13 сојева БМК) биле су испод границе детекције аналитичке методе (0,1 mg L^{-1}), док су код хистамина, тирамина и спермина биле изнад границе детекције, али још увек без статистичке значајности. Тирамин је био једини амин који је имао измерену концентрацију од 59,89 ± 0,06 mg L^{-1}. Већина тестираних сојева БМК производи тирамин у бујону испод 26 mg L^{-1}, са изузетком сојева *Enterococcus faecalis* (59,89 ± 6,66 mg L^{-1}) и сојева *Enterococcus faecium* (47,33 ± 8,58 mg L^{-1}). Ниске концентрације биогених амина се сматрају незначајним, како са технолошког аспекта, тако и са аспекта безбедности.

***Кључне речи**:* биогени амини, бактерије млечне киселине, српска традиционална ферментисана храна, течна хроматографија са тандем масеном спектрометријом.

1. Introduction

Modern production of fermented meat and milk products, as well as other fermented foods and beverages, relies on the high quality of starter cultures. A basic prerequisite for including microorganisms in starter cultures is to demonstrate (through extensive research) their genetic, biochemical and functional properties (Martinović and Vesković Moračanin, 2005). It is necessary that starters should meet food safety criteria, shelf-life requirements, technological effectiveness, including economic feasibility criteria (Wang et al., 2015; Grujović et al., 2020). The ability to produce toxic substances, biogenic amines or other harmful substances could disqualify a culture from entering the technological process (Latorre-Moratalla et al., 2010; EFSA, 2011). Therefore, starter cultures used in production process of raw fermented meat products (as well as other fermented foods) are usually selected among amine-negative bacteria (without ability to convert to biogenic amines by decarboxylation of amino acids) or amine-oxidizing bacteria (with ability to oxidize biogenic amines and convert them into aldehyde, hydrogen peroxide and ammonia) (Suzzi and Gardini, 2003). This is because fermented food products are of concern due to its intensive microbial activity and high potential to form biogenic amines (BAs). In the context of toxicity and food safety histamine and tyramine are the most relevant BAs (EFSA, 2011).

By definition, BAs are basic nitrogenous compounds of low molecular weight, mainly formed by microbial decarboxylation of amino acids and through other forms of biological activities, e.g. amination and

transamination of aldehydes and ketons. They are ubiquitous compounds commonly found in animals and humans. BAs have different roles in variety of biological processes including control of blood pressure, synaptic transmissions, allergic responses, as well as control of cellular growth (EFSA, 2011). BAs may be hazardous to consumers` health if their concentrations in fermented foods reach a critical thresholds, as observed by Ladero et al. (2010). Foods that are most commonly associated with higher levels of BAs are as follows: fish and fish products, as well as variety of fermented foodstuffs of animal and plant origin (meat, dairy products, vegetables, beers and wines).

The production of BAs in various food commodities has been associated with specific metabolic activity of some microbial groups.

It is observed by Coton and Coton (2009) that some microbial groups have specific capacity to produce BAs and this can be considered as a strain-specific characteristic connected with certain genera or species. Furthermore, it is suggested that such ability of certain microbial group to produce BAs can be acquired by horizontal dissemination of genes between strains (EFSA, 2011).

In a study carried out by Suzzi and Gardini (2003) it was concluded that production of BAs in fermented foods depends on amino acids (the precursors), the presence of decarboxylase-positive microorganisms, and on optimal growth conditions supporting decarboxylation activity.

Enzymes performing decarboxylation of amino acids can be found in different microorganisms (e.g. family of *Enterobacteriaceae* and *Micrococcaceae*) and they show substrate-specific amino acids` decarboxylation, which is stated by Linares et al. (2011). Kučerová et al. (2009) observed that *Enterobacteriaceae* are particularly active in the production of BAs. In addition to that, many Lactic Acid Bacteria (LAB) that regularly present the microbiota of fermented foods, belong to the *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Lactococcus* and *Leuconostoc* genera and are also able to decarboxylate amino acids (Lonvaud-Funel, 2001; Buňková et al., 2009). The highest concentrations of BAs are generally observed in fermented foods, on the one hand, and spoiled foods, on the other (Gardini et al., 2016; Miclotte, 2016).

