Lactobacillus: Potential Probiotics Isolated from Milk Sample

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ABSTRACT

Introduction: The rising incidences of antimicrobial resistance among gastrointestinal pathogens is a current global acumen regarding public health menace due to the unjustified usage of commonly available antibiotics and raised healthcare expenditures. Complementary and alternative medicine has drawn interest and has contemporary insights for which the capacity of probiotic bacteria, Lactobacillus species, to inhibit pathogenic microorganisms has shown promising results and is in great demand.The potential of Lactobacillus species as potent substitutes for traditional antibiotics is further demonstrated by their capacity to create antimicrobial substances such as hydrogen peroxide, bacteriocins, and organic acids. By preserving healthy microbiota and boosting the host immune system, these probiotics prevent harmful bacteria and promote gut health. Therefore, integrating probiotic-based therapies into dietary and clinical procedures provides a novel and durable way to fight antibiotic resistance while enhancing general health outcomes.

Objective: The study was conducted to characterize Lactobacillus lactis and its potential to be used as probiotics.

Methodology:

Isolation

Raw milk samples were collected from a local market in Karachi. The Lactobacillus spp were isolated on de Man, Rogosa, and Sharpeagar (MRS) media. The isolated colonies were then characterized based on morphology and biochemical reactions.

Gram Staining

On a grease-free cleaned slide, prepare an isolated culture smear. Gently reheat the smear to fix it. Then, using a crystal violet dye, carefully cover the smear and keep it for a minute. Next, use distilled water or clean tap water to remove the discoloration. Subsequently, a drop of Grams iodine is applied to the smear and left to rest for a minute or two. After that, rinse with water and the decolorizing agent, diluted alcohol, and then rinse with water immediately. Finally, let the smear dry completely. Then examine it under a microscope, under an immersing lens. [Rokon-Uz-Zaman *et al.*, 2023].

Biochemical Identification

Using established protocols, colonies on the primary or subculture plates that were suspect and resembled those of lactobacillus species were chosen for additional identification. A biochemical test was performed on each of the isolates to identify the isolates. Based on the results of the catalase, oxidase, and triple sugar tests, identification was completed. The catalase activity test was conducted using a 3% hydrogen peroxide solution. [Ogodo, *et al.*, 2022]. The oxidase test was performed by placing a piece of filter paper in a petri dish, moistening it with an oxidase reagent, and smearing an isolated bacterial colony onto the paper. A positive result, indicated by a blue color change within 10-60 seconds, signifies the presence of oxidase activity. A sugar fermentation test used glucose, fructose, mannose, galactose, maltose, xylose, and arabinose. Durham tubes were included in the test to detect gas production as a byproduct of fermentation. [Kandou, *et al.*, 2024].

Molecular Characterization

The molecular characterization of Lactobacillus species based on the 16S rRNA gene was performed using 16S rRNA universal primers [awd *et al.*, 2020].

Antimicrobial Activity by Bacteriocin Method

The ability to produce bacteriocin was analyzed using the agar well diffusion method. The bacteriocin activity was observed against *Escherichia coli, Enterococcus fecalis, Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, Bacillus cereus, Candida, Bacillus Subtilis* [Shabaan *et al.*, 2020].

Biofilm Production

Biofilm synthesis of Lactobacillus was evaluated through the tube assay method, which yielded definitive insights into the extent of biofilm formation.

The organism was grown in MRS broth for 48 hrs. The inoculum was placed in separate tubes and incubated for one week at 37 ℃. The cells were separated from each tube and washed twice with PBS. The 1% crystal violet was added and incubated for 30 minutes. Optical density was measured at 540 nm through a spectrophotometer [Samtiyael *et al.*, 2024].

Antioxidant Production

Antioxidant properties of intact cells were observed via DPPH (2,2-Diphenyl-1-picrylhydrzyl) assay, which allowed for precise measurement of its free radical scavenging capacity.Overnight grown (48hr) active lactobacillus lactis were harvested by centrifugation at 10000 rpm for 7 minutes. For intact cell (IC) preparation, cell pellets were washed with PBS (7.2) two times, after centrifugation of cell 4000rmp for 5 minutes, the pellets were resuspended in PBS and the $OD_{600}$was adjusted at 0.8 by use of the same PBS and used for 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

The DPPH is a violet-colored, stable free radical, which is reduced to 2,2-diphenyl-1-picrylhydrazyl (pale yellow) by reacting with antioxidants. DPPH standard solution is made by weighing DPPH powder o. oo4g into 100ml methanol. Then in 3.5ml DPPH add 0.5ml methanol and incubate it for 30min at room temperature and the OD_{600} was adjusted at 1.2. then in 3.5ml DPPH add a 0.5ml sample of intact cell (IC) that formed above and incubate at room temperature for 30 minutes in the dark after mixing vigorously. And the OD_{517}nm using a spectrophotometer [Vougiouklaki, 2023]. To compute the radical scavenging activity, the following equation was used:

$$\text{Radical scavenging activity Radical (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100\%$$

Where Asample = absorbance of sample; Acontrol = absorbance of control.

Antibiotic Susceptibility by Agar Kirby Bauer Method

The antibiotic susceptibility of the Lactobacillus species was determined by the Kirby Bauer method, against ampicillin, cephalosporin, clindamycin, and erythromycin. The standardized culture was spread on Muller Hinton agar and discs of antibiotics were placed on it. Following incubation, inhibition zones surrounding the disks were measured to assess the organism's sensitivity [Sharma *et al.*, 2024]

Result: From 15 raw milk samples, two isolates of Lactobacillus species were selected for this study. The organisms were identified as gram-positive, large rods. The organism showed variable results of carbohydrate fermentation. The 16SrRNA genes were amplified and found to be 1500 bp in length. The organisms can produce bacteriocin against Enterococcus fecalis, E.coli, Klebsiella, Staph aureus, and Pseudomonas aeruginosa. The organism demonstrates the capacity for biofilm production, which is 2.24% exhibiting robust formation under suitable conditions. The organism exhibits a notable capacity for antioxidant activity,at 38.2% contributing to the neutralization of free radicals. This property underscores

its potential role in reducing oxidative stress under specific conditions. The organism exhibited notable sensitivity to various antibiotic disks, demonstrating its susceptibility to multiple antimicrobial agents. This indicates that certain antibiotics can effectively target the organism, which could be utilized in therapeutic interventions.

Conclusion: The field of probiotics holds significant emerging potential in addressing the growing concerns related to antimicrobial resistance. By leveraging beneficial microorganisms, probiotics offer a promising alternative to traditional antibiotics, helping to restore the balance of the microbiota and prevent infections. They can potentially reduce the reliance on chemical antibiotics, which contribute to the development of resistant strains. With further research, probiotics could be optimized for specific therapeutic uses, enhancing their efficacy in treating a variety of infections. The growing interest in probiotic-based therapies also opens up new avenues for commercial-scale applications in medicine, agriculture, and food industries. Ultimately, probiotics represent a sustainable and innovative approach to overcoming the global challenge of antibiotic resistance.

Keywords: Probiotics, Bacteriocin, Antibiotic Resistance, Antioxidant Attributes.