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Antibacterial Bioactivity of Some Lactic Acid Bacteria Isolated from Various Egyptian Products

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Abstract

The current research aimed to isolate and screening of antibacterial bioactive lactic acid bacteria (LAB) from different foods such as raw milk, dairy products and fermented meat and evaluate their potential for production of antibacterial substances, namely bacteriocins. Ten samples were collected from different market foods, examined and used for LAB isolation on MRS medium. A total of 71 bacterial isolates were selected based on some variation properties, such as color, shape, size and oxygen requirements. These isolates were confirmed as lactic acid bacteria based on their microscopic observation and chemical properties, i.e. catalase negative and acid producing from glucose fermentation. All selected isolates were grown on MRS medium at 37°C for 48 h, and compared to identified LAB strains, i.e. Lactococcus lactis, Streptococcus thermophilus, Befidobacterium sp, Lactobacillus casi, Lb rhamnosus, Lb. acidophilus, Lb. helveticus and Lb. plantarum. Isolates and strains were surveyed for bacteriocin production as inhibitory activity test. Partial purification of bacteriocin was carried out by salt precipitation method; this method was done by ammonium sulphate 40-60% saturation and centrifuged at 9500 rpm for 20 minutes at 4°C. Some of microbial indicator strains found to test by inhibitory biological activity substance (partial purification of bacteriocin) produced by lactic acid cultures. Indicator strains such as staphylococcus aureus, Listeria monocytogenes and others were grown by nutrient agar at 37° C for 24h. An inhibitory activity test was executed by paper diffusion

method. The inhibition effect was examined by form clear inhibition zone around indicator strains. Twenty-eight isolates and the 8 LAB strains showed bioactivities against the tested pathogens. Gram positive indicator bacteria were found to be the most sensitive to inhibitory substances produced by LAB culture as compared to gram negative indicator strains. Staphylococcus aureus was exhibited the highest sensitivity to it. The highest bacteriocins activities were produced by strain Lactobacillus rhamnosus while R5 and S6 from LAB isolates. They were shown broad spectrum against gram positive and gram-negative pathogenic bacteria. Diameters of inhibition zone refer to degree of sensitive between indicator strains and bacteriocin activity.

Keywords: Lactic acid bacteria, MRS medium, antibacterial agent, Bacteriocin, Paper diffusion method.

1 Introduction

Lactic acid bacteria (LAB) are Gram-positive, bacilli, cocci, or poly-form shaped, in pairs, tetrad or in chain, catalase-negative, devoid cytochrome, non-respiring but aero-tolerant, and the optimum growth is in micro-aerophilic or anaerobic condition, with optimum pH between 5.5 and 6 (Sadishkumar and Jeevaratnam 2016). They play very important role in food fermentation and preservation, they are generally recognized as safe (GRAS) bacteria and either as normal microflora or used as starter cultures in some food products under controlled conditions (Mirnejad et al 2013). Some species are Probiotic, this term remain promoting good human health and safe for human consumption. Probiotics refer to microorganisms that are consumed as a part of nutrition (directly or indirectly), producing useful effect and refining the microbial balance in gastrointestinal tract for human and animal (Quinto et al 2014, Nallala et al 2016).

Lactic acid bacteria (LAB) are classified based on cellular morphology, mode of glucose fermentation, utilization of sugar and rang of temperature growth. They are classified in phylum Firmicutes, class Bacilli and order Lactobacillus (Quinto et al 2014). Based on fermentation end products from organic acid, LAB are categorized into two groups, homo-fermentative group, where lactic acid is the main product, and hetero-fermentative group produce lactic acid, acetic acid, alcohol and carbon dioxide (Mokoena et al 2016).

LAB produces various antimicrobial substances such as organic acids, diacetyl, carbon dioxide, hydrogen peroxide and bacteriocins (Puniya et al 2012).

