TLC Identification of Bacteriocin from Different LAB Clinical Isolates of Najaf Hospitals and in Vitro Evaluation of Its Effectiveness Against three Pathogenic Bacterial Isolates

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Abstract

Probiotics are useful microorganisms that are effective in protecting against pathogenic microorganisms, used to support food to provide beneficial effects to human health by maintaining the natural balance of the intestinal flora and reducing diseases, especially those related to the gastrointestinal tract.The ability of the Lactic acid bacteria to produce bacteriocin. In this study 74 samples were collected from various clinical sources include 31 samples of mouth and 43 samples from Vaginal swabs, for the period July 2018 until December 2018. The results of the isolation and laboratory diagnosis and biochemical testes the ownership of 43 isolates from lactic acid bacteria in vaginal swab and the highest percentage isolates bacterial (52%) of the samples of the vagina. All isolates showed lactic acid bacteria effectiveness of the microbial agents toward some negative bacterial species to dye grams diameters ranged between inhibition zones (14 – 22mm). Findings showed that RF bacteriocin values produced by the bacterium LAB isolates ranged from (0.45 – 0.57).

***Keywords**: Lactic acid bacteria LAB, bacteriocin, TLC.*

Introduction

Probiotics are useful microorganisms that are effective in protecting against pathogenic microorganisms, used to support food to provide beneficial effects to human health by maintaining the natural balance of the intestinal flora and reducing diseases, especially those related to the gastrointestinal tract (4). Species belonging to genera Lactobacillus and Bifidobacterium are the most important biological enhancers possessing high tolerance to storage conditions and manufacturing of fermented milk products (16). Lactobacillus species are prevalent in the natural flora of the small and large intestine of the healthy human body in various age groups (14). Beside their use to protect against many pathogens and support metabolic processes, LAB can also be a therapy for people with allergies to glucose and different type of diarrhea (14). It is therefore preferred to be used as alternative to antibiotics and other chemicals with adverse side effects C. W Tannok, (4).

LAB are known to produce a sort of metabolites namely 'bacteriocins' which was first studied in 1925 with the discovery of a plasmid carrying gene encoding a protein produced by E.coli.

LAB bacteriocins are characterized by low molecular weights and antibacterial activity, often extending to species other than those closely related to producing bacteria (8). Yeasts and molds, but have an effect in some cram-positive bacteria and the possible presence of strains of insensitive cells within the sensitive strain (10).

Bacteriocin has been described as a bacterially lethal protein with a narrow range of inhibition limited to the type of bacteria it produces or closely related species, and that its coding genes should be carried on sensitive cells. These properties are generally produced by many bacteria, but not all of them, especially those

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produced by Cram positive bacteria in general, by LAB in particular [9].

Bacteriocytes are ribosomia-made bacterial peptides and have antimicrobial and antimicrobial properties and thus differ from antibiotics, which are secondary metabolites produced in the phase of Idiophase. Although bacteria are produced by positive and negative Cram bacteria, the most important are bacteria produced by Lactic acid bacteria LAB because of their large use in food processing as amino preservatives [5]. The amount of bacteria produced depends on the type of environment in which these species grow. Bacteriocytes are secondary metabolites that are synthesized in stationary growth phase, but the maximum yield in the farm can occur at some growth stages. [7], have found that the production of Streptocin (STH) is better. Logarithmic reaches higher after 24 and 48 hours of incubation.

Bacteriocytes are antimicrobial compounds of a protein nature produced by a large selection of bacteria and have a killer or developmental effect against their sensitive bacteria, which are often related or genetically related to the producing bacteria. It has anti-bacterial activity and often extends to species other than those related to the producing bacteria, where it is characterized by its ability to eliminate some pathogenic bacteria and maintain the quality of the product added to it. Recent studies have suggested the possibility of the use of these bacteriocins instead of preservatives to keep the product for a long time without adding preservatives manufactured [15].

Materials and Methods

SAMPLING

74 random samples including 31 oral samples and 43 vaginal samples were collected. Samples were cultured in LAB selective medium for the presence of LAB bacteria. Routine tests were performed to confirm the presence of LAB.

Pathogenic isolates used in the study

Three pathogenic bacterial isolates, Pseudomonas aerugenasa, E.coli and Proteus mirabilis, previously diagnosed with Vitek were obtained after being isolated from wounds in hospitals in Najaf province. The sensitivity test was performed on these isolates to assess their resistance to five antibiotics. Biological activity of LAB bacteriocin against these pathogenic isolates was tested and compared with the results of antibiotics sensitivity test.

