International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Isolation & Identification of Microbiota Present in Different Probiotic Samples & Test Against Commercially Available Antibiotics

Abhijit Panda[1], Disha Mohanty[2], Dharitri Priyadarsini[3], Rajat Kumar Nayak[4], Pritismita Mahalik[5]

[1]M.Sc. Biotechnology, Reva University, Bangalore, Karnataka.
[2]Research Scholar, Department of Biotechnology & Medical Engineering, NIT, Rourkela, Odisha
[3,4]Senior Research Associate, SBio Science Pvt Ltd, Bhubaneswar, Odisha.
[5]M.Sc. Biotechnology, Trident Academy of Creative Technology, Bhubaneswar, Odisha.

Abstract

Probiotic bacteria are primarily offered in fermented foods, and dairy products which plays an important role as probiotic carriers. For various reasons, functional dairy foods are highly adapted to promote probiotics' favorable health image: (i) Fermented foods, particularly dairy products, have a positive health image due to their centuries-long use; (ii) people are aware that fermented foods contain living microorganisms; and (iii) probiotics are used as a starter to combine the positive images of fermentation and probiotic cultures. Probiotics are live bacterial formulations (food or medication) that have been clinically proven to improve human health. The majority of probiotics work by regulating mucosal barrier function and immunological activity. The human digestive tract and intestinal microbiota are specialized targets and possess certain characteristics of probiotics. In this research, commercially available antibiotics and separated microorganisms from them by culturing them in various nutritional media. The antibacterial activity was then observed. Different biochemical tests have been performed on samples to identify the types of bacteria present. The bacteria identified from pickle, lassi, watermelon, soy sauce were *Micrococcus varians*, *Pseudomonas aeruginosa*, *Vibrio* species, *Lactobacillus fermenti*, respectively. The antimicrobial activity of different antibiotics was tested against the isolated microbes from which cefolac-200 shows greater zone of inhibition against *Pseudomonas aeruginosa* (26.75 mm) lowest zone of inhibition against *Lactobacillus fermenti* (11.5 mm). Azithromycin and CV-625 does not show any zone of inhibition against *Micrococcus varians*. Some future studies should be performed to use these isolates reliably including molecular techniques like 16S rRNA sequencing for accurate identification of lactic acid bacterial species.

Keywords: Probiotics, *Micrococcus varians*, *Pseudomonas aeruginosa*, *Vibrio* species, *Lactobacillus fermenti*, cefolac-200, CV-625, Azithromycin.

Introduction:

Probiotics are beneficial microorganisms that provide health benefits when consumed. They can help digestion by fostering healthy gut microbes. Probiotics may aid with digestive issues such as diarrhoea,

IBS, and constipation. They can also improve nutrient absorption, increase immune function, and reduce the risk of certain ailments **[1]**. Probiotics have also been studied for their capacity to reduce the symptoms of allergies, eczema, and some mental health concerns. However, further research is needed to fully appreciate the benefits of probiotics and their optimal utilization. Before commencing any new supplement, contact a healthcare practitioner. **[2]**.

Probiotics may have the following benefits:

- Better Digestive Health: Probiotics can help maintain a healthy gut by managing intestinal flora. They help in nutrition digestion and absorption, reduce the risk of diarrhoea (especially antibiotic-induced diarrhoea), and may even alleviate the symptoms of gastrointestinal diseases such as IBS and inflammatory bowel disease (IBD) **[3]**.
- Enhanced Immune Function: The immune system and the gut are inextricably linked, and probiotics can aid in immune function. They aid in immune response regulation, the production of chemicals that prevent hazardous infections, and the production of natural antibodies. 3-Antibiotics have the potential to disrupt the usual balance of bacteria in the stomach, resulting in issues such as diarrhoea and yeast infections **[4]**.
- Lactose intolerance, allergies, eczema, and urinary tract infections are just a few of the problems that probiotics may aid with. The effectiveness, however, may vary based on the specific probiotic strains and individual conditions.
- Support for Mental Health: Recent research indicates a relationship between gut flora and mental health. According to certain studies, specific probiotic strains may improve brain function, reduce anxiety and depressive symptoms, and improve mood **[5]**.

Probiotics may have certain advantages, but it's crucial to remember that different strains, doses, and reactions may apply to different people. Before beginning any probiotic supplements, it is advised to speak with a healthcare provider, especially if you have any particular health issues or illnesses. Additionally, while probiotic supplements are usually thought to be safe for the majority of people, some people may suffer moderate stomach issues when first taking them. It is essential to speak with your healthcare professional before using probiotics if you have a compromised immune system or are really unwell **[6]**.

Probiotics have attracted increasing interest from product developers, academic investigations, and consumers in the twenty-first century. Their origins may be found in the early usage of cheese and fermented foods, which the Greeks and Romans were familiar with and encouraged people to eat. One of the oldest methods of food preservation is the fermentation of dairy products **[7]**.

