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ANTIBACTERIAL SPECTRUM OF PRODUCED REUTERIN FROM NEW ISOLATES OF *Lactobacillus reuteri*

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ABSTRACT

A total number of 20 samples were collected from different local Iraqi sources. The samples were diluted and inoculated on MRS agar medium. After growth, the isolates were transferred on the modified MRS selective agar medium in order to get isolate of *Lactobacillus reuteri* which able the reuterin production. Five isolates were obtained from the human infant feces (1-3 months), which were tested by morphological, physio-biochemical assays and molecularly confirmed as *L. reuteri* by detection of the species gene specific. Electrophoresis of PCR products showed bands at the position 303pb while the sequencing and blasting results of these products were revealed 100% identity of three isolate products to a reference strain *L. reuteri* ATCC53608 and *L. reuteri* BPL-36. The fifth residual isolate was limited on physio-biochemical identification as *L. reuteri* due to failing in sequencing.
Infrared spectrum (FT-IR) of extracted reuterin from local isolated was matching with FT-IR of standard reuterin. The antimicrobial inhibitory spectrum of all five isolates was determined against 16 species of gram positive and gram-negative pathogens and food spoilage for their application in food bio-preservation. *Escherichia coli* and *Staphylococcus aureus* were observed to be the most affected among the tested organisms, whereas *L. acidophilus* and *L. plantarum* were less affected among them.

Keywords: Reuterin, *Lactobacillus reuteri*, antibacterial activity, FT-IR

INTRODUCTION

In recent years, many biologists were appeared a wide interest to study antibiotics produced by lactobacilli. This interesting was due to many reasons such as their wide inhibition spectrum for growth of pathogens and food damaging bacteria. In addition, lactobacilli antibiotics represent a natural inhibitors used in food preservation, specially acidic and neutral foods as well as foods needed for heat treatments. The antibiotics and bacteriocins of lactobacilli used commercially as a replacement natural materials against chemical preservatives, which mostly causes a great harms at the level of food safety and human health **(Niamah, 2018)**. This interesting was due to many reasons such as their wide inhibition spectrum for growth of pathogens and food damaging bacteria. In addition, lactobacilli antibiotics represent a natural inhibitors used in food preservation, specially acidic and neutral foods as well as foods needed for heat treatments **(Soomro *et al.*, 2002)**.
One of lactobacilli species is *Lactobacillus reuteri*, which found naturally in gastrointestinal tract of human, chicken and other animals. *L. reuteri* can produces of reuterin compound which is an intermediate metabolic compound produce from glycerol fermentation under anaerobic conditions and has a wide inhibition spectrum against other gram positive and gram negative bacteria , molds , yeasts and some proto-organisms **(Talapico *et al.*, 1988; Mishra, 2012) .** *L. reuteri* is a Gram-positive, non-spore forming, non-motile, facultative anaerobic, rod shaped bacillus, their cells are slightly irregular, bent rods with rounded ends, generally 0.7-1.0× 2.0-3.0 µm in size **(Kandler and Weiss, 1986)**, occurring singly, in pairs and in small clusters. The optimum growth temperature is between 37-42 °C and the optimum growth pH is about 6.5 (no growth occurs below pH 4.5). *L. reuteri* is an obligate heterofermentetive bacteria can ferment glucose in phosphoketolase metabolic pathway to produces lactate, ethanol, acetic acid and CO_2 **(Ganzle *et al.*, 2007)**. *L. reuteri* is found in types of environmental niches like sour meat, milk, dairy fermentations, and fermented vegetables and in the digestive tract as well as the urogenital tract of humans and warm-blooded animals **(Jin *et al.*, 2007; Van Coilie *et al.*, 2007)**.
Reuterin has a broad spectrum of antimicrobial activity against certain Gram-positive and Gram-negative bacteria, yeast, mold and protozoa. The Spoilage organisms also are sensitive to the reuterin such as species of *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, *Candida* and *Trypanosoma*. Reuterin is a hydrosoluble compound, active in a wide range of pH and has resistance against both of 'proteolytic' and 'lipolytic' enzymes **(Da Silva *et al.*, 2009).** The aims of this study is to determine the antibacterial activity of produced reuterin from new isolates of *Lactobacillus reuteri*.