Microbiological and biochemical tests

Microbial, microscopic and biochemical tests of lactic acid bacteria LAB were carried out based on the morphological characteristics of the bacterial colonies, their forms and appearance under electron microscopy, and their pigmentation with Cram stains [13].

Based on the method by [6], the bacteriocin produced by LAB was deposited on the Brain heart infusion broth using 24-hour-old young colonies for the LAB isolates, which were selected based on their growth efficiency and efficacy towards gram-negative bacterial species. The final sediment product was left to dry to be tested as bacteriocin.

TLC was used to detect bacteriocin in sediment extracted from LAB for all developing isolates. The value of (Rotation factor) was calculated. TLC results were photographed using a Sony camera [20].

Antagonistic Effect of bacteriocin against Gram-negative pathogenic bacteria

The detected bacteriocins from each producing LAB were tested for their biological activity against pathogenic isolates under test. Muller Hinton Agar medium was prepared according to the manufacturer's instructions, autoclaved and poured into Petri dishes. The dishes were allowed to cool at 37C ° and cultured with the pathogenic bacteria using Cotton swab. Five holes with a diameter of 5 mm were made in each dish using a sterile cork-borer. 100µL of bacteriocin was carefully added in each hole and the plates were incubated at 37 C ° for 24 hours after which the inhibition diameter was measured for each hole.

Antibiotics Sensitivity test of pathogenic isolates

The sensitivity test was carried out according to the method used. The samples were spread on Muller Hinton Agar medium as in the previous method. The

manufactured antibiotic tablets were then placed five tablets in each dish. The plates were incubated at 37 ° C for 24 hours and the inhibition diameter was measured and compared with the bacteriocinase inhibition diameter

Results and Discussion

Isolation and diagnosis

74 samples were collected, including 31 oral samples and 43 vaginal samples, which were grown on MRS agar medium, a differential medium for LAB. 18 oral samples (28%) were LAB and 13 non-developing samples were non-developing isolates whereas all vaginal isolates (52% of total samples) were LAB (Figure1).

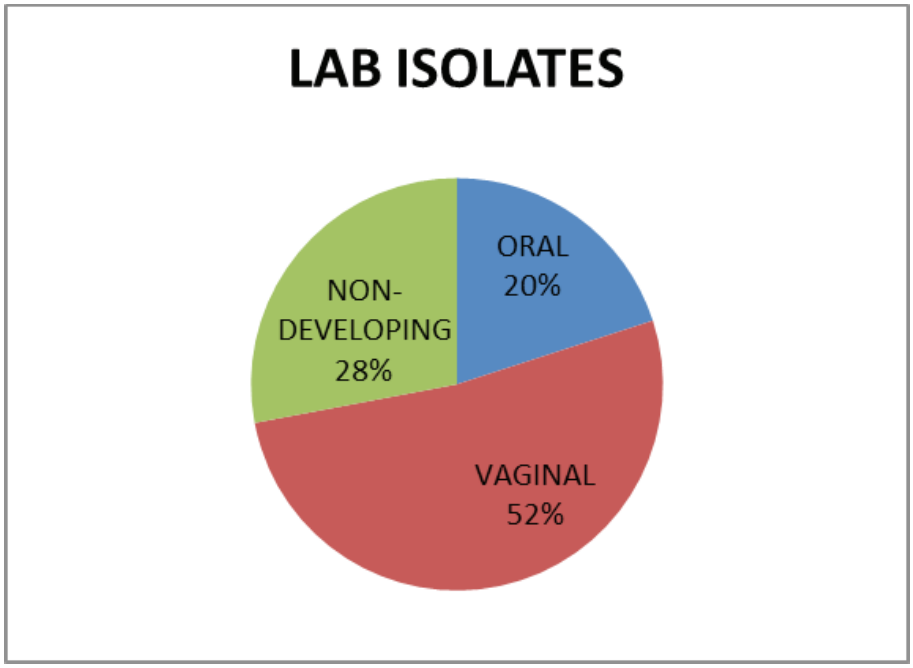

Figure1. Distribution of LAB growth from a total of 74 samples under study

The diagnosis of the isolates was confirmed using microscopic examination and its acceptance of the Cram stain, in addition to the initial routine tests for oxidase and catalase. The agricultural properties were also based on MRS agar medium. Oral isolates were labeled from LAB1 to LAB31 and vaginal isolates were from LAB32 to LAB74. Molecular detection results of the TLC test showed the presence of bacteriocin in one of the oral isolates (LAB22) and in three vaginal isolates, LAB43, LAB54 and LAB72 (Table 1,2) and (Figure2).

