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A REVIEW- APPROACH TO ANTIMICROBIAL ACTIVITY OF PROBIOTICS AGAINST PATHOGENIC ORGANISMS

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ABSTRACT

Probiotics are unique bacteria with crucial traits. They can withstand stomach acid and bile salts. These microorganisms have the potential to bind to intestinal cells and compete with pathogen microorganisms for those cells. They can create antimicrobial substances like lactic acid, bacteriocin, etc. The word "probiotic" is derived from the Greek word for "life."

Evaluation of potential *Lactobacillus* species activities was the study's goal. Each *Lactobacillus* isolate's probiotic qualities and antibacterial activity against pathogens were then assessed in vitro. Diarrhoea is one of the main causes of paediatric illness and mortality in developing countries. The disc diffusion method evaluated the antibacterial activity of two commercially available probiotics, *Lactobacillus acidophilus* and *Escherichia coli* against the clinical bacterial isolates.

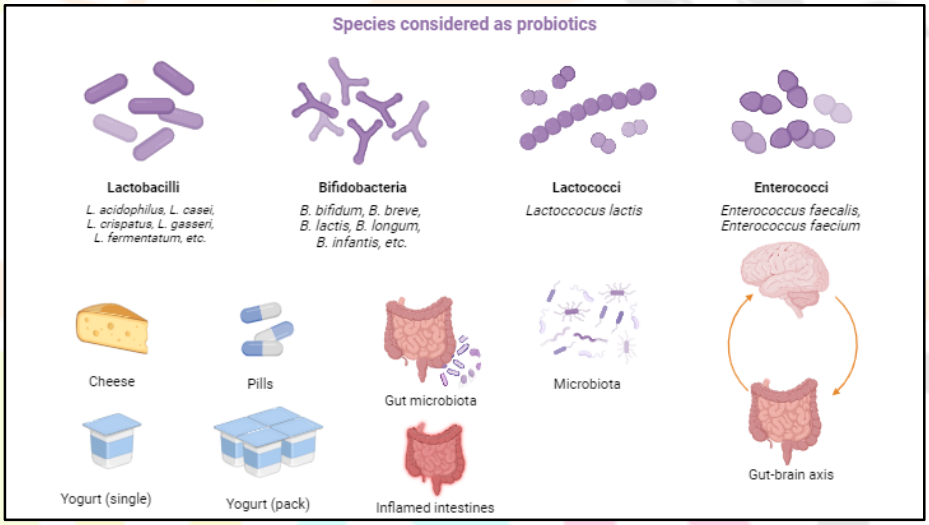
Based on the results of this study, present probiotic bacteria in natural resources can be used for the inhibition and reduction of pathogens, including enteric pathogens and the antibacterial effects of their metabolites are active and stable under different temperature and acidity conditions.

Keywords: Probiotics, *Lactobacillus, E .coli*, Disk diffusion method, Zone of inhibition.

INTRODUCTION

Probiotics are unique bacteria with crucial traits. They can withstand stomach acid and bile salts. These microorganisms have the potential to bind to intestinal cells and compete with pathogen microorganisms for those cells. They may be a member of the gut microflora. They can create antimicrobial substances like lactic acid, bacteriocin, etc. The word "probiotic" is derived from the Greek word for "life." Following the most recent data from the FAO and WHO, probiotics are live bacteria that, when consumed in sufficient amounts, have positive impacts on health (**S**occol *et al*., 2010). Several studies showed their impact on the prevention and treatment of numerous ailments.

The type of food consumed has a significant impact on the gut microbiota. As a result, it might replace dangerous germs with beneficial ones. Probiotics either increase the number of healthy intestinal bacteria or inhibit the growth of dangerous germs. In impoverished nations, diarrhoea is one of the leading causes of paediatric sickness and mortality. Bacteria are the second cause of diarrhoea after viruses (Keeratikunakorn *et al*., 2023).



These days, people drink probiotics to prevent diseases and lengthen the shelf life of their food. They exude antimicrobial substances that combat pathogens (such as lactic acid, bacteriocin and hydrogen peroxide) and boost the host's immunological response. Since the beginning of time, humans have consumed milk and milk products, which are also well-known sources of lactobacilli. Research on the health benefits of *Lactobacillus* spp. as dietary supplements has increased significantly. In order to isolate probiotic *Lactobacillus* species, yoghurt samples from different companies were obtained (Coombs *et al*., 2005).

