Research Article

Antimicrobial Activity of Probiotic Lactobacilli Against Pathogenic Bacteria

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ABSTRACT

The antimicrobial or antagonistic activity of probiotics is an important property that includes the production of antimicrobial compounds, competitive exclusion of pathogens, enhancement of the intestinal barrier function and others. Probiotics are viable lactic acid bacteria (LAB) that are 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 strains were tested for their antibacterial activity against the clinical bacterial isolates: Escherichia coli, Klebsiella pneumoniae, 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: Probiotics, Lactobacillus, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, Cell-free supernatant, Lactobacilli strains.

INTRODUCTION

The term Probiotics is derived from a Greek word meaning "for life" and used to define living non-pathogenic organisms and their derived beneficial effects on hosts. The term Probiotics was first introduced by Vergin, when he was studying the detrimental effects of antibiotics and other microbial substances, on the gut microbial population. He observed that probiotics were favourable to the gut microflora[1]. Probiotic were then redefined by Lilly and Stillwell as "A product produced by one microorganism stimulating the growth of another microorganism. Subsequently the term was further defined as Non-pathogenic microorganisms which when ingested, exert a positive influence on host's health or physiology by Fuller. The latest definition put forward by FDA and WHO jointly are live microorganisms when administered in adequate amounts confer a health benefit on the host. The guidelines make it necessary to perform the following activities:

1. Strain identification.
2. Functional characterization of the strain(s) for safety and probiotic attributes.
3. Validation of health benefits in human studies.
4. Honest, not misleading labelling of efficacy claims and content for the entire shelf life.

Characteristics and Classification of Probiotics:

Should not be hydrolysed or absorbed in the upper part of G.I tract. Should be a selective substrate for one or a limited number of potentially bacterial commercial to the colon culture protagonist. Should be able to alter the colonic microflora towards a healthier composition or selectively stimulates the growth and or activity of intestinal bacteria associated with health and wellbeing. Should help increase the absorption of certain minerals such as calcium and magnesium. Favourable effect on the immune system and provide improved resistance against infection. Produce lactic acid- lowers the pH of intestines and inhibiting bacterial villains such as Clostridium, Salmonella, Shigella, E. coli, etc. Decreases the production of a variety of toxic or carcinogenic metabolites[2,3]. Aid absorption of minerals, especially calcium, due to increased intestinal acidity. Production of β- D- galactosidase enzymes that break down lactose. Produce a wide range of antimicrobial substances - acidophil in and bacteriocin etc. help to control pathogenic bacteria. Produce vitamins (especially Vitamin B and vitamin K) Act as barriers to prevent harmful bacteria from colonizing the intestines.

Probiotic Food Products:

The main products existing in the market are dairy-based ones including fermented milks, cheese, ice cream, buttermilk, milk powder, and yogurts, the latter accounting for the largest share of sales[4]. Non-dairy food applications include soya-based products, nutrition bars, cereals, and a variety of juices as appropriate means of probiotic delivery to the consumer.

Types of Probiotics:

Probiotics may contain a variety of microorganisms. The most common are bacteria that belong to groups called Lactobacillus and Bifidobacterium. Other bacteria may also be used as probiotics, and so may yeasts such as Saccharomyces boulardii[5]. Taking into consideration their definition the number of microbial species which may exert probiotic properties is impressive.

Properties of Probiotics

In order for a potential probiotic strain to be able to exert its beneficial effects, it is expected to exhibit certain desirable properties[6]. The ones currently determined by in vitro tests are

1. Acid and bile tolerance which seems to be crucial for oral administration.
2. Adhesion to mucosal and epithelial surfaces, an important property for successful immune modulation, competitive exclusion of pathogens, as well as prevention of pathogen adhesion and colonization.
3. Antimicrobial activity against pathogenic bacteria.
4. Bile salt hydrolase activity.

Mechanism of Probiotic activity

Probiotics have various mechanism of action although the exact manner in which they exert their effects is still not fully elucidated. These range from bacteriocin and short chain fatty acid production, lowering of gut pH, and nutrient competition to stimulation of mucosal barrier function and immunomodulation[7]. The latter in particular has been the subject of numerous studies and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T cell responses, enhancing Th 1 responses, and attenuating Th2 responses[8].

Health Benefits of Probiotics

There is increasing evidence in favour of the claims of beneficial effects attributed to probiotics, including improvement of intestinal health, enhancement of the immune response, reduction of serum cholesterol, and cancer prevention. While some of the health benefits are well documented others require additional studies in order to be established[9,10]. In fact, there is substantial evidence to support probiotic use in the treatment of acute diarrhoeal diseases, prevention of antibiotic associated diarrhoea, and improvement of lactose metabolism, but there is insufficient evidence to recommend them for use in other clinical conditions.