Although episodes of foodborne incidents related to consumption of foods with high levels of BAs are relatively rare, the intake of higher amounts poses a serious health risk due to their synergistic effect on gastrointestinal and nervous system, as well as impact on blood pressure. The symptoms most commonly associated with toxic effect followed by ingestion of high concentrations of BAs are nausea, respiratory distress, hot flushes, perspiration, heart palpitations, headache, rashes, oral burning, hypo- and hypertension, vomiting, etc. (Spano et al., 2010; Jeon et al., 2018). The most frequently reported food borne intoxications with BAs are caused by histamine (acting as vasoactive mediator) and also having a role in allergies and inflammations (Gonzaga et al., 2009). Other BAs involved in food borne intoxications are as follows: tyramine, putrescine and cadaverine whose high concentrations may cause nausea, vomiting, migraine, hypertension and headaches (Stratton et al., 1991). Although there are no harmonized threshhold values regarding the maximum levels for the majority of biogenic amines in foods for human consumption, there are certain recommended thresholds suggested by several authors: tyramine, 100–800 mg kg^{-1}; β-phenylethylamine, 30 mg kg^{-1}; and total biogenic amines, 1000 mg kg^{-1} (Jeon et al., 2018); total BAs, 750–900 mg kg^{-1} (Ladero et al., 2010). The only exception is histamine since its maximum level in certain fish species is strictly regulated by national and international regulations.

Besides toxicity, another aspect of the harmful properties of BAs is food safety, keeping in mind that their presence is the result of dynamic growth and metabolism of microorganisms. Therefore, BAs can be also seen as reliable indicators of microbial quality of raw materials or food spoilage (Ruiz-Capillas and Jiménez-Colmenero, 2004), including the hygiene of the production process, since it is recorded that BAs concentrations increase as the hygienic quality of a product decreases (EFSA, 2011).

Therefore, it would be beneficial to monitor production process of fermented foods regarding BAs levels, along the whole food chain to ensure the safety of products, as well as to create data for evidence-based knowledge. This implies, in particular, on toxicity, process-based control measures, process hygiene verification, food safety criteria and validation of detection methods.

One of the most important steps in the food industry manufacturing fermented foods is to identify BAs-producing bacteria to avoid and/or minimize the risk of amine formation and, subsequently, prevent them from entering the food chain and causing harmful effects on consumers` health, as well as economic losses.

The aim of this study was to determine the ability of lactic acid bacteria (LAB) isolated from several Serbian traditionally fermented sausages (Sremska, Uzička and Levačka) and Zlatar cheese to produce biogenic amines, *in vitro,* by amino acid decarboxylation.

2. Materials and methods

2.1. Bacterial cultures

A total of 156 strains of LAB isolated from Serbian traditionally fermented sausages and cheese were used in this study.

The identification of LAB isolates was based on their phenotypic (morphological and biochemical) characteristics (Gram affiliation, cell morphology, catalase test, oxidase test, relation to different salt concentrations, different ambient temperatures and different pH values of the medium, formation of exopolysaccharides). The preliminary identification of LAB isolates was performed using the biochemical kits API CHL 50 and Rapid ID 32 Strep test (Bio-Mérieux, France) (Vesković Moračanin et al., 2013).

Upon isolation by conventional microbiological techniques, LABs were molecularly identified by sequencing their 16S rDNA (Veskovic Moracanin et al., 2013).

Data on the number of strains of each tested species are given in Table 1.

LAB strains were kept frozen at −20°C in appropriate media (MRS or M17 broth) and supplemented with 20% glycerol as a cryoprotective

agent as previously described (Vesković Moračanin et al., 2014, 2015). LAB activation is performed in 10 mL of MRS or M17 broth (1% inoculum, 24 h, 37°C) as previously described (Vesković Moračanin et al., 2014, 2015). Activated cultures LAB contain cca 10^7–10^8 colony-forming unit per mililitre of sample (CFU/mL).

