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MOLECULAR CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA ISOLATED FROM SOME FERMENTED MEAT PRODUCTS

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ABSTRACT

In this investigation, 123 lactic acid bacterial strains (LAB) were isolated and identified from 30 of each type of fermented meat products (sausage, salami and hotdog). Colony and cell morphology, Gram reaction, biochemical assessments and sequencing of the 16S rRNA gene using PCR technique were principally employed to identify some of the LAB strains. According to the molecular characterization data, *Lactobacillus plantarum*, *Lactococcus lactis* spp. *Lactis* and *Leuconostoc mesentroides* spp. *mesentroides* were the three most common LABs. Additionally, the results revealed that the mean inhibition zone of antibacterial activity of pure bacteriocin of LAB was expressed as 21.50±3.55, 25.71±2.18, 20.07±5.16, and 2.93±3.97 in millimeter against *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Salmonella Typhimurium* ATCC 14028, respectively. So, pure bacteriocin is shown by its capability to inhibit the growth of both Gram-positive and Gram-negative bacteria. Currently, bacteriocins producing LAB are frequently utilized as an alternative to antibiotics in the food sector as a food preservation agent. In terms of promoting food safety, extending shelf life, and upgrading product quality, it will further expand the application area of bacteriocin. In the categories of fermented food sectors, they are economically very important.

***Keywords**:* Fermented meat products, Lactic acid bacteria, PCR, Bacteriocin.

INTRODUCTION

Fermented meat products are a kind of meat products with special flavor, color, texture, high nutritional value and prolonged shelf life, produced from a variety of natural and artificially controlled and regulated processing techniques (such as curing, fermentation, drying, and/or smoking). The most common varieties of fermented meat items include fermented sausage, fermented ham, smoked meat and cured goods, etc. (Cui and Fan, 2019). Additionally, it's well acknowledged that meat and meat products like sausage, hotdogs and salami are excellent sources of group B vitamins, proteins, minerals, and trace elements like iron and zinc as well as certain other bioactive compounds (Decker and Park, 2010).

Occasionally, LAB are significant organisms valued for their capacity for fermentation as well as their advantages in terms of nutrition and health. They are micro-aerophilic, Gram-positive, catalase-

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negative, oxidase-negative and non-sporulating bacteria that produce lactate as their main product of fermentation process from carbohydrates (Gilliland, 1990; Sablon *et al.,* 2000 and Shaheen *et al.,* 2019). Normally, LAB are typically facultative anaerobic bacteria that primarily be a member of the genera *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc* and *Pediococcus*. They are employed as starters in fermented meat products (Fraqueza *et al.,* 2016 and Laranjo *et al.,* 2019). They are considered as generally recognized as safe (GRAS) organisms and can be safely used as probiotics for medical and veterinary applications (Eid *et al.,* 2016).

Additionally, LAB's antimicrobial properties results from the competition with other organisms for nutrients and/or space, as well as the production of antimicrobial active metabolites such as organic acids (mainly lactic and acetic acid), di-acetyl, hydrogen peroxide and other substances such as bacteriocins and antifungal peptides (Kavitha *et al.,* 2020).

Bacteriocins are ribosomally synthesized peptide toxins produced by bacteria as well as with the express purpose of eradicating their own bacteria. One of bacteria's natural defense mechanisms that can inhibit other narrow- or broad-spectrum microorganisms is the synthesis and release of toxic peptides (Cotter *et al.,* 2005 and Yang *et al.,* 2014). Application of these powerful bio-preservative bacteriocins, in food is presently and actually the focus of extensive and intense investigation. Finding novel bacteriocins that have a wider range of activities and compatibility with various food systems is being investigated (Eid *et al.,* 2016).

Foodborne pathogens (bacteria, etc.) are biological agents that cause food poisoning. Moreover, pathogens that multiply in the host after the consumption of food and the toxins they generate in food products those are consumed by the host cause foodborne (FDB) disease. Accordingly, FDB is divided into two categories: infection and intoxication (Suvarna *et al.,* 2022). Therefore, the main focus of the current work was to isolate, screen, molecular identification and LAB's antagonistic activities from various fermented meat products (sausage, salami, and hotdog).

