

Antimicrobial Effect of *Lactobacillus* as a Probiotic Isolated from Yoghurt Products Against *Staphylococcus aureus* and *Escherichia.coli*

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Abstract

Probiotic bacteria using in commercially produced foods are at most members of the genera *Lactobacillus* and *Bifidobacterium*. This research was aimed to study the antimicrobial effect of lactobacillus bacteria as probiotic against some pathogenic bacteria, strains of *lactobacillus* bacteria were isolated from two types of yoghurt in Baquba city, Iraq. Total of 60 yoghurt samples from 2 origins (30 locally and 30 commercial produced yoghurt) were used to isolate probiotic bacteria which were identified phenotypically and biochemically. 30 (100%) of locally produced yoghurt samples include with probiotic bacteria while 12 (40%) commercially types of yoghurt samples were contained with probiotic, the method of modified agar overlay was used to determine the antibacterial activity among the *lactobacillus* isolates. Results indicated spectrum of their antibacterial effects which were varied against the selected pathogenic bacterial isolates (*Staphylococcus aureus* and *Escherichia.coli*). The results of this study encourage people to consume more probiotic dairy products instead of using antibiotic drugs as prophylactics which lead to health problems also encourage local industry for producing bioyoghurt products.

Key words: Probiotics, *Lactobacillus* spp., Antibiotic effect, Yoghurt, Pathogenic bacteria.
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1. Introduction

Probiotics are live microorganisms mainly bacteria thought to be safe and useful to the host according to the currently approved definition by the United Nations Food and Agriculture Organization and the World Health Organization FAO/WHO (2001:2002) [1]. Most probiotic microorganisms belong to Lactic Acid Bacteria (LAB), such as *Lactobacillus* spp., *Enterococcus* spp and *Bifidobacterium* spp. [2]. Beneficial effects accorded by lactobacilli include inhibition of pathogenic organisms, such as *Helicobacter*, *Salmonella* and *Shigella*. Although the notion of using bacteria as bio therapeutic factors is not new, probiotic lactic acid bacteria (LAB) have attracted attentiveness only in recent years [3,4]

Probiotic LAB include (*L. casei*, *L. johnsonii*, *L. rhamnosus*, *L. reuteri*, *L. acidophilus* and *L. gasseri*) and Bifidobacteria include (*B. lactis*, *B. bifidum*, *B. infantisa* and *B. longum*) are the most common types of bacteria used as probiotics and are almost used in food industry, but certain yeasts and some bacilli may also be used [1].

Most reports have described the advantageous effects from different aspects on important pathogenic cases, like inflammatory bowel disease (IBD), intestinal infections and allergy by supplementing of some selected strains to food products, often together with a fiber or prebiotic substance [2]. A number of reports have found probiotic exhaustion to be useful in the treatment of many forms of diarrhea, including diarrheal diseases in young children caused by rotaviruses, travelers' diarrhea and antibiotic-associated diarrhea in adults [5].

Lactobacilli can produce various antimicrobial ingredient including organic acids (Propionic acid, lactic acid and acetic acid) carbon dioxide, hydrogen peroxide, low-molecular weight antimicrobial substances, bacteriocins and adhesion inhibitors have obtained significance as probiotics [6]. Probiotics are implicated in synthesis of biotin, vitamin K and B and in ions absorption as Mg^{+2} , Ca^{+2} , and Fe^{+3} [7]. Furthermore, some LAB have antimicrobial properties against pathogens, spoilage bacteria, molds, yeasts and viruses by different mechanisms [1,8].

Increasing in bacteria resistant to antibiotic has awakened the scientists to the therapeutic and prophylactic uses of probiotics and to use them as alternatives to antibiotic drugs [9]. The use of naturally produced antimicrobial agents without any counter effects on human health to inhibit the generation of pathogenic agents in food is a good option to overcome the problems related with food contamination. [10,11].

