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Research Article

Antimicrobial Activity of Probiotic Lactobacilli against Some Pathogenic Bacteria

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ABSTRACT

Probiotics are viable Lactic Acid Bacteria (LAB) that is believed to provide health benefits when administered in appropriate quantities. *Lactobacillus* is one of the most important genera of LAB that are known to produce substances including bacteriocins which can inhibit the growth of pathogenic bacteria. The current study determined the antibacterial activity of probiotic lactobacilli against some clinical bacterial isolates. A commercially available probiotic lactobacilli product which contains *Lactobacillus acidophilus* and *Lactobacillus plantarum* strains were tested for their antibacterial activity against the clinical bacterial isolates. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, following disk diffusion method. The cell-free supernatant of Lactobacilli strains isolated in the present study exhibited very remarkable and noticeable antimicrobial activity against pathogenic bacteria.

Keywords: Antimicrobial activity; *Lactobacillus*; Probiotics; Pathogens; *Invitro* diagnostics; Clinical strains

INTRODUCTION

Throughout the history of microbiology, most human studies have been focused on the disease-causing organisms found on or in people, whilst fewer studies have examined the benefits of the bacteria. However, we are surrounded by beneficial microorganisms that live in or on the human body. Probiotics are defined as live microorganisms when administered in adequate amounts confer a health benefit on the host [1,2]. Lactic Acid Bacteria (LAB) are considered as Generally Recognized as Safe (GRAS) organisms and can be safely used as probiotics for medical applications [3]. Members of the genus *Lactobacillus* are the most common probiotics used in commercial fermented and non-fermented products, such as in some yogurts and fermented milk drinks, as well as dietary supplements, which are available in the market in the form of capsules, liquid/gel, and powdered, to combat the diseases due to their ability to inhibit the pathogenic microorganisms [4]. Lactobacilli, primarily facultative or strict anaerobes generally have fastidious growth requirements. They prefer an acidic environment by producing lactic and other acids.

In general, Lactobacilli have not been associated with disease and have been regarded as non-pathogenic and isolates were able to tolerate the acidic condition of the environment, Nacl concentration, and resistance to bile. The reported health benefits of probiotics include: boosting of the immune system, inhibition of the growth of pathogenic organisms, prevention of diarrhea from various causes, improvement of digestion of proteins and fats and synthesis of vitamins [5]. This study was conducted to detect the antimicrobial activity of the probiotic lactobacilli isolated from a commercial probiotic product against some bacterial pathogens. Probiotics, a word derived from Latin, that means 'for life', have been with us for as long as people have eaten fermented milk, but their association with health benefits dates only from the turn of the last century. Growing awareness of the health benefits of consuming microorganisms such as probiotics have encouraged consumers worldwide. These probiotic bacteria are essential for their beneficial effect on a particular organism's health and host nutrition for healthy gastrointestinal function.

The original modern hypothesis of the positive role played by certain bacteria was first introduced by Russian scientist Elie Metchnikoff, who in 1907 suggested that it would be possible to modify the gut microbiota and replace harmful microbes with useful microbes [6]. On the other hand, the rise in antibiotic antibiotic-resistant has awakened the scientific community to the prophylactic and therapeutic uses of probiotics and to reconsider them as alternatives to antibiotics [7]. Over the last years, there has

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been increasing public and scientific interest in the administration of these live micro-organisms to prevent or treat disease. Twenty-three publications were retrieved from PubMed for the year 1995 using the search term 'probiotic' compared with about 200 in the year 2000 and more than 600 for the first half of 2012.

Much of the focus of this research has been on the use of probiotics for the prevention or treatment of gastrointestinal conditions such as inflammatory bowel disease and inflammatory bowel syndrome [8]. Antimicrobial activity of *Lactobacillus* strains against bacterial pathogens emerges to be multifactorial and includes the production of hydrogen peroxide, lactic acid, bacteriocin-like molecules, and unknown heat-stable, non-lactic acid molecules [9]. Other mechanisms proposed for their activity are competition for nutrients [10,11], adhesion inhibition of pathogens to the surface, and stimulation of the immune system [12]. One of the important features of probiotic lactobacilli to achieve antagonistic activity against bacterial pathogens because of their capacity to produce lactic acid and other organic acids that lower the pH in the human intestine, and to produce H_2O_2 and bacteriocin, thereby establishing a hospital environment for the growth and survival of various human pathogenic bacteria.