Table 1. LAB isolates and bacteriocin-producing isolates from total oral and vaginal samples under study

Sample type	N0. Of samples	No. of LAB isolates	No. of Failed isolates	No. of bacteriocin producing isolates	% Bacteriocin producing isolates
Oral	31	18	13	1	5.5%
Vaginal	43	43	None	3	7%

Figure2. Diagnosis of bacteriocin molecules produced by LAB using TLC technique

Table2. RF values for bacteriocin extracted from four LAB isolates under study

Sample No.	Bacteriocin running distance	RF
LAB22	3.9	0.45
LAB43	4.7	0.55
LAB54	4.9	0.57
LAB72	4.8	0.56

Antibiotic sensitivity test

The results of the antibiotic sensitivity (susceptibility) test for the three pathogenic isolates were studied. Isolates differed in their sensitivity to antibiotics (Table 3). The highest inhibition diameter (25mm) was recorded with E. coli in the treatment of LEVOFLOXACIN. *E. coli* and *P. aerugenasa* were generally sensitive to all antibiotics used except CEFTRIAXONE, with inhibition diameters between 15 to 25mm for the first and 13 to 22mm for the second, respectively. *Proteus mirabilis* was sensitive only to AMIKACIN, NORFLOXACIN and MASTDISCS, with inhibition diameters of 18, 20 and 21 mm, respectively (Table3).

Antimicrobial activity of bacteriocin of different LAB isolates

The results of table (3) showed that bacteriocin for all isolates under study resulted in growth inhibition of all three isolates. The highest inhibition diameters were recorded in E. coli with inhibition diameters between 17 to 22 m, followed by Proteus Maarbeles Vrahaf 17-21 mm, and finally *Pseudomonas aerugenasa* With relatively lower diameters, it ranged from 14 to 19 AD. The results of the study showed the bacteriocin ability to inhibit the growth of the pathogens under study *Pseudomonas aerugenasa*, *E.coli* and *Proteus mirabilis*. There was a significant inhibition of bacteriocin depending on the inhibition diameter.

Table3. Antibiotic sensitivity and antibacterial effect of bacteriocin of different LAB isolates against three pathogenic bacterial isolates

Antibiotic (1-6) or bacteriocin (7-10)	Pseudomonas aerugenasa	Proteus mirabilis	E.coli
AMIKACIN	19 mm	18 mm	15 mm
CEFTRIAXONE	Zero	Zero	Zero
CIPROFLOXACIN	13 mm	Zero	mm15
LEVOFLOXACIN	21 mm	Zero	25mm
NORFLOXACIN	17 mm	20mm	15mm
MASTDISCS	22 mm	21mm	21 mm
LAB22	17 mm	15 mm	17 mm
LAB43	14mm	18mm	22 mm
LAB54	19 mm	17 mm	19 mm
LAB72	14 mm	16 mm	22 mm

