EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 11
176-179
Year: 2023

ISOLATION, CHARACTERISATION, AND SCREENING OF GUT BACTERIA FOR ESSENTIAL PROBIOTIC CHARACTERISTICS

Nishi J. Patel[1]*, Dr. Murtaza Hajoori[1] and Dr. Piyush Desai[1]

[1]Ph.D. Research Scholar, B.Sc. Microbiology, M.Sc. Microbiology, Bhagwan Mahavir University, Surat.
[2]Head of Department, B.Sc. Microbiology, M.Sc. Bioinformatics, Ph. D Microbiology, Bhagwan Mahavir University, Surat.
[3]Prior Research Dean, B.Sc. Microbiology, M.Sc. Microbiology, Ph. D Microbiology, Bhagwan Mahavir University, Surat.

***Corresponding Author: Nishi J. Patel**
Ph.D. Research Scholar, B.Sc. Microbiology, M.Sc. Microbiology, Bhagwan Mahavir University, Surat.

Article Received on 08/09/2023 **Article Revised on 29/09/2023** **Article Accepted on 20/10/2023**

ABSTRACT
The human gastrointestinal system is home to a wide variety of microbes that collectively comprise a unique microbiota characteristic of every individual. Healthy microbiota are those that are normal and sustain and promote overall health and the absence of disease, particularly in the gastrointestinal system. The basis for probiotic therapy is the improvement of the native microbiota's unbalanced characteristics. Here we have identified 4 such bacteria isolated from the gut that can be used as potential probiotics. The bacteria have been screened for probiotic characteristics like resistance to pH, resistance to bile salts, resistance to phenol, Haemolytic test, DNase test, antibacterial test, and antifungal test. Various biochemical tests have characterized the isolates. Four isolates have been considered to have good probiotic properties on the basis of biochemical characterization two of the isolates are presumed to be *Bacillus subtilis sp.* and the other two belong to *Lactobacilli sp.*

KEYWORDS: Gut bacteria, Probiotics, Gut modulation.

INTRODUCTION
10^{13}–10^{14} microorganisms, or 1–10 times more than the body's eukaryotic cells, can be found in the human gut, typically containing 500–1000 different bacterial species.[1]

The beneficial bacteria are retained in the caecum, a pocket of the large intestine connected to the appendix. This way, if the good bacteria go completely due to situations like diarrhea, they can repopulate the digestive system. Within the first three years of life, the gut begins to colonize. Our individual microbiomes are as distinctive as our fingerprints. From the moment you are born, practically everything you do and experience influences the development of your particular gut flora composition. While bacteria are found throughout the human body, including in the oral cavity, placenta, vagina, skin, and GIT, the colon is home to the bulk of anaerobic bacteria. The gut microbiome expresses 3.3 million prokaryotic genes, compared to the human body's 20,000 eukaryotic genes, to put the size of the bacterial population and its possible impact on the host into perspective. The primary functions of the intestinal barrier and the prevention of the colonization of harmful microorganisms are supported by the gut commensals. While performing its duty of warding off invasive pathogenic microbes, the immune system has also co-evolved to live in a collaborative partnership with the healthy microbiota. Thus these gut bacteria can be explored for their probiotic characteristics and used for gut modulation.[2]

Probiotics are live, nonpathogenic microorganisms that are given to patients to help with microbial balance, especially in the digestive system.[3]

METHODS AND MATERIAL
1.1 Sampling and Isolation: Faecal samples were collected from volunteers after obtaining a signed consent form. The samples were collected in a sterile container by direct sampling technique and stored at -3°Celcius for further use. The bacteria were isolated on Nutrient agar medium and Gut Microbiome medium[4] to isolate all possible bacteria. The samples were serially diluted and spread and plates were incubated at 37°Celcius. After incubation each type of different bacterial colony was isolated by streaking and pure cultures were obtained.

1.2 Screening: the isolates were screened for various probiotic characteristics.[5,6,7,8]

- Resistance to bile salts: Bacteria were grown in broth supplemented with 0.3% oxgall. The bile salt condition control was the NA broth free of ox gall. The samples were incubated at 37°C before being removed and tallied. The colonies (CFU/ml) of each culture acquired from the trials were counted on NA agar and cultivated at 37°C for 24 hours. The survival rate (%) was calculated using the formula below:

Survival rate (%) = Biomass at time (t)/Biomass at initial time (0) × 100.

- Resistance to pH: Injecting various species into NA broth with a pH of 2 worked. NA broth that had been previously pH-adjusted to 6.5 served as the control. The samples were incubated at 37°C before being removed and tallied. The colonies (CFU/ml) of each culture acquired from the trials were counted on NA agar and cultivated at 37°C for 24 hours. The survival rate (%) was calculated using the formula below:

Survival rate (%) = Biomass at time (t)/Biomass at initial time (0) × 100.