MATERIAL AND METHODS

Chemicals

The essential Chemicals for genetic assays used in this study were agarose, Lysozyme, Master mix (Promega Co. ,USA); Boric acid,Tris-HCl, Tris base, Triton x-100, Ethedium bromide (fisher Co.,USA) ; DNA ladder 100-1500pb , Primers (Bioneer Co., Korea) ; EDTA (ethylene diamine tetra acetate),BDH Co.UK; Genomic DNA mini kit, blood /cultured cell (Geneaid Co., Taiwan). Another chemicals and pigments used in the study were Absolute ethanol, Lab M, UK; formalin, BDH, UK; Phosphate buffer saline, Oxoid,UK; Congo red, DAB.6,Germany; Gram's stain, Merck, Germany.

Commercial culture media

The commercial cultural media used in this study were MRS Agar, MRS broth, Blood agar base(Himedia,India), Simmon citrate agar (Oxoid, England), Nutrient agar (LAB,UK), Nutrient broth (CDH, India), Tryptone soy agar (Alpha, USA), Mueller Hinton agar (DCM, Netherland). All these media were prepared according to their companies and sterilized at 121°C for 15 min.

Strains of bacteria test

The bacterial cultures used in this study are shown in table 1 and used as indicator in the screening of activity and antibacterial spectrum of local *L. reuteri* isolates.

Culture conditions and assay procedure

A total number of 20 samples (infant stool and dairy products) were collected as a source of bacterial isolates. The samples were diluted at 0.9% with sodium phosphate buffer from 10^{-1} to 10^{-8}, then 0.1ml of each dilution tube were exposed to MRS agar plates by streaking method.
All isolated grown colonies were sub cultured on the selective medium (MRS agar in with 2 % sodium acetate added. The pH adjusted to 6.2 and sterilized at

121°C for 15 min. After that, 50 g/ L of vancomycin was added), and incubated anaerobically at 37ºC for 48 hour. At the same time , a sterilized mixture of 1% agar and 2% glycerol was prepared and stored at 50ºC in water bath on order.

Table 1 bacterial cultures used in the study

No.	Bacteria straians	sources
1	*E.coli*	postgraduate laboratories at the College of Agriculture, University of Basrah
2	*Staphylococcus aureus*	
3	*Klebsiella pneumoniae*	
4	*Bacillus subtilis*	
5	*Pseudomonas aeruginosa*	
6	*Micrococcus* sp.	
7	*Lactobacillus acidophilus*	
8	*Lactobacillus plantarum*	
9	*Staphylococcus epidermidis*	AL-Sadr educational hospital at Basrah
10	*Streptococcus. pyogenes*	
11	*Bacillus* sp.	
12	*Diplococcus* sp.	
13	*Klebsiella* sp.	postgraduate laboratories at the College of Science, University of Basrah
14	*Enterococcus* sp.	
15	*Listeria* sp.	
16	*Clostridium* sp.	Research laboratory at the College of nursing , University of Basrah

The agar-glycerol mixture was then poured directly above grown colonies in selective medium and the plates incubated anaerobically at 37ºC for 1 h. After that, 5ml of DNPH (2,4-Dinitrophenylhydrazine, Oxoid, England) solution for 3 min. and 5mol/L of potassium hydroxide for 30 sec. were added to the medium respectively. The appearance of reddish brown zone around colonies indicate to a positive result **(Ortiz-Rivera *et al.*, 2017)**.