E.coli bacteriocin

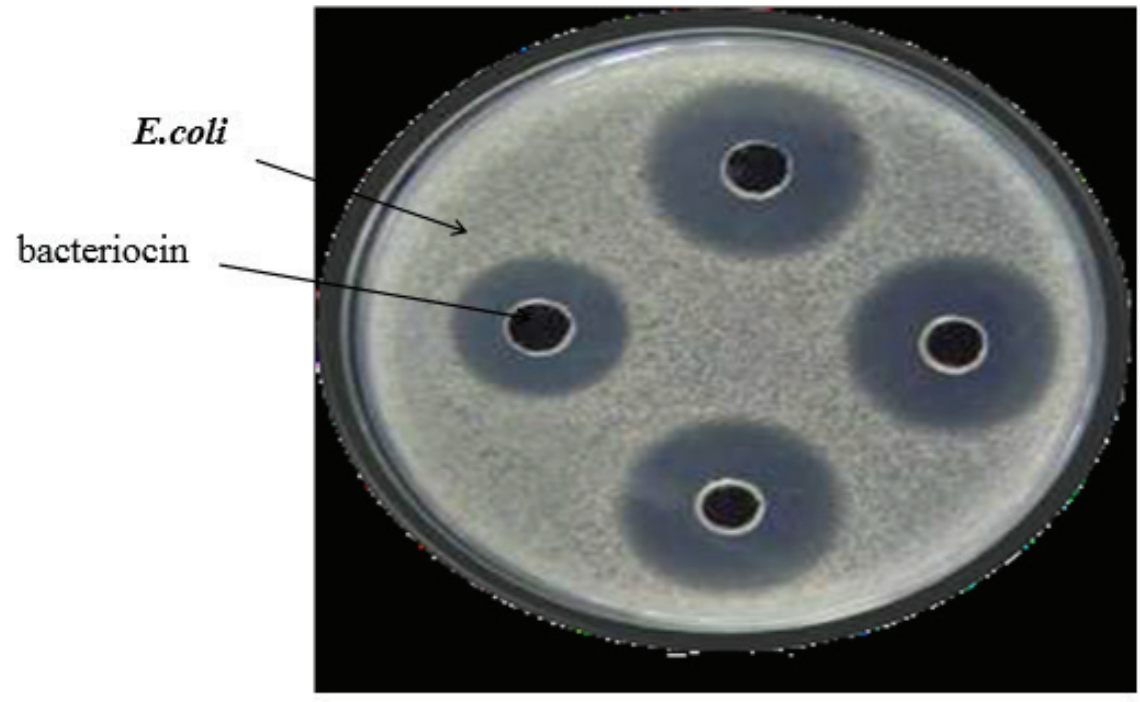

Figure3. Inhibitory effect of bacteriocin isolates on E. coli

The results showed that there was a clear inhibition of bacteriocin on the pathogenic bacteria negative for Gram stain compared to inhibition of antibiotics where the inhibition diameters resulting from bacteriocin were greater or equal to the diameters of antibiotic inhibition of the same isolates under test.

The results of the study showed that LAB isolated from the vagina had a higher efficacy against Cram-negative bacteria compared to the isolated bacteria from the mouth. This result was consistent with several studies that showed that the materials produced by LAB bacteria, especially bacteria, are capable of inhibiting the growth of many microorganisms such as *Pseudomonas aerugenasa, E. coli, K. pneumoniae and E. faecalis* [18,19].

Ali et al. [1] show that LAB isolated from the vagina has the potential to secrete a bacteriocin that has the potential to inhibit the growth of many microorganisms and that these strains are used as an alternative to antibiotics in the treatment of urinary and reproductive tract infections, while the bacteria produced from Pre-isolated LAB from the mouth has a broad spectrum of activity against many positive and negative microorganisms of the Cram dye. [19].

On the one hand, lactic acid bacteria have antibacterial activity against many positive and negative Cram bacterial species due to their production of many antibacterial effects of other microorganisms. Or, it may be due to the production of CO2 during the fermentation process, which leads to anaerobic conditions, causing an obstacle to the growth of compulsory aerobic microorganisms [3]. Lactic acid bacteria have the ability to produce many compounds such as acetylsalicylic acid and diacetyl which have the inhibitory power against many microorganisms.

The inhibitory action of lactic acid bacteria in microorganisms can be attributed to the synergistic action of bacteria and lactic acid produced by bacteria. Or, it may be attributed to the lactic acid produced and the low pH that increase the permeability of the outer membrane, especially in Cram negative bacteria, which makes them weak and more sensitive to the bacteriocin produced [15,3].

The high efficacy of bacteria produced by lactic acid bacteria against bacterial species is due to the ability of bacteria to form openings in Phosphate bilayer found in the plasma membrane of the bacterium, thereby blocking the movement of protons [17].

The variation in the efficacy of the extracted and partially purified bacteriocin compared with that excreted by living cells can be attributed to the effect of the extract. Bacteriocin produced by lactic acid bacteria in vivo is highly active against Cram-negative bacteria. Cram negative bacterial species outside the in vivo are insensitive to bacteriocin produced by Cram positive species [11].

The results of the molecular diagnosis of bacteriocin produced from lactic acid bacteria by thin layer chromatography technique in the present study are consistent with a number of studies which indicated that the value of Rf in bacteriocin LAB isolates ranged from 0.54 to 0.11 [17,21].

Lactic acid bacteria are very important biological enhancers because of their wide spectrum of pathogenic antibacterial effect. It is also preferred to use in the treatment of genital and urinary infections. Therefore, it is significant to highlight the compounds resulting from the vital enhancers and to study the possibility of utilizing them in the treatment of outbreaks in hospitals in the country.

Conclusion

The ability of bacteria produced by lactic acid bacteria is very high on negative bacteria compared to Cram positive bacteria. Because bacteriocin is equivalent to the inhibitory action of known antibiotics, it is preferable to use them as safe alternatives to reduce the side effect of commercial antibiotics. However, this requires extraordinary efforts to find appropriate manufacturing methods, pills or capsules, to deliver pure bacteriocin to the bio-effect area as alternative therapies to antibiotics.

Conflict of Interest: None

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Ethical Clearance: Not required

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