Materials and Methods:

SAMPLE PREPARATION:

Four different samples viz., pickle, lassi, watermelon and soy sauce were collected from the local market of Cuttack and Bhubaneswar, Odisha. Different concentrations of samples were prepared by using serial dilution method.

ISOLATION OF MICROBIOTA:

The concentrations of diluted samples were used for pickle 10^{-4}; for lassi 10^{-5}; for watermelon 10^{-3}; and for soy sauce 10^{-4} and then directly inoculated in the nutrient agar media aseptically, and after

inoculation samples were incubated at 37°C for 24 hours to get the primary culture, sub-culture, pure culture and broth culture **[8]**.

IDENTIFICATION OF BACTERIA:

After isolation of bacteria, colony morphology and Gram's staining was done and various selective media were prepared to identify the specific microorganism followed by various biochemical tests viz., catalase test, motility test, salt tolerance test, carbohydrate fermentation test, urease test, indole test, MR test, VP test, citrate utilization test.

Catalase test was used to identify organisms that produce the enzyme, catalase and detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. Production of oxygen gas with bubbles indicates positive result. Motility test was used to determine the ability of an organism to swim randomly with flagella monitored under microscope. Turbidity in the entire tube against the current of water streaming indicates that bacteria are motile in a wet mount and vice versa **[8]**.

Indole test was conducted for the identification of organisms having tryptophanase enzymes which convert amino acid tryptophan into indole. The organism was inoculated in a test tube containing culture broth with the addition of 0.5 mL of Kovac's reagent and incubated at 37°C for 24 hours. Development of red-violet ring layer indicates positive whereas yellow ring layer indicates negative test **[8]**.

ANTIMICROBIAL ACTIVITY:

It is described as the process by which any activity of the microbe is restricted or the microorganism's development is impeded. Here we have used commercially available antibiotics to assess antimicrobial activity viz., Cefolac-200, CV-625, and Azithromycin.

Nutrient Agar (NA) plates were plated with 24 hours broth culture of different bacteria. In each of these plates, 3 wells (8 mm) were cut out. Using sterilized dropping pipettes, 0.1 ml of extracts (Cefolac-200, CV-625, and Azithromycin) were carefully added into the wells and then incubated at 37°C for 24 hours. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated **[9]**.

Results and Discussion:

Not all microorganisms obtained from probiotics are useful and health promoting, some of them might be disease and spoilage causing. In the present study total four samples viz., pickle, lassi, watermelon, and soy sauce were collected from different sources located in Bhubaneswar. In total four isolates were selected characterized through colony morphology on specific media, gram staining and biochemical tests shown in table 1.

Biochemical Tests	Pickle	Lassi	Watermelon	Soy sauce
Catalase	+ve	-ve	-ve	-ve
Motility	-ve	-ve	+ve	-ve
Urease	+ve	+ve	-ve	+ve
Carbohydrate Fermentation	+ve	-ve	+ve	+ve
Salt Tolerance	-ve	-ve	+ve	-ve

Indole	+ve	-ve	-ve	+ve
Methyl Red	+ve	+ve	-ve	-ve
Voges-Proskauer	-ve	-ve	+ve	+ve
Citrate Utilization	-ve	-ve	-ve	+ve

[Table.1: Biochemical tests done for the bacteria isolates from Pickle, Lassi, Watermelon, and Soy Sauce]

The most common bacterial species found are *Micrococcus varians*, *Pseudomonas aeruginosa*, *Vibrio species*, *Lactobacillus fermenti* shown in table 2.

Probiotics	Concentration of Samples	Gram's Staining Result	Microorganism Identified
Pickle	10^{-4}	Positive	*Micrococcus varians*
Lassi	10^{-5}	Negative	*Pseudomonas aeruginosa*
Watermelon	10^{-3}	Negative	*Vibrio species*
Soy Sauce	10^{-4}	Positive	*Lactobacillus fermenti*

[Table.2: Gram's staining of the isolates and microbial identification]

In this study, the antimicrobial activity of different antibiotics was tested against the isolated microbes (Table.3) from which cefolac-200 shows greater zone of inhibition against *Pseudomonas aeruginosa* (26.75 mm) lowest zone of inhibition against *Lactobacillus fermenti* (11.5 mm). Azithromycin and CV-625 does not show any zone of inhibition against *Micrococcus varians*.

Commercial Antibiotics	Zone of Inhibition (mm)			
	Micrococcus varians	***Pseudomonas aeruginosa***	***Vibrio species***	***Lactobacillus fermenti***
Cefolac-200	23.75	26.75	16.75	11.50
CV-625	0	25.75	24.25	12.50
Azithromycin	0	21.25	20.00	14.50

[Table.3: Zone of inhibition created by different fruit peel extracts against different bacteria]

Although the discovery of antibiotics was an important breakthrough in the 20th century and drugs such as penicillin and streptomycin have saved millions of lives, there is always collateral damage as our commensal microbiota is also affected.

