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Antibacterial activity of bacteriocin produced by lactic acid bacteria (lab) isolated from different raw milk samples

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Abstract: Lactic acid bacteria effects are due to the production of antimicrobial agents such as bacteriocin or related substances. The point of this examination was to disconnect the Lactic Acid Bacteria creating bacteriocin from various crude milk tests and halfway clean them to inspect the counter bacterial movement against the pathogenic microbes. Lactic Acid Bacteria (LAB) were confined from camels, sheep, Cows and Goats milk from Jeddah - Saudi Arabia on (MRS) medium. By agar well dissemination strategy, Forty-two secludes of LAB demonstrated antibacterial exercises against marker Gram positive microscopic organisms *S. aureus* ATCCBAA977, *ST. pneumonia* ATCC49619, and Gram-negative microscopic organisms *E.coli* ATCC35218, *P. aeruginosa ATCC27853*, results demonstrated that 15 disconnects of 42(35.7%) of the tried disengages have antibacterial exercises against all marker microscopic organisms. The most elevated LAB secludes were distinguished based on its morphological and biochemical attributes and even by hereditary investigations.
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Key words: Bacteriocin, Lactic Acid Bacteria

Introduction

Lactic Acid Bacteria (LAB) are generally utilized as starter societies for maturation in the dairy, meat and other nourishment businesses. Their properties have been utilized to fabricate items like cheddar, yoghurts, aged milk items, drinks, hotdogs, and olives (Junnarkar et al., 2019). The LAB are viewed as commonly perceived as protected (GRAS) due to their omnipresent use in nourishment and their one of a kind job in the sound microflora of human mucosal surfaces (George et al., 2018). They are Gram-positive, non-spore-framing, microaerophilic or anaerobic microscopic organisms that produce lactic corrosive as the significant final result of sugar maturation. LAB are normally catalase and cytochrome negative, critical, aerotolerant, and corrosive tolerant (Papadimitriou et al., 2016). The nearness of 21 species having a place with the genera Enterococcus, Fructobacillus, Lactobacillus, Lactococcus, Leuconostoc, and Weissella was prove by utilizing society subordinate techniques (Ruiz Rodriguez et al., 2019).

Despite the fact that there are a few microorganisms that produce bacteriocins, those delivered by the lactic corrosive microbes (LAB) are exceptionally compelling to the dairy business (Egan et al., 2016). LAB have for some time been utilized in an assortment of nourishment maturations by changing over lactose to lactic corrosive, just as delivering extra antimicrobial particles, for example, other natural acids, diacetyl, acetoin, hydrogen peroxide, antifungal peptides, and bacteriocins (Egan et al., 2016) Lactic corrosive microscopic organisms bacteriocins are regularly dynamic over a scope of pH esteems, impervious to high temperatures and dynamic against a scope of nourishment pathogenic and deterioration microbes (Ahmad et al., 2017). Also, LAB bacteriocins are touchy to stomach related proteases, for example, pancreatin complex, trypsin and chymotrypsin, and along these lines don't affect adversely on the gut microbiota (Egan et al., 2016).

LAB bacteriocins can hinder foodborne pathogens like Clostridium botulinum, Bacillus spp., Enterococcus faecalis, Listeria monocytogenes and Staphylococcus aureus (Rodrıguez et al., 2003, Tahiri et al., 2004). There are at any rate three manners by which bacteriocins can be fused into a nourishment to improve its wellbeing, i.e., utilizing a purged/semi-refined bacteriocin arrangement as a fixing in nourishment, by consolidating a fixing recently aged with a bacteriocin-creating strain, or by utilizing a bacteriocin-delivering society to supplant all or part of

a starter culture in aged nourishments to deliver the bacteriocin in situ (Deegan et al., 2006). The creation of bacteriocins by LAB is worthwhile for endurance of the delivering microscopic organisms in a serious biological specialty; in this manner, they could be misused by the nourishment business as a device to control unfortunate microorganisms in a nourishment evaluation and normal way, which is probably going to be increasingly satisfactory to buyers (Deegan et al., 2006).