The objective of this study was to present some data on isolation of *lactobacillus* bacteria from two types of yoghurt and study their antimicrobial activity as a probiotic bacterium against *Escherichia coli* and *Staphylococcus aureus* as an example to gram negative and gram positive pathogenic bacteria sequentially.

2. Material and Methods

Sampling:

Sixty samples of yoghurt were collected from different local markets in Baquba city, Iraq. Samples were put into sterile plastic containers and transferred to the laboratory of

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Microbiology at Department of Pathological Analysis, Baquba Technical Institute, Middle Technical University. They were from two origins (30 of commercially imported types and 30 of locally yoghurt) these samples are kept in the refrigerator for 2-4 h until analysis was proceeded.

Preparation of yoghurt samples:

For suspending the bacterial content, samples of yoghurt were shake strong. Then, ten grams of each sample were dissolved into 90 ml of normal saline 0.9% w/v [12].

Isolation of lactic acid bacteria (LAB):

De Man Rogosa Sharp (MRS) medium is the standard media which have been accepted by the International Dairy Federation for isolation of lactic acid bacteria. After arrival of the samples to the laboratory within 3-6 h . The prepared yoghurt samples were inoculated into MRS agar medium. plating was done then incubated anaerobically in anaerobic jar at 35°C for 48 h. The suspected colonies were enumerated (cfu/ml) and purified by multiple streaking on MRS agar medium before identification. [12,13].

Phenotypic Characterization of LAB:

The purified isolates were identified on the basis of their morphological, cultural and biochemical characteristics. Stock culture were kept on MRS agar slant at 4°C and have been streaked every 4 weeks. [13, 14] .

Pathogenic bacterial isolates :

The pathogenic isolates used in this study were preserved in brain heart infusion (BHI) agar butt-slants in tubes kept at 4°C. They included both Gram-negative (*E.coli*) and Gram-positive (*S. aureus*) provided by Microbiological laboratory in the Department of PATHOLOGICAL ANALYSIS, BAQUBA TECHNICAL ENISTITUTE –MIDDLE TECHNICAL UNIVERSITY (MTU).

Determination of antibacterial effects :

The stock cultures including probiotic Lactobacilli bacteria were inoculated into brain heart infusion (BHI) broth. The turbidity of this broth was adjusted to equal of 1 standard McFarland.

The chosen pathogenic bacterial isolates taken from the stock cultures and then subcultured in BHI broth under aerobic condition for 18h at 37°C. The turbidity of broth cultures was adjusted to 0.5 standard McFarland. To determine the antibacterial effects of probiotics isolates, modified agar overlay method have been used. The prepared probiotic Lactobacilli was inoculated into the plates by swabbing in the center of plate, then were anaerobically incubated for 72 h at 37°C. Then the growth in the plate was overlaid with 10 ml of molten and cooled BHI Agar inoculated previously with 1 ml of the selected cultures of pathogens. The plates were aerobically incubated for 24 h at 37°C after being allowed to solidify. The plates were examined for the presence of growth inhibition. For more determination whether the selected pathogens inhibited or killed by probiotics, the zone of growth inhibition was swabbed. Then the swab was inoculated into BHI broth and aerobically incubated for 24 h under 37°C. The BHI broths were checked for growth. The presence of growth in the broth was accepted as an inhibitory property in the agar plate, while no growth was cleared to be as a result referring to the bactericidal effect. For determination of antibacterial effects of the probiotics each test was duplicated. [15,16,17,18].

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3. Results and Discussion

The interesting in foods that are fresh, natural and healthy are increased by consumer after being aware of processing loss of nutritional value and the possible health risk of foods concluded with variety of chemical preservatives. Probiotics could be a beneficial strategy in prevention of antibiotic side effects which may lead to disturbances of the intestinal homeostasis and disorders [3].