Table 1.
The strains of LAB isolated from Serbian traditionally fermented sausages and cheese

LAB – genus	Number of isolates	LAB – species	Number of isolates
Lactococcus spp.	51	*Lc. lactis* ssp. *lactis*	43
		Lc. garviae	4
		Lc. lactis ssp. *cremoris*	3
		Lc. lactis	1
Lactobacillus spp.	38	*Lb. plantarum*	12
		Lb. brevis	8
		Lb. sakei	10
		Lb. curvatus	4
		Lb. carnosum	2
		Lb. alimentarius	1
		Lb. inhae	1
Enterococcus spp.	35	*E. faecalis*	32
		E. faecium	3
Leuconostoc spp.	25	*Ln. mesenteroides*	18
		Ln. mesenteroides ssp. *mesenteroides*	6
		Ln. gasicomitatum	1
Pediococcus spp.	6	*P. pentosaceus*	6
Weissella spp.	1	*W. hellenica*	1
The total number of isolates of LAB			156

2.2. Analysis of biogenic amines produced by LAB

The analytical standards of BAs (histamine - HIS, tyramine - TYR, putrescine - PUT, cadaverine - CAD, tryptamine - TRY, spermidine - SPD and spermine - SPE) were purchased from Sigma-Aldrich (St. Louis, MA, USA).

The analytical method for the determination of BAs after 24-hour incubation LAB in MRS and M17 broth, using liquid chromatography tandem mass spectrometry (LC-MS/MS), is based on the modified procedure for the determination of these compounds in fish (Sagratini et al., 2012) as previously described (Vesković Moračanin et al., 2014, 2015).

LC-MS/MS system consisted of Waters Acquity™ UPLC pump and autosampler coupled with a Waters TQD detector (Waters, Milford, MA, USA). Separation was performed on Thermo Scientific (Waltham, MA, USA) Hypersil Gold, 100 x 2.1 mm, 3µm. The mobile phase consisted of 10 mM ammonium acetate in 0.1% formic acid (mobile phase A) and ACN (mobile phase B) at a flow rate of 0.3 mL/min. The gradient elution program was as follows: 0 min - 20% B, 0–10 min - 85% B, 15–25 min - 20% B. Mass spectrometer's ion source operated in positive electrospray (ESI+) mode with capillary volrage of 3.5 kV. Ion source and dessolvation temperatures were 120°C and 400°C, respectively. Dessolvation gas was (N_2) with the flow of 550 L/h. Cone gas was also nitrogen at flow rate of 50 L/h. Quadripoles of mass spectrometer were set to oprate in multiple reaction monitoring (MRM) mode.

MRM conditions for each compound are provided in Table 2.

Table 2.
Multiple reaction monitoring conditions

Biogenic amines	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)	Dwell time (ms)
HIS	112	95	10	12	150
PUT	89	86	10	12	150
CAD	103	72	10	12	150
SPE	203	112	10	12	150
SPD	146	112	10	12	150
TYR	138	121	10	12	150
TRY	161	144	10	12	150

The traditional chromatographic analysis of BAs based on UV or fluorescent detection after pre- or post-column derivatization has been extensively used and has provided satisfactory results. However, the lack of specificity as well as the erratic and time – temperature dependent derivatization step might have a significant influence over precision and quantification in general. Mass spectrometry in multiple reactions monitoring (MRM) mode offers absolute specificity (monitoring of two fragments) and does not require the chemical alteration of the analytes, resulting in significant improvements in quantification. Furthermore, the ability to considerably shorten the chromatographic run contributes to higher sample throughput as well.

All measurements were performed in triplicate on duplicate trials. Concentrations of BAs are given in mg/mL. All data were presented as means ± standard deviation (SD) of means.

3. Results and discussions

The obtained results on BAs production by 156 LAB strains showed very low concentrations, which were considered non-significant from both a technological and safety viewpoint (Table 3). In fact, very low contents were determined for all seven BAs, which is in a correlation with numerous studies (Vesković Moračanin et al., 2014, 2015; Thakkar et al., 2015; Stadnik and Dolatowski, 2015).