Evidence from well-conducted observation research and a lot of randomised controlled research confirms the potential influence of probiotics on human health. However, extending the term "probiotic" to include bacteria isolated from traditionally, spontaneously fermented foods seem justified. Microorganisms isolated from fermented products constitute the microflora of an environment in which

the products were produced. If they are tested, particularly in terms of their probiotic properties and safety, they may constitute an interesting alternative to gut bacteria **[10]**.

Conclusion:

Probiotics have been shown to promote a variety of biological effects in a number of physiological conditions and pathologies, including allergy, organ diseases, urinary and upper respiratory infections, and AIDS. In addition, multiple studies are necessary to evaluate the actual role of probiotics in the improvement of symptoms for many diseases.

In this study, the isolation and identification of microbes were determined by colony morphology, gram staining and different biochemical tests. The bacteria identified from pickle, lassi, watermelon, soy sauce were *Micrococcus varians*, *Pseudomonas aeruginosa*, *Vibrio* species, *Lactobacillus fermenti*, respectively. The antimicrobial activity of different antibiotics was tested against the isolated microbes from which cefolac-200 shows greater zone of inhibition against *Pseudomonas aeruginosa* (26.75 mm) lowest zone of inhibition against *Lactobacillus fermenti* (11.5 mm). Azithromycin and CV-625 does not show any zone of inhibition against *Micrococcus varians*.

Recently it has been discovered that probiotics have effect on anticancer agent. Therefore, some future studies should be performed to use these isolates reliably including molecular techniques like 16S rRNA sequencing for accurate identification of lactic acid bacterial species and multiplex RAPD-PCR technique could be used to reveal the complete metabolic potential of each of the probiotic strains **[11]**.

Acknowledgment:

We are grateful to SBio Science Pvt. Ltd. for supporting us for well-established laboratory facilities and helping us in paper writing.

Conflict of Interest:

Nil

References:

1. Huang, M. L., Huang, J. Y., Kao, C. Y., & Fang, T. J. (2018). Complete genome sequence of Lactobacillus pentosus SLC13, isolated from mustard pickles, a potential probiotic strain with antimicrobial activity against foodborne pathogenic microorganisms. *Gut Pathogens*, *10*, 1-6.
2. Lata, P., Kumari, R., Sharma, K. B., & Rangra, S. (2022). In vitro evaluation of probiotic potential and enzymatic profiling of *Pichia kudriavzevii* Y33 isolated from traditional home-made mango pickle. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 132.
3. Ragul, K., Syiem, I., Sundar, K., & Shetty, P. H. (2017). Characterization of probiotic potential of Bacillus species isolated from a traditional brine pickle. *Journal of food science and technology*, *54*, 4473-4483.
4. Pant, R., Sharma, N., Kabeer, S. W., Sharma, S., & Tikoo, K. (2023). Selenium-enriched probiotic alleviates western diet-induced non-alcoholic fatty liver disease in rats via modulation of autophagy through AMPK/SIRT-1 pathway. *Biological Trace Element Research*, *201*(3), 1344-1357.
5. Behera, S. S., Panda, S. H., Panda, S. K., & Kumar, A. (2019). Biochemical analysis of elephant foot yam (Amorphophallus paeoniifolius) lacto-pickle with probiotic Lactobacillus plantarum. *Annals of Microbiology*, *69*(6), 577-590.

6. Al-Shawi, S. G., Swadi, W. A., & Hussein, A. A. (2019). Production of probiotic (Turshi) pickled vegetables. *J Pure Appl Microbiol*, *13*(4), 2287-2293.
7. Bahaddad, S. A., Almalki, M. H., Alghamdi, O. A., Sohrab, S. S., Yasir, M., Azhar, E. I., & Chouayekh, H. (2023). Bacillus Species as direct-fed microbial antibiotic alternatives for monogastric production. *Probiotics and antimicrobial proteins*, *15*(1), 1-16.
8. Mishra, Omkar., S, Pavithra., Priyadarsini, D., & Nayak, R. K. Isolation and Identification of Complex Microbiota from Milk and Milk Products and Anti-Microbial Activity of Fruit Peels Against Isolated Bacteria. *IJFMR-International Journal For Multidisciplinary Research*, *5*(4).
9. Kittibunchakul, S., Yuthaworawit, N., Whanmek, K., Suttisansanee, U., & Santivarangkna, C. (2021). Health beneficial properties of a novel plant-based probiotic drink produced by fermentation of brown rice milk with GABA-producing Lactobacillus pentosus isolated from Thai pickled weed. *Journal of Functional Foods*, *86*, 104710.
10. Zielińska, D., & Kołożyn-Krajewska, D. (2018). Food-origin lactic acid bacteria may exhibit probiotic properties. *BioMed research international*, *2018*.
11. Rahman, S. M. K. (2015). Probiotic properties analysis of isolated lactic acid bacteria from buffalo milk. *Arch Clin Microbiol*, *7*(1), 5-10.