The purpose of the study was to assess the potential activities of *Lactobacillus* species. The probiotic properties and antibacterial activity against pathogens of each *Lactobacillus* isolate were then determined in vitro. Probiotics either increase healthy gut flora or inhibit dangerous microbes. In impoverished nations, diarrhoea is one of the leading causes of paediatric sickness and mortality. Bacteria are the second most common cause of diarrhoea after viruses. *E. coli* is one of the major bacteria in the gut microflora and accounts for around 30% of cases with high treatment expenses. Children's diarrhoea cases are caused by intestinal pathogenic *E. coli* in 8–10% of cases (Jamalifar *et al*., 2011). The antibacterial activity of two commercially available probiotics, *Lactobacillus acidophilus* and *Escherichia coli*, was evaluated against the clinical bacterial isolates using the disc diffusion approach (Nataro *et al*., 2006)

Probiotics' inhibitory mechanisms against *Listeria monocytogenes*

One of the worst foodborne diseases is *Listeria monocytogenes,* and few microbes are able to effectively stop its growth. The best sources of antagonistic bacteria are safe lactic acid bacteria that have probiotic and antibacterial qualities. Through this work, a strain of *Lactobacillus plantarum* 4–10 that eradicated *L. monocytogenes* entirely from sauerkraut from northeastern China was identified. Following characterisation, 16 Gram-positive, Gram-negative, and fungal species were shown to be antagonistic to probiotics, exhibiting broad-spectrum bacterial suppression. Following tolerance to stomach and intestinal simulated fluids, the survival rate exceeded 45%. *L. plantarum* 4–10 showed good hydrophobicity, auto-aggregation, and co-aggregation properties, and was susceptible to erythromycin, doxycycline, tetracycline, and chloramphenicol. When co-cultured with *L. monocytogenes*, it may cause structural disruptions to the cells and become fatal in 15 hours. Lactobacilli spots are surrounded by an inhibitory zone that *L. monocytogenes* strains exhibit. The strains of *L. rhamnosus* (LR-B19, LR-10, LR-B5, LR-B20, LR-A3, LA-A20, LR-C44, LR-52), *L. paragasseri* (LA-B17, LDD-C1), *L. crispatus* LC-C1, and the strain *L. gasseri* LG-C45 had the most antilisterial action. Nevertheless, no growth inhibition of *L. monocytogenes* was seen in the agar-well diffusion assay when the pH-neutralized CFS of the LAB strains were employed (Xinyu *et al*., 2024).

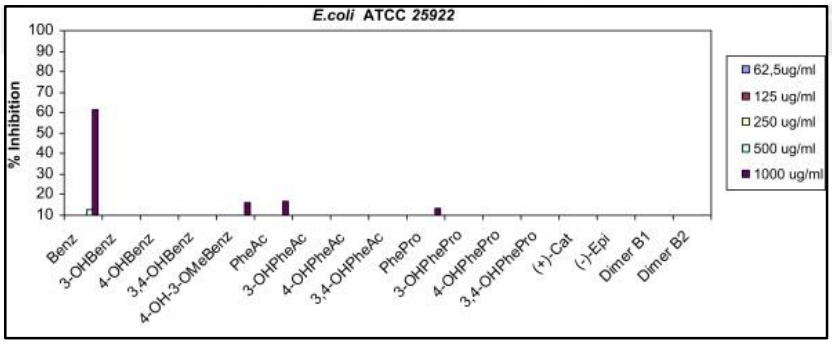
Isolation and Identification of bacteria

For the separation of probiotic bacteria, samples are collected from dairy products including (yoghurt, cheese and milk) and vegetables including carrots and cabbages (red and white) were collected from different areas. The probiotic properties and antibacterial activity against pathogens of each *Lactobacillus* isolate were then determined in vitro. The isolates grew well in acidic conditions and were resistant to NaCl (1-6%), bile salt (0.5-3%), and other chemicals.

Maximum growth was seen at a pH of about 6.0. All of the isolates were amenable to therapeutic antibiotics, and they also demonstrated successful aggregation and hydrophobicity investigations. Selected *Lactobacillus* isolates were thought to be unique and promising probiotic bacteria as a result of the findings (Chen *et al.*, 2019).