Bacteriocins are antimicrobial peptides produced by some gram-negative and gram-positive bacteria including members of Lactic acid bacteria, active against similar or closely related bacterial species. They are primary metabolite and ribosomally synthesized (Belicova et al 2013). They are like antibiotic substance which are encoded often in the plasmids but sometimes encoded in chromosomes or transposons. They are extracellular biological active peptides or peptide complexes that are bacteristatic or bactericidal due to strategy action of the bacteriocin and the host autolysin strain (Kleanthous 2010).

The first discovered bacteriocin was Colicine in 1952 from *E. coli* by Andre Gratia and his work group, and subsequently a lot of bacteriocins have been successively identified (Settann and Corsetti 2008).

Bacteriocins have two type, first type has narrow spectrum whereas its activity only against similar species while the second type, less common, has broad spectrum activity. Nisin which produces by *Lactococcus lactis* subsp *lactis* is the best example of second type (Mills et al 2011).

Bacteriocins of LAB are classified by Jeevuratnam et al (2005) and Murua et al (2013) into four classes. Class I: (Lantibiotics) they are small active peptides, contain unusually amino acids and heat stable. Class II: they are small active peptide, heat stable but its amino acids are usually unmodified. Class III: they are large active peptides, heat labile proteins. Class IV: (circular peptides) they are large protein complexes with other moieties, lipids or carbohydrate required for activity.

Bacteriocins target energized membrane to lack the proton motive force (PMF) and then inhibit amino acids transport. PMF is very important in the cell membrane in several processes, such as the accumulation of ions and ATP synthesis (Guilhelmelli et al 2013). Negatively-charged phosphate groups on target cell membranes play important role in forming initial binding with the positively-charged bacteriocin molecules, thus depleting the transmembrane potential and pH gradient. Bacteriocins are hydrophobic peptides inserts into membrane, forming pores through common two strategies, barrel-stave or wedge (Snyder and Worobo 2013).

Bacteriocins are small cationic hydrophobic molecules of about 30-60 amino acids, forming amphiphilic helices. LAB strains protect themselves from their produced bacteriocins by a specific immunity protein, which encoded in same of the bacteriocin operon (Parada et al 2007).

Lan I and Lan EFG protein specific immunity protein are preventing pore formation by pushing back the bacteriocin molecules which are inserted into the cytoplasmic membrane, therefore the bacteriocin concentration in the membrane is kept under control (Zacharof and Lovit 2012).

Bacteriocins are suitable as biopreservatives of food products since they are inactivated by protyolitic enzymes when consumed in gastrointestinal tract. In addition, they are also non-toxic, non-immunogenic, inactive against eukaryotic cells, resistant to high temperature, active at low pH and have broad spectrum activity against some food-borne and spoilage microorganisms (Nath et al 2014).

The application of bacteriocin can increase shelf-life of many foods. It is considered as an extra protection during temperature treatment; decreasing the risk of food-borne pathogens through the food handling, and reducing the need for heat treatments and chemical preservatives in food products, without reducing food safety (Güllüce et al 2013).

Although several bacteriocins have been discovered, only Nisin has been used as commercial food preservative widely in about fifty countries. The limited utilization of bacteriocins as preservatives in

the commercial industry is mainly derived from narrow spectrum inhibitory activity and the high cost of production (Fahim et al 2016).

In this paper we report on isolation and screening antibacterial bioactivities LAB isolated from various Egyptian foods against gram-positive and gram-negative pathogenic bacteria.

2 Materials and Methods

2.1 Sampling

Certain sources were tapped for isolation of lactic acid bacteria, composed of ten samples of raw milk, dairy products, pickles and fermented meat, procured from different local markets, and were immediately transferred in cool boxes to the microbiology laboratory and kept refrigerated (≈ 4 °C) until used for isolation.

Sample's physical and chemical characteristics, including color, taste, texture, pH, electrical conductivity and TDS, were examined.

2.2 Strains

All strains of LAB, (*Lactococcus (Lc) lactis, Streptococcus (St) thermophilus, Befidobacterium* sp, *Lactobacillus (Lb) casi, Lb rhamnosus, Lb. acidophilus, Lb. helveticus and Lb. plantarum).* And indicator pathogenic strains (*Escherichia coli, Salmonella typhi, Shigella sp, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, and B. subtilis),* were obtained from Cairo Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2.3 Media

MRS medium was used to isolate and propagate LAB strains (Mohammed et al 2016) and Pathogenic strains were grown on nutrient medium (Rashmi et al 2017).