Morphological and biochemical tests

The morphological characteristics of the colonies were determined on different agar plates as well as the preparation of gram-stained smears from the active cultures, according to standard gram staining procedure, to determine the shape, arrangement and size of individual bacterial cells. Identification of pure colonies was carried out by performing growth at (10, 30, 37 and 42)°C for 72 h, growth of pH values (4.2, 6.2, 7.2 and 9.2), growth in 6.5% NaCI, growth on Tellurite Agar (TA). Citrate utilization, gelatinase production, catalase production, resistance to 60°C, motility and resistance to vancomycin. The methods that reported in both of **Mishra, 2012; Ariff *et al*., 2015**.

16S rRNA of isolates

PCR method for amplifying the 16S rRNA gene of bacterial isolates was accomplished according to that reported in **Garg *et al*., (2009).** The primer used to amplify the specific gene was F-CAGACAATCTTTGATTGTTTAG for forward and R-GCTTGTTGGTTTGGGCTCTTC for reverse. The PCR reaction was carried out in PCR tubes having 25µl reaction mixture including PCR ready to use master mix (12.5 µl), template DNA (5µl), forward primer (1µl), reverse primer (1µl) and Nuclease free water (5.5µl). The amplification program involved an initial denaturation at 95˚C for 10 min, followed by 40 cycles of denaturation at 94˚C for 15 sec, annealing at 60˚C for 1 min, extension at 72˚C for 15 sec and followed by a final extension at 72˚C for 10 min. After detection of PCR product by electrophoresis, the preparation, purification and sequencing of products were done at BIONEER Company, South Korea. All sequencing products were then exposed to treatment and re-correction before they tested in the "BLAST" providing by the National Center for Biotechnology Information Service (NCBI) http://www.ncbi.nlm.nih.gov **(Kerbauy *et al*., 2011)**.

Production of reuterin supernatant

Reuterin was produced from *L. reuteri* cultures using methods described elsewhere **(Schaefer *et al.*, 2010)** with slight modifications. Briefly, overnight grown cultures of *L. reuteri* were inoculated in MRS broth tubes and incubated at 37°C for 20 h in anaerobic conditions. After that, cells from each single broth tube were harvested by centrifugation and washed twice with 50 mM sodium phosphate buffer (pH 7.4). The cells were then re-suspended in 15 ml 250 mM glycerol-water solution and transferred to 15 ml screw-capped tubes with making a solution of water-glycerol without reuterin was used as negative control. The tubes of cell-glycerol suspension were then incubated at 37 °C for 2 h. in anaerobic conditions. After that, cells were removed from the suspension by centrifugation and filtering the supernatants through 0.22mm pore-size filters (Millipore). The resulting cell-free supernatant was then stored in refrigerator at -4°C before it analyses by FT-IR test to be contain reuterin and glycerol.

Inhibitory activity spectrum of reuterin

The activity of reuterin-containing cell-free culture supernatants were determined for their antibacterial inhibitory spectra against a broad range of Gram-positive and Gram-negative indicator strains (table 1). 0.1 milliliter (10^6 CFU/ml) of 20 hours old cultures of each indicator bacterial cultures was spread on Mullar Hinton agar plates independently, and then 6-millimeter wells were accomplished onto agar plates. The agar wells were then filled with 0.05 milliliter of the reuterin cell-free supernatants and kept undisturbed for 2 hours before the plates were subsequently incubated at 37ºC for 24 hours. All these experiments for assaying inhibitory activity were performed in triplicate and the zones of growth inhibition around the agar wells were measured by taking the mean of diameters (mm) for each indicator bacterial strains **(Niamah,2010)**.

Statistical analysis

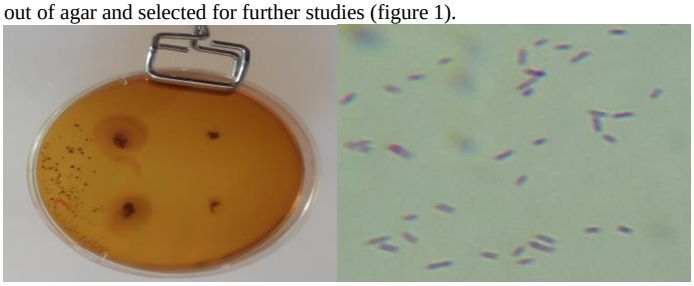
Statistical analysis of treatments was proceed by using the SPSS Statistics V22.0 (Statistical Package for Social Sciences, USA). Analysis of variance (ANOVA table) of the data was conducted and means for treatments values were analyzed ($P \leq 0.01$) with Least Significant Difference (LSD). Differences were considered significant at $P \leq 0.01$.(**Montgomery, 2017**).

