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Probiotic potential of autochthone microbiota from dry-cured sheep ham

Probiotisches Potenzial der autochthonen Mikrobiota aus trocken gereiftem Schafschinken

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Summary

This study assessed the potential of probiotic characteristics of bacterial strains isolated from dry-cured sheep ham. It is one of the most common autochthonous processed meat products made in a traditional way on the Pešter plateau (Western Serbia). Isolates were identified as *Lactobacillus curvatus* (9 strains), *Lactobacillus sakei* (3 strains), and *Enterococcus faecium* (4 strains) using MALDI-TOF mass spectrophotometry. The study of probiotic characteristics of 16 dry-cured sheep ham isolates included survival rate through the gastrointestinal tract (GI), the possibility of biogenic amine synthesis, growth on medium with different concentrations of phenol, and antimicrobial activity. The results showed that in simulated gastric juice conditions, the cell number decreased after the first hour of incubation in the tested strains of *Lb. curvatus*, *Lb. sakei* and *En. faecium* except in the case of *Lb. curvatus* IIos19 where the number of cells remained approximately the same. After the second hour of incubation, the number of cells generally remained at the level of the first hour except in the case of the following isolates: *Lb. sakei* Ios12, *Lb. curvatus* IIos18 and *En. faecium* IIos24, where an increase in the number of cells was noticed after the second hour of incubation.

In simulated small intestine conditions, an increase in the number of vital cells after 4 and 6 hours of incubation was observed in the isolates *Lb. curvatus* IIos4, *Lb. sakei* (Ios12, IIIos13), and *En. faecium* Ios1a. Synthesis of biogenic amines was not observed in investigated lactobacilli and enterococci. Analyzed isolates exhibited growth on media with 0.1% and 0.2% phenol, while 5 isolates exhibited decarboxylase activity. Six *Lactobacillus*

strains, *Lb. curvatus* (IIos6, IIos17, and IIIos1), *Lb. sakei* (IIIos16, Ios12, and IIIos13) and *En. faecium* Ios4 inhibited the growth of tested pathogens, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus cereus* ATCC 14579.
Keywords: Lactic acid bacteria (LAB); probiotic properties; simulated gastric juice conditions; bile salt tolerance.

Zusammenfassung

Diese Studie untersuchte das Potenzial von probiotischen Eigenschaften von Stämmen, die aus trocken geheilten Schafschinken isoliert wurden. Es ist eines der am meisten verbreiteten autochthonen Fleischprodukte, die traditionell auf dem Pester Plateau (Westserbien) hergestellt werden. Die Untersuchung der probiotischen Eigenschaften von 16 Isolaten von trocken geheilten Schafen beinhaltete die Überlebensrate durch den Gastrointestinaltrakt (GI), die Möglichkeit einer biogenen Aminsynthese, das Wachstum auf einem Medium mit verschiedenen Konzentrationen von Phenol und antimikrobielle Aktivität. Die Ergebnisse zeigten, dass unter simulierten Magenbedingungen alle Stämme von *Lactobacillus curvatus* und *Lactobacillus sakei* eine hohe Resistenz gegen 0,3% Konzentration von Gallensalzen aufwiesen, während *Enterococcus faecium* Isolate einen signifikant geringeren Grad an Resistenz zeigten. Unter simulierten Dünndarmbedingungen wurde in den Isolaten *Lb. curvatus* IIos4, *L*b. *sakei* (Ios12, IIIos13) und *En. faecium* Ios1a eine Zunahme der Anzahl vitaler Zellen nach 4 und 6 Stunden Inkubation beobachtet. Die Synthese von biogenen Aminen wurde in untersuchten Laktobazillen und Enterokokken nicht beobachtet. Die analysierten Isolate zeigten ein Wachstum auf Medien mit 0,1% und 0,2% Phenol, während 5 Isolate Decarboxylase-Aktivität zeigten. Acht Milchstämme, *Lb. curvatus* (IIos6, IIos17 und IIIos1), *L*b. *sakei* (IIIos16, Ios12 und IIIos13) und *En. faecium* Ios4 hemmten das Wachstum getesteter Pathogene, einschließlich *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 27853 und *Bacillus cereus* ATCC 14579.