2.4 Isolation of LAB

For isolation of LAB, ten ml (or 10g, where appropriate) of each sample was homogenized with 90 ml MRS broth medium and incubated at 37°C for 48 h for enrichment the total LAB (Somashekaraiah et al 2019). Then each sample was serially diluted to 10⁻⁸, using sterile solution (Khalil and Anwar, 2016). One ml from each dilution of 10⁻⁵ to 10⁻⁸ was poured onto MRS agar plates, then overlaid with MRS soft

agar (0.75% agar) and incubated after solidification at 37°C for 48 h. After appearance of separated visible growth in the MRS agar plates, well-isolated colonies with distinct cultural differences, such as differences in color, shape, size and had microaerophilic or anaerobic, were carefully picked from each plate and transferred to MRS broth (Nikita and Hemangi 2012). Each isolated colony was sub-cultured for purification.

2.5 Conformation tests of LAB

Overnight cultures of LAB pure isolates were examined by Gram staining and microscopically for morphological shape; Catalase test was carried out by adding few drops up to 1 ml of 3% hydrogen peroxide to clean test tube containing 1 ml overnight cultures within 5-10 seconds (Sangita et al 2013), and acid production from glucose fermentation was also tested (Mahantesh et al 2010). The isolates conforming to the notable characteristics of LAB (viz. Gram-positive, catalase-negative, rods or cocci and glucose fermentation-acid production) were selected for antagonistic bioactivity against chosen indicator pathogenic bacteria.

2.6 Glucose fermentation test

Fermentation test was performed in MRS broth medium containing 1% glucose (w/v) and bromothymol blue 0.1% as pH indicator and incubation at 37°C for 48h without shaking (Khalil and Anwar 2016).

2.7 Preparation of the cell-free supernatants

Inocula were prepared as follow: 0.1 m of each overnight LAB culture was transferred to 10 ml of MRS broth medium and incubated at 37°C for 48h without shaking (Al-Zahrani and Al-Zahrani 2006).

Extraction of bioactive substances was carried out according to the method described by Gautam and Sharma (2009) with minor modifications. The culture of LAB was centrifuged at 6000 rpm for 15 minutes at 4°C, and pellets were decanted while the supernatant was designated as the crude extract of bacteriocin. The crude extract pH was adjusted to 6.5 using NaOH to avoid the effects of organic acids and keep away from the isoelectric point, and then heated at 60-70°C for 10 minutes to inactivate any present enzymes, especially proteases (Simova et al 2009).

2.8 Partial purification of bacteriocin

For detection of antibacterial bioactivity of each isolate, bacteriocin was partially purified by salt saturation strategy. Solid ammonium sulphate was slowly added to the crude extract to reach 60-70% saturation and held overnight at 4°C without stirring. The mixture was centrifuged at 9500 rpm for 20 minutes at 4°C, and the pellets were re-suspended in 1 ml phosphate buffer pH 6 (Dhewa 2012).

2.9 Determination of antimicrobial bioactivity

Antimicrobial activity of bacteriocin was tested by disc diffusion method against gram-positive and gram-negative food-borne pathogens, which listed above (Savadogo et al 2004). 100 μ l of the partially purified bacteriocin were placed on a filter paper disc of 5mm in diameter. All the tests were performed on nutrient agar plates inoculated with 0.5% overnight culture of pathogenic bacteria (Rashmi et al 2017) and left for 2h at room temperature to allow suitable diffusion of the bacteriocin through the medium. Plates were incubated at 37°C for 24 h. At the end of incubation time, diameters of inhibition zones were measured (Daniel et al 2016).