Materials And Methods

2.1. Samples Collection

Forty-two raw milk samples were used in this research (nearly 150 ml) of cows, sheep, camels and goat milk were collecting from different regions of Jeddah Province, Kingdom of Saudi Arabia, these samples were used for isolation of lactic acid bacteria.

2.2 Isolation of lactic acid bacteria

Serial dilution of the milk samples was made 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 sub culturing on solid MRS medium, the pure cultures were transferred to slants and preserved at 4°C. for the following studies.

LAB isolates were preserved in (MRS) broth medium which contained 30% (v/v) glycerol as frozen stocks at -80°C for long term preservation and for short term in -20°C.

2.3. Quantitative assay of the antibacterial activity by agar well diffusion assay:

The MRS broth medium was used to grow LAB isolates in liquid media. The inoculated flasks were incubated at 35°C for 24 h. The MRS broth medium was used to grow the LAB isolates in liquid media. The culture filtrate of the LAB was centrifuged at 10,000 rpm to obtain the Cell-free filtrate (CFF) of the cultures.

The agar diffusion method for quantifying the antibacterial activity of the LAB against the indicator pathogenic bacteria is based on the assumption that antibacterial agents diffuse freely in the solid nutrient medium (Bonev et al., 2008). The solid nutrient medium was inoculated with the indicator bacteria. Wells were made using sterile cork borers (diameter 5 mm) and were filled with 100 µl of the CFF of the LAB. Plates were left for 30 min in the refrigerator to allow proper diffusion of the supernatant in the medium. The plates were incubated at 35°C for 24 h. Inhibition zone diameters in (mm) of bacterial growth was measured. All experiments were carried out in triplicate. Three LAB isolates that recorded as the highest antimicrobial activity were selected for the next studies.

2.4. Identification of the highest bacteriocin production isolates:

Morphological properties: The highest bacteriocin production isolates were further purified by streaking repeatedly on MRS agar plates, and the colony morphology (color, shape and size) were examined by sight. Gram staining discovered by the Danish scientist and physician **Hans Christian Joachim Gram** in 1884 (Cantey and Doern, 2015)**.** Motility test Hanging-drop wet method described by (MacFaddin, 2000) was used for motility test of the selected isolates for identification (Lakhanpal and Gupta, 2017).

Cultural properties: Conventional methods of hemocytolytic assay are carried out either using commercial sheep blood agar according to Rodrigues et al., (2006). Determination of bacteriocin production at different culture conditions (temperatures and pH described by (Sarika et al., 2010)

Physiological tests: Catalase test was performed as described by (Wang et al., 2017). The CO2 production was evaluated in test tubes containing MRS broth and inverted Durham tubes As a method by (Amer et al., 2017).

Results:

3.1 Isolation of LAB bacteria:

Forty-two samples of fresh milk (cows, sheep, camels and goats milk) were collected from different regions at Jeddah Province, in Saudi Arabia. All the samples were used to isolate lactic acid bacteria using MRS agar medium, twelve bacteria isolates were obtained from camel milk, whereas ten isolates were obtained from sheep, Cows and Goats milk.

3.2. Quantitative assay of the antibacterial activity by agar well diffusion assay: Quantitative assay of the antimicrobial activity of LAB isolates using agar well diffusion method against different indicator bacteria, the antimicrobial activities of the isolates were estimated by the mean diameter of the inhibition zones, data summarized in Figures (1-5).

The mean diameters of inhibition zones were ranged from (15.5-24mm) after 24h of incubation. The highest antibacterial activity was by isolate CM4 was (24mm) and by isolates S4 was (23.5mm) against *E. coli* ATCC35218.

About 15 isolates of 42 isolates (35.7%) have antagonistic activities against four indicator bacteria. The isolates (CM4 and S4) were the most active and inhibited the growth of all the indicator bacteria with the highest inhibition zone diameters.