Schlüsselwörter: Milchsäurebakterien (LAB); probiotische Eigenschaften; Simulierte Magen- und Darmsäfte

Introduction

In the food industry, starter cultures that have probiotic properties are used in a large number of products to preserve quality, sustainability, and organoleptic characteristics. The addition of selected strains of probiotic bacteria to food products, often along with fibers and prebiotics, enhances the beneficial probiotic effects on human health. Probiotic strains should be present in the product in at least 10^6 CFU/g to have an impact on the health of consumers (Kołozyn-Krajewska and Dolatowski, 2012). Foods containing probiotic bacteria fall within the category of functional foods (Pavli et al., 2016). Probiotic bacteria are mainly lactic acid bacteria (LAB), safe organisms that have a beneficial effect on both humans and animals (Gerez et al., 2012). It should be emphasized that the preventive role of probiotics is far more important than the therapeutic one. Lactic acid bacteria (LAB) that has probiotic features is added to fermented milk products, used as a part of the bread-making process, vegetable and fruit juices, and other foods, including fermented meat. Probiotic meat products are obtained by the addition of probiotic microorganisms to fermented meat products. It is known that meat is an excellent medium for the growth of probiotic microorganisms and could be a suitable carrier to support and deliver probiotics to the host (Gänzle et al., 1999; Khan et al., 2011).

Research on LAB from meat products as possible sources of probiotic cultures is becoming more and more interesting today, therefore special attention must be paid to autochthonous isolates as potential probiotics. Bacterial strains that can be used in the manufacturing of fermented meat products should be capable of surviving in conditions found within those products as well as dominate other microorganisms found in the finished product (Kołozyn-Krajewska and Dolatowski, 2012). The acid and bile tolerance, resistance to degradation by hydrolytic enzymes, and bile salts in the small intestine are fundamental properties that indicate the ability of probiotic microorganisms to survive the GI (gastrointestinal) tract (Pieniz et al., 2014). The LAB genera that have been identified from meat products include *Lactobacillus, Pediococcus, Leuconostoc, Weissella,* and *Enterococcus* and are well adapted to the ecological niche of meat fermentation (Olaoye and Idowu, 2010). Dry-cured sheep ham is among the traditional fermented food products with autochthonous microbial populations.

Dry-cured sheep ham or Sjenica sheep ham is one of the oldest and most popular meat products in Western Serbia. This product is prepared in an exceptionally complex way from mutton carcasses of an autochthonous breed of sheep.

An essential part of its production is the sanitary safety of raw materials which must meet all veterinary and sanitary conditions of production. Production of dry-cured sheep ham includes

several phases: a selection of raw materials, salting and brining, smoking, drying, and aging, in which the product does not change and receives its trademark smell, consistency, and texture (Stamenković and Dević, 2006).

Dry-cured sheep ham fermentation is a long-lasting process caused and helped by autochthonous LAB, which defines the taste of texture as well as the nutritional properties of the product. As the probiotic potential of this product so far has not been explored, the goal of this study was to determine the probiotic characteristics of 16 autochthonous LAB isolates from dry-cured sheep ham through the research of survival rate through the GI tract, the possibility of biogenic amine synthesis, growth on medium with different concentrations of phenol and antimicrobial activity towards pathogens *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 27853 *Bacillus cereus* ATCC 14579. These observations present a first step towards establishing logical criteria for screening and selecting foodborne microorganisms that exhibit probiotic properties beneficial to humans.

1. Materials and methods

Bacterial strains and growth conditions

The research used 16 isolates of LAB isolated from 9 samples of dry-cured sheep ham. Isolation of LAB samples was performed using serial 10-fold dilution technique. 1 gram of meat sample was mixed in 9 mL of quarter strength ringer solution (Hemofarm, Vršac, Serbia) and further serially diluted. Then, 0.1 ml of sample was placed on selective medium de Man Rogosa Sharpe (MRS) agar (Torlak, Belgrade, Serbia). After the incubation period of 24-48 hours, initial growth at 37°C was checked. Enumerated *Enterococcus* spp. colonies were screened for growth on modified MRS (6.5% NaCl, 9.6 pH), at 10 and 45°C as well their growth on kanamycin aesculin azide (KAA) agar (Merck, Darmstadt, Germany). The purity of these isolates was confirmed by microscopic observation of homogeneity and their biochemical reactions were evaluated for: arginine hydrolysis, growth ability on MRS agar at different temperatures (15°C and 45°C), growth ability on MRS agar with 4 and 8% NaCl, CO_2 production from glucose, lipolytic activity and proteolytic activity. Gram-positive, catalase-negative, non-spore-forming isolates were considered as LAB and further distinguished by their carbohydrate fermentation profile using an API 50CH and API 20 Strep tests, bacterial identification system (Bio Merieux, S.A., France). An analysis of biochemical profiles of isolates was performed to species and subspecies level using