3 Results and Discussion

3.1 Samples' Characteristics

Examined characteristics of the samples are listed in **Table 1**. Samples showed different physical and chemical properties, colors were found to be white for raw milk and dairy products but Luncheon and Pickles were red. Mostly tastes were sour, whereas textures were Liquid, soft or solid. The chemical properties as sample's pH values were between neutral to acidic. All samples had natural properties for each product and not expired so that they were valid for human consumption.

3.2 Isolation of LAB

This experiment aimed to isolate a number of lactic acid bacteria with antimicrobial bioactivity agent. All isolates were grown on MRS medium and a total of 71 isolates were recovered from different sources, (raw milk, dairy products, pickles and meat fermented). Products used as sources of LAB isolates are as follow:

Local yoghurt (Y): 6 isolates, Luncheon (B): 9 isolates, Raw Milk (M): 11 isolates, Commercial sweeten yoghurt (H): 5 isolates, Commercial yoghurt (R): 7 isolates, pickles (L): 11 isolates, White cheese(C): 6 isolates, Sour milk(S): 6 isolates, Processed cheese (N): 5 isolates and Fresh cheese (D): 5 isolates.

Samples	Code	Color	taste	texture	рН	E.C mS/cm	TDS ppm
Local yoghurt	Y	White Sour		soft	3.77	1.22	7.8 x 10 ³
Luncheon	В	Red	Slightly sour	solid	5.7	4.82	3.0 x 10 ⁴
Raw milk	М	White	//	liquid	6.7	3.54	2.26 x 10 ³
Commercial sweeten yoghurt	н	White	Sweeten	eten soft		0.79	5.0 x 10 ³
Commercial yoghurt	R	White	Sour	soft	4.08	1.11	7.1 x 10 ³
Pickles	L	Red	Sharp	liquid	3.4	63.2	4.9 x 10 ⁴
White cheese	С	White	Slightly sour	Liquid+ soft	4.14	37.5	2.4 x 10 ⁴
Sour milk	S	White	Extremely Sour	Semi liquid	3.91	6.2	3.9 x 10 ³
Processed cheese	N	White	Slightly sour	soft	5.5	12	1.3 x 10 ⁴
Fresh cheese	D	White	Sour	soft	4.8	49	3.6 x 10 ⁴

Table 1. Physical and chemical properties of samples

3.3 Conformation tests of Lactic acid bacteria isolates

All isolates were found to be Gram-positive and catalase negative, their shapes under the light microscope were bacilli, cocci or pleomorphic. The color of the medium changed from blue to yellow due to the formation of acids as results of glucose fermentation. Amount of acid production was compared between LAB isolates and strains **Table 2**. These are characteristics of LAB; these results were found to be similar to those reported by Khalil and Anwar (2016).