MATERIALS AND METHODS

Sample collection:

Ninety samples of various brands of some fermented meat products (sausage, smoked salami and hotdog) (30 of each) were purchased from different local supermarkets in Assuit City during the period from January 2022 to August 2022. Samples were immediately put in a sterile ice box with dry ice or freeze packs. Every sample was individually wrapped within a sterilized and aseptic plastic container and then it was carefully sealed and labeled. After sampling, the samples were quickly transferred to the Laboratory of Meat Hygiene, Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt where they underwent immediate microbiological investigations. In exceptional circumstances, the samples were kept at refrigerator temperature (±4ºC) until used for isolation.

I. Isolation procedures of LAB (Zdolec *et al.,* 2009):

Using the dilution agar technique, a variety of bacterial strains was isolated from such dietary sources. The samples were aseptically removed from their casings, and then 10 grams of the sample were taken and diluted into 90 ml of sterile saline peptone water media (Oxoid, UK) in a stomacher (Stomacher 400 circular, Seward, Worthing, West Sussex, UK) for 2 minutes at 200 rpm. After that, the homogenate was then made for each sample in a series of sequential decimal dilutions under aseptic conditions. LAB populations were observed using a pour plate of selected dilutions on De Man, Rogosa and Sharpe agar (MRS agar media, Hi-media, India) and for 24-72h incubated at 37°C under anaerobic environment in a

Co_2 incubator. After incubations, characteristic colonies of LAB were counted, enumerated, and expressed as colony-forming units (cfu/g). Three to five typical isolates from each sample were picked up, cultivated, purified and kept at MRS slants for future grouping.

II. Morphological identification (David *et al.*, 2019):

For phenotypic characterization, colony and cell morphology, Gram staining, arrangement, as well as biochemical identifications were used. Colony size, consistency, color and shape (both margin and elevation) were all noted. Colonies opacity (transparent, translucent, or opaque) and roughness (dull, pale, gritty, bumpy, or matte surface). Whether the colonies are smooth and soft (shiny sparkling surface), or mucoid (slimy, gooey or gummy semblance) were also recorded. Randomly selected isolated colonies were chosen for purification. The obtained purified colonies were examined for:-

1. Microscopic examination using Gram stain (Narender *et al.*, 2010):

A colony was fixed onto a slide while being passed through a Bunsen flame, and then stained with crystal violet for one minute. Then the stain was removed by holding the slide at an angle over the sink and extra crystal violet was washed off with water. The slide was placed back on the rack and was covered with the fresh Lugol's iodine for one minute. Following this, the slide was rinsed with tap water, drained, and treated with alcohol for ten seconds, which also serves to decolorize Gram-negative cells. The slide was counterstained for two minutes with safranin or carbol fuchsin, followed by a tap water wash and blotting to remove excess water. The slide was looked at under the microscope with oil immersion lens (100 x objectives with oil).

III. Biochemical characterization:

1. Catalase test (Land *et al.*, 1991):

A drop of 3% hydrogen peroxide was applied on a spotless microscopic slide. A discernible quantity of bacterial growth was placed with a sterile inoculating loop. Both were mixed, and the formation of gas bubbles was monitored.

2. Oxidase test ((Dharmappa *et al.*, 2022):

On the oxidase disc, pick up and distribute a well-isolated colony. At 25-30°C, the reaction is observed within 5-10 seconds. A change that occurs more than 10 seconds later or does not occur at all is deemed as a negative reaction.

3. Growth at 15°C and 37°C (Collins and Lyne's, 1989):

Well-isolated and purified organisms were cultured in tubes of MRS broth media (Himedia, India) and their growth was monitored between 15°C and 37°C.