Table-1 showed that out of 60 examined samples of yoghurt, 30 (100%) of locally produced yoghurts and 12 (40%) of imported yoghurt samples, were contained with LAB that isolated depending on macroscopic and microscopic properties of isolates. They produced convex, yellow, round and moist colonies with smooth edges. Smears after stained with Gram stain, bacteria appeared as gram-positive bacilli in pairs or chains, proportionate with the morphology of *Lactobacillus* spp. [5]. These findings almost agree with Abdallah et al. (2013) [1]. 100% of locally produced yoghurt samples included with probiotic bacteria while 40% of commercially types of yoghurt samples were contained with probiotic bacteria. As shown in table 1: the viable numbers of the LAB present in the examined samples the mean count (Log cfu/ml) of total LAB in examined samples were 7.00 ± 0.70 and 5.00 ± 0.40 in locally produced and imported yoghurt samples respectively. To some extent higher findings were stated by (Dardir 2012) [19].

The minimum amount of probiotics needed to obtain a clinical effect has not been established. Some researchers recommended dosage of 10^9 – 10^{10} cfu/g as a minimum consideration for healthy enteric system [20].

Table (1): Occurrence and frequency distribution of examined yoghurt samples (n=60)

Yoghurt sample type	No. of positive samples	Percentages of positive samples	No. count of lactobacillus
Locally produced yoghurt	(30) 30	100%	7.00±0.70
Imported types of yoghurt	(30) 12	40%	5.00±0.40

Determination of Antibacterial Effects:

Determining the antibacterial effects of probiotics is one of the most selected important standards, which supports the development of foods containing these bacteria as possible solutions to improve health of public consumer.

The colonization or infection of the intestine by pathogenic bacteria such as *Shigella*, *Clostridium*, *Campylobacter*, *Klebsiella*, *Escherichia*, *Pseudomonas*, *Streptococcus*, *Salmonella*, *S. aureus*, *Enterococcus* and coagulase-negative staphylococci increases the risk of necrotizing enterocolitis. Many reports showed that the incidence of necrotizing enterocolitis has been decreased when nonpathogens, such as lactobacilli and bifidobacteria colonize the intestine. [21].

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All tested human *Lactobacillus* strains were able to inhibit the growth of strains of anaerobic gastrointestinal pathogens [22].

Table (2): Antibacterial effects of *Lactobacillus* spp. against *Staphylococcus aureus* and *E.coli* .

Type of yoghurt sample	Antibacterial effects	
	<i>E.coli</i>	<i>Staphylococcus aureus</i>
Locally produced yoghurt	Bacteriostatic	Bacteriocidal
Imported types of yoghurt	Bacteriostatic	Bacteriostatic

Results of antibacterial effects using the modified agar overlay method as shown in table-2 results which refer that all isolates of probiotic which isolated from the different yoghurt samples were able to inhibit the growth of some, if not all of the selected pathogens. Because of lacking standard bacteriostatic and bacteriocidal effects as rates for probiotics, we used culturing of microorganisms from inhibition zone to determine whether the effect of probiotic was bacteriostatic or bacteriocidal for the pathogenic bacteria used in this study.

There was variation in bacteriostatic and bacteriocidal effects. *Lactobacillus* as probiotics isolated from locally yoghurt samples appear to have bacteriocidal effect for *S. aureus* while inhibited the growth of *E. coli*. Similar findings were described by Abdallah et al. 2013[1]. who showed that isolated probiotic strains from dairy products were able to inhibit the growth of pathogenic *E. coli* , also agree with Catherine and Cabrera (2011) who relay almost the same results [23].

The adhesion of LAB to host intestinal epithelium which result in the competitive adhesion of pathogenic bacteria is one of the factors giving the property of antibacterial activity to probiotics [24]. The mechanism of inhibition on the pathogen invasion might also be due to obstruct enterocytic pathogen receptors by whole *Lactobacilli* rather than to a specific blockade of receptors [25]. Many studies reported that the antagonistic activities against intestinal pathogens are produced by antimicrobial substances contained by several probiotic strains. They might reduce both the duration of illness and the frequency of stools. A study showed that lactic acid bacteria isolated from dairy products suppress the growth of some pathogens like *Salmonella typhimurium* and *Salmonella enteritidis*, which considered as the main cause of *Salmonella* food infection [26] .