Isolates	Morphology shape	Change color	pН	Isolates	Morphology shape	Change color	pН
St. thermophilus	cocci	+++	4.6	C2	Cocci	+++	4.38
Lc. lactis	cocci	+++	4.39	C3	Cocci	++	5.05
Lb. acidophilus	Bacilli	+++	4.53	C4	Cocci	+++	4.63
Lb. casi	Bacilli	+++	4.3	C5	Bacilli	+++	4.03
Lb. rhamnosus	Bacilli	+++	4.35	C6	Cocci	+++	4.15
Lb. Plantarum	Bacilli	+++	4.5	R1	Cocci	+++	4.66
Lb. helveticus	Bacilli	++	5.05	R2	Cocci	++	4.81
Befidobacterium sp	Pleomorphic	+++	4.42	R3	Cocci	+++	4.31
Y1	Cocci	+++	4.66	R4	Bacilli	++	4.9
Y2	Bacilli	+	5.56	R5	bacilli	+	5.3
Y3	Bacilli	+++	4.49	R6	bacilli	++	4.94
Y4	cocci	+++	4.12	R7	Bacilli	+	5.32
Y5	Bacilli	+++	4.4	L1	Bacilli	+++	4.02
Y6	bacilli	+++	4.3	L2	cocci	+++	4.15
B1	Bacilli	+	5.7	L3	Bacilli	+++	4.29
B2	Bacilli	++	5.2	L4	cocci	+++	4.01
B3	Bacilli	+++	4.17	L5	Bacilli	+++	4.29
B4	Pleomorphic	+++	4.27	L6	cocci	+++	3.89
B5	cocci	+++	4.51	L7	Bacilli	+++	4.25
B6	Bacilli	+++	4.13	L8	Cocci	+++	4.00
B7	Bacilli	++	4.85	L9	Bacilli	++	4.91
B8	cocci	+	5.6	L10	Bacilli	+++	4.25
B9	Pleomorphic	+++	4.36	L11	cocci	+++	4.62
M1	cocci	+++	4.39	S1	Bacilli	+++	4.38
M2	cocci	+++	4.31	S2	Cocci	+++	4.35
M3	Bacilli	+++	4.65	S3	Bacilli	++	4.72
M4	Bacilli	+++	3.94	S4	Bacilli	+++	4.52
M5	Pleomorphic	+++	4.41	S5	Cocci	+++	4.55
M6	Bacilli	+++	4.52	S6	Pleomorphic	+++	4.36
M7	Bacilli	+	5.75	N1	Cocci	++	5.02
M8	cocci	+++	4.31	N2	Cocci	++	5.11
M9	Pleomorphic	+++	4.44	N3	Bacilli	+	5.75
M10	Bacilli	+	5.65	N4	Bacilli	+	5.59
M11	cocci	+++	4.01	N5	Bacilli	+	5.81
H1	Cocci	+++	4.41	D1	Bacilli	+++	4.04
H2	Cocci	+	5.52	D2	Pleomorphic	+++	4.29
H3	Bacilli	+	5.67	D3	Cocci	+++	4.51
H4	Cocci	+++	4.18	D4	Bacilli	+	5.7
H5	Bacilli	+++	4.22	D5	cocci	+++	3.9
C1	Bacilli	+	5.34		•	•	

Change color of pH indicator: +++ extremely, ++ moderate and + slightly

3.4 Bacteriocin purification

3.1.1 Characterization of the crude extract of bacteriocin

The bacteriocins of LAB strains and isolates were preceded for the extraction. LAB cultures were centrifuged and cell free supernatants were collected to potential display antimicrobial bioactivity. LAB secreted many substances that may, including Bacteriocins, hydrogen peroxide, enzymes, lactic acid, and aldehydes. The antimicrobial activity due to these substances must be eliminated before detection of antimicrobial bioactivity. Therefore, the cell free supernatants were subjected to the following treatments: adjusting the pH=6 to avoid acidity effect of lactic acid production, heating at 60-70°C for 10 minutes to exclude effect of enzymes then addition of ammonium sulphate to precipitated partially purified bacteriocins. Ammonium sulphate has an important role in protein precipitation; the water molecules become attracted to the salt because it's higher charge, and thus it decreases bacteriocin solubility in water, and thus can be easily removed (Gautam and Sharma 2009).

3.1.2 Characterization of partially purified bacteriocin

The partially purified bacteriocin was precipitated from cell free supernatant by 60-70 % ammonium sulfate. Partially purified bacteriocin was precipitated as a pellet after second centrifugation. The pellet showed complete insolubility in Potassium phosphate buffer (pH 7.0). This solution was used in following experiments.

4.1. Antibacterial spectrum of bacteriocins

Antagonistic bioactivity of the LAB isolates and strains against indicator pathogenic bacteria was carried out using the partially purified bacteriocin. Inhibition test of target organisms was conducted by disc diffusion method. All LAB strains and twenty-eight isolate produced antimicrobial bioactive substances. Results in **Fig 1** clearly show that Percentage of twentyeight LAB isolates from Various Egyptian Products exhibited antibacterial activity of partially purified bacteriocin against microbial indicator strains.

Raw milk contained the highest percentage of antimicrobial bioactivity LAB isolates (22%), followed by Luncheon (14%) and sour milk (14%). while white cheese had the least percentage (4%) of total bioactivity LAB isolates.