4. Motility assay (Baron *et al.*, 1994):

Using a bacteriological needle, pure cultures of probable isolates were stabbed into tubes of semisolid Motility Indole Ornithine Medium (MIO Medium, TM MEDIA, India). Mobility or movability was visible as a mist of growth spreading out from the stabbing line into the agar after overnight incubation at the proper temperature of 37°C.

5. Urease test (Aryal, 2022):

Streak a portion of a well-isolated colony to the surface of a urea agar slant (Urea Agar Base, TM MEDIA, India) or inoculate the slant with one to two drops from an overnight brain-heart infusion broth culture. After that, place the cap loosely back on and incubate the tube for 48 to 7 days at 35 to 37^{o}C in free air. Following that, check for up to 7 days to see if a pink color develops.

6. Sugar fermentation (Collins and Lynes, 1989):

Through MRS broth tubes containing 1% concentrations of lactose, glucose, sucrose, and xylose sugars as well as 0.05% phenol red (indicator), inoculate well-isolated colonies. The presence of yellow color showed that sugar was being assimilated following incubation of tubes at 37ºC and findings were observed every day for up to 7 days.

7. Conservation of LAB (Pure Culture) (Gálvez *et al.*, 2008):

The working cultures were kept on MRS agar slants at 4°C with streaking monthly. The purified strains were kept at -40°C within cryotubes with MRS broth (80%) supplemented with glycerol (20%) for long-term preservation.

IV. Molecular characterization by 16S-rRNA sequencing of PCR technique (Sambrook *et al.*, 1989):

Sequencing of PCR products was performed by the Animal Health Research Institute at Dokki, Cairo, Egypt. According to closer and intensified phenotypic profiling, only 14 representative species were picked out for molecular typing. Therefore, the chosen LAB isolates were cultivated in MRS broth media (HI MEDIA, India) and incubated for 24h at 30°C. According to QIAamp DNA mini kit instructions, DNA was extracted from each isolate analyzed. The LAB strains were then identified using the approach given by Sambrook *et al.* (1989) through sequencing analysis of the 16S rRNA region. Amplification of the entire 16S rRNA gene was carried out with Oligonucleotide primers sequences (Metabion, Germany): *Lactobacillus plantarum*: CAGAATTGAGCTGGTGGTGG and recA gene; TGTTACTTTCGCAACCAGAT, *Lactococcus lactis subsp. Lactis*: ATGCGTAAACTTGCAGGAC; recA gene CAACCTTGAATGGTGGAG and *Leuconostoc mesenteroides* ATACAGGCGAACAGGGGATTA recA gene; GGGTGTAGTTTCTGGGTTTC. Using 1.5% agarose gel electrophoresis, PCR products were visualized.

V. Bacteriocin Activity Assay:

Evaluation of the antibacterial activity of pure bacteriocin through agar well diffusion technique:

1.Preparation of indicator test microorganisms (Bacterial strains and growth condition):

Foodborne pathogens were provided by the Animal Health Research Institute, Assiut, Egypt and stored at the Laboratory of Meat Hygiene at refrigerator temperature (±4 °C). For investigation of the antibacterial activity, pathogens that were utilized as indicator organisms including *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 25922 and *Salmonella Typhimurium* ATCC 14028.

2. Preparation and extraction of cell free-supernatant:

With some modifications, Mostafa *et al.* (2019) approach was used for the purification and extraction of bacteriocin. The pure bacteriocin was extracted and taken away through incubation for 18h of inoculated isolate culture into 250 ml MRS broth media (1% v/v) at 37ºC under aerobic/anaerobic environment and then at 10,000 rpm of centrifugation at 4ºC for 10 minutes (cold centrifuge, Laboratory Centrifuge, Sigma- Sartorius 3K30, USA) at the Central Laboratories at Faculty of Agriculture, Assiut University, Assiut, Egypt. Subsequently, in sterile falcon tubes, supernatants were added and kept in the refrigerator until use.