identification software (API WEB, V 1.1.0, Biomeriuex, Marcy I"Etoile, France). LAB isolates were identified as *Lb. curvatus, Lb. sakei,* and *En. faecium*. Identification of isolates was confirmed with MALDI-TOF mass spectrophotometry**,** using the Bruker Microflex LT instrument (Bruker Daltonics, Bremen, Germany) 34/5000 which is equipped with a nitrogen laser (337 nm) under control of Flexcontrol software ver. 3.1 (Bruker Daltonics). Isolates of LAB were analyzed using the protein extraction method with modifications described in detail in Muruzović et al. (2018). Measurement results for each isolate were expressed by results from MALDI-biotyper (from 0.000 to 3.000), and comparisons were made with similarities to known bacterial profiles available on the MALDI-biotyper software database, with values ≥ 2.000 taken as the correct identification of species levels.

Survival in the gastrointestinal (GI) tract

Tolerance to simulated gastric juice conditions. Overnight cultures of isolates were inoculated in 1:10 ratio into the solution of 0.5% NaCl and 0.22% pepsin with pH value 2.0 and incubated at 37°C for 1 and 2 hours (Radulović et al., 2008). Growth was monitored at OD_{620} (Bassyouni et al., 2012). Isolates that showed tolerance to simulated gastric juice conditions were further subjected to bile salt testing.

Bile salt tolerance. Overnight cultures of the isolates were inoculated into a solution of 0.2% pancrelipase, 0.4% bile salts and 0.5% NaCl with pH 8. Tubes were incubated for 4 and 6 h at 37°C (Radulović et al., 2008). Growth was monitored at OD_{620} (Bassyouni et al., 2012).

Synthesis of biogenic amines

The ability of isolates to synthesize biogenic amines from histidine and tyrosine has been tested using the method of Do-Won and Jong-Hoon, (2015). Seeded and modified substrates with the addition of amino acids were incubated at 37°C / 24h. The appearance of a purple color in the histidine substrate or the appearance of sediment in the tyrosine substrate confirms the presence of decarboxylase.

Growth on medium with different quantity of phenol

The growth capacity of isolates in the presence of phenol was determined by the inoculation of overnight culture onto the MRS agar plates with the addition of 0.1%, 0.2%, and 0.3% of phenol. Physiological levels of phenol compounds in human intestines are low; that is why it is important to analyse the sensitivity of potential probiotics against these substances in smaller concentrations of 0.1, 0.2, and 0.3%. The appearance of colonies after 48 h of

incubation at 37°C indicates the growth ability of isolates in the presence of a certain concentration of phenol (Šusković et al., 2001).

Antimicrobial activity

Antimicrobial activity of the tested isolates has been examined using the method described in Vesković-Moračanin et al. (2010). Diffusion method with small wells meant that 5 ml of soft (0.7%) nutrient agar (Torlak, Belgrade, Serbia), containing indicator strains, were poured on firm MRS media which was inoculated with 10^5-10^6 of cells of indicator culture/ml medium. In the soft agar small wells of 5 mm diameter were formed into which 100 µl of partially purified bacteriocin was poured. Partial purification of bacteriocin was performed in the following way: after 18 h of growth, the cultures were spin-dried at 10000 revolutions for 30 minutes at 4°C. After separation and neutralization of the supernatant with 10 M NaOH, up to pH 6.5 – 7.0, the precipitation of bacteriocin was done by ammonium sulfate (472.2 g/l) until a saturated solution was obtained. Separated bacteriocin in the form of a white deposit was dissolved in 25 ml 0,05 M of sodium phosphate buffer with pH 7. The sterilization of partially purified bacteriocin was made by filtration through 0.22 µm microfilter (Acrodisc, Germany). Antimicrobial activity was detected based on the appearance of light zones around small wells as a consequence of growth inhibition in sensitive bacteria strains.