Table 3.
Biogenic amine production by LAB isolated from Serbian traditionally fermented food

Lactic acid bacteria	Biogenic amine production* (mg L^{-1})						
	HIS	TYR	PUT	CAD	TRY	SPD	SPE
Leuconostoc mesenteroides							8.35 ± 0.02
F	15.80 ± 0.20	16.39 ± 0.05	-	-	-	-	6.15 ± 0.13
IMAU 60148	12.76 ± 0.45	21.83 ± 0.23	-	-	-	-	8.21 ± 0.05
KLDS 5.0606	15.42 ± 0.18	18.58 ± 0.03	-	-	-	-	23.62 ± 0.06
SK kimchi 6	2.20 ± 0.50	25.96 ± 0.07	-	-	-	-	5.75 ± 0.05
F 170	1.15 ± 0.05	-	-	-	-	-	3.21 ± 0.03
Leu 1	-	-	-	-	-	-	-
CTC 6578	2.36 ± 0.05	-	-	-	-	-	3.53 ± 0.10
LHICA-5331	8.00 ± 0.25	-	-	-	-	-	-
SK kimchi 6	15.63 ± 0.08	15.16 ± 0.03	-	-	-	-	3.60 ± 0.04
AaD8	-	22.58 ± 0.07	-	-	-	-	3.14 ± 0.04
C 12	-	-	-	-	-	-	3.52 ± 0.23
F 170	2.44 ± 0.14	-	-	-	-	-	12.17 ± 0.03
LHICA-53-3	17.94 ± 0.08	-	-	-	-	-	3.57 ± 0.09
AaD8-2	18.52 ± 0.08	15.62 ± 0.03	-	-	-	-	5.19 ± 0.05
IMAU60148-2	16.84 ± 0.23	-	-	-	-	-	6.58 ± 0.08
MBF5-9	13.49 ± 0.29	-	-	-	-	-	4.96 ± 0.04
MU10	21.85 ± 0.13	15.85 ± 0.05	-	-	-	-	9.94 ± 0.03
SK kimchi 6-2	17.31 ± 0.26	-	-	-	-	-	4.97 ± 0.02
Ln. mesenteroides* ssp. *mesenteroides							
IMAU:10231	16.04 ± 0.06	21.95 ± 0.03	-	-	-	-	5.58 ± 0.04
J9	20.41 ± 0.04	18.62 ± 0.03	-	-	-	-	9.82 ± 0.05
J9-2	14.72 ± 0.03	-	-	-	-	-	7.55 ± 0.05
3 strains	14.69 ± 2.86	18.36 ± 2.20	-	-	-	-	6.83 ± 1.34
Ln. gasicomitatum							
J 13	-	16.61 ± 0.07	6.36 ± 0.06	-	-	-	6.74 ± 0.04
Lactobacillus plantarum							
IMAU:40091	-	-	-	-	-	-	3.99 ± 0.17
11 strains	-	-	-	-	-	-	-
Lb. inhae							
IH 101	19.46 ± 0.06	-	-	-	-	-	4.80 ± 0.18
Lb. brevis							
IMAU 80121	12.48 ± 0.03	15.33 ± 0.50	-	-	-	-	7.17 ± 0.15
bh 1	17.16 ± 0.15	25.42 ± 0.58	-	-	-	-	8.17 ± 0.33
IMAU:10206	17.76 ± 0.33	17.59 ± 0.26	-	-	-	-	8.60 ± 0.19
NRIC 0134	21.76 ± 0.46	15.80 ± 0.11	-	-	-	-	9.97 ± 0.41
b 4	16.79 ± 0.36	18.83 ± 0.07	-	-	-	-	9.48 ± 0.12
T 10	17.37 ± 0.14	18.54 ± 0.23	-	-	-	-	12.47± 0.12
12-2	18.74 ± 0.05	-	-	-	-	-	10.37 ± 0.27
T10-3	17.23 ± 0.16	-	-	-	-	-	12.54 ± 0.11
Lb. sakei							
IMAU 80189	22.57 ± 0.07	19.26 ± 0.14	5.74 ± 0.16	1.26 ± 0.21	-	-	15.03 ± 0.06
D	11.42 ± 0.17	19.77 ± 0.07	5.83 ± 0.16	1.14 ± 0.04	-	-	7.48 ± 0.08
FLEC 01	16.49 ± 0.70	15.83 ± 0.78	-	-	-	-	2.72 ± 0.48
23 K	22.26 ± 0.29	20.35 ± 1.47	-	-	-	-	14.31 ± 1.04
IMAU 80168	19.25 ± 0.31	19.68 ± 1.14	-	-	-	-	10.04 ± 0.90
JS 3	23.75 ± 1.10	18.45 ± 0.63	-	-	-	-	13.74 ± 0.46
CRL 1467	17.46 ± 0.51	-	-	-	-	-	6.40 ± 0.27
3 strains	17.11 ± 0.63	19.34 ± 1.16	-	-	-	-	9.42 ± 1.77