RESULTS AND DISCUSSION

In the present study we obtained a results included; the locality isolation of four *L. reuteri* strains, from human infant stool samples, which identified by morphological, physiochemical and molecular assays; studying the antimicrobial activity of these isolates. A total number of 20 samples were collected from different local sources included of 8 breast fed human infant feces (1-3 months), 3 specimens of human women milk, 3 specimens of white cheese, 3 specimens of yoghurt, and 3 specimens of vinegar.

Cultural morphology and microscopically characters

All samples plated on MRS agar were resulted to growing of small, cream color, sticky and oval shape colonies. The isolated bacterial cells were found to be short middle in size, purple colored, Gram-positive rods with rounded ends might be found as single cells, pairs or small aggregations under microscope. Hanging drop technique was also performed to test the ability of rod bacterial cells to motile where no motility observed for all five isolates. After transferring of grown colonies from MRS agar and cultivation on selective agar (modified MRS) by stabbing method , the process resulted to obtain of 5 positive grown isolates based on colonial morphology (brown in color with reddish- brown zone around colonies). **Rodriguez *et al*. (2003)** was illustrated that this color is due to the acrolein formed by dehydration of β-hydroxypropionaldehyde (reuterin), so provides a fast and simple tool for screening of reuterin-producer isolates. The five-reuterin producing colonies, each from a different isolate, were picked up out of agar and selected for further studies (figure 1).



A **B**

Figure 1 (A) Reddish brown zone around some colonies grown on a selective MRS medium and (B) microscopically features of isolated *Lactobacillus* bacterial cells.

Physical and biochemical characteristics of isolates

Table (2) shows the most physical and biochemical characteristics for identification of the five selected isolates to be most likely belonged to the *Lactobacillus* group, in comparing with previous studies and Bergey's manual of systematic bacteriology **(De.Vos and Garrity, 2009)**.

The five selected bacterial cultures were gave a turbidity after incubation in MRS broth at (10, 30, 37 and 42)**°C** for 72 h. As an indication of microbial growth. The best absorbance of growth turbidity was at 37°C. When the same isolates were incubated at 30°C and 42°C for 72 h. The absorbance of growth turbidity was at lowest degree than 37°C; whereas growth at 10°C was weaken than other previous test temperatures. These growth results at four different temperatures gave an indicates that the isolates could belong to *Lactobacillus* group.

The bacterial cultures of selected isolates were also tested for their growth at different pH levels, where a turbidity appeared after incubation in MRS broth of pH (4.2, 6.2, 7.2 and 9.2) for 72 h referring microbial growth. The best absorbance of growth turbidity was at pH 6.2 whereas the absorbance of growth

turbidity at pH 4.2 was at lowest degree than pH 6.2. On the other hand, absorbance of growth turbidity at pH (7.2 and 9.2) was weaken than previous test pH. These growth results at four different pH gave further indicates that the isolates could belong to *Lactobacillus* group **(Zhoa and Ganzie, 2018)**. All five selected isolates were appeared the ability of their growth at 6.5% NaCl in MRS broth where the absorbance of turbidity referred to a positive results. After incubation at 60ºC for 30min, spectrophotometric measurements of MRS broth cultures of all study isolates revealed a very little absorbance, equal to zero, of growth turbidity which indicate that no cells were grown due to heating treatment. This further test gave positive results of study isolates to be belong to *Lactobacillus* group where a black colored colonies grown on all Tellurite agar plates isolates after incubation period **(Ganzle, 2015; Pallin *et al.*, 2016).**

All study isolates were negative results for this test where utilization of citrate was done from these isolates. Inoculated with study isolates, was returned to solidify when it leaved for a period in the laboratory temperature. This referred to a negative result and the isolates were not producing of gelatinase enzyme. All study isolates were of α- hemolysis type depending on green zone that appeared around growing colonies on each TSA plates. The five selected isolates were found to be negative for catalase enzyme production **(De.Vos and Garrity, 2009)**.