Recently, many researches indicated the applicability of probiotic bacteria as an alternative bio-therapeutic therapy for and protection against some pathogenic infections. Although, probiotics have a totally safety record, they should be used with caution in certain patient groups particularly with immune deficiency patients, neonates born or prematurely [1, 5]. A number of criteria must be studied in selecting bacterial strains which used as functionally probiotic in foods, one of the most important among them is having the ability to produce antimicrobial substances leading to increase the natural defenses of the host against enteropathogens and through competitive inhibition of these pathogens [27]. Some strains of LAB bacteria like *Lactobacillus paracasei* could be used as a good barrier to prevent or treat infections caused by enteric staphylococcal bacteria, and might extend its effect on the *S.*

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aureus toxins or their target, probably by having the ability to produce bacteriocin-like substances, which might participate to the antibacterial effect [28].

Increasing rate of multi-drug resistant strain lead researchers to search about probiotics as alternative therapies decreasing our dependence on antibiotics. In conclusion, present study showed that the traditional made yoghurt products can be used as a good source of potentially probiotic bacteria unlike the imported types which may contain preservatives which prevent the growth and may lack any probiotic bacteria. Also give us evidence on having such beneficial bacteria to the antibacterial effects on pathogens and there is prospect for health benefits by consuming food containing probiotics. However, we recommended additional researches to confirm data on a number of these health benefits in humans and animals determining the amount of probiotics needed to minimize the clinical effects.

References

- [1]. M.I.M.1 , Abdallah; A.M.M. Nasr and M.S. Abd Elaziz, Antibacterial Effects of Probiotics Isolated from Milk Products Against some Common Bacterial Pathogens. Egypt . J. of Appl. Sci., 28 (8): 272-294,2013
- [2]. A. Ljungh and T. Wadström. Lactic Acid Bacteria as Probiotics. Curr. Issues Intestinal Microbiol, 7: 73–90,2012.
- [3]. M.F. Bernet-Camard; V. Lievin; D. Brassart; J.R. Neeser; A.L. Servin and S. Hudault; The human *Lactobacillus acidophilus* strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo. Applied Environ. Microbiol., 63: 2747-2753. FROM 51997.
- [4]. D., P.A. Sgouras; K. Maragkoudakis; B. Petraki; E. Martinez-Gonzalez; S. Eriotou; G. Michopoulos; Kalantzopoulos, Tsakalidou and A. Mentis,. In vitro and in vivo inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. Applied and Environ. Microbiol., 70: 518-526,2004.
- [5]. R.D. Brown ;A.C, and A. Valiere, Probiotics and Medical Nutrition Therapy. Nutr Clin Care, 7(2): 56–68, 2004.
- [6]. A.C. Quwehand, and S. Vesterlund. Antimicrobial components from lactic acid bacteria. Lactic Acid Bacteria Microbiological and Functional Aspects, Marcel Dekker Inc., New York, USA. 2004
- [7]. W.H. Holzapfel; P. Habere; R. Geisen; J. Bjorkroth, and S. Ulrich. Taxonomy and important features of probiotic microbes in food and nutrition. Am. Clin. Nutr, 37: 365–373,2001.
- [8]. S.D. Todorov; M. Wachsman; E. Tome; X. Dousset; M.T. Destro; L.M.T. Dicks; B.D.G.M Franco. ; M. Vaz-Velho and D. Drider.. Characterisation of an antiviral pediocin-like bacteriocin produced by *Enterococcus faecium*. Food Microbiology, 27: 869-879,2010.
- [9]. F.E. Ahmed, Genetically modified probiotics in foods. Trends Biotechnol, 21: 491–497, 2003.
- [10]. M.E. Sanders,. Considerations for Use of Probiotic Bacteria to Modulate Human Health. American Society for Nutritional Sciences. J. Nutr, 130: 384S–390S. 2000