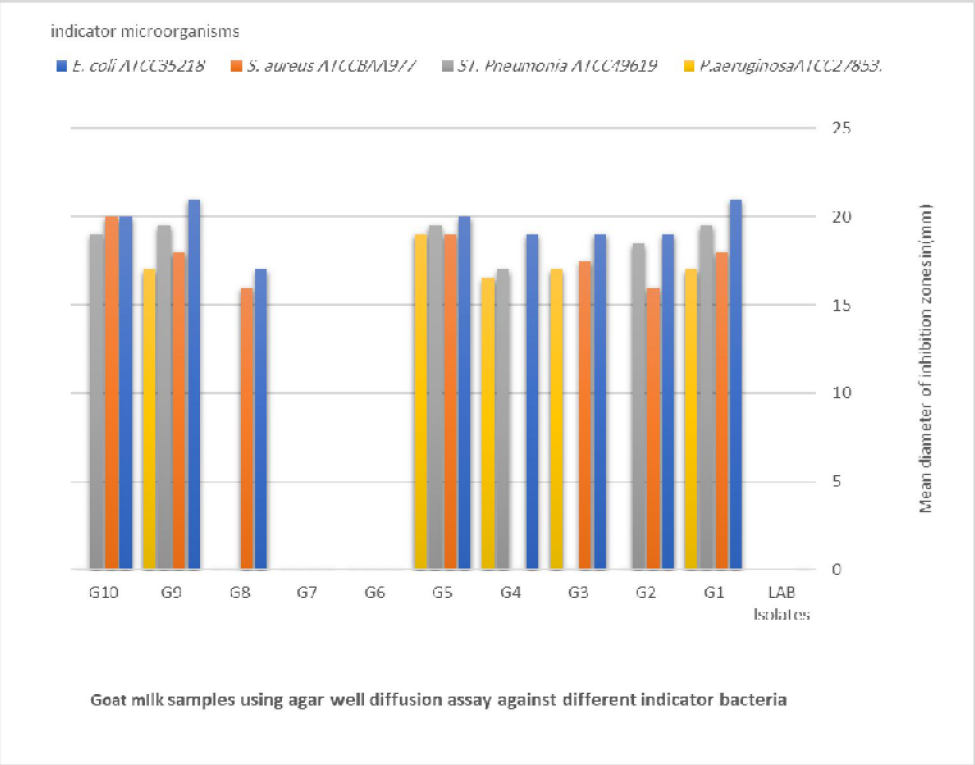


Figure (1): Screening and estimation of antimicrobial activity of LAB isolates obtained from Goat milk samples using agar well diffusion assay against different indicator bacteria.

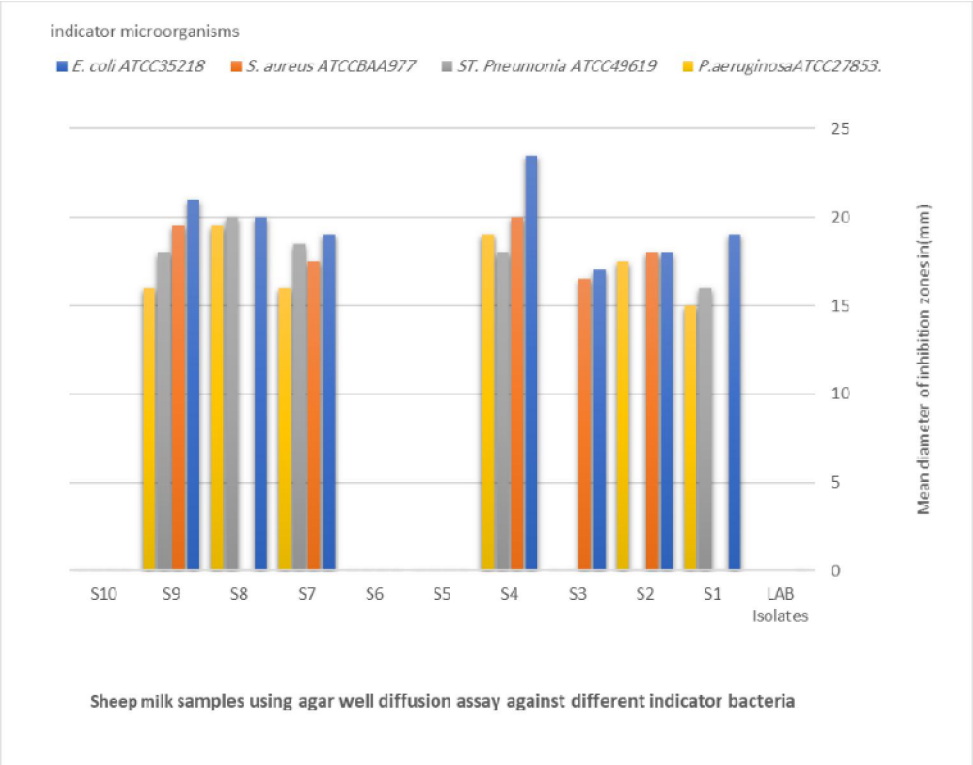


Figure (2): Screening and estimation of antimicrobial activity of LAB isolates obtained from Sheep milk samples using agar well diffusion assay against different indicator bacteria.