Table 2 Physiological and biochemical tests of five selected *Lactobacillus* isolates

No	Tests		Bacterial Isolates				
			Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
1	°C	10	+	+	+	+	+
		30	++	++	++	++	++
		37	+++	+++	+++	+++	+++
		42	++	++	++	++	++
2	pH	4.2	++	++	++	++	++
		6.2	+++	+++	+++	+++	+++
		7.2	+	+	+	+	+
		9.2	+	+	+	+	+
3	Growth in 6.5% NaCl		++	++	++	++	++
4	Resistance to 60°C		–	–	–	–	–
5	Growth on TA medium		+	+	+	+	+
6	Citrate utilization		–	–	–	–	–
7	Gelatinase production		–	–	–	–	–
8	Hemolysis		+ (α)	+ (α)	+ (α)	+ (α)	+ (α)
9	Catalase		–	–	–	–	–
10	Motility		–	–	–	–	–
11	Resistance to vancomycin		+++	+++	+++	+++	+++

+++ =V. good growth, ++ = good growth, + = weak growth, - =Negative growth,(+ α)= positive (alfa)

16S rRNA of *Lactobacillus* isolates

The total DNA was used as template for molecular identification of the five isolates. PCR products for the genus specific primer showed bands on agarose gel at the position 303bp compared with the standard molecular DNA ladder of 100-1000 pb (Figure 2). These results were in agreement with **Petrova *et al.* (2017)** who also demonstrated the amplification of 303 bp products in the PCR assay with all isolates of used *Lactobacillus reuteri*.

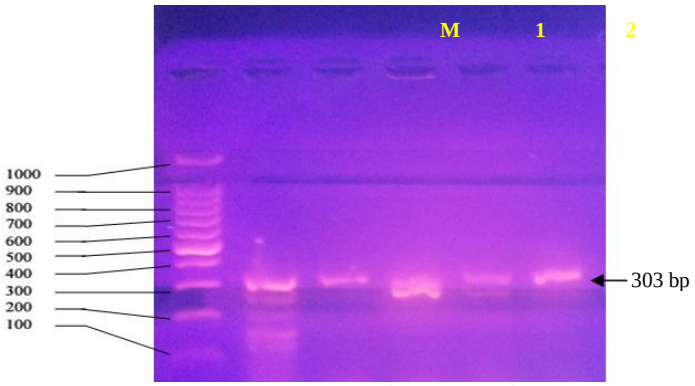


Figure 2 Agarose (2%) gel electrophoresis of 303 bp specific gene PCR products for *L.reuteri* isolates under UV transilluminator. Lane (M): 1Kb (100 bp – 1000 bp) DNA ladder. Lane 1: isolate1 . Lane 2: isolate 2 . Lane 3: isolate 3 . Lane 4: isolate 4 . Lane 5: isolate 5 .

Table (3) showed out of alignments specific gene sequences for five isolates of *L. reuteri*. Four isolates from infant's stool were identified depending on their specific gene sequencing in comparing with identical reference strains. Three isolates were revealed 100% identity to a reference strain *L. reuteri* ATCC 53608 and one isolate revealed 100% identity to a reference strain *L. reuteri* BPL36 when treated and tested in European Nucleotide Archive (ENA), National Centre for Biotechnology Information (NCBI) and Gene Bank .