Bacteriocin activities, as diameter of clear inhibition zone, of LAB strains and isolates against the indicator strains are shown in **Figs 2, 3, 4 and 5**, these diameters were recorded after cut down 5mm which the diameter of paper disc, results were in agreement with those obtained by Mohammed et al (2016). Some isolates showed inhibitory bioactivity against only gram-positive pathogenic bacteria, others had inhibitory bioactivity against both of gram-positive and gram-negative bacteria.

Most of the inhibitory strains and isolates showed inhibition zones after incubated for 18-24h and several inhibition zones demonstrated during 48-72h incubation, this may be due to the development of resistance in Indicator microbial strains.

Data presented in **Figs 2 and 3** show evaluation of the bacteriocin as bioactive agent of LAB strains by disc diffusion method (diameter of inhibition zone), against gram-negative and gram-positive pathogenic bacteria, respectively. These data illustrate that *streptococcus thermophilus*, *Lactococcus lactis*, *Lactobacillus casi* and *Lactobacillus rhamnosus* were highly active against the tested indicator bacteria, while *Lactobacillus helveticus* produced slight bioactive substance against gram-positive pathogens and no bioactivity reaction against gramnegative pathogens.

Bioactivities of LAB isolates against the pathogenic bacteria are presented in **Figs 4-9**. Isolates which did not give any activity, i.e. no inhibition zone against any indicator pathogen, are not included in the charts.

Isolates demonstrating highly bioactivity against gram-negative and gram-positive indicator pathogens were B1, Y4, M8, M10, C1, R5, S4, S6, N4 and N5. While R5 and S6 were the isolates with the highest bioactivities against indicator pathogens.

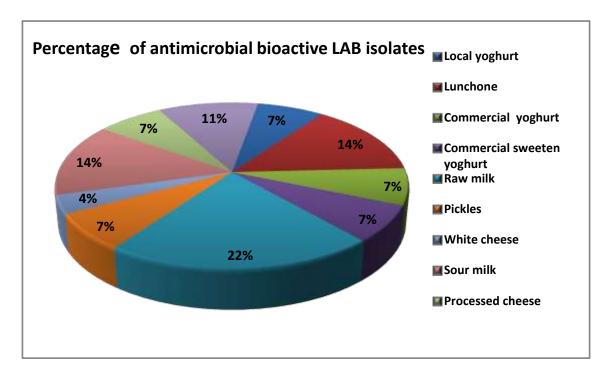


Fig 1. Percentage of antibacterial bioactive LAB isolates obtained from various foods

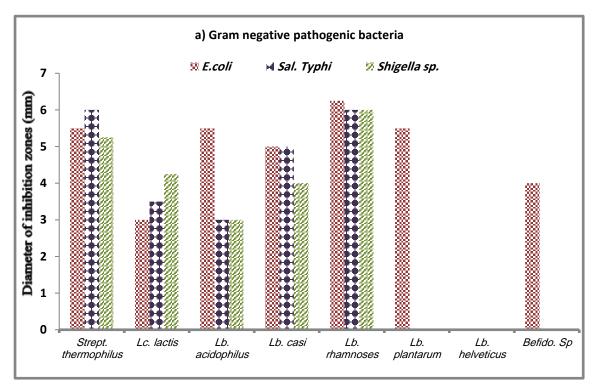


Fig 2. Antibacterial bioactivity (diameter of clear zone) of LAB strains against gram-negative pathogenic bacteria

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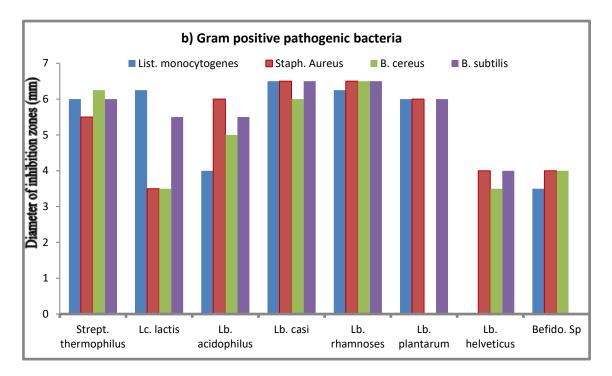


