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Screening and estimation of antibacterial activity of bacteriocins produced by *Enterococcus faecium* LR isolated from raw horse milk samples

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Abstract: Lactic acid bacteria are microorganisms that can be present in meat, milk and fermented products, as well as fermented drinks and vegetables, and they inhibit the growth of pathogenic and decaying microorganisms. Our aim was isolating, screening, characterization and estimation of bacteriocin/s of Lactic acid bacteria isolated from Horses milk in Jeddah city, Saudi Arabia. That have antagonistic activity against a wide range of pathogenic bacteria. On MRS agar medium 16 LAB isolates were examined using the agar well diffusion method against the pathogenic bacteria (Gram-positive bacteria: *S. aureus* ATCCBAA977, *ST. Pneumonia* ATCC49619) and (Gram-negative bacteria: *E. coli* ATCC35218, *P. aeruginosa* ATCC27853). The results showed that 12 isolates out of 16 isolates (75%) worked to inhibit all types of pathogenic bacteria used. The best isolate was identified using molecular methods; the results showed that the isolate (H6) matched 100% with (*Enterococcus faecium* LR135401.1). This isolate produced the highest bacteriocin at 35°C/24 h/pH (6.5).

Keywords: *Enterococcus faecium*, antibacterial, bacteriocins, milk

INTRODUCTION

The significant benefit of the lactic acid bacteria probiotics on food preservation has inspired significant awareness due to the nutritional qualities of raw materials throughout the long shelf life of foods and their capability to restrained deterioration and food borne pathogens, which are of interest to the food manufacture (Reis *et al*., 2012. The term lactic acid bacteria refer to these microorganisms are found in milk, meat and fermented products, as well as in fermented vegetables and beverages inhibiting the growth of pathogenic and deteriorating microorganisms. LAB belonging to *Leuconostoc* and *Weissella* genus (Barbieri *et al*., 2019).

Lactic Acid Bacteria (LAB) are widely used as starter cultures for fermentation in the dairy, meat and other food industries. Their properties have been used to manufacture products like cheese, yoghurt, other fermented milk products, beverages, sausages and olives (Junnarkar *et al*., 2019). The LAB are regarded as generally recognized as safe (GRAS) because of their ubiquitous use in food and their unique role in the healthy microflora of human mucosal surfaces (George *et al*., 2018). The presence of 21 species belonging to the genera Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Lactococcus, Lactosphaera, Melissococus, Oenococcus, Tetragenococcus, Vagococcus and Weissella was evidenced by using culture-dependent techniques (Agriopoulou *et al*., 2020; Kusdianawati *et al*., 2020; Ruiz rodriguez *et al*., 2019; Mokoena *et al*., 2017). Most members of this genus are found in fermented milk products such as cheese, yogurt, and butter (Mirhadizadi and Mohsenzadeh, 2021; Fusco *et al*. 2019). Human breast milk (Kang *et al*., 2020), Horse milk Kusdianawati *et al*., 2020; Manguntungi *et al*. (2018). Food fermentation (Putra *et al*. 2018) and fermented fish products (Manguntungi *et al*., 2020). LAB can improve the quality of the products. The main bioactive compounds produced by LAB during fermentation are vitamins, γ-aminobutyric acid, bioactive peptides, bacteriocins, enzymes, conjugated linoleic acid, and exopolysaccharide. These compounds are the main biopreservative and have functional properties such as antioxidant and anti-diabetic (Speranza *et al*. 2017; Muryany *et al*. 2017; Linares *et al*. 2017; Mokoena *et al*. 2016). Lactic acid bacteria in dairy products are the important bacteriocin-producing group with antibacterial activity effect (Mirhadizadi and Mohsenzadeh, 2021). The presence of various probiotic and bacteriocin-producing LABs is an effective step in the food and pharmaceutical industries (Kusdianawati *et al*., 2020).

LAB also has the ability to inhibit pathogen growth by disrupting the outer membrane of bacteria that causes lysis. LAB produces bacteriocins such as nisin, lactococcin and lactacin by *Lactobacillus lactis*, pediocin by *L. plantarum* and garvieacin by *Lactobacillus garvieae* and *Enterococcus faecalis* and *Enterococcus faecium*., produce enterocins (Erginkaya *et al*., 2019; Sabo, *et al*.,2020; Hernández-González *et al*., 2021), in essential suitable culture condition of medium for LAB (Endo *et al*., 2019).

Bacteriocins are peptides synthesized by bacteria that can inhibit the growth of other bacteria and fungi, parasites,

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and viruses. Lactic acid bacteria (LAB) are a group of bacteria that produce bacteriocins; their mechanism of action can replace antibiotics and prevent bacterial resistance (Hernández-González *et al*., 2021). An essential attribute of bacteriocins is the antimicrobial activity against different bacteria, fungi, parasites, viruses, and even against natural resistant structures, such as bacterial biofilm (Torres *et al*., 2013, Martín-Escolano *et al*., 2019).