Statistical analysis

The results represent the mean ± standards deviations. Statistical analysis was conducted with SPSS 11.0 Bivariate Correlation Analysis (Chicago, Illinois, USA).

2. Results and Discussion

The indigenous microbiota of traditional dry-cured meat products today represents a significant field of research on wild strains and the product itself as functional food (Arihara, 2006). Our study results of dry-cured sheep ham microbiota indicated the dominance of LAB strains with high probiotic potential. The results presented in Žugić-Petrović et al., (2020), indicated that the number of viable LAB was in range from 2.6 x 10^2 to 9.2 x 10^3 CFU/g of dry-cured sheep ham. According to the pattern of sugar fermentation, these isolates were identified as *Lb. curvatus* (9 strains), *Lb. sakei* (3 strains) and *En. faecium* (4 strains) (Table 1). The MALDI-TOF application in the study confirmed the preliminary API identification of the strains. Almost all LAB strains had a high level of identification, biotyper database and software were 100% correct in assigning the isolates to species. Four enterococci were

identified at a level of highly probable species identification (score values ≥ 2.4), and 12 isolates (*Lb. curvatus* and *Lb. sakei* strains) were identified at a level of secure genus and species identification (score values between 2.25 - 2.28). By exploring dry-cured meat products from Eastern Himalayas, Rai et al. (2010) came up with identification results showing the dominance of *Lb. curvatus* and *Lb. sakei* in the studied samples. The results of the probiotic potential of identified *En. faecium* isolates from dried Tunisian meat "Dried Ossban" were presented by Zommiti et al. (2018). They indicated the safety aspect of the strains themselves. Our study showed that MALDI-TOF can be an effective, and sustainable method in classifying *Lactobacillus* and *Enterococcus* strains.

TABLE 1. *Isolated species of LAB from the sheep ham*

MALDI-TOF Identification	*Lb. curvaus* (9)	*En. faecium* (4)	*Lb. sakei* (3)
Growth at:			
15ºC	+	+	+
45ºC	-	+	-
Growth in:			
4.0% NaCl	+	+	+
8.0% NaCl	+	+	+
Gas from glucose	-	-	-
NH_3 from arginine	-	+	-
Lipolytic activity	-	-	-
Proteolytic activity	-	-	-
Biochemical (API)			
L-arabinose	-	+	-
Cellobiose	+	+	+
Ribose	+	+	+
Esculin	+	+	+
Galactose	+	+	+
Lactose	+	+	-
D-mannose	+	+	+
Melezitose	-	-	-
Melibiose	-	+	+
D-raffinose	-	+	-
Sucrose	+	+	+
Trehalose	-	+	+
D-xylose	-	+	-
Rhamnose	-	-	-
Mannitol	-	+	-
Maltose	+	+	+
Sorbitol	-	-	-
Salicin	+	+	+

"+" - positive reaction; "-" – negative reaction

Survival in simulated gastrointestinal tract conditions

Falagas et al. (2006) point out that probiotics are living microorganisms which when administered in adequate amounts confer a health benefit on the host. One of the necessary conditions that manifest their probiotic characteristics is the ability of survival through the GI tract. Gastrointestinal tract possesses very harsh conditions for the survival of microorganisms which need to remain viable in population levels of 10^6-10^7 CFU/g of product in order to deliver the health benefits (Pavli et al., 2016). The tested strains isolated from dry-cured sheep ham from western Balkans showed a good survival rate in the artificial gastric and bile juice conditions, and the results are presented in Table 2.