One PCR product from the five that sent to BIONEER Company was failed in sequencing. The peaks of sequence of this product were overlapped with each other so it was difficult to read and treatment. Hence, the identification of this product's isolate was restricted to morphological and biochemical test results. The reason of fail of their sequences may be due to the purification step of PCR product from salts and minerals before sequencing or may be a result to colorization step during sequencing process, where the non-good staining PCR product will causes of interaction between nitrogen bases which be undistinguished by sequencing device leading to obtaining of rubbished sequence. Purification of PCR product was very important to obtain a clear single band in electrophoresis, so the unpurified gene will cause problems during sequencing which lead to difficult reading in Blast program **(Ma and Difazio, 2008)**.

Infrared spectrum of extracted reuterin

Several distinguished peaks were appeared in the IR absorption spectrum of extracted reuterin compound, table (4) and figure (3). In addition, analysis of these peaks were illustrated their agreement with what reported in **Burg´e *et al.* (2015)**. The most important bands were ; the stretch broad band of alcohol hydroxyl (-OH) group at the frequency position 3423 cm^{-1} and are medium to strong ; the stretch band of (-CH) bond at the position 2856 cm^{-1} , which represent Aldehyde group and are weak, sharp. As well as (C=O) stretch band of aldehyde was distinguished at the frequency 1724 cm^{-1} which mostly are medium , sharp and saturated. In addition, a stretch band of (CH) bond deal with Alkane was found at 2925 cm^{-1} were medium to strong, sharp. Moreover, a stretch band of methyl (-CH) bond was appeared at frequency 1406 cm^{-1} which has variable appearance in the spectrum. The stretch band of (C-O) appeared in three frequencies represents, alcohol at 1260 cm^{-1} which was strong and sharp, Ether at 1101 cm^{-1} was strong, and finally, Ester group was appeared at 1026 cm^{-1} and was medium. The most functional groups that are clearly determine the reuterin compound structure from other near compound are the alcohol hydroxyl group and the aldehyde carbonyl group. The alcohol OH stretch is broader because single bonds can stretch and bend While the aldehyde C=O stretch is sharp and "well-defined because double bonds can stretch, but do not bend very well.

Table 3 Sequencing results of specific gene and universal *16SrRNA* for *L. reuteri* isolated from local sources.

No.	No. of isolate	Bacterial specie	Nucleotide sequence	Source	Identical with reference strain	bp
1	3	*L. reuteri*	GCGCATGGTGAATGCCTTGGTACTAGGAG CCGATGAAGGACGGGACTAACACCGATA TGCTTCGGGGAGCGGTAAGTACGCTTTGA TCCGGAGATTTCCGAATGGGGCAACCCAA TCAGCTTAGTCGCTGATTACTTGACTAGT GAATACATAGCTAGCAAGAGGTAGACGC AGTGAACTGAAACATCTTAGTAGCTGCAG GAAGAGAAAGAAACATCGATTCCCTGAG TAGCGGCGAGCGAAAAGGGAAGAGCCCA AACCAACAAGCAGGATTTATATTTTTGAT TGTTTTTTGAATTATTTTGATTTCAATA	infant's stool	ATCC 100%	268
2	1	*L. reuteri*	ATGGTGAATGCCTTGGTACTAGGAGCCGA TGAAGGACGGGACTAACACCGATATGCTT CGGGGAGCGGTAAGTACGCTTTGATCCGG AGATTTCCGAATGGGGCAACCCAATCAGC TTAGTCGCTGATTACTTGACTAGTGAATA CATAGCTAGCAAGAGGTAGACGCAGTGA ACTGAAACATCTTAGTAGCTGCAGGAAGA GAAAGAAACATCGATTCCCTGAGTAGCG GCGAGCGAAAAGGGAAGAGCCCAAaCCA ACAAGCATGC	infant's stool	BPL36 100%	264
3	1	*L. reuteri*	Failed to sequencing	infant's stool	-	-

Table 4 The bands and their structural groups in the IR spectrum of extracted and standard reuterin.