Bacteriocins are a powerful weapon that can be exploited in veterinary medicine. The administration of these antimicrobial peptides in domestic animals eliminates potentially pathogenic undesirable microorganisms without causing cytotoxicity on cells or tissues (Hernández-González *et al*., 2021). Bacteriocin purification and use as an alternative to chemical antibiotics can be useful in the treatment of antibiotic-resistant infections and as bio preservative in controlling foodborne and fermentative pathogens (Mirhadizadi and Mohsenzadeh, 2021). The potency, specificity, and stability of these bacteriocins presented promising perspectives for application as biopreservatives in the food industry. (Sonbol *et al*., 2020). As they have no potential health risks, bacteriocins, either in crude/purified form or excreted by the bacteriocin producing strains, represent a fundamental alternative to the chemical preservatives in various dairy and food products (Silva *et al*., 2018). Identification and isolation of probiotic bacteria from such rich sources as well their preservation is necessary (Mirhadizadi and Mohsenzadeh, 2021). The aim of the present study is isolating, screening, characterization and estimation of bacteriocin/s from LAB isolates isolated from Horse milk, in Jeddah city, Saudi Arabia.

MATERIALS AND METHODS

Samples Collection

In this study, fourteen raw milk samples (almost 150 ml) of horse's milk were collected from various districts of Jeddah Province, Kingdom of Saudi Arabia, and were used to isolate lactic acid bacteria.

Isolation of lactic acid bacteria from milk Samples

The milk samples were serially diluted to 10-4. Of each dilute, 0.1 ml was transferred over solid MRS medium the plates were incubated at 35C. After 24h of incubation at 35°C, bacterial colonies were purified and subcultured on solid MRS medium; the pure cultures were transferred to slants and kept at 4°C for the next investigations. Lactic acid bacteria isolates have been kept as frozen stocks in MRS broth medium containing 30% (v/v) glycerol at -80°C for long-term conservation and at -20°C for short-term conservation.

Screening bacteriocin production from LAB isolates by agar well diffusion assay

As test organisms, four ATCC bacteria have been used: *S. aureus* ATCCBAA977, *S. pneumonia* ATCC49619, *E. coli* ATCC35218 and *P.* aeruginosa ATCC27853. The bacteria used in the study were acquired from the International Medical Center (IMC) in Jeddah, Saudi Arabia. All cultures were checked up again in terms of purity.

To study bacteriocin production and antibacterial action in the cell-free filtrate (CFF), a 250 ml Erlenmeyer flask experiment was carried out with 25 MRS ml of broth medium injected by oblique from preculture of LAB isolates at 35°C after 24 hours of incubation, and CFF was collected during centrifugation at 5000 rpm for 20 min at 4°C. Evaluation of bacteriocin activity employing agar-well diffusion (Bonev *et al*., 2008). A sterile swap was used to pour and spread 0.1 mL of inoculum suspension. Wells were created with a sterilised cork borer and were filled with supernatant (100 l). Refrigerated plates for 30 minutes. Plates were incubated for 24 hours at 35°C. LAB isolate that recorded highest antimicrobial activity were selected for the next studies.

Effect of the cultural factors on the production of bacteriocin using the selected bacterial isolate

Effect of incubation temperatures on the production of bacteriocin (s) by the selected isolate

The chosen bacteria were inoculated into MRS broth medium at different incubation temperatures (25, 30, 35, 40, 45ºC) and inoculated into 250 mL Erlenmeyer flasks with 25 mL MRS broth medium. For 24 hours, the cultures have been incubated at different temperatures. Following 24 hours of incubation, the antibacterial activation was measured in the inhibition zone in mm as described before.

Effect of incubation period on the production of bacteriocin(s) by the selected isolate

About 25 ml of MRS broth medium had been prepared in 250 ml Erlenmeyer flasks and incubated at 35ºC for various periods (24, 48, and 72 hr). Antibacterial activity was measured as described before.

Effect of pH on the production of bacteriocin(s) by the selected isolate

The MRS broth medium was prepared in 250 ml Erlenmeyer flasks with 25 ml of sterile medium at various pHs of 4.5, 5.5, 6.5, 7.5, and 8.5 with 1 M HCl or 1 M NaOH and then autoclaved. Every flask was injected with the chosen isolates and incubated for 24 hours at 35°C without excitation. Following a 24-h incubation period, the inhibition zone's antibacterial activity versus indicator bacteria was measured in mm

Production of antimicrobial agents (Bacteriocins) by selected Lactic Acid Bacteria isolate using organic wastes whey medium

Whey medium was prepared using the following steps: pH adjusted to 4.5 using 5N HCI, medium was warmed to 121°C for 15 minutes to deteriorate proteins and the residue was eliminated using cooling centrifuge at 10,000 rpm/15 min/4° percentage. The supernatants have been pH-adjusted to 6.5, autoclaved at 121ºC for fifteen minutes, and utilized as a fermentation medium.

Dates medium

Wastes of the industry of Alkhlas dates in Saudi Arabia were used in this study for the production of bacteriocin(s) by LAB isolated from milk. To prepare the Dates medium, 100 g of Date was added to 300 ml distilled water, after filtering, sterilized at 121ºC for 15 min and this solution was used as Date medium. 250 ml Erlenmeyer flasks with 25 mL date or whey or 50 mL each of 25 mL whey and 25 mL date broth medium inoculated with the selected isolates. The cultures were incubated at 35°C. Following 24 hours of incubation, the antibacterial activity against indicator bacteria was measured. The agar diffusion examination is one process for quantifying the capability of antibiotics to prevent bacterial growth (Bonev *et al*., 2008).