Fig 3. Antibacterial bioactivity (diameter of clear zone) of LAB strains against gram-positive pathogenic bacteria

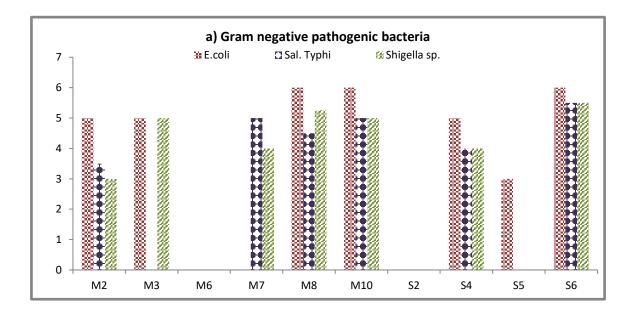


Fig 4. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from raw milk (M) and sour milk (S) against gram-negative pathogenic bacteria

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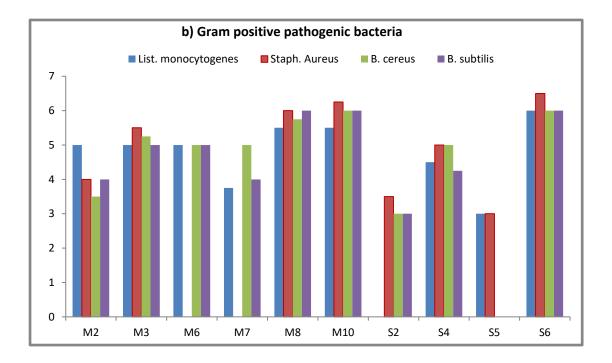


Fig 5. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from raw milk (M) and sour milk (S) against gram-positive pathogenic bacteria.

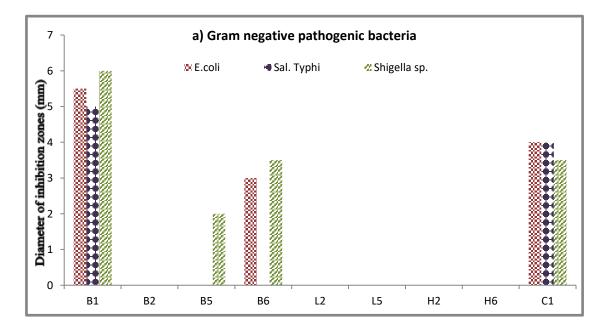


Fig 6. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from Luncheon (B), Pickles (L), Commercial sweeten yoghurt (H) and White cheese (C) against gram-negative pathogenic bacteria

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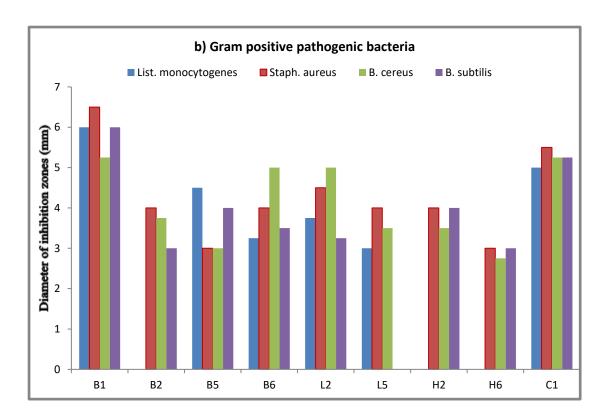


Fig 7. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from Luncheon (B), Pickles (L), Commercial sweeten yoghurt (H) and White cheese (C) against gram-positive pathogenic bacteria.