Functional group	Bonding	Vibration type	Band frequency (cm^{-1})	
			Band of extracted reuterin	Wavelength range (cm^{-1})
Alcohol	-OH	Stretch.	3423 cm^{-1} S.Br.	3200–3600
Aldehyde	-CH	Stretch.	2856 cm^{-1} W.	2850–3000
Alkanes	-CH	Stretch.	2925 cm^{-1} M	2878–2990
Aldehyde	C=O	Stretch.	1724 cm^{-1} S.	1720–1740
CH of CH_3	-CH	Stretch.	1406 cm^{-1} V.	1350–1480
Alcohol	C-O	Stretch.	1260 cm^{-1} S.	1000–1300
Ether	C-O	Stretch.	1101 cm^{-1} S.	1031–1118
Ester	C–O	Stretch.	1026 cm^{-1} M.	1000–1300

*** Br = broad; W=weak; M= medium; S= strong; V = variable**

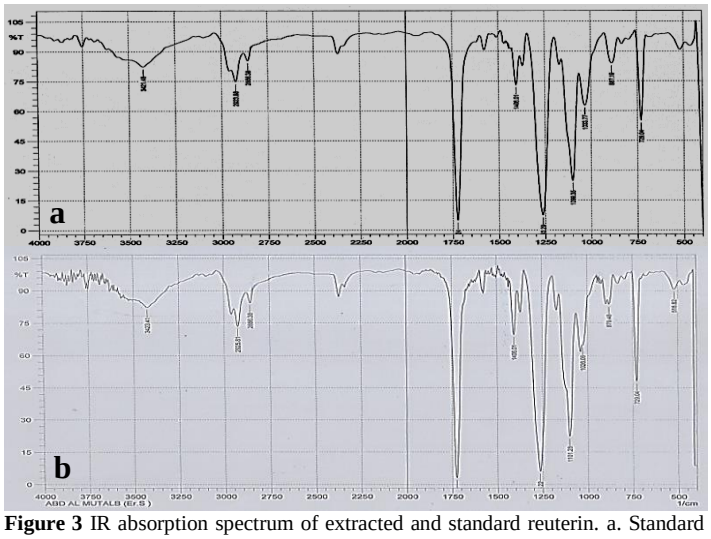


Figure 3 IR absorption spectrum of extracted and standard reuterin. a. Standard reuterin, b. extracted reuterin produced from local isolates.

Antibacterial activity spectrum of *L. reuteri* isolates

Table (5) and figure (4) show the inhibition effect of the culture supernatant, of the five *L. reuteri* isolates, against a wide range of Gram positive and Gram negative indicator bacterial isolates. The test was included *L. reuteri* itself and two bacterial species that classically related to it, as well as, 16species of food spoilage and food borne pathogen as indicator bacteria. The spectrum was showed a weak inhibition zones (< 10 mm) to strong inhibition zones (>20mm) while the range in between was divided to intermediate inhibition zones (from 10mm up to 15mm) and less strong inhibition zones (from 16 mm up to 20mm). On the other hand, no sensitivity shown when we subjected the five *L. reuteri* isolates to the culture supernatants of each to other. Therefore, this refer to reuterin has no effect on the producer *L. reuteri* itself. Our results were agreement with many other studies, which indicated the antibacterial ability of reuterin. **Mishra (2012)** was stated *L. reuteri* as all other lactic acid bacteria has been reported to produce various organic acids during fermentation, such as lactic acid and acetic acid , which lead to lowering of pH in the gastrointestinal tract. The organic acids besides the production of reuterin gave *L. reuteri* a strong antagonistic effect, where they served as potent antibacterial agents against pathogenic bacteria **(Cleusix *et al.*, 2007)**. The antagonistic effect of *L. reuteri* as well as their ability to survive at lower pH were considered beneficial in maintaining general health of the gastrointestinal tract and female genital tract of the host. The reuterin produced from *L. reuteri* DPC16 (Human source) had potent antibacterial activity against both Gram positive and Gram negative pathogenic bacteria such as *S. typhimurium*, *E. coli* 157:H7, *S. aureus* and *L. monocytogenes* **(Bian *et al.*, 2011)**. The supernatant of 14 *L. reuteri* (Iranian source) had antimicrobial activity against two indicator bacteria (*Salmonella enteritidis* and *S. typhimurium*) **(Royan *et al.*, 2018)** . Reuterin is a mixture of three dimeric forms of the 3-hdroxypropionaldehyde (3-HPA) compound. This compound can be transformed in aqueous solution to the compound acrolein , which has been considered as a toxic material and is able to reacts with other compounds that present in the food products .The mechanism of action of reuterin compound, spends its antimicrobial effects, has stay elusive. In study, we supply evidence that reuterin induces oxidative stress in microorganism cells, most probable by changing thiol groups in protein compounds and other small molecules in the cells. The action mode of reuterin's antimicrobial activity has been suggested to be an imbalance in cellular redox state produced from reaction of 3-hdroxypropionaldehyde with thiol groups of proteins, rising the reduction of glutathione and changes in proteins complex in clusiving functional enzymes. In addition to its well investigated inhibition activities, reuterin compound is