Molecular Identification: of selected Lactic Acid Bacteria isolate

Genomic DNA extraction

For all methods, genomic DNA has been produced overnight in Luria-Bertani medium from an exponential stage. To harvest aliquots of 10 ml of bacterial cultures, centrifugation at 12,000 rpm for 15 minutes was used, followed by a wash in sterile distilled water. Genomic DNA isolated from bacteriocin-producing LAB isolates cultures were identified as positive to produce bacteriocin-producing LAB isolates. Thermo Fisher Scientific protocol for isolating genomic DNA with the GeneJET Genomic DNA Purification Kit:

Polymerase chain reaction (PCR)

After extracting gDNA from selected bacterial isolates, PCR was performed for amplification of the 16S rRNA gene for each isolate. Edwards *et al*. (1989) described an amplicon of 1500 bp pieces reflecting the whole length of the 16S rRNA gene that was amplified utilizing well-preserved universal primers. Utilizing the BLAST algorithm as well as the RDP database, DNA sequences have been phylogenetically analyzed and contrasted to those in Gen Bank to look for near evolutionary relatives (table1).

Table 1: Primers designed for PCR amplification.

Primer name	Nucleotide sequence
27F	5'-AGAGTTTGATCCTGGCTCAG-3'
1492R	5'TACGGYTACCTTGTTACGACTT-

STATISTICAL ANALYSIS

For statistical analysis, the variability degrees of the data are calculated as mean ± standard deviation (Mean ± SD).

RESULTS

Isolation of Lactic Acid Bacteria

In the Saudi Arabian province of Jeddah, forty samples of fresh horse milk have been obtained from various regions. Whole samples were selected to isolate LAB utlizing MRS agar medium.

Screening and estimation of antimicrobial activity of LAB isolates by agar well diffusion assay against indicator bacteria

By using an agar well diffusion assay, all of the acquired LAB isolates have been tested for antibacterial activity versus four distinct ATCC pathogenic bacteria. The antimicrobial activity of those was estimated across the mean inhibition zone diameters. Following 24 hours of incubation, the average diameters of inhibitory zones varied from 16.1 to 25.66 mm. Isolate H6 had the best antibacterial activity against *St. Pneumonia* ATCC49619 (25.66±0.57) and against *E. coli* ATCC35218 (24.16±0.28), *S. aureus* ATCCBAA977 (24.33±0.57) and *P. aeruginosa* ATCC27853 (24.66±0.57) fig. (2).

Molecular Identification

Nucleotide BLAST alignment tools were used to analyze DNA sequences and revealed the highest bacteriocin producing isolate, which was identified as *Enterococcus faecium* 100%. The sequence has been assigned the entry number LR135401.1 in the Bacterial or Archaeal 16S ribosomal RNA Sequences database (table 2).

Effect of the cultural factors on the production of bacteriocin using Enterococcus faecium isolate

The effect of different culture conditions on the synthesis of bacteriocin using the chosen isolates was tested.

The influence of incubation temperatures on bacteriocin (s) synthesis by *Enterococcus faecium* isolate results in fig. (3) Showed that the selected isolates and cultures had the greatest antibacterial activity versus all indicator bacteria when incubated at 35ºC. The inhibitory zone ranged from 15.5 to 24 mm. The greatest antibacterial activity was 24mm by *Enterococcus faecium* against *E. coli* ATCC35218 and *ST. Pneumonia* ATCC49619, 22.5mm against *S. aureus* ATCCBAA977 and 23mm against *P. aeruginosa* ATCC27853.

The influence of the incubation period on the synthesis of bacteriocin(s) using *Enterococcus faecium* showed that the selected isolated incubation for 24 hours had the greatest antibacterial activity versus all indicator bacteria. The inhibitory zones ranged from 16 to 25.5 mm. The

greatest antibacterial activity by *Enterococcus faecium* versus *ST. Pneumonia* ATCC49619 was 25.5mm. The antibacterial activity by the selected isolates decreased against indicator bacteria when the incubation period increased (fig. 4).

Table 2: Identity percentage of 16S rDNA of H6

Bacterial isolate	Name and Accession of No. of the most related strain in NCBI GenBank.		Identity	Coverage	Suggested Name and Accession No. of the isolates obtained in the work.	
H6	LR135401.1	*Enterococcus faecium*	100%	100%	LR135401.1	*Enterococcus faecium*