3. Agar well diffusion method procedure (Geis *et al.*, 1983):

100 ml of molten Muller Hinton agar media (Oxoid, UK) were cooled at 47 °C then 1% of each indicator organism's overnight culture was seeded separately. Into a sterilized petri dish, seeded agar was added and let up to settle down at room temperature. A sterile metal cork borer (diameter: 7 mm; Sigma-Aldrich, USA) or sterile tips were used to create 7 mm-diameter wells in the hardened and cohesive agar. Individual LAB isolates' cell-free supernatants (100 L) were added into cut wells. The petri dish was kept for 2h at 4-5ºC to let the compounds diffuse before being incubated in an aerobic environment for 24h at the ideal temperature for each indicator organism 37ºC. Inhibition zones' presence or absence as well as their diameters were measured and registered.

As a control, an else Muller Hinton plates with 1% of each indicator organism's

overnight culture. For 24 hours, the wells were incubated at 37 ºC with sterile distilled water. Inhibition zones' presence or absence as well as their diameters was noted. The measurements were carried out in triplicates.

VI. Statistical analysis:

Data represented as mean and slandered deviation (SD), the variables were tested using GraphPad Prism 9.5.1 software (GraphPad Software Inc., San Diego, CA, USA). Where Kruskal-Wallis test ANOVA, two-way ANOVA test, one-way ANOVA test, Unpaired T-test and Chi-square test were used to evaluate whether a significant variation between them, statistical significant was regarded when "P" value of <0.05.

RESULTS

Table 1: Incidence, macroscopic and microscopical appearance of LAB isolates.

Type of Sample	No. of Samples	Shape	Arrangement	
			Gram +ve bacilli	Gram +ve** cocci
Sausage	30	White creamy, opaque, small, convex, rounded, small and large	21	27
Smoked Salami	30	White creamy, opaque, small, convex, rounded, small and large	20	24
Hotdog	30	White creamy, small and large	27	4
Total (Percentage %)	90 (100%)	-	68 (55.28%)	55 (44.72%)

** High significant statistical difference between types of samples ($p < 0.01$, chi 2 = 43.8).

Table 2: Incidence and viable count of LAB isolates as well as their sources.

Fermented meat products	No. of Samples	No. of isolated LAB	Count		Total percentage
			Mean	±SD	
Sausage	30	48	8.67×10^{7} **a,b**	2.93×10^{8}	39.0%
Smoked salami	30	44	1.69×10^{8} **a**	2.89×10^{8}	35.8%
Hotdog	30	31	3.05×10^{8} **b**	4.15×10^{8}	25.2%
Total	90	123	1.86×10^{8}	3.47×10^{8}	100%

a, b means with the same superscript in the identical column is statistically significant ($p < 0.05, 0.01$).

Table 3: Biochemical properties of some LAB isolates:

Criterion isolate	Hypothetical Name	Gram Staining	Catalase Test	Oxidase Test	Motility Test	Oxidase Test	Growing at temperature (°C) 15	37	Carbohydrate fermentation
1	*L. plantarum*	+	-	-	-	-	+	+	+
2	*L. plantarum*	+	-	-	-	-	+	+	+
3	*L. plantarum*	+	-	-	-	-	+	+	+
4	*L. plantarum*	+	-	-	-	-	+	+	+
5	*L. plantarum*	+	-	-	-	-	+	+	+
6	*L. plantarum*	+	-	-	-	-	+	+	+
7	*L. plantarum*	+	-	-	-	-	+	+	+
8	*Lac. lactis* ssp. *lactis*	+	-	-	-	-	+	+	+
9	*Lac. lactis* ssp. *lactis*	+	-	-	-	-	+	+	+
10	*Lac. lactis* ssp. *lactis*	+	-	-	-	-	+	+	+
11	*Lac. lactis* ssp. *lactis*	+	-	-	-	-	+	+	+
12	*Leuc. mesentroides* ssp. *mesentroides*	+	-	-	-	-	-	+	+
13	*Leuc. mesentroides* ssp. *mesentroides*	+	-	-	-	-	-	+	+
14	*Leuc. mesentroides* ssp. *mesentroides*	+	-	-	-	-	-	+	+