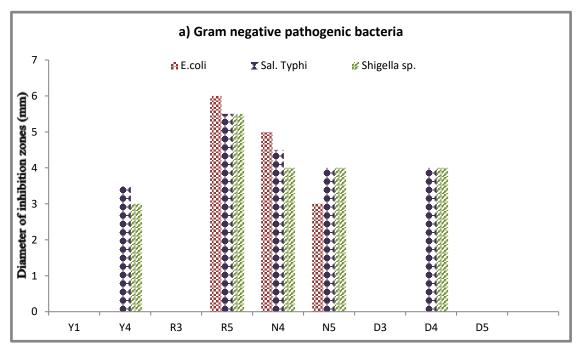


Fig 8. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from Local yoghurt (Y), Commercial yoghurt (R), processed cheese (N) and Fresh cheese (D) against gram-negative pathogenic bacteria

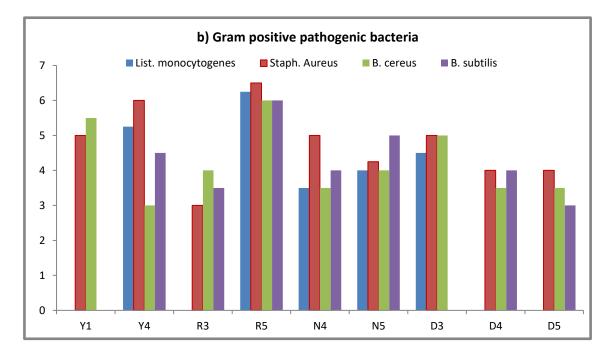


Fig 9. Antibacterial bioactivity (diameter of clear zone) of LAB isolated from Local yoghurt (Y), Commercial yoghurt (R), processed cheese (N) and Fresh cheese (D) against gram-positive pathogenic bacteria

4 Conclusion

The current investigation showed that, some Lactic acid bacteria isolates, isolated from various Egyptian foods had antibacterial bioactive potential against some gram-negative and gram-positive food borne pathogen and spoilage microorganisms. Results showed that *Lactobacillus rhamnosus* strain, R5 isolate (isolated from Commercial yogurt) and S6 isolate (isolated from sour milk) had high active antibacterial bacteriocins. Therefore, it is recommended to further identify these isolated and do more experiments to investigate utilizing them or their bacteriocins in food product and probiotic bacteria.

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النشاط الحيوى المضاد للبكتريا لبعض بكتريا حمض اللاكتيك المعزولة من منتجات مصرية متنوعة

[79]

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أستهدف هذا البحث عزل 71 عزله من بكتريا حمض اللاكتيك من عشرة عينات مأخوذة من مصادر غذائية مختلفة وهى اللبن الخام وبعض منتجات الالبان والمخلل واللحوم المتخمره فى الأسواق المحليه المصريه من اماكن مختلفه، ومقارنة هذه العزلات بسلالات من بكتريا حمض اللاكتيك المعرفه. واجراء بعض التجارب لتأكيد ان تلك العزلات ضمن بكتريا حمض اللاكتيك بصبغ جرام وبالفحص المجهرى، اختبار الكتاليز وانتاج الاحماض بتغير لون البيئه من الأزرق إلى الأصفر. واتبع ذلك أنتخاب العزلات والسلالات التى لها القدره على انتاج البكتريوسين تجاه بعض البكتريا المرتبطه بسلامه الغذاء.

المعامله بالبكتريوسين المنقى جزئياً وظهور هالات التثبيط حول البكتريا المستهدفه. حيث وجد ان هناك 28 عزله قادره على احداث هذا التثبيط باختلاف قطر هاله التثبيط والتى تدل على مدى حساسيه السلاله المستهدفه تجاه البكتريوسين المفرز فقد اختلف مدى التثبيط من ضعيف الى قوى وكذلك اختلف تجاه البكتريا الموجبه لجرام فقط او البكتريا الموجبه والسالبه معا. تم اجراء هذه التجارب مقارنه ببعض السلالات المعرفه من بكتريا حمض اللاكتيك. وقد وضح خلال هذا البحث ان افضل سلالات بكتريا حمض اللاكتيك قدرة على التثبيط كانت سلالات بكتريا حمض اللاكتيك قدرة على التثبيط كانت R5 من الزيادى المصنع تجارياً وكذلك عزله S6 من اللبن الرايب المصنع تجاريا.