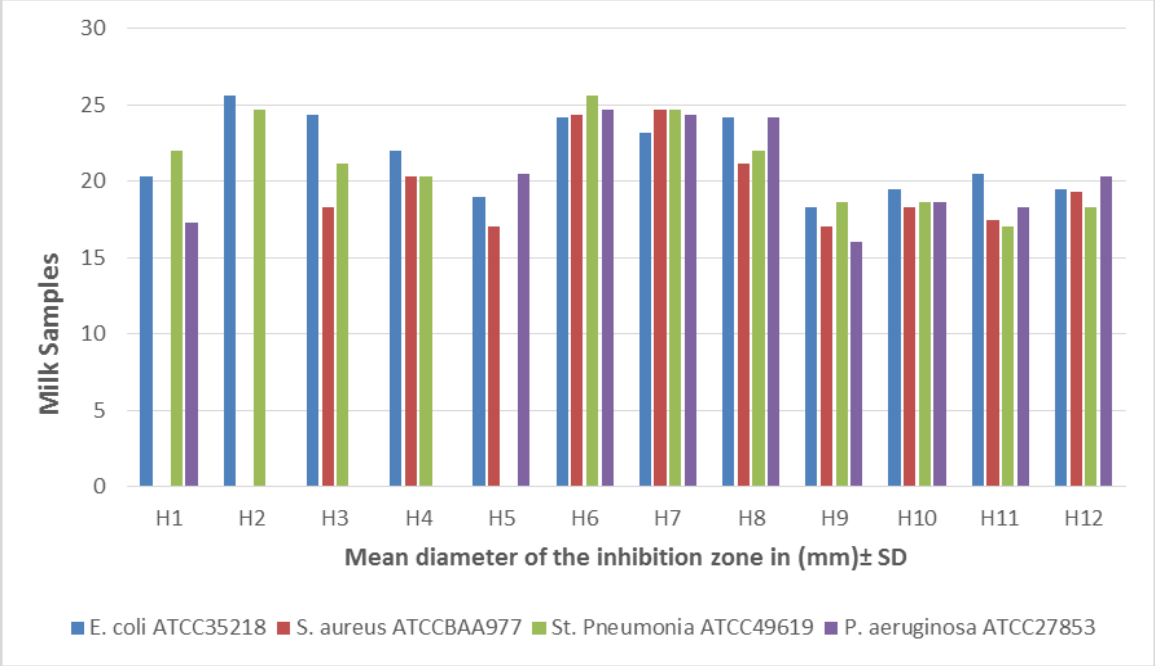

Fig. 1: Estimation of the antimicrobial activity of LAB isolates obtained from (Horse milk) samples using agar well diffusion assay against different indicator bacteria.

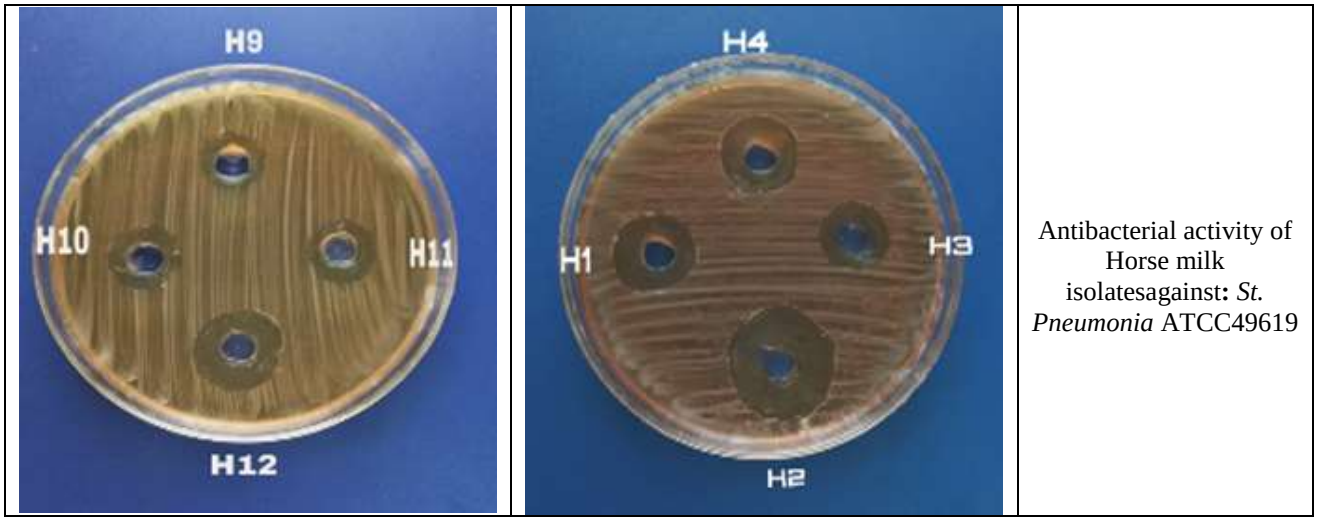

Fig. 2: Screening of the antimicrobial activity of LAB isolates obtained from (Horse milk) samples using cell difusion method Against: *St. Pneumonia* ATCC49619.