involved in the conjunction of heterocyclic amines, apparition of possible contacts to the bioavailability of toxicant compound against microorganisms in the human intestine **(Engels *et al*., 2016)**. The supernatants exhibited a broad spectrum of inhibitory action against aerobic and anaerobic spore-forming bacteria **(González *et al*., 2019)**.

Table 5 Antibacterial activity spectrum of the five *L. reuteri* culture supernatants

No.	Strains test	LR1	LR2	LR3	LR4	LR5
1	*E.coli*	++++	++++	++++	++++	++++
2	*Staphylococcus aureus*	++++	+++	+++	++++	++++
3	*Klebsiella pneumoniae*	++	++	++	++	++
4	*Bacillus subtilis*	+++	+++	+++	+++	+++
5	*Pseudomonas aeruginosa*	+++	+++	+++	+++	+++
6	*Micrococcus* sp.	++	++	++	++	++
7	*Lactobacillus acidophilus*	+++	++	++	++	++
8	*Lactobacillus plantarum*	+++	++	++	++	++
9	*Staphylococcus epidermidis*	+++	+++	+++	+++	+++
10	*Streptococcus. pyogenes*	++	++	++	++	++
11	*Bacillus* sp.	+	+	+	+	+
12	*Diplococcus* sp.	+	+	+	+	+
13	*Klebsiella* sp.	++	++	++	++	++
14	*Enterococcus* sp.	++	++	++	++	++
15	*Listeria* sp.	++	++	++	+++	++
16	*Clostridium* sp.	++	++	++	++	++

- = no inhibition; + < 10mm ; ++ = 10-15mm ; +++ =16-20mm ; ++++ >20mm

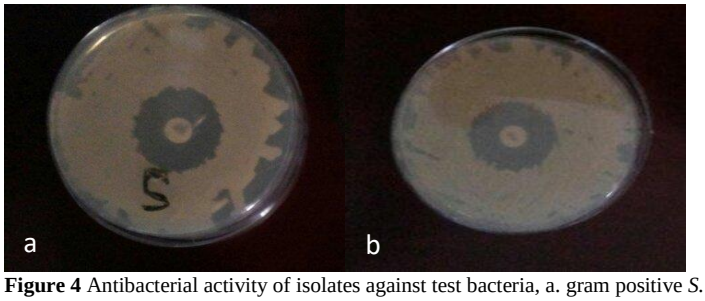


Figure 4 Antibacterial activity of isolates against test bacteria, a. gram positive *S. aureus* b. gram negative *E. coli*.

CONCLUSION

Lactobacillus reuteri strains are among the primary microbiological barriers in human body against the infection by intestinal pathogens. They were isolated from infant feces. 16S rRNA test shows to match between local isolates and strains *L. reuteri* ATCC53608 and *L. reuteri* BPL-36. They have a strong potential to produce of inhibitory substance, such as reuterin, which have a wide antimicrobial spectrum including those have near genetic relationship. The produced reuterin from local isolates had higher inhibition spectrum against 16 types of bacteria strains.

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