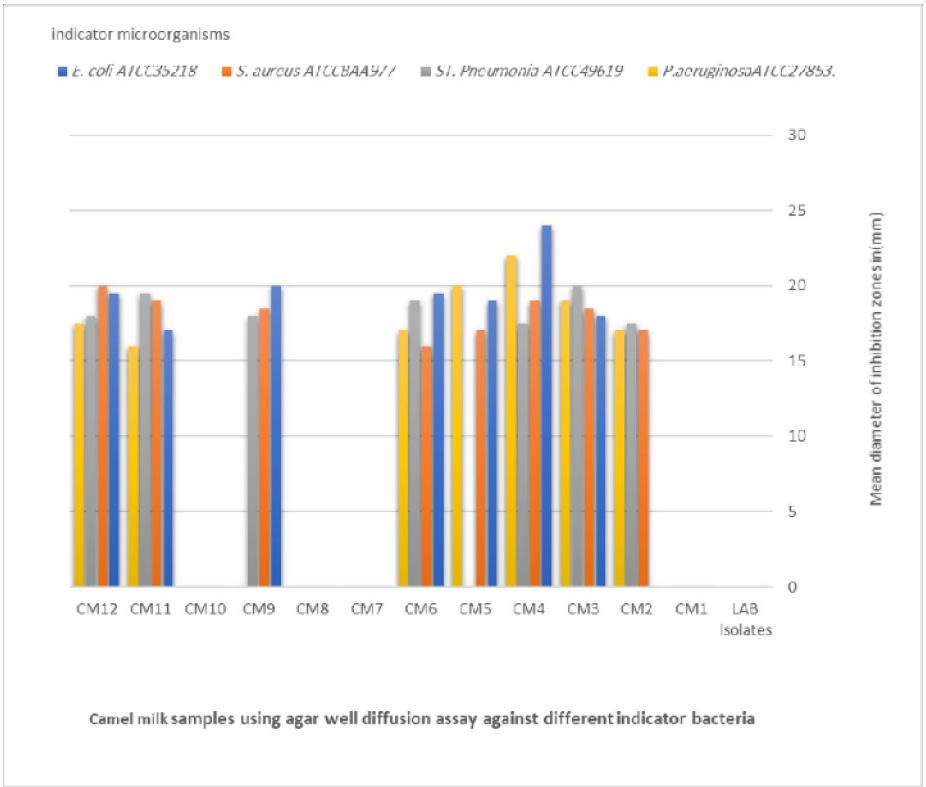


Figure (3): Screening and estimation of antimicrobial activity of LAB isolates obtained from Camel milk samples using agar well diffusion assay against different indicator bacteria.