L. = Lactobacillus Lac. = Lactococcus Leuc. = Leuconostoc

Molecular typing of some selected LAB isolates by PCR technique through 16S rRNA gene sequencing:-

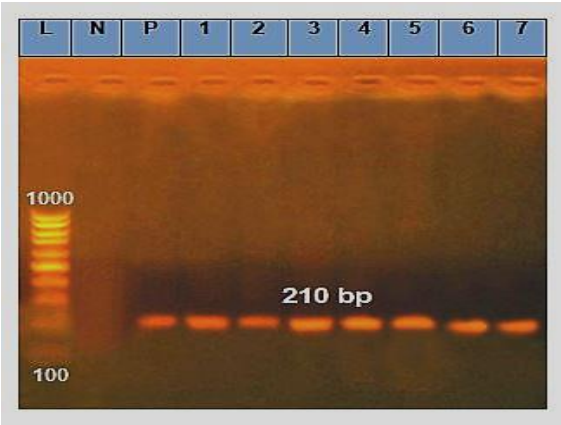

Fig. (1): Agarose gel electrophoresis of PCR products with 1000 bp target sequence. Well N: negative control (non-DNA); well P: amplified DNA of *Lactobacillus plantarum* as positive control; and wells 1-7 amplified DNA of tested *Lactobacillus plantarum.*

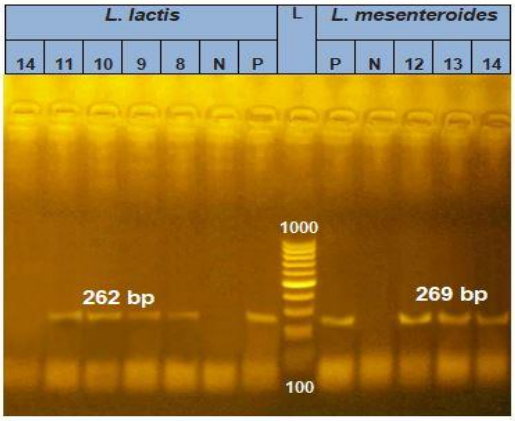

Fig. (2): Agarose gel electrophoresis of PCR products with 1000 bp target sequence. Well N: negative control (non-DNA); well P: amplified DNA of *Lactococcus lactis and Leuconostoc mesentroides* as positive control; Wells 8-11 of amplified DNA of *Lactococcus lactis* spp. *lactis* and wells 12-14 *Leuconostoc mesentroides* spp. *mesentroides.*

Bacteriocin activity assay:

Table 4: Antibacterial activity of cell-free extracts of some LAB isolates against different indicator organisms in terms of the inhibitory zone's diameter (mm):

CFS of LAB Isolates	***L. monocytogenes* ATCC 7644**	***Staph. aureus* ATCC 6538**	***E. coli* ATCC 25922**	***Sal. Typhimurium* ATCC 14028**
Pathogens	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Minimum	6.67 ± 11.55	19.33 ± 5.86	15.00[a, b] ± 3.0	0.00 ± 0.00
Maximum	26.67 ± 4.16	27.33 ± 2.31	24.67[a] ± 3.0	9.33 ± 8.14
Mean	21.50 ± 3.55	25.71± 2.18	20.07 ± 5.16	2.93 ± 3.97

A high level of antimicrobial activity was detected if the diameters of inhibition zones were over 6 mm, these highlighted by Pan *et al.* (2009).
a, b, c: There is a statistical significant difference, SD: standard deviation

DISCUSSION

In order to meet the demand for meat storage, fermented meat products were developed. Because throughout the fermentation procedure, a number of biochemical and natural or physical alterations brought on by microbial leavening or fermentation or enzymes give the meat products a distinctive color, texture, special flavor, antibacterial, and antioxidant qualities, enhancing the edible quality of the meat (Shen *et al.*, 2023).