- Resistance to Phenol: Bacterial isolates were added to the nutritious broth, which also included phenol, at concentrations of 0.4 and 0.6% v/v. The cultures were diluted and distributed over Nutrient agar plates after 24 hours at 37°C. The plate count method was used to calculate cell viability (log CFU/ml).
- Antifungal activity: The antifungal efficacy of the LAB isolates against Aspergillus spp. was evaluated using the agar overlay method. LAB isolates were dispersed in two different, equally spaced patches on Nutrient agar plates and incubated anaerobically at 37°C for 24 hours. A 20 l suspension of a fungal pathogen's spores (106 spores/ml) that had been mixed with 0.7% soft potato dextrose agar (PDA) was then spread over the NA agar plates. After 4 days of aerobic incubation at 28 2°C, the plates were examined for apparent inhibitory zones around the LAB colonies' spot areas.
- Haemolytic test: After being streaked onto blood agar plates with 5% (w/v) blood, all of the studied isolates were allowed to develop for 48 hours at 37°C. After incubation, the plates were examined for α-hemolysis, β-hemolysis, and non-hemolytic activities.
- DNase test: The LAB isolates were streaked onto a deoxyribonuclease (DNase) agar medium to see if the DNase enzyme had formed. After a 48-hour incubation period at 37 °C, the DNase activity zone is checked. A distinct pinkish zone surrounding the colonies was considered a sign of DNase activity.
- Antibacterial activity: Using the microplate method, the antibacterial potency of the LAB isolates was examined against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. Fill a sterile 96-well plate with 50 l of CFS/nCFS and 50 l of bacterial solution to achieve 108 CFU per well. Then, you can make up to 200 l of NA broth. The negative control is just NA broth, while the positive control is NA soup with a bacterial suspension. After a day of incubation at 37 °C, check the optical density (OD) at 600 nm.

Calculate the total per cent inhibition of bacterial growth using the following formula: [(OD of the test sample(A) - OD of control(B))/OD of control(C)] × 100.

A = OD of test sample after incubation with pathogenic strain.

B = OD of test sample before incubation with pathogenic strain.

C = OD of pathogenic strain before incubation with the test sample.

1.3 Characterisation of isolates: The final selected isolates were characterized by Gram's staining, colony characteristics, growth on various selective and differential agar media, biochemical tests and oxidase test and catalase tests.[9]

RESULTS

2.1 Sampling and isolation

Figure 1: Isolation on Nutrient agar plate and Gut microbiome medium.

2.2 Screening

- Resistance to bile salts: Survival rates obtained for the four main isolates were HFS 10.2 TM – 87%, HFS 2.1 TM – 84%, HFS 11.1 PDA- 67%and HFS 11.1 TM - 67%.
- Resistance to pH: Survival rates obtained for the four main isolates were HFS 10.2 TM – 93%, HFS 2.1 TM – 51%, HFS 11.1 PDA- 56%and HFS 11.1 TM - 62%.
- Resistance to phenol: All the isolates were quite resistant to phenol. Values of Log CFU/ml were obtained mostly around 7.

Table 1: Resistance to phenol.

Sr. No.	Sample code	Log CFU/ml					
		Broth Without phenol		Broth with 0.4% phenol		Broth with 0.6% phenol	
		0 hour	24 hours	0 hour	24 hours	0 hour	24 hours
1	HFS 2.1 TM	7.46	8.54	7.45	7.39	7.44	7.14
2	HFS 10.2 TM	8.72	9.19	8.7	8.43	8.71	8.21
3	HFS 11.1 TM	7.77	7.94	7.80	7.57	7.76	7.31
4	HFS 11.1 PDA	8.66	9.50	8.65	8.32	8.61	8.19

- Antifungal activity: Antifungal activity was exhibited by all 4 isolates.

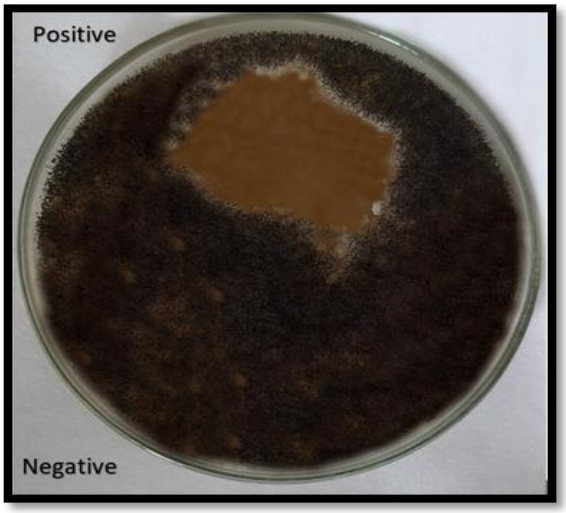

Figure 2: Antifungal activity.