Lb. curvatus							
IMAU:10284	-	13.76 ± 0.58	-	-	4.06 ± 0.15	-	20.71 ± 1.92
IMAU:10189	23.84 ± 1.77	20.19 ± 2.06	5.94 ± 0.08	-	1.18 ± 0.29	-	14.72 ± 0.43
L5	12.43 ± 0.31	9.62 ± 1.41	3.33 ± 0.69	-	-	-	6.84 ± 0.23
IMAU:10284-2	29.89 ± 0.09	24.18 ± 0.16	-	-	-	-	10.36 ± 0.12
Lb. alimentarius							
IMAU 80147	2.11 ± 0.12	-	-	-	-	-	-
Lb. carnosum							
SACB 703a	-	-	-	-	-	-	2.66 ± 0.16
KNUC 23	18.40 ± 0.04	-	-	-	-	-	6.94 ± 0.06
Lactococcus lactis							
zmjh 8	3.61 ± 0.06	-	-	-	-	-	-
Lc. lactis* ssp. *lactis							
43 strains	7.89 ± 2.43	-	-	-	-	-	7.06 ± 1.34
Lc. garvie							
4 strains	7.33 ± 3.96	-	-	-	-	-	8.89 ± 0.73
Lc. lactis* ssp. *cremoris							
IMAU 60131	17.19 ± 0.18	25.30 ± 0.06	11.15±0.06	-	-	-	7.95 ± 0.08
IMAU 50150	16.86 ± 0.51	-	-	-	-	-	8.61 ± 0.04
IMAU 60024	-	-	-	-	-	-	5.76 ± 0.05
Enterococcus faecalis							
32 strains	15.87 ± 8.22	59.89 ± 6.66	-	-	-	-	-
En. faecium							
3 strains	11.03 ± 6.64	47.33 ± 8.58	-	-	-	-	-
Pediococcus pentosaceus							
CTSPL 1	15.86 ± 0.14	-	-	-	-	-	7.36 ± 0.06
YTX 32 BMX	15.01 ± 0.80	18.72 ± 0.20	-	-	-	-	8.61 ± 0.08
L 2-2	-	-	-	-	-	-	4.12 ± 0.03
YTX4BMX	15.10 ± 0.02	15.56 ± 0.10	-	-	-	-	4.19 ± 0.02
FB-301	13.42 ± 0.51	-	-	-	-	-	6.19 ± 0.27
CTSPL1-2	19.60 ± 0.44	-	-	-	-	-	11.51 ± 0.24
Weissella hellenica							
1402	15.31± 0.17	44.04 ± 0.96	13.48±0.44	5.20 ± 0.30	-	7.05 ± 0.05	16.65 ± 0.25

* All data were presented as means ± SD

\- Not detected

The measured amounts of PUT, CAD, TRY and SPD (except in 13 LAB strains) were below the quantification limit of the analytical method (0.1 mg L^{-1}), whereas the amounts of HIS, TYR and SPE were above the limit of quantification, but were not significant from both a technological and safety viewpoint (Jeon et al., 2018).

Moreover, all LAB strains (87.18%) produced very small amounts of HIS (ranging from 1.15 ± 0.05 mg L^{-1} to 29.89 ± 0.09 mg L^{-1}). HIS was not produced by only 20 of 156 LAB strains, primarily, *Lactobacillus plantarum* (12 strains), *Lb. curvatus* (IMAU:10284), *Lb. carnosus* (SACB 703 a), Pediococcus pentosaceus (L 2-2), Leuconostoc mesenteroides (Leu 1, AaD8, C 12) and one strain of *Ln. gasicomitatum* (J 13) and *Lc. lactis* ssp. *cremoris* (IMAU 60024). Histamine is the most important amine in food-borne intoxications, due to its strong biological activity (Cabanis 1985), derived from its vasoactive and psychoactive properties (EFSA, 2011). The histidine decarboxylase activity is characteristic of some *Lactobacillus*, *Oenococcus*, *Pediococcus* and *Tetragenococcus* strains (EFSA, 2011). Kanki et al. (2007) suggested that the enzymatic activity of histidine decarboxylase was responsible for histamine formation and that its action could continue even after bacterial autolysis.