The introduction of probiotic strains in fermented meat products represents an opportunity for innovation in the meat industry and a new way for consumers to ingest probiotics (Jofré *et al.*, 2015). Bacteriocins are antibacterial peptides that can inhibit both food spoilage and pathogenic bacteria, making them a potential alternative to antibiotics (Simons *et al.*, 2020) and also, probiotics are considered an excellent candidate for the sector of food bio-preservation (Surati, 2020). So, studies in the field of probiotics are a never-ending path of isolation,

identification and examination of the probiotic properties of different strains including their antimicrobial activity against pathogenic and conditionally pathogenic microorganisms. In this concept, studies are needed to describe the microbial ecosystem of Egyptian foods in order to clarify the role of probiotics in food technology and preservation (Denkova *et al.,* 2017).

I. Isolation and preliminary identification of LAB:

Ultimately, out of 90 samples, 123 strains were preliminarily considered as LAB. The achieved findings of the current work are discussed in Table 1 which depicts the incidence, phenotypic and microscopic morphology of LAB. Most of them showed the typical characteristics of LAB: milky white colony, creamy, opaque, convex, rounded, small and large colonies with Gram-positive bacilli and cocci, this is in agreement with Le *et al.* (2019). In detail, according to the data presented in this table declared that the incidence of total Gram-positive bacilli and cocci were 68 (55.28%) and 55 (44.72%) among LAB isolates, respectively. From the statistical point of view, there is a highly significant statistical difference between Gram-positive cocci and bacilli ($p < 0.01$, chi 2 = 43.8). In addition, 141 strains with LAB characteristics were isolated when examined various fermented sausages in the research conducted by Castro *et al.* (2011). Moreover, 30 LAB isolates from 20 various fermented meat products (sausage, salami), in the Turkish province of Afyonkarahisar were characterized as Gram (+) and catalase (-) bacteria by Erdoğmuş *et al.* (2021).

Additionally, the data documented in Table 2 pointed out that the total number of LAB isolates isolated from each type of meat product were 48 (39 %), 44 (35.80 %), and 31 (25.20 %) from sausage, smoked salami, and hotdog, respectively. Besides, this table declared that the average LAB counts at sausage, smoked salami, and hotdog were in the range of $8.67 \times 10^7 \pm 2.93 \times 10^8$, $1.69 \times 10^8 \pm 2.89 \times 10^8$, and $3.05 \times 10^8 \pm 4.15 \times 10^8$ cfu/g, respectively and the total LAB count in all fermented meat products was $1.86 \times 10^8 \pm 3.47 \times 10^8$. Other investigations discussed that LAB count in the range from 10^8 to 10^{10} cfu/g on MRS agar at nem chua sausage by Nguyen *et al.* (2013), while the results summarized the viable cell count of probiotic strains in the salami was from 10^8 to 10^9cfu/g/day by Ruiz et al. (2013). The count of *lactobacilli* found in the final product varies in the range from 8 to 9 logs cfu/g assessed by Milicevic *et al.* (2014). Also, the total count of LAB in Sai-krog-prieo (fermented sausage) ranged between 2.05×10^8 - 1.71×10^{10} cfu/g recorded by Tanasupawat *et al.* (2015). In addition, from 16 distinct commercially available, conventionally processed sausage samples, the viable LAB counts ranged from $3.3\ 10^1$ to $5.8\ 10^5$ cfu/g registered by Laslo *et al.* (2019).

II. Biochemical properties of some LAB isolates:

The findings outlined in Table 3 cleared the biochemical properties of some LAB isolates. These findings revealed that all LAB isolates fit LAB categorization as Gram (+), catalase (-), oxidase (-), urease (-), non-motile bacilli and cocci, as well as being able to ferment carbohydrate sugars (lactose, sucrose, glucose and xylose) and all strains grow at 37 °C. However, strains of *lactobacilli* and *lactococci* grown at 15 °C. This is in agreement with the review performed by Bansal *et al.* (2013).