TABLE 2. *The survival rate of selected strains in simulated gastric and bile juice conditions*

Isolates	Initial cell	Tolerance to gastric juice conditions		Tolerance to bile juice conditions	
	Incubation time 0 h	Incubation time 1 h	Incubation time 2 h	Incubation time 4 h	Incubation time 6 h
Lb. curvatus IIos17	1.1 ± 0.07	0.7 ± 0.00*	0.7 ± 0.00*	0.7 ± 0.00*	0.7 ± 0.04*
Lb. curvatus IIos11	1.0 ± 0.00	0.8 ± 0.06*	0.8 ± 0.00*	0.7 ± 0.02*	0.7 ± 0.05*
Lb. curvatus IIos4	1.0 ± 0.05	0.7 ± 0.00*	0.7 ± 0.05*	0.8 ± 0.00*	1.0 ± 0.04
Lb. curvatus IIos3	0.9 ± 0.00	0.5 ± 0.01*	0.5 ± 0.03*	0.5 ± 0.04*	0.6 ± 0.02*
Lb. curvatus IIos19	1.0 ± 0.06	1.0 ± 0.02	0.9 ± 0.01*	0.8 ± 0.03*	0.5 ± 0.02*
Lb. sakei IIIos16	0.8 ± 0.05	0.5 ± 0.00*	0.5 ± 0.01*	0.6 ± 0.00*	0.6 ± 0.01*
Lb. curvatus IIos18	1.0 ± 0.00	0.8 ± 0.17*	0.9 ± 0.00*	0.9 ± 0.00*	0.6 ± 0.00*
En. faecium Ios4	1.0 ± 0.02	0.9 ± 0.01*	0.9 ± 0.03*	0.9 ± 0.02*	0.8 ± 0.04*
Lb. curvatus IIos6	1.0 ± 0.06	0.8 ± 0.02*	0.8 ± 0.00*	0.8 ± 0.04*	0.8 ± 0.00*
En. faecium Ios5a	1.1 ± 0.00	1.0 ± 0.04*	0.9 ± 0.05*	1.0 ± 0.07*	0.9 ± 0.02*
Lb. curvatus IIos17a	1.0 ± 0.05	0.9 ± 0.04*	0.8 ± 0.09*	0.8 ± 0.13*	0.8 ± 0.12*
Lb. sakei Ios12	1.0 ± 0.05	0.7 ± 0.02*	0.8 ± 0.03*	0.8 ± 0.09*	1.0 ± 0.04
En. faecium Ios1a	1.0 ± 0.05	0.6 ± 0.04*	0.6 ± 0.01*	0.6 ± 0.09*	0.8 ± 0.01*
Lb. curvatus IIIos1	1.0 ± 0.00	0.9 ± 0.01*	0.9 ± 0.29*	0.9 ± 0.14*	0.8 ± 0.03*
En. faecium IIos24	0.9 ± 0.02	0.6 ± 0.01*	0.7 ± 0.09*	0.6 ± 0.16*	0.3 ± 0.11*
Lb. sakei IIIos13	1.0 ± 0.00	0.9 ± 0.02*	0.8 ± 0.21*	1.0 ± 0.03*	1.0 ± 0.28*

OD at 620nm at different time interval (hour); Values marked with asterisks are not significantly different from the control group (0 h), according to the Duncan's test ($p<0.05$).

As shown in Table 2, in simulated gastric juice conditions, the number of cells after the first hour of incubation decreased in all of the tested *Lb. curvatus* and *Lb. sakei* isolates except in isolate *Lb. curvatus* IIos19 where the number of cells remained approximately at the initial level. After the second hour of incubation, the number of cells for the studied *Lb. curvatus* and *Lb. sakei* did not decrease compared to the first hour in simulated gastric juice conditions while the number of cells in isolates *Lb. curvatus* IIos18 and *Lb. sakei* Ios12 showed a slight increase after the second hour of incubation. The obtained results are in accordance with the work of Bacha et al. (2009) on the probiotic characteristics of lactobacilli isolated from beef sausage, which showed a high rate of survival in simulated stomach conditions. The studied enterococci isolates showed a decrease in the number of cells in the first hours of incubation relative to their initial number in simulated gastric juice conditions. In the second hour of incubation, the number of cells in the examined *En. faecium* isolates remained approximately the same as in the first hour, except for *En. faecium* IIos24 in which the number of cells increased slightly.