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- [11]. J. Nowroozi; M. Mirzaii and M. Norouzi. Study of Lactobacillus as Probiotic Bacteria. Iranian J Publ Health, (2) 3:1 -7, 2004.
- [12]. I.D.F. "International Dairy Federation" .Yoghurt: Enumeration of characteristic microorganisms colony count technique at 37°C. IDF Standard 117 International Dairy Federation, Brussels. 1983.
- [13]. J.D. De Man; M. Rogosa and M.E. Sharpe. "A Medium for the Cultivation of Lactobacilli". J. Appl. Bact. (23): 130–135, 1960.
- [14]. A. Florez; T. Lopez-Diaz; P. Alvarez-Martin and B. Mayo Microbial characterization of the tradition Spanish blue-veined cabrales cheese: identification of dominant lactic acid bacteria. Food Technol, 223: 503-508, 2006.
- [15]. N.P. Shah, and R. Dave, Antimicrobial substances including bacteriocins produced by lactic acid bacteria. Bioscience Microflora, 21 (4): 217-223, 2002.
- [16]. Jr. E.L. Chuayana; C.V. Ponce; M.R.B. Rivera and E.C. Cabrera, Antimicrobial activity of probiotics from milk products. Phil J. Microbiol. Infect. Dis., 32: 71-74, 2003.
- [17]. S. Lim, and I.M. Dong-Soon, Screening and characterization of probiotic lactic acid bacteria isolated from Korean fermented foods. J. Microbiol. Biotechnol., 19: 178-186, 2009.
- [18]. Millette, M.; F.M. Luquet and M. Lacroix, In vitro growth control of selected pathogens by Lactobacillus acidophilus and Lactobacillus casei fermented milk. Lett. Appl. Microbiol., 44(3): 314-319, 2006.
- [19]. Dardir H.A. In vitro Evaluation of Probiotic Activities of Lactic Acid Bacteria Strains Isolated From Novel Probiotic Dairy Products Global Veterinaria, 8 (2): 190-196, 2012.
- [20]. M. E. Sanders, and Veld, J. Huis "Bringing a probiotic containing functional food to the market: microbiological, product, regulatory and labeling issues," Antonie van Leeuwenhoek, 76 (1-4): 293–315, 1999.
- [21]. G. Reid; J. Jass; M.T. Sebulsky and K. McCormick J. Potential Uses of Probiotics in Clinical Practice, Clinical Microbiology Reviews, 4(16): 658–672, 2003.
- [22]. J. Nowroozi ; M. Mirzaii and M. Norouzi, Study of Lactobacillus as Probiotic Bacteria. Iranian J Publ Health, 33 (2): 1 -7, 2003.
- [23]. A.M.M. Catherine, and C.E. Cabrera, Probiotics from Philippine dairy products are bactericidal for pathogens with transferable multiple drug resistance, 4: (2). 91-97, 2011.
- [24]. M. Velraeds; H. Van Der Mei; G. Ried and H. Busscher, Inhibition of initial adhesion of uropathogenic Enterococcus faecalis by biosurfactants from Lactobacillus isolates. App. Environ. Microbiol, 62: 1958-1963, 1996.
- [25]. M.H. Coconnier; M.F. Bernet ; S. Kerneis ; G. Chauviere ; J. Fourniat and A.L. Servin, Inhibition of adhesion of enteroinvasive pathogen to human intestinal Caco-2 cells by Lactobacillus acidophilus strain LB decreases bacterial invasion. FEMS Microbiology Letters, 110: 299-305, 1993.
- [26]. M. Thirabunyanon; P. Boonprasom and P. Niamsup, Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells", Biotechnol. Lett, 31: 571-576, 2009.

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[27]. R. Denkova; Z. Denkova; V. Yanakieva and D. Blazheva, Antimicrobial activity of probiotic lactobacilli, bifidobacteria and propionic acid bacteria, isolated from different sources, Microbial pathogens and strategies for combating them: science, technology and education 857-864,2013.

[28]. k. Kailasapathy and J. Chin. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunology and Cell Biology 78: 80–88,2000

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