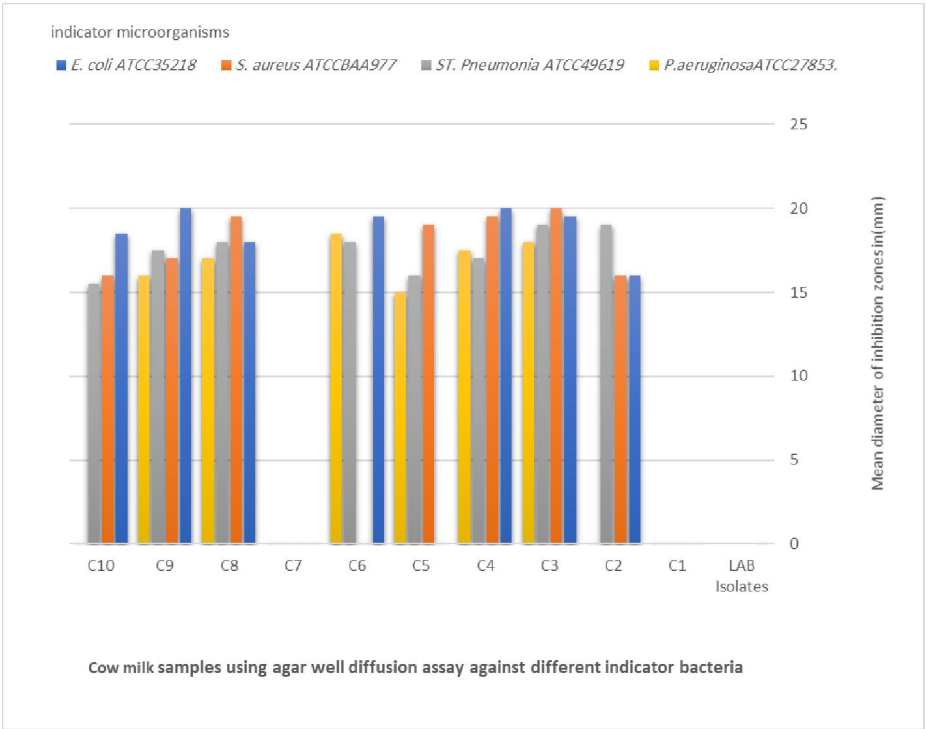
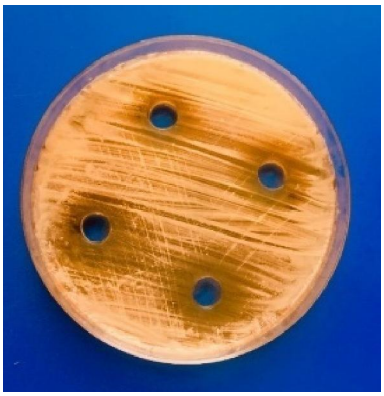
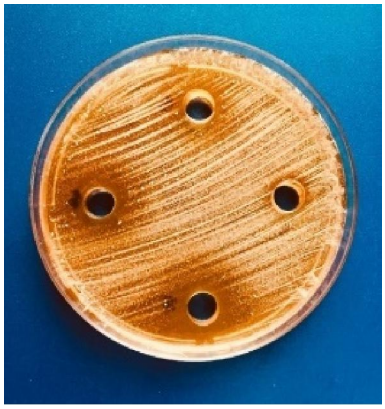


Figure (4): Screening and estimation of Antimicrobial activity of Cow Milk Isolates obtained from Cow milk samples using agar well diffusion assay against different indicator bacteria.

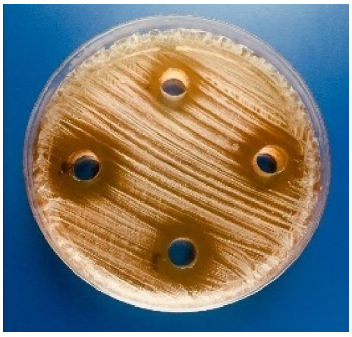


Camel milk isolate **Against** *E. coli* ATCC35218

Sheep milk isolate **against** *E. coli* ATCC35218



Cow milk isolate **Against** *P. aeruginosa* ATCC27853



Goat milk isolate **against** *S. aureus* ATCCBAA977

Figure (1-5): Production of bacteriocin by LAB using agar well diffusion assay against different indicator bacteria.

Identification of selected LAB isolates:

1Morphological characters: Two selected LAB isolates were obtained from camel and sheep milk (CM4 and S4). The isolates were grown on MRS medium within 24 h at 35°C (de Mann *et al*., 1960). The cells were examined under light microscope. Morphological properties showed all isolates gram-positive. S4 isolate appeared creamy color, while CM4 appeared white color, smooth colonies flat and round edges, they were about 1-2 mm in diameter. Isolates were Gram-positive, cocci shape, no flagella and no capsule. The isolates were no spore forming bacteria. The cultural characteristics of the bacterial isolates which were obtained from different milk samples, non-hemolytic activity, selected isolate of LAB after growth at MRS agar media at 35°C for 24 h are catalase negative. CM4 didn't ferment glucose after 24 h of incubation, so late S4 and CM4 grow well at 10°C, 15°C, 20°C, 40°C at pH 6.5.