There is no statistically significant decrease in the number of cells in the first and second hours of incubation. Hosseini et al. (2009) point out that the survival rate of *En. faecium* increases with increasing pH of the environment. In simulated bile juice conditions, isolates of *Lb. curvatus* mainly showed a mild decrease in the number of cells (isolates: IIos11, IIos19, IIos18, IIos17, IIos6, and IIIos1) or maintained approximately the same number of cells (isolate IIos17), except in the case of *Lb. curvatus* IIos4 which showed an increase in the number of cells after 6 h of incubation but without significant statistical differences. *Lb. sakei* isolates showed the same results in relation to the number of cells under gastric juice conditions. Maragkoudakis et al. (2006), researching the probiotic potential of Lactobacillus strains isolated from dairy products, concluded that all strains were resistant to pancreatin, as even after 4 h of exposure they retained viability. The number of viable cells of enterococci in simulated bile juice conditions after incubation for 4 and 6 h decline, in all the tested isolates. However, *En. faecium* isolate Ios1a showed an increase in the number of cells, but this was not statistically significant. According to Ruiz-Moyano et al. (2009), *En. faecium* isolate SE906 from Iberian dry-fermented sausages has a good survival capability in the simulated GI tract conditions, which characterizes it as a good potential probiotic.

Synthesis of biogenic amines

Biogenic amines are organic compounds that may be created in the meat processing and fermentation. They can cause headaches, circulatory disorders, and intoxication (Virgill et al.,

2007). Synthesis of biogenic amines test results on dry-cured sheep ham isolates showed that in the case of histidine there is no appearance of synthesis of biogenic amines in both lactobacilli and enterococci isolates. In the case of a tyrosine substrate, synthesis of biogenic amines in investigated lactobacilli and enterococci was not detected, which is in disagreement with the results presented by Landeta et al. (2013) that indicate that most *En .faecium* and *Lb. sakei* strains showed the production of tyramine. Similar results of tyramine production in enterococci were reported by Muñoz-Atienza et al. (2011).

Growth on medium with different quantity of phenol

According to Šušković et al. (2001), phenols can be formed in the intestines as a product of bacterial deamination of some aromatic amino acids derived from foods or endogenously produced. Phenols have distinct bacteriostatic properties, so phenol-tolerant bacteria have a greater chance of surviving the conditions of the GI tract than those bacteria that are more susceptible to this compound. As physiological levels of phenols in the human organism are low, it is important to analyze the sensitivity of potential probiotics to this substance precisely at concentrations in which phenols can be expected in the human body, in the range of 0.1%, 0.2%, and 0.3%. The results of research on the growth medium with different quantity of phenol showed good growth of investigated strains in substrates with a phenolic concentration of 0.1%, 0.2%, and 0.3%. Isolate *Lb. curvatus* IIIos1 showed no growth in the substrate with 0.3% phenol, while isolates *Lb. curvatus* IIos11 and *Lb. curvatus* IIIos1 showed no growth at any tested phenol concentration. The results obtained by Aswathy et al. (2008) indicated that many investigated strains of enterococci, lactobacilli, and leuconostoc have successfully tolerated a low level of phenol of 0.2-0.3%, which qualified them as possible probiotics. Vizoso Pinto et al. (2006) presented survival results on the phenol resistance of *Lb. plantarum* strains which suggest that these are generally moderately tolerant to a phenol concentration of about 0.4%.

Antimicrobial activity

Probiotic bacteria can antagonize pathogens using several mechanisms that involve the production of antimicrobial compounds such as fatty acids, organic acids, hydrogen peroxide, diacetyl, acetone, bacteriocins, as well as competition for the substrate and co-aggregation with a pathogen (Todorov et al., 2011).

TABLE 3. *Antimicrobial activity of the isolated LAB against pathogenic bacteria*