MATERIALS AND METHODS

Materials, Ingredients and instrument

Table 1:

S.NO	Ingredients	Instruments
1	Ricesoaked water	Autoclave
2	Milk	Refrigerator
3	Jaggery	Incubator
4	Muller- hinton agar	Scanning electron microscopy

Methods for Preparation of Product:

Isolation of Probiotic Strains: Fermentation:

Fermentation is an anaerobic process in which microorganisms like yeast and bacteria break down food components (e.g. sugars such as glucose) into other products (e.g. organic acids, gases or alcohol). This gives fermented foods their unique and desirable taste, aroma, texture and appearance[11]. Fermented foods are foods and beverages that have undergone controlled microbial growth and fermentation.

Probiotics are usually defined as microbial food supplements with beneficial effects on the consumers[12]. Most probiotics fall into the group of organisms' known as lactic acid-producing bacteria and are normally consumed in the form of yogurt, fermented milks or other fermented foods. Probiotics strains can be isolated directly from natural fermented milk products or milk and can then be added as starter cultures for fermentation in products such as cheese, yogurt, and butter[13,21].

Procedure for the Isolation of Probiotic Strains:

Rice washed water was used to isolate the lactobacilli used in the study. Culturing of lactobacilli in rice washed water is carried out by adding 1 cup of organic rice into 250 ml of water, then fermenting it for 3-5 days in a closed container[1,14] (Figure 1). Activation of LAB in milk is carried out by adding 50 ml of LABS from fermented rice washed water into 500 ml of milk by extracting 50 ml of LABS from the middle part of the fermented rice washed water and waiting for 48 -72 hours (Figure 2). For stabilizing the LAB culture add 250 ml of molasses or Brown sugar or Muscovado sugar in 1:1 ratio with 250 ml of LAB extracted and shake well.

Figure 1: Collected rice washed water

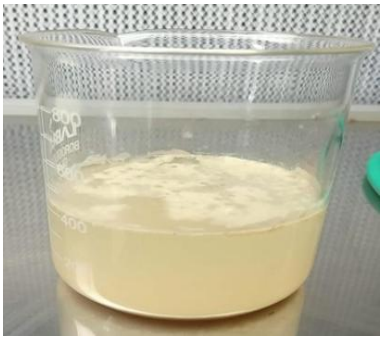

Figure 2: Fermented rice washed water

Figure 3: Activated lab collected from middle part of processed sample water adding to the milk

Figure 4: Activated lab in fermented milk.

Figure 5: Added molasses into the extracted solution for stability

Zone of Inhibition Test Method[16,17]

1. A swab of pure bacterial culture is evenly spread over Mueller-Hinton agar plates.
2. Using a sterile forceps, the treated product sample is placed on the media plate.
3. This petri plate is kept for incubation for 18-24 hours at 36°C along with other optimal conditions for bacterial growth.
4. After the incubation period, a clear area (zone of inhibition) around the antibacterial product sample is observed and measured.
5. Treated products with strong antibacterial activity form a larger zone of inhibition or vice versa.

Procedure For Detection of Antimicrobial Activity

Detection of antimicrobial activity: The antimicrobial activity of probiotic lactobacilli from a fermented rice washed water was investigated against Staphylococcus aureus, Escherichia coli using the 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[15]. Zones of inhibition were measured after incubation at 37°C for 24 hours and the results were recorded.

RESULTS

Isolation and Identification of Lactobacillus spp:

Probiotic lactobacilli were isolated from "the rice washed water" and identified by noticing their colony morphology (FIG 7A), and cultural as welcome biochemical characteristics. Microscopically they Gram-positive (FIG 7B), rod-shaped-motile, and absence of Endospore.

Antimicrobial activity:

Lactobacilli strains were tested for their antimicrobial activity against some pathogenic bacteria by the disk diffusion method[18,20]. The results revealed that the cell-free supernatant of Lactobacillus Plantarum and Lactobacillus acidophilus, in combination exhibited the of test pathogens: *Staphylococcus aureus* and *Escherichia coli*.