Although the proportion of decarboxylase-positive examined LAB strains was high in this study, their histaminogenic potential was weak. All concentration values, including TYR and HIS, were very low and were obtained due to the high sensitivity of the analytical technique used in the experiment.

Seventy-two (46.15%) of the LAB isolates were decarboxylated tyrosine. TYR amounts were between 9.62 ± 1.41 mg L^{-1} and 59.89 ± 6.66 mg L^{-1}. Most of the positive tested LAB strains produced TYR in broth at concentrations below 26 mg L^{-1}. Exceptions included *Enterococcus faecalis* strains (a total of 32), which produced TYR at concentrations of 59.89 ± 6.66 mg L^{-1}, and three strains of *Enterococcus faecium* at levels of 47.33 ± 8.58 mg L^{-1} TYR. For these reasons, strains may indirectly have a potential risk to consumer health. The amount of TYR produced by the tested strains of *Enterococcus faecalis* is consistent with the findings of other authors (Kučerová et al., 2009), which confirms the fact that they are highly efficient in converting tyrosine to tyramine. Decarboxylating acitivity was lower in strains of *E. faecium*. The production of TYR is a commonly detected characteristic of enterococci from dairy products (Martín-Platero, 2009). In this study, TYR was not produced by all strains of *Lactobacillus plantarum*, *Lb. inche*, *Lb. carnosus*, *Lb. alimentarius*, *Lactococcus lactis*, *Lc. lactis* ssp. *lactis*, and *Lc. garvie*.

With regard to tryptophan, lysine, arginine and ornithine decarboxylation, this kind of metabolism was very rare in the tested LAB strains (only 8.33%). PUT amounts were between 3.33 ± 0.69 and 13.48 ± 0.44 mg/L, while CAD amounts ranged from 1.17 ± 0.04 to 5.20 ± 0.30 mg/L. TRY concentrations were 1.18 ± 0.29 and 4.06 ± 0.15 mg L^{-1} (*Lb. curvatus* IMAU:10284 and *Lb. curvatus* IMAU:10189, respectively) and SPD content was 7.05 ± 0.05 mg L^{-1} (only *Weissella hellenica* 1402). Similar results have been reported by Romano et al. (2013).

Research on the presence of BAs in food (especially fermented products) as well as gaining knowledge on microorganisms, especially the conditions favoring BA formation in food, are of great importance for toxicity prevention. In addition to their biological role (source of nitrogen and precursors to hormones, alkaloids, nucleic acids and proteins), BAs are known to act as important aromatic components of food and potential precursors to carcinogenic N-nitroso compounds (De Mey et al., 2015; Karovičová and Kohajdová, 2005). The intake of food containing high amounts of BAs can lead to toxic effects, with histamine toxicity being the most common toxicity caused by biogenic amines (EFSA, 2011; Hungerford, 2010; Karovičová and Kohajdová, 2005).

4. Conclusions

The production of biogenic amines is an important point in the safety evaluation of autochthonous LAB with the potential to be used as functional strains (probiotic or starter cultures). Therefore, there is a need to consider their presence in functional LAB with high precision. The results of the present study indicate that amino acid decarboxylating enzymes were not uniformly present in the tested species/strains of LAB. Concurrently, the results presented that LAB isolated from Serbian traditionally fermented sausages and cheese are not significant producers of BAs *in vitro*. In addition to their other favorable properties (protective and technological roles), the use of the tested LAB in terms of the safety of biogenic amine production is possible. The established LAB collection, which exhibits technologically positive properties, would serve as a basis for further research towards the potential production of national starter cultures. Therefore, this study may serve as a proposal of a suitable strategy in developing autochthonous starters for the manufacture of typical fermented food. The next step should be to estimate the effective influence of the selected strains on the hygienic and sensory characteristics of the final products in real-life industrial production.

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Declaration of competing interest

Authors declare that no personal and/or financial relationship with other people or organizations is present in this paper.

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