Isolates	Indicator strains				
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Listeria monocytogenes* ATCC 19115	*Pseudomonas aeruginosa* ATCC 27853	*Bacillus cereus* ATCC 14579
Lb. curvatus IIos17	10.2 ±0.64*	12.0 ±1.70	10.3 ± 1.10	10.0 ± 1.10	10.0 ± 1.70
Lb. curvatus IIos11	10.0 ± 1.90	-	8.1 ± 0.20	-	-
Lb. curvatus IIos4	25.6 ± 0.20	20.0 ± 1.80	18.0 ± 0.50	-	-
Lb. curvatus IIos3	16.1 ± 1.70	12.3 ± 1.50	-	10.0 ± 2.10	10.0 ± 0.10
Lb. curvatus IIos19	12.0 ± 1.00	10.0 ± 2.00	12.0 ± 0.50	-	-
Lb. sakei IIIos16	20.0 ± 1.70	18.3 ± 0.57	18.0 ± 1.70	20.0 ± 0.80	20.0 ± 0.30
Lb. curvatus IIos18	-	16.0 ± 4.10	15.0 ± 1.50	-	16.0 ± 0.50
E. faecium Ios4	23.0 ± 1.70	23.0 ± 1.70	25.0 ± 0.00	20.0 ± 3.00	18.0 ± 0.20
Lb. curvatus IIos6	25.6 ± 0.50	23.3 ± 0.50	25.0 ± 0.90	23.0 ± 0.00	22.0 ± 0.60
E. faecium Ios5a	20.0 ± 1.73	18.0 ± 0.00	20.0 ± 0.00	-	14.0 ± 0.80
Lb. curvatus IIos17a	10.0 ± 2.78	12.3 ± 0.50	10.0 ± 2.60	10.0 ± 0.00	-
Lb. sakei Ios12	22.0 ± 2.90	19.5 ± 2.10	20.0 ± 1.80	15.0 ± 3.20	20.0 ± 1.30
E. faecium Ios1a	10.0 ± 4.50	8.0 ± 1.70	-	-	8.0 ± 2.00
Lb. curvatus IIIos1	15.0 ± 2.17	13.1 ± 0.20	10.0 ± 0.80	10.0 ± 0.50	12.0 ± 1.70
E. faecium IIos24	-	14.0 ± 0.10	10.0 ± 3.00	-	10.0 ± 0.05
Lb. sakei IIIos13	15.0 ± 1.90	13.0 ± 0.10	14.0 ± 0.00	15.0 ± 2.20	14.0 ± 0.00

*Diameter of inhibitory zone (mm)

As shown in Table 3. *Lb. curvatus* IIos6, *En. faecium* Ios4, *Lb. sakei* IIIos16, *Lb. sakei* Ios12, *Lb. curvatus* IIos17, *Lb. curvatus* IIIos1 and *Lb. sakei* IIIos13 are isolates that showed an inhibition zone to all tested pathogens. All the isolates showed the largest inhibition zone towards *E. coli* ATCC 25922 where in the mean values of the inhibition zone ranged from 25.6 to 10.0. Similar results were also obtained by Brink et al. (2006), who investigated the probiotic potential of LAB, wherein the isolates showed a strong inhibition related to *E. coli*. In the case of *L. monocytogenes* ATCC 19115, potential probiotics showed good antimicrobial properties, wherein the isolate *Lb. curvatus* IIos6 also had an inhibition zone of about 25 ± 0.9. Benito et al. (2007) in their study, highlighted the antimicrobial activity of LAB strains from Iberian dry-fermented sausages against *L. monocytogenes. Lb. curvatus* IIos11 had the lower antimicrobial activity, with no inhibition zone to *S. aureus, P. aeruginosa* and *B. cereus*. *P. aeruginosa* ATCC 27853 is a pathogen for which the highest number of investigated strains of LAB (43.75%) did not show an inhibition zone. Antagonistic effects against *B. cereus* had not been shown by 25% of isolates. Žugić-Petrović et al. (2020) indicated that LAB showed high

or moderate sensitivity to clinically relevant antibiotics (tetracycline, cephalexin, amoxicillin, ceftriaxone, and erythromycin).

3. Conclusion

The emerging demand that traditional foods should have health benefits beyond nutritional ones has provided ample opportunity to explore unexplored foods for isolation of lactic acid bacteria and their potential role as probiotics. The results presented in this study demonstrated that isolates of LAB from dry-cured sheep ham exhibited favorable probiotic characteristics as the ability to grow and survive through the GI tract, the possibility of synthesis of biogenic amines, growth on medium with different quantity of phenol and antimicrobial activity. Further studies if investigated strains need to evaluate their potential health benefits, and their performance as novel probiotic or starters cultures.

Conflict of interest

The authors declare that no conflict of interest among authors.

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