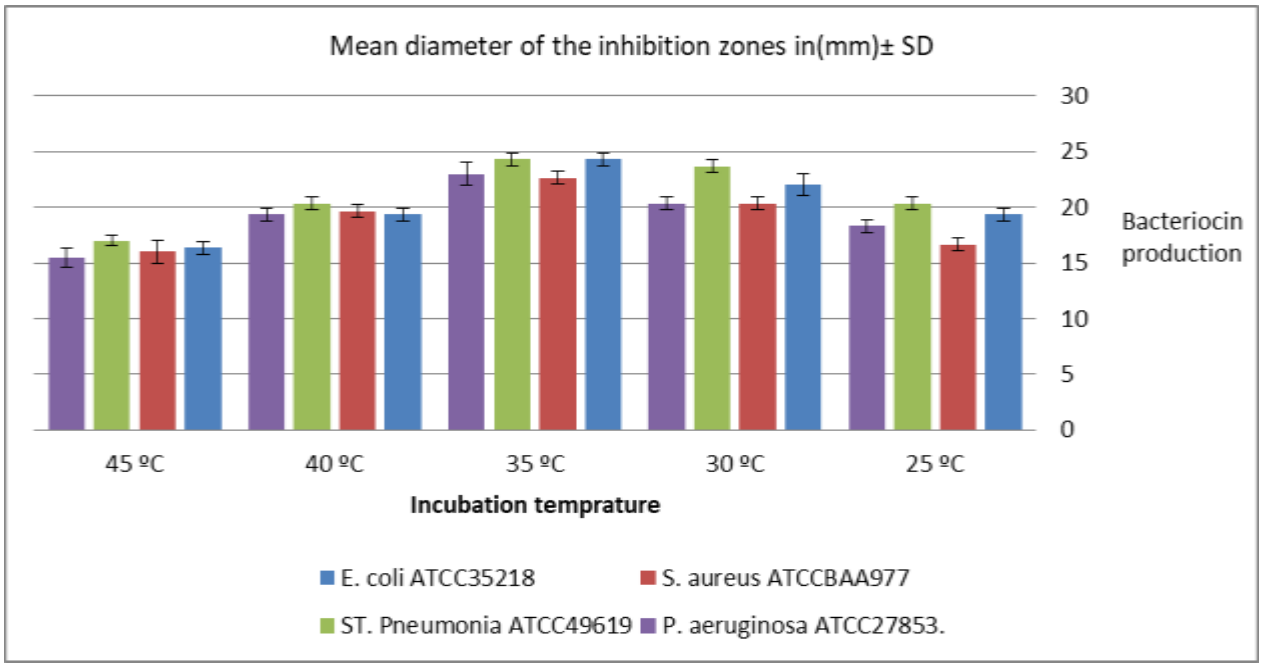

Fig. 3: Effect of incubation temperatures on synthesis of bacteriocin(s) using the *Enterococcus faecium*chosen isolate.

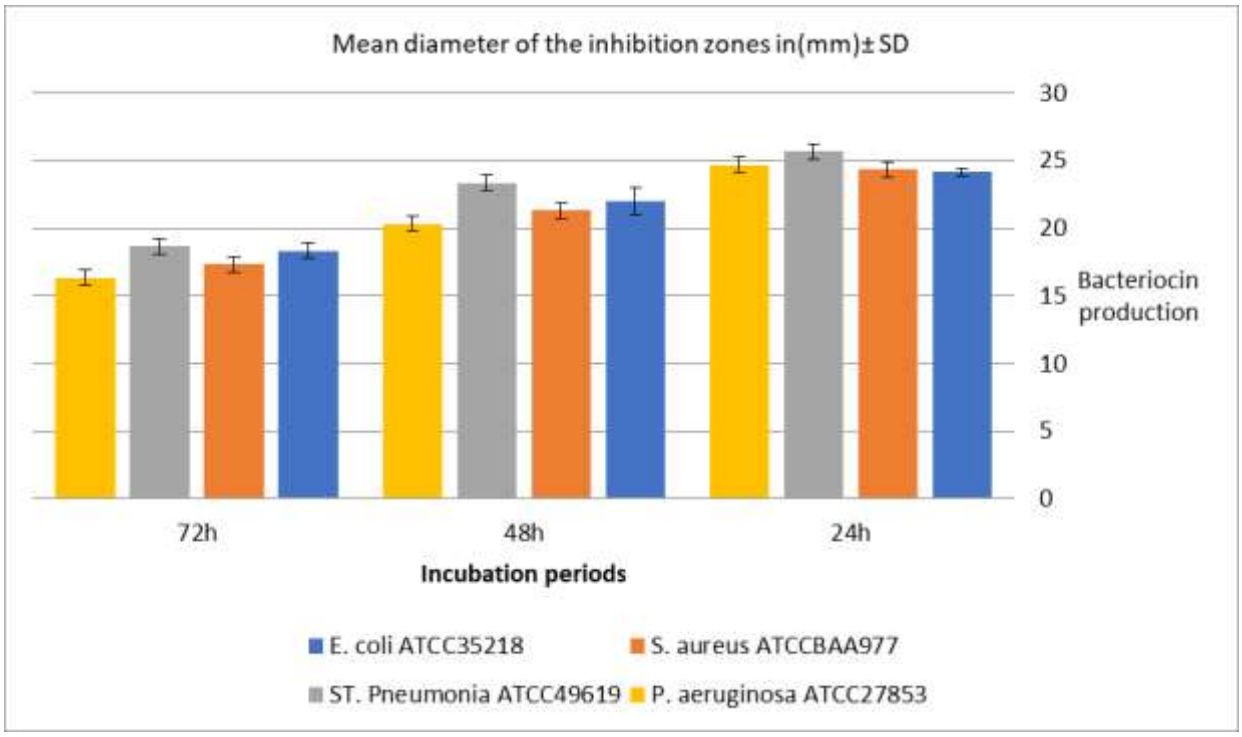

Fig. 4: Influence of incubation period on the production of bacteriocin(s) by *Enterococcus faecium* selectedisolate.