III. Molecular typing of some selected LAB isolates by PCR technique through 16S rRNA gene sequencing:-

Molecular techniques have been successfully employed to recognize and characterize LAB isolates from various fermented food items (Samelis *et al.,* 1995 and Sacilik *et al.,* 2000). Furthermore, the results demonstrated in Fig. 1 & 2 illustrated that only 14 representative randomly selected LAB strains undergo genetic and molecular identification by PCR technique through 16S rRNA sequence amplification.

The information obtained from these figures described that all seven representative strains of Gram-positive bacilli were *L. plantarum*, which was also the most frequently isolated species 68 (55.28%) and has been found to predominate in some fermented meat products. There were also another seven gram-positive cocci isolates, including *Lac. lactis* spp. *lactis* (4 isolates) and *Leuc. mesenteroides* spp. *mesentroides* (3 isolates).

Other evaluations noted that certain isolates (0.4% each) were classified as *Lac. lactis* subsp. *lactis* by Nguyen *et al.* (2013) using PCR method. Adding to that, 30 LAB isolates by 16S rRNA gene amplification declared that 4 *Leuc. mesenteroides* isolates and 1 *L. plantarum* isolate were recovered from samples of salami and sausage, respectively by Erdomuş *et al.* (2021). Nine *Lactobacillus* bacteria and three *Leuconostoc* spp. were recovered from 20 packs of fresh vacuum-packed organic pork sausages, according to Moslem *et al.* (2022).

IV. Bactriocin activity evaluation:

Food-borne pathogenic bacterial reaction against different pure bacteriocins was assessed and evaluated by using the agar well diffusion technique to determine the millimeter size of the inhibitory zone. According to the results discussed in Table 4 which illustrated the *in-vitro* antibacterial activity evaluation of cell-free supernatant or pure bacteriocin of some selected LAB strains antagonistic toward numerous indicator organisms in terms of the inhibitory zone's diameter (mm).

In the current study, the minimum and maximum inhibition zones for *L. monocytogenes* ATCC 7644 were 6.67 ± 11.55 and 26.67 ± 4.16 mm, in *Staphylococcus aureus* ATCC 6538 were 19.33 ± 5.86 and 27.33 ± 2.31 mm, while in *Escherichia coli* ATCC 25922 were 15.00 ± 3.0 and 24.67 ± 3.0 mm and in case of *Salmonella Typhimurium* ATCC 14028 were 0.00 ± 0.00 and 9.33 ± 8.14 mm. The results revealed that bacteriocin produced by the LAB isolates displayed broad-spectrum antibacterial activity against all indicator species and the high level of antimicrobial activities was identified if inhibition zone diameters were more than 6 mm, these highlights were stated by Pan *et al.* (2009).

Meanwhile, the wide range of antibacterial action of pure bacteriocin of LAB was shown by its capability to suppress the growth and development of both Gram-positive and Gram-negative germs. However, all isolates were able to exhibit a wide range of antibacterial action against Gram-positive higher than Gram-negative germs. This is in agreement with Moreno-Arribas *et al.* (2000) who stated that while Gram-negative bacteria display high resistance to bacteriocins, only Gram-positive germs, like *L. monocytogenes* ATCC 7644 and *Staph. aureus* ATCC 6538, are inhibited by them.

According to earlier research by Rzepkowska *et al.* (2017), *L. monocytogenes* ATCC 19111 had the largest zones of inhibition, measuring 18.55 ± 1.8 mm. According to Laslo *et al.* (2019), the Gram-positive bacteria (*S. aureus*) with the largest inhibitory zone of 16.63 mm in the instance of the CCP 10-1/1 isolate had the highest suppressive impact. While the examined Gram-negative bacteria (*E. coli*) with largest inhibitory zones that were 14.47 mm in diameter. Erdoğmuş *et al.* (2021) used the agar well diffusion technique to assign and know the antibacterial action of the LAB isolates. LAB isolates indicated higher antibacterial action antagonistic toward *L. monocytogenes* ATCC 19115 and *S. aureus* ATCC 25923 than against *E. coli* ATCC 35218.