Molecular Identification:

According to, the 16S rDNA sequence comparison analysis of the bacteriocin production isolates with the sequences of nearest type species retrieved by NCBI BLAST tool, these strains showed taxonomic affiliation with *lactobacillus gravieae* strains. The partial 16S rDNA sequence of the selected isolate were submitted into the Bacterial or Archaeal 16S ribosomal RNA sequences database under the accession numbers: LC145570.1 for strain Cm4 isolated from Camel milk, and S4 from sheep milk *Enterococcus faecalis* under accession number LC096215.1(Figure6-7).

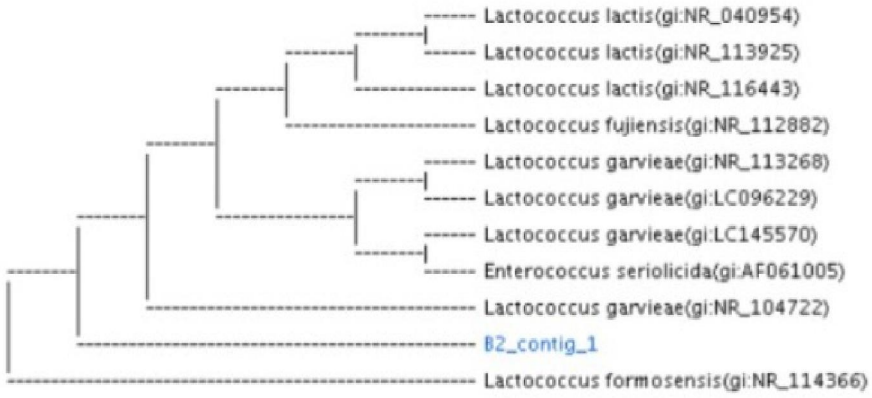


Figure (6): Phylogenetic tree analysis of *Lactococcus gravieae* isolate based on 16S Rdna.

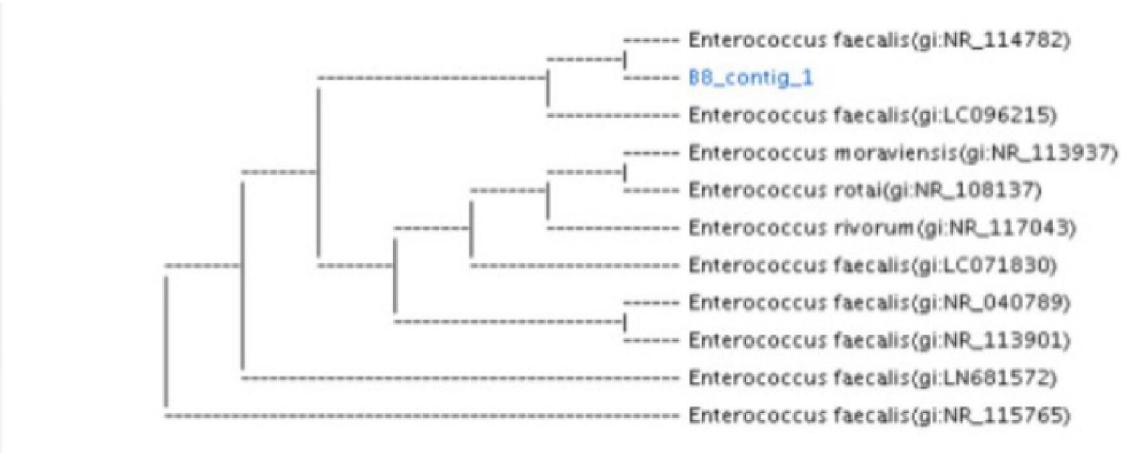


Figure (7): Phylogenetic tree analysis of *Enterococcus faecalis* isolate based on 16S rRNA.

Neighbor-joining tree showing the phylogenetic position of *Lactococcus gravieae* CM4 showed as name B2 and *Enterococcus faecalis* S4 isolate showed as name B8 and their related species based on partial 16S rRNA gene sequences. GenBank accession numbers of nucleotide sequences LC1455700.1 and LC096215.1 were shown along with the name of the bacterial strain.