While the results of the impact of different pH values on the synthesis of bacteriocin(s) by *Enterococcus faecium* were shown in fig. (5), growing this isolate in medium at pH6.5 revealed the greatest antibacterial activity versus all indicator bacteria after 24 hours of incubation at 35ºC. *Enterococcus faecium* has the greatest antibacterial activity of 25.5mm versus *ST. Pneumonia* ATCC49619. The synthesis of antibacterial activity is reduced when the pH value rises and falls.

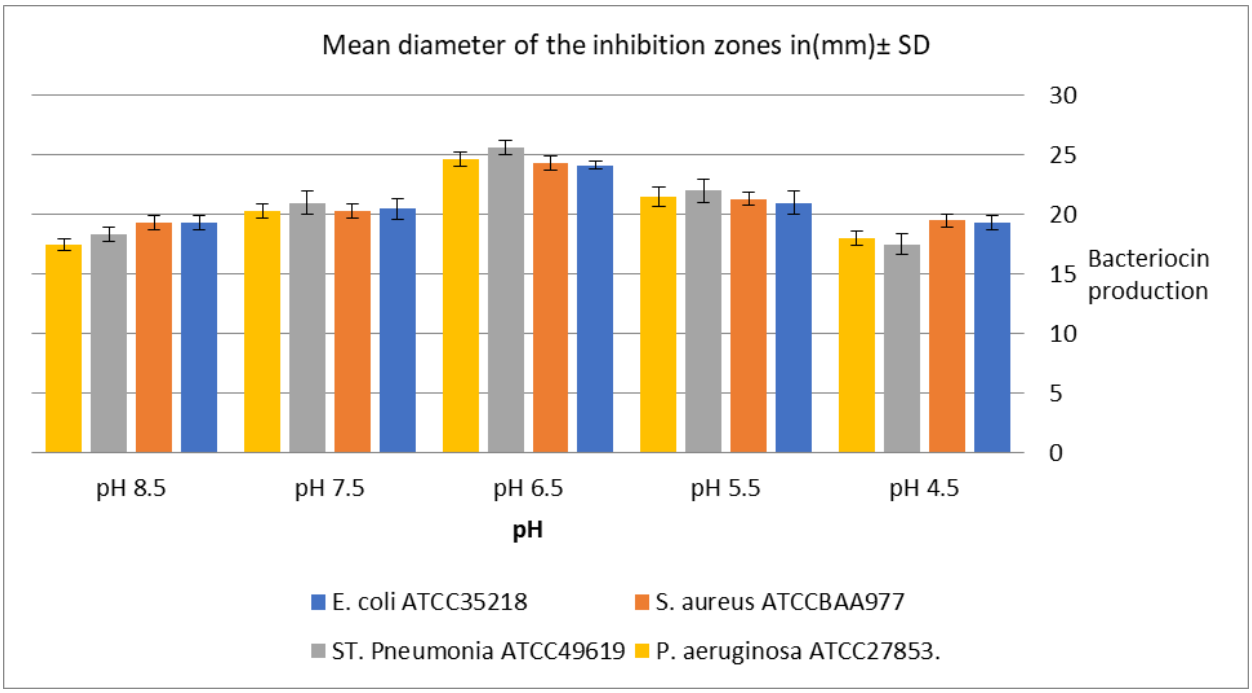

Fig. 5: Effect of pH values on the production of bacteriocin(s) by *Enterococcus faecium* selected isolate.