Assessment of probiotic bacteria's antibacterial activity

The antibacterial activity of isolated probiotic bacteria was investigated using Mueller-Hinton Agar (MHA). Using the Disk Diffusion Agar and Well Diffusion Agar procedures, the inhibitory and antagonistic effects of probiotic bacteria on two pathotypes of *E. coli* were assessed for this aim. Every test was run twice. The bacteria *Lactobacillus* spp. were isolated using DeMan Rogosa and Sharpe medium (Fijan *et al*., 2023). Each sample containing 5–10 g of yoghurt was homogenised with sterile phosphate solution (2% w/v) at 30–40°C in a Stomacher 200–400 circulator (Remi make, India). Later, 1–2 ml of diluted samples were inoculated in MRS broth tube, prepared as per (De Man *et al*, 1960) under aseptic conditions. A control was also run with each batch of samples by pouring distilled water instead of yoghurt samples. The inoculated MRS broth tubes were incubated at 37°C for 24– 48 hours.

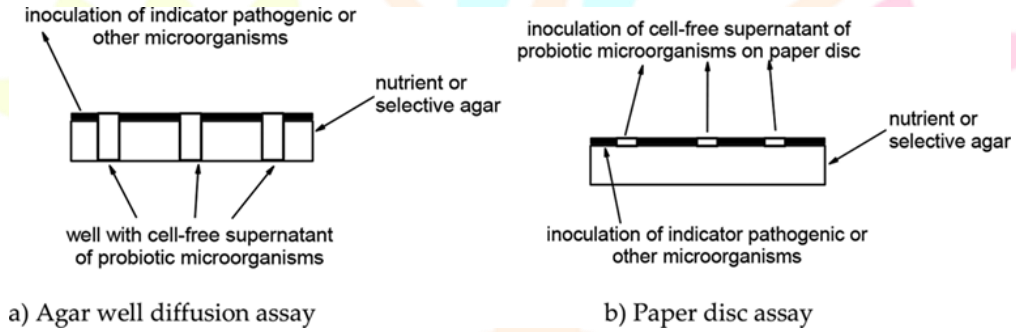


Inhibition (%) of growth of *E. coli* strains (Carolina Cueva *et al*, 2010).

Disk Diffusion Agar method

The discs were heated to 37°C for 15 minutes to dry them. On plates with MHA medium, the pathogenic bacterial suspension was applied. Supernatant impregnated discs were positioned 2.5cm apart from MHA. After 24 hours of incubation on each plate at 37 °C, the growth inhibition zone of probiotics against *E. coli* pathotypes was measured with a ruler.

For *Lactobacillus* the activated cultures were swabbed onto the agar plates. Dodeca discs (Hi Media, India) containing the following antibiotics were used: tetracycline (30 mg), ampicillin (30 mg), erythromycin (15 mg), chloramphenicol (30 mg), gentamicin (10 mg), ciprofloxacin (5 mg), cephalotin (30 mg), cotrimoxazole (25 mg), ceftriaxone (30 mg), amoxicillin (10 mg), amoxyclav (10 mg), clindamycin (30 (Hi-Media). After 24 hours of incubation at 37 °C, the zones of inhibition were identified. Resistance and sensitivity pattern data were interpreted (Gharbi *et al*, 2019)



Scheme of the agar well diffusion assay and paper disc assay (Fijan *et al.*, 2016).

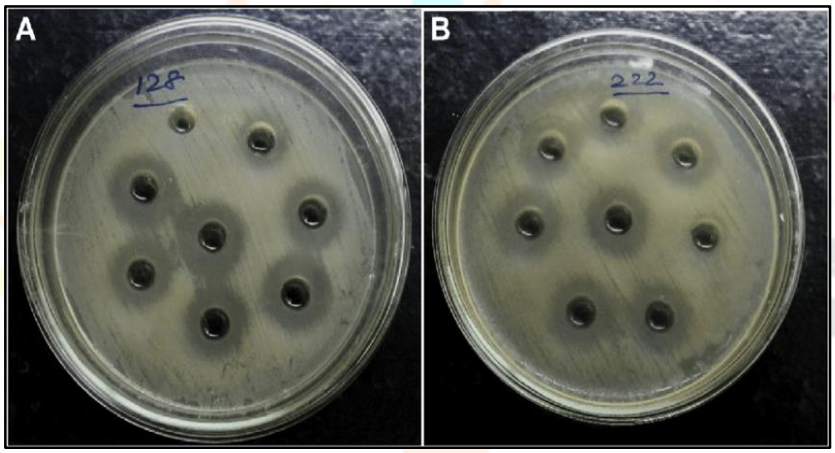
Antagonistic activity of *Lactobacillus* isolates against pathogens