Discussion:

Lactic corrosive microorganisms have been for quite some time set up as the ordinary vegetation in aged nourishment, since they are accepted to be protected; consequently, they have extraordinary potential for use in biopreservation. The safeguarding impacts of lactic corrosive microorganisms are because of the creation of antimicrobial operators, for example, bacteriocin or related substances (Cocolin et al., 2007)

The guidelines concerning expansion of lactic corrosive microorganisms in nourishment change broadly globally between countries (Wessels et al., 2004). In biotechnological angles, the wild strains of the LAB are imminent bacteriocins producers (Wessels et al., 2004) and probiotics (Rinkinen et al., 2003). These days, buyers request sheltered, sound, tasting, long timeframe of realistic usability, and insignificantly handled nourishment items. LAB are nourishment grade microorganisms that have been widely utilized in matured food sources, and huge numbers of them have GRAS and QPS status (Alvarez-Sieiro et al., 2016)

In the current work, Forty-two LAB bacterial disconnects were recouped from various environments sources in Jeddah city, realm Saudi Arabia, and all the past examples were utilized to separate lactic corrosive microscopic organisms utilizing MRS agar medium to segregate the probiotic microorganisms (De Man et al., 1960). Results uncover that LAB ruled the microbial vegetation of tests: twelve confines from Camels' milk and ten secludes from Sheep, Cows and Goats milk. It may be because of the explanation that

particular medium MRS agar was utilized to contemplate the morphological qualities of LAB disconnects, this particular media permits just explicit kind of microorganisms to develop along these lines the capacity of bacterial species to develop on explicit media is viewed as a significant trademark in recognizable proof. MRS probably the best medium appropriate for the separation of LAB as announced before by (Godousi, 2002) various papers have revealed that MRS medium is a superior mechanism for cell development and bacteriocin creation than other media (Daba et al., 1991).

Bacteriocin framed by E. faecium RZS C5(Leroy and De Vuyst, 2002) and enterocin P (Herranz et al., 2001), which is produced by Enterococcus spp. The more prominent level creation of bacteriocin has been distinguished at problematic development circumstances (Todorov and Dicks, 2005). A decrease of the bacteriocin creation at 25^{O}C and vanished at 40, 45^{O}C for 24 hours was seen when contrasted with hatching at 35^{O}C, (Leroy and De Vuyst, 2002) considered the creation of bacteriocin by Enterococcus faecium RZS C5 under various temperatures and acquired comparative outcomes with less bacteriocin creation beneath 35°C; most likely it is because of cell condition guideline and development related procedures. The distinction of ideal development temperature on cell development and bacteriocin creation has been recently revealed by Matsusaki et al. (1996).

The detach exhibited ideal restraint action against the test microorganisms at brooding occasions somewhere in the range of 24 and 48h. The opposing action of disengages was diminished after 72 h of hatching time. Balasubramanyam and Varadaraj (1998) found that, the antibacterial movement of the way of life filtrate from L. delbruecki ssp. bulgaricus against B. cereus happens at a hatching time of 24 h and that the restraint action increment until 48 h. When all is said in done bacteriocin is one of the antimicrobial operators ideally created somewhere in the range of 48 and 60 h (Ogunbanwo et al., 2003). As per Campos et al. (2006) the most extreme creation of bacteriocin from the chose LAB strains is seen in the fixed period of development, which for the most part goes from 21 to 72 h. These outcomes were like those revealed by Campos et al. (2006), it was found in this examination that a brooding time of 24-48 hours give most extreme bacteriocin creation. Then again, the yield of bacteriocin was low in societies hatched for over 30 hours comparable perceptions have been made already. This reduction could be because of the impact of extra cell endogenous proteinase advanced during delayed incubation (Piard et al., 1990).

The morphological, cultural, physiological and molecular identification using DNA sequencing of 16S, and according to Nocker et al (2004) of the highest bacteriocin producing isolates (CM4 isolated from Camel milk and S4 isolated from sheep milk), DNA sequences analyzed using Blast alignment tools of GenBank and showed that one isolate was identified as *lactobacillus gravieae* LC145570.1 and the second as *Enterococcus faecalis* LC096215.1. with similarity percentages 99%.

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