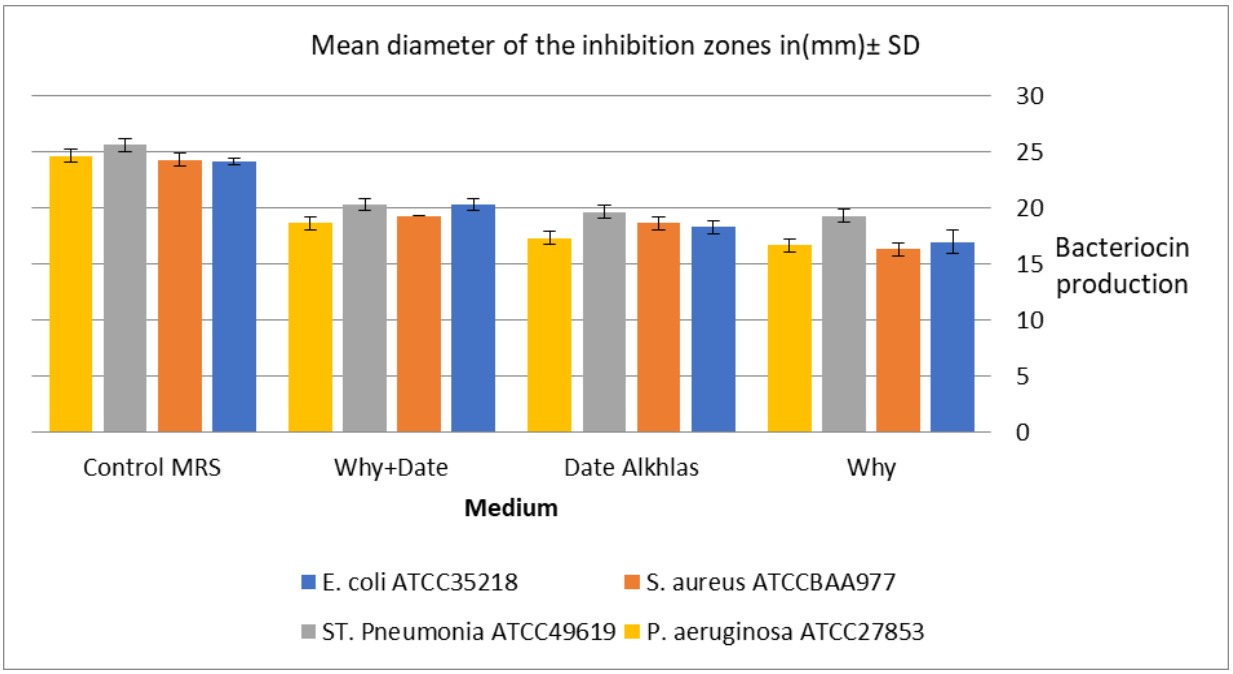

Fig. 6: Effect of different medium on the production of bacteriocin(s) by *Enterococcus faecium* selected isolate.

Production of antimicrobial agents (Bacteriocins) by Enterococcus faecium using organic wastes

Using Whey medium, Dates medium and mixture medium of Whey with Date encouraged the selected isolates to produce antibacterial agents (bacteriocin/s), the antibacterial activity produced when why with Date medium was used. *Enterococcus faecium* had the greatest antibacterial activity versus *E. coli* ATCC35218 and *ST. Pneumonia* ATCC49619 and the inhibition zone was 24 mm. The inhibition of other isolates in fig. 6.

DISCUSSION

Isolation of lactic acid bacteria (LAB) is the first and crucial step to study possible roles of LAB in the environment, especially in food fermentation. This is also

important to use the organisms for further application. LAB are diverse bacterial group and have diverse growth characteristics. Culture condition of LAB is thus varied, and selection of a suitable culture medium is essential for the purposes (Endo *et al*., 2019). In the present work, 54 LAB bacterial isolates were recovered in MRS medium from different habitat sources in Saudi Arabia's Jeddah province. Twelve LAB isolates have been detected for antagonistic activity in MRS medium and agar well diffusion processes, which are commonly utilized to assess the antibacterial action of plants or microbial extracts (Balouiri *et al*., 2016; Madduluri *et al*., 2013), the highest inhibition zone diameters varied between 16 to 25.5mm with great agreement with De Niederhäusern *et al*. (2020), MRS is one of the best medium proper for the isolation of lactic acid bacteria capable of producing bacteriocin as reported earlier by (Moraes *et al*., 2010). Manguntungi *et al*. (2018) reported that LAB isolated from Sumbawa horse milk had antimicrobial activity against pathogenic bacteria, including Staphylococcus epidermidis, *S. aureus*, *Escherichia coli* and *Vibrio cholerae*.

Eight LAB isolates from Sumbawa horse milk identified as different strains, including *E. faecium* DSM 20477, *E. faecium* NBRC 100486, *E. faecium* ATCC 19434, *E. durans* 98D, *E. faecalis* ATCC 19433, *E. faecalis* NRBC 100480, *L. lactis* subsp. hordniae NBRC 100931, and L. garvieae JCM 10343(Fidien *et al*., 2021) and bacteriocin supernatants produced *by E. faecium* and *L. curvatus* had antibacterial activity to both Gram-positive and Gram-negative bacteria and also reduces the growth of pathogenic strains (Mirhadizadi and Mohsenzadeh, 2021). *Enterococcus faecalis* (OE-7 and OE-12) and *Enterococcus hirae* (OE-9), from Egyptian raw cow milk and homemade dairy products were show the highest antibacterial activity with narrow spectrum against multidrug-resistant (MDR) Gram-positive foodborne bacteria: *Enterococcus faecalis* and *Staphylococcus aureus* (Sonbol *et al*., 2020). Enterococcus strain showed antimicrobial effect against *S. Enteritidis, B. cereus, E. coli* and *S. aureus*. One of food origin Enterococcus (*E. faecium*) exhibited the antimicrobial effect on all of the pathogen microorganisms used. Enterocins from food and clinical isolates were very effective against *Salmonella enteritidis* (Erginkaya *et al*., 2019). The optimal conditions for bacteriocin production by the three selected isolates were observed after 16–24 h of cultivation in MRS broth, at pH 6–6.5 and incubation temperature 37°C *S. aureus* (Sonbol *et al*., 2020).

When *E. faecium* DB1 was inoculated on a modified MRS medium with sucrose as a carbon source, it produced the most bacteriocin activity (Choi *et al*., 2011). As indicators in this study, gram-positive and gram-negative bacteria have been used. The majority of isolated lactic acid bacteria exhibited strong inhibitory activity on pathogenic indicator strains that are gram positive or gram negative. Some LAB species have a great capability to prohibit *Staphylococcus* species development and propagation through contest with different pathogenic microorganisms for nutrient element requests (Cadieux *et al*., 2002) and have been noticed to have stronger antimicrobial features versus Gram positive bacteria like *S. aureus* (Al-Zahrani and Al-Zahrani, 2006). Khochamit *et al*. (2015) reported LAB prevents the growth of some pathogenic bacteria, involving *P. aeruginosa, Salmonella mtyphimurium, Vibrio cholera, E. coli, B. cereus and S. epidermidis*.