Lactobacillus isolate was filled in 7 mm diameter well in nutrient agar containing test pathogens. The diameter of the inhibition clear zone was noted after 24 h of incubation. Antagonism in Action by using the agar overlay and agar-well diffusion method, the antagonistic activity of four lactobacilli isolates (designated as LMEM6, LMEM7, LMEM8, and LMEM9) against the indicator strains: *Salmonella enterica serovar typhi* (*S. typhi* from blood culture), *Escherichia coli* (*E. coli* from urine culture), *Proteus vulgaris* (*P. vulgaris* from pus culture), and *Acinetobacter baumannii* (*A. baumannii* from pus culture) was assessed using these techniques (Vineetha *et al*., 2015).

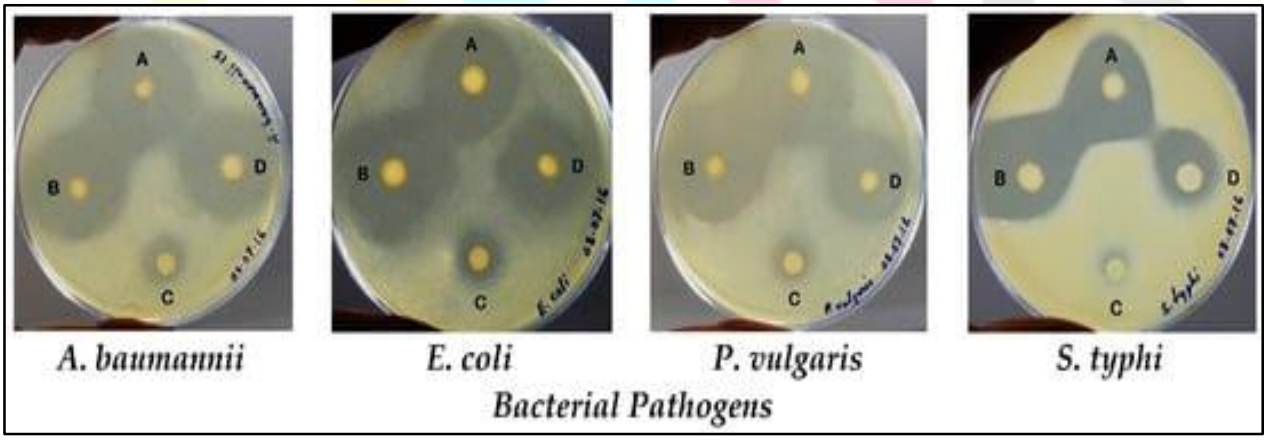
Probiotic Characteristic

The ability of the isolated lactobacilli to withstand low pH, bile salt, and sodium chloride was used to assess their probiotic qualities. The methodology of Liong and Shah was followed to test the bile salt and low-pH (acid) tolerance (Haghshenas *et al*., 2023)The protocol of was used to test the sodium chloride tolerance, with minor adjustments as previously indicated. In short, the isolated lactobacilli were grown in MRS broth for 24 hours at 37 degrees Celsius, supplemented with 2, 4%, and 6.5% sodium chloride.

The growth of the lactobacilli was then observed after the MRS broth cultures were subcultured on MRS agar for another 24 hours at 37 degrees Celsius, indicating their tolerance to sodium chloride. The experiments were duplicated twice and carried out in atmospheric carbon dioxide.



Antagonistic activity of *Lactobacillus* isolates against test pathogens (Zhou *et al*., 2005)



Agar overlay technique demonstrates the antibacterial activity of natural lactobacilli isolates against four bacterial pathogens (Halder *et al*., 2017*)*.