CONCLUSION

This study focuses on the isolation, identification and molecular characterization of LAB from different types of fermented

meat products (sausage, smoked salami and hotdog). Regarding, the macroscopic and microscopical morphology of LAB strains were preliminarily classified as LAB, most of them displayed milky white colonies, creamy, opaque, convex, rounded, small and large colonies with Gram-positive bacilli and cocci which was the typical characterization of LAB. As well as, all LAB isolates fit the classification of LAB as Gram (+), catalase (-), oxidase (-), urease (-), non-motile bacilli and cocci, as well as being able to ferment carbohydrate sugars (lactose, sucrose, glucose and xylose) and all strains were capable of growing at different incubation temperatures.

Concerning, genetic and molecular typing by amplification of the 16S rRNA gene sequencing using the PCR method, *L. plantarum*, *Lac. lactis* spp. *lactis* and *Leuc. mesentroides* spp. *mesentroides* were the three most common LABs. *L. plantarum* was the most pre-dominate isolated species in some fermented meat products.

In the current study, LAB isolates exhibited broad-spectrum antibacterial activity against all indicator organisms (*L. monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 25922, and *Salmonella Typhimurium* ATCC 14028) and the high level of antimicrobial activities of pure bacteriocin shown by its capability to inhibit the growth of Gram-positive bacteria higher than Gram-negative bacteria in vitro assays, because, Gram-negative bacteria display high resistance to bacteriocins.

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التوصيف الجزيئى والنشاط المضاد البكتيرى للبكتيريوسين المنتج من بكتيريا حمض اللاكتيك المعزولة من بعض منتجات اللحوم المخمرة

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في هذا البحث تم عزل وتشخيص ١٢٣ سلالة بكتيرية لحمض اللاكتيك من ٣٠ نوع من منتجات اللحوم المخمرة (السجق والسلامي والنقانق). حيث تم استخدام مورفولوجيا المستعمرة والخلية، وتفاعل الجرام، والتقييمات البيوكيميائية، وتسلسل جين الحمض النووي الريبي ١٦ أس باستخدام تقنية تفاعل البوليميراز المتسلسل بشكل أساسي لتحديد بعض سلالات بكتيريا حمض اللاكتيك. وفقا لبيانات التوصيف الجزيئي، لاكتوباسلس بلانتاروم، المكورات اللبنية لاكتيس سلالات لاكتيس، وليوكونوستوك المساريقية سلالات المساريقية وكانت بكتيريا حمض اللاكتيك الثلاثة الأكثر شيوعًا. بالإضافة إلى ذلك، كشفت النتائج أن متوسط منطقة تثبيط النشاط المضاد للبكتيريا للبكتيريوسين النقي المنتج من بكتيريا حمض اللاكتيك تم التعبير عنه بـ ٢١,٥٠ ± ٣,٥٥، ٢٥,٧١ ± ٢,١٨، ٢٠,٠٧ ± ٥,١٦، و ٢,٩٣ ± ٣,٩٧ ملم ضد الليستيريا المستوحدة ATCC 7644، المكورات العنقودية الذهبية ATCC 6538 ، الإرشيشكية القولونية ATCC 25922، والسالمونيلا التيفيميوريام ATCC 14028، على التوالي. ولذلك فإن البكتيريوسين النقي يظهر قدرته على قمع نمو كل من البكتيريا الموجبة والسالبة لصبغة الجرام. وحاليًا، تُستخدم البكتيريوسينات المنتجة من بكتيريا حمض اللاكتيك بشكل متكرر كبديل للمضادات الحيوية في قطاع الأغذية كعامل لحفظ الأغذية. من حيث تعزيز سلامة الأغذية، وتمديد مدة الصلاحية، وتحسين جودة المنتج، فإنه سيزيد من نطاق استخدام البكتيريوسين في الصناعات الغذائية المخمرة، فهي ذات أهمية اقتصادية كبيرة.