The molecular identification of lactic acid bacteria is accomplished using 16S DNA sequencing, and amplification of bacterium 16S sequences can create a 750bp amplicon, as per Nocker *et al*. (2004). The DNA sequences were examined using Gen Bank's Blast alignment equipment, and one isolate has been recognized as *Enterococcus faecium* LR135401.1 (H6) with 100% similarity. 16S rRNA sequencing has performed an essential function in the delicate recognition of microorganisms and the detection of new isolates in microbiology laboratories (Senthilraj *et al*., 2016). The development of molecular characterization based on the genotypic characteristic could be done for LAB diversity analysis (Kusdianawati *et al*., 2020). Therefore, the molecular method-based identification of probiotic strains is increasing significantly (Mirhadizadi and Mohsenzadeh, 2021). The diversity of indigenous bacterial species was investigated by the 16S rRNA gene-targeted metagenomic approach from bacterial DNA isolated from Sumbawa horse milk (Fidien *et al*., 2021; Kusdianawati *et al*., 2020 and Manguntungi *et al*., 2020). Mirhadizadi and Mohsenzadeh (2021) also demonstrate the high ability of the 16S rRNA marker in detecting the different strains of E. faecium and *L. curvatus*.

Following 24 hours of incubation, the optimal cultural conditions for the highest synthesis of bacteriocin by *Enterococcus faecium* LR135401.1 (H6) were reported at pH 6.5 at 35°C. These data were identical to those described by Campos *et al*. (2006), was noted in this investigation that an incubation time of 24-48 hrs gives more bacteriocin synthesis. Campos *et al*. (2006) showed that the high synthesis of bacteriocin from the chosen lactic acid bacteria strains is discovered in the stationary stage of growth, which commonly ranges from 21 to 72 hrs.

Kumar *et al*. (2012) found that temperatures between 33.5 and 34.5°C were efficient for bacteriocin synthesis by *L. casei* LA-1. According to Barman *et al*., (2018), the best proper temperature demands by the 3 strains of *Enterococcus faecium* for bacteriocin synthesis were 28°C, followed by 20°C, while bacteriocin synthesis was

considerably reduced at 37°C. Malheiros *et al*. (2015) showed that maximum bacteriocin production was achieved at temperatures between 25 and 30°C.

The antibacterial factors were stable within a broad range of pH values from 4.5 to 8.5. The suppression influences were even further apparent in the pH range of 5.5 to 7.5. These results were similar to those of Danial *et al*. (2016), who found that static culture incubation at 35°C for 24 h and pH 6.2 were the optimal conditions for *Leuconostoc mesentroides* to produce bacteriocin. Bacteriocin is also stable over a broad range of pH and possibly utilized in acid in addition to non-acid foods. Furthermore, it is stable at low temperatures (de Niederhäusern *et al*., 2020).

Optimum conditions for bacteriocin production were cultivation in MRS broth at 37°C, pH 6–6.5 for 16–24 h. The tested bacteriocins exhibited bactericidal activity on *S. aureus* subsp. aureus ATCC 25923; such activity was further investigated by transmission electron microscopy that revealed leakage and lysis of treated cells. (Sonbol *et al*., 2020)

By using Whey medium, Date medium, and Whey and Date medium encouragement the selected isolates to produce antibacterial agents, the antibacterial activity produced when why and Date medium was used. These observations are in agreement with Alzahrani and Alzahrani (2006), they found that specific synthesis of bacteriocin by four isolates of lactic acid bacteria belonged to *Lactococcus lactis* sp. lactis which isolated from goat's and Camel's milk, increased by using a mixture of Whey and Date broth medium at 1:1, in compared with Whey and Date broth medium separately, apparently there would be a dependence between bacteriocin production and presence of different saccharides in Date as fructose and sucrose and glucose (Alzahrani and Alzahrani, 2006). Cheese whey, a low-cost milk by-product, was employed as a substrate for bacteriocin synthesis by *E. faecium* by Schirru *et al.* (2014), with skimmed milk and MRS broths as reference media. Use of cheaper culture medium (e.g. wastes from food industry) and an cultivation method could be an appropriate alternative to produce probiotic culture highly concentrated at a low production cost (Guerra and Castro, 2003; Guerra *et al* 2007; Bernardez *et al*., 2008)

CONCLUSION

Lactic acid bacteria were isolated from the milk of Horses from Jeddah province, Saudi Arabia. *Enterococcus faecium* LR135401.1 LAB isolates have been detected for antagonistic activity in MRS medium and agar well diffusion processes against the on a wide range of pathogenic bacteria (Gram-positive bacteria: *S. aureus* ATCCBAA977, *ST. pneumonia* ATCC49619) and (Gram-negative bacteria: *E. coli* ATCC35218, *P. aeruginosa* ATCC27853).The influence of incubation temperatures, incubation period and pH on bacteriocin(s) synthesis showed the greatest antibacterial activity when incubated at 35^{o}C incubation for 24 hours in medium at pH6.5.By using Whey medium and Dates, the antibacterial activity produced when mixture of why with Date medium was used. So we can improve the production of the amount of bacteriocin by improving environmental conditions as well as the possibility of taking advantage of food wastes. The LAB bacteria can be raised for the production of various kinds of food and pharmaceutical products. They can also be used for the production of new functional foods. Therefore, increasing use of dairy products containing probiotics, identification and production of foods containing LAB in daily diet.

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