Figure 6: Detection of Antimicrobial Activity by Zone of Inhibition Test

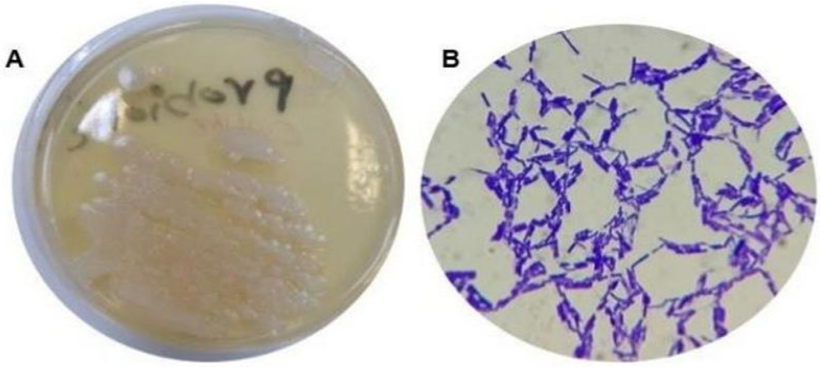

Figure 7A: Characteristic colonies of *lactobacillus spp*

Figure 7B: Microscopically appearance of lactobacilli with Gram's stain

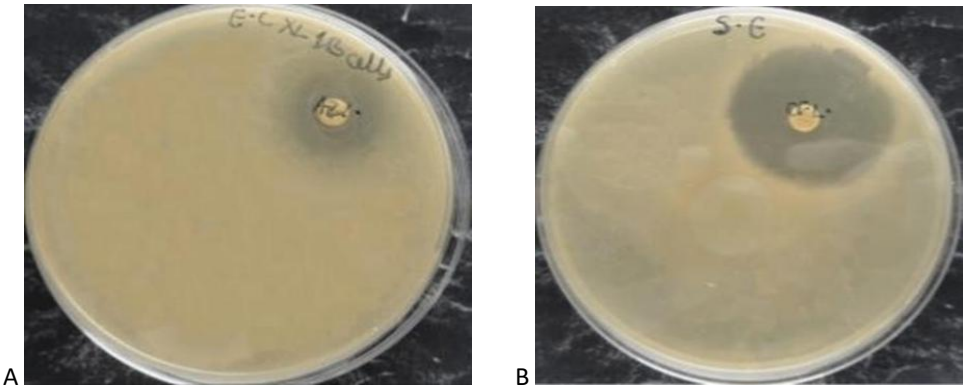

Figure 8: Antimicrobial activity of probiotic Lactobacillus against different test pathogen *Escherichia coli* (A), *Staphylococcus aureus* (B)

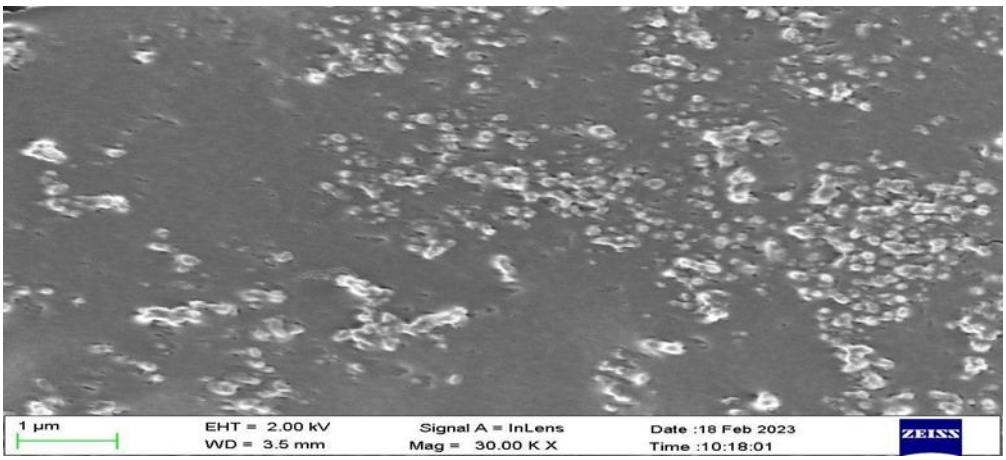

Figure 9: Morphology of lactobacillus by scanning electrone microscopy

Table 2: Antimicrobial activity of probiotic lactobacillus in terms of zone of inhibition using agar disk diffusion method

Pathogenic Bacteria	Zone of Inhibition
Escherichia coli	+(15mm)
Staphylococcus aureus	+(20 mm)

DISCUSSION

It is a long time since scientists have been trying to formulate alternative therapeutic protocols with nonantibiotic 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 oral that plays an important role in human health.

Probiotic Lactobacilli produces 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 and Escherichia coli the latter was in the highest inhibitory effect, which comes in compatible with a study by16 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[19] (TABLE NO 2) and (FIG 8A, 8B).

CONCLUSION

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. Further, studies are needed to explore probiotic interactions. Additional research data are needed to confirm a number of the health benefits in humans.

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