There are also other physiological benefits of probiotics that have been published as it helps in the removal of carcinogens, lowering of cholesterol, immune-stimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance [13]. One important limitation is that only one kind of probiotic bacteria may not exert protection against all harmful strains that cause gastrointestinal pathogenic infections [14].

MATERIALS AND METHODS

Isolation of probiotic strains

Commercial probiotic product 'Vitalactic B' was used to isolate the probiotic lactobacilli used in the study. According to the product content information given on the "Vitalactic B" packet, two probiotic strains, *Lactobacillus plantarum* and *Lactobacillus acidophilus* were present in it. "Vitalactic B" is in form of a capsule so the powder from the capsule were suspended in Brain Heart Infusion Broth (BHIB) and kept in aerobic and anaerobic conditions at 37°C for 24 hrs. The probiotic lactobacilli were subjected to cultural and morphological characterization. Then, the whole broth was centrifuged at 3000 rpm for 10 minutes and the was-free supernatant was collected and tested for its antibacterial activity against the clinical bacterial isolates.

Bacterial cultures and growth conditions

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with their case profiles were obtained from the laboratory of Nanakali hospital, Erbil, and maintained in Brain Heart Infusion (BHI) agar and MacConkey agar [2,3] isolated colonies of each bacterial pathogen were sub-cultured onto BHI broth under aerobic conditions for 24 hours before testing. These bacteria served as test pathogens for antibacterial activity assay.

Detection of antimicrobial activity

The antimicrobial activity of probiotic lactobacilli from a commercial probiotic product was investigated against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using the method. For the purpose of production of paper discs, Whatman filter paper No.3 was used. Using an ordinary office hole punching machine, holes of approximately 6 mm diameter were punched. The discs were then autoclaved at 15 lbs pressure for 20 minutes [15]. Susceptibility of the pathogens to 2 antibiotics including Gentamicin (GEN) and Ampicillin (AMP) was also determined by the diffusion method. The Muller-Hinton agar plates were examined for the presence of inhibition zones around the paper disks. The result was considered positive when a clear zone around the paper disk was present whereas, negative was defined as the absence of a growth inhibition zone around the disk. Zones of inhibition were measured after incubation at 37°C for 24 hours and the results were recorded.

RESULTS

Isolation and identification of *Lactobacillus* species

Probiotic lactobacilli were isolated from the commercial probiotic product "Vitalactic B" and identified by noticing their colony morphology as shown in Figure 1A, and cultural as welcome biochemical characteristics. Microscopically they were gram-positive as shown in Figure 1B, rod rod-shaped-motile, and absence of endospore (Figure 1).

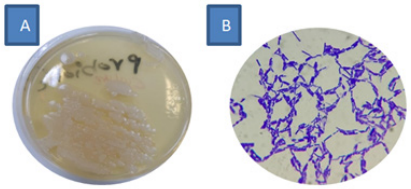

Figure 1: Isolation and identification of *Lactobacillus* species. A: Characteristic colonies of *lactobacillus* species on BHIA; B: Microscopically appearance of *Lactobacilli* with gram stains.

Antimicrobial activity

Lactobacilli strains were tested for their antimicrobial activity against some pathogenic bacteria by the disk diffusion method. The results revealed that the cell-free supernatant of *Lactobacillus Plantarum* and *Lactobacillus acidophilus*, in combination, exhibited the average inhibition (15-35 mm) on the growth of test pathogens: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, but had no activity against *Klebsiella pneumoniae*. On the other hand, all the test pathogens were resistant to Ampicillin (AMP) and varieco Gentamycin (GEN) (Figure 2 and Table 1).

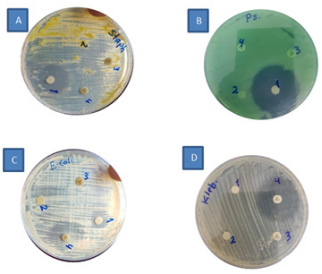

Figure 2: Antimicrobial activity. A: *Staphylococcus aureus*; B: *Pseudomonas aeruginosa*; C: *Escherichia coli*; D: *Klebsiella pneumoniae* by the disk diffusion method. Note: (1) Probiotic Lactobacillus strains supernatant; (2) BHIB (negative control); (3) Ampicillin; (4) Gentamicin against different test pathogens.