CONCLUSION

The obtained results showed, growth inhibition effects of all probiotic strains on *E. coli* in the method subjected to biochemical and molecular identification. Inhibition zones in disk diffusion were considerable. The disc diffusion method was used to test different Lactobacilli strains for their antibacterial activity against a few pathogenic bacteria. The combined cell-free supernatant of *Lactobacillus acidophilus* showed an average (15–35 mm) inhibition on the growth of the test pathogens However, all of the test infections were resistant to ampicillin (AMP), and the susceptibility of the pathogens to gentamycin varied (GEN). Based on the results of this study, present probiotic bacteria in natural resources can be used for the inhibition and reduction of pathogens, including enteric pathogens and the antibacterial effects of their metabolites are active and stable under different temperature and acidity conditions. *Lactobacillus* strains of the isolates showed attractive probiotic properties, such as excellent pH, NaCl and bile tolerance, aggregations, and suppression of pathogen growth under in vitro conditions. Also, several clinically useful antibiotics were effective against all strains that were examined. These findings imply that yoghurt isolates may possess the qualities needed for brand-new probiotics. As the *Lactobacillus* species has shown more effective probiotic properties than earlier reports suggest, more research is required to take advantage of other possible probiotic properties. Also, this work supports the development of novel probiotics as biotherapeutic agents or nutritional supplements for the treatment of bacterial gastrointestinal illnesses and other related enteric infections, temperature and acidity conditions.

REFERENCES

1. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, Tang HJ. Antimicrobial Activity of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. Front Microbiol. 2019 Apr 18; 10:789.
2. Carolina Cueva, M. Victoria Moreno-Arribas, Pedro J. Martín-Álvarez, Gerald Bills, M. Francisca Vicente, Angela Basilio, Concepción López Rivas, Teresa Requena, Juan M. Rodríguez, Begoña Bartolomé, Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria, Research in Microbiology, Volume 161, Issue 5,2010, Pages 372-382,
3. Combs BG, Raupach JC and Kirk MD. Surveillance of Shiga toxigenic *Escherichia coli* in Australia. *Commun Dis Intell Q Rep*. 2005; 29 (4):366–9.

4. Fijan, S. Probiotics and Their Antimicrobial Effect. *Microorganisms* 2023, *11*, 528. https://doi.org/10.3390/microorganisms11020528

5. Fijan. Antimicrobial Effect of Probiotics against Common Pathogens. InTech. 2016, doi: 10.5772/63141

6. Gharbi, Y. In-vitro characterization of potentially probiotic *Lactobacillus* strains isolated from human microbiota: interaction with pathogenic bacteria and the enteric cell line HT29. *Ann. Microbiol.* 2019; 69, 61–72.

7. Haghshenas, B., Kiani, A., Mansoori, S. *et al.* Probiotic properties and antimicrobial evaluation of silymarin-enriched *Lactobacillus* bacteria isolated from traditional curd. *Sci Rep* 13, 10916 (2023). https://doi.org/10.1038/s41598-023-37350-3.

8. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous Probiotic Lactobacillus Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines. 2017 Jun 16; 5 (2):31. doi: 10.3390/biomedicines5020031. PMID: 28621711; PMCID: PMC5489817.

9. Jamalifar, H., Rahimi, H., Samadi, N., Shahverdi, A., Sharifian, Z. and Hosseini, F. (2011). Antimicrobial activity of different *Lactobacillus* species against multi-drug resistant clinical isolates of *Pseudomonas aeruginosa*. Iran J. Microbiol. 3.

10. Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R and Tirrell S. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New haven, Connecticut. *Clin Infect Dis.* 2006; 43(4):402–7. doi: 10.1086/505867.

11. Keeratikunakorn, K., Kaewchomphunuch, T. and Kaeoket, K. Antimicrobial activity of cell free supernatants from probiotics inhibits against pathogenic bacteria isolated from fresh boar semen. *Sci Rep* 13, 5995 (2023). https://doi.org/10.1038/s41598-023-33062-w

12. Vineetha N, Vignesh RA and Sridhar D. Preparation, standardization of antibiotic discs and study of resistance pattern for first-line antibiotics in isolates from clinical samples. Int J Appl Res. 2015;1 (11):624-631.

13. **S**occol CR, Vandenberghe LP, Spier MR, Medeiros AB, Yamaguishi CT and Lindner JD. The potential of probiotics: a review. *Food Technology and Biotechnology.* 2010; 48 (4):413–34.

14. Xinyu Yang, Zheng Peng, Mengni He, Zhibin Li, Guihua Fu, Shaolei Li, Juan Zhang, Screening, probiotic properties, and inhibition mechanism of a *Lactobacillus* antagonistic to *Listeria monocytogenes*, Science of The Total Environment, Volume 906, 2024,167587, ISSN 0048-9697.

15. Zhou, J. S., Pillidge, C. J., Gopal, P. K. and Gill, H. S. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *Int. J. Food Microbiol.* 2005; 98, 211–217.