Table 1: Antibacterial activity of probiotic *Lactobacillus* strains supernatant compared with AMP and GEN in terms of zone of inhibition using the agar disk diffusion method.

Pathogenic bacteria	Source	Zone of inhibition			
		Probiotic *Lactobacillus* strains supernatant	AMP	GEN	Control
Escherichia coli	Stool	+(15 mm)	-	+(20 mm)	-
Klebsiella pneumoniae	Urine	-	-	+(30 mm)	-
Pseudomonas aeruginosa	Wound	+(35 mm)	-	-	-
Staphylococcus aureus	Urine	+(20 mm)	-	-	-

DISCUSSION

It is a long time since scientists are trying to formulate alternative therapeutic protocols with non-antibiotic agents against bacterial infection. Nowadays, various natural products and methods are used to prevent or treat diseases. The use of probiotics is one of these methods. Lactobacilli are normal intestinal flora that plays an important role in human health. Probiotic Lactobacilli produce antimicrobial compounds such as bacteriocin, which can be used to prevent the growth of many bacteria. In this study, it was found that the cell-free supernatant of these bacteria, was able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, the latter was in the highest inhibitory effect, which comes in compatible with a study by 16 which study the inhibitory activity of bacteriocin producing Lactic Acid Bacteria (LAB) against *P. aeruginosa* and *Escherichia coli* using free cell supernatant and disk diffusion method. Jamalifar et.al., [16,17] showed that *Lactobacillus acidophilus* exhibit significant inhibitory activity against clinical isolates of *P. aeruginosa* by different mechanisms. Similarly, a bacteriocin from L. plantarum was found to be active against pathogenic bacteria including *Cl. sporogeneses*, *E. faecalis*, *E. coli*, and *S. aureus* [18,19]. Antibacterial activity of bacteriocin produced by isolated probiotics showed that *L. plantarum* has the strong antibacterial effect against enteric bacterial pathogens [20].

In our study, the cell-free supernatant of *Lactobacillus plantarum* and *Lactobacillus acidophilus*, have no antimicrobial effect against *Klebsiella pneumoniae*. According to previous studies, a large number of lactic acid bacteria strains showed different bioactive potentials, especially in the form of antibacterial properties. The spectrum of antimicrobial activity for the species suggested that the inhibitory components were differ [21]. Similarly, Hami [22] and Hassan Ali Maarof et al., [23] observed varying degrees of inhibition of various pathogens by the culture filtrate of lactic acid bacteria, although these inhibitory substances produced by the lactic acid bacteria strains, they act differently on the pathogenic reference indicator strains. On the other hand, the inhibitory activity of probiotic *Lactobacillus* supernatant was compared with the inhibitory activity of AMP and GEN against the clinical pathogens. None of the antimicrobial agents was effective against all the tested pathogens demonstrating the current problem in the treatment of multi-drug resistant bacteria. In a previous study, *P. aeruginosa* isolates showed intermediate or full resistance to antimicrobial agents [24].Unfortunately, *P. aeruginosa* and *S. aureus* showed complete resistance against gentamicin and Ampicillin (AMP) and varies in their susceptibility to Gentamycin (GEN) as shown in Figure 2 and Table 1.

CONCLUSIONS

The results of the present study revealed the following:

1. The probiotic bacteria isolated in our study possess varying degrees of inhibition towards tested pathogenic bacteria.

2. Lactobacilli strains are potentially promising because they generate bactericidal bioactive agents that can control the growth of pathogens.

3. Results from our present study are expected to encourage people to consume probiotic products.

4. Given the increasing use of probiotics as health supplements and therapeutic agents, clinicians need to be aware of the risks and benefits. Although probiotics have an excellent overall safety record, they should be used with caution in certain patient groups particularly neonates born, prematurely or with immune deficiency.

5. *Lactobacillus* strains isolated in this study from the different probiotic medicine have *in vitro* properties that make them potential against pathogenic bacteria which were susceptible to all the strains of *Lactobacillus*. These results collectively suggest that probiotics particularly commercially available types are important for combating pathogens.

COMPETING INTERESTS

No competing interests.

FUNDING

The research was self-funded. The authors' independently design the study, analyze and interpret the data, prepared the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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