- Haemolytic test: All four isolates were found to be non-hemolytic.

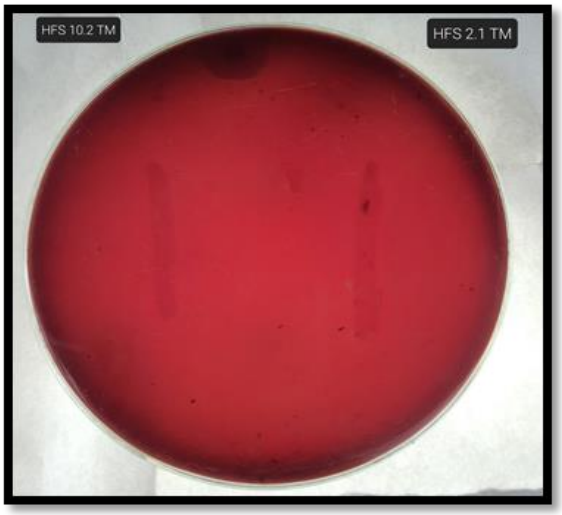

Figure 3: Haemolytic test.

- DNase test: All four isolates showed negative results for DNase activity.

Figure 4: DNase test.

- Antibacterial activity: Maximum antibacterial activity was observed against *Pseudomonas aeruginosa* least antibacterial activity was observed against *Escherichia coli* and almost equal antibacterial activity was obtained against *Staphylococcus aureus* and *Staphylococcus aureus.*

2.3 Characterization

Table 2: Gram's reaction and colony characteristics.

Sample Code	Gram's Reaction	Colony characteristics
HFS 2.1 TM	Gram positive rods	Large, rough, opaque, fuzzy white or slightly yellow with jagged edges
HFS 10.2 TM	Gram positive rods	Large, rough, opaque, fuzzy white or slightly yellow with jagged edges
HFS 11.1 TM	Gram positive rods	Medium, round, smooth, translucent, entire, no pigmentation, slightly raised.
HFS 11.1 PDA	Gram positive rods	Medium, round, smooth, translucent, entire, no pigmentation, slightly raised.

No growth was observed on EMB agar plate, Mannitol salt agar, and Mackonkey agar plates. No fishy smell or pigmentation was produced on King's agar plate. HFS 2.1 TM and HFS 10.2 TM showed rhizoid growth on Potato.

Table 3: Catalase and Oxidase test.

Sample Code	Catalase test	Oxidase test
HFS 2.1 TM	Positive	Positive
HFS 10.2 TM	Positive	Positive
HFS 11.1 TM	Negative	Negative
HFS 11.1 PDA	Negative	Negative

Table 4: Biochemical tests.

Sample Code	Indole Test	MR Test	VP Test	Citrate Test	Gelatine Test	Urease Test	Nitrate Reduction test	Lead Acetate Test	TSI Slant/butt/gas/H_2S
HFS 2.1 TM	-	+	-	+	+	-	+	-	R/R/-/-
HFS 10.2 TM	-	+	-	+	-	-	-	-	Y/R/-/-
HFS 11.1 TM	-	-	-	-	-	-	-	-	Y/R/-/-
HFS 11.1 PDA	-	-	-	-	-	-	-	-	Y/R/-/-

DISCUSSION

The research offers 4 bacterial isolates that can be used in probiotic formulations. All 4 isolates are nonpathogenic as they have shown negative results for the hemolytic test and DNase test, and are resistant to pH, bile, and phenol so can survive in gut conditions and also exhibit antifungal and antibacterial activity thus will help eliminate harmful, spoilage and pathogenic strains from the gut.

REFERENCES

1. Thursby, E., & Juge, N. Introduction to the human gut microbiota. *Biochemical Journal*, 2017; *474*(11): 1823-1836.
2. Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Nageshwar Reddy, D. Role of the normal gut microbiota. *World journal of gastroenterology*, 2015; *21*(29): 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787
3. Nancy Toedter Williams, Probiotics, *American Journal of Health-System Pharmacy*, 15 March 2010; 67(6): 449–458. https://doi.org/10.2146/ajhp090168
4. Ito T, Sekizuka T, Kishi N, Yamashita A, Kuroda M. Conventional culture methods with commercially available media unveil the presence of novel culturable bacteria. Gut Microbes, 2019; 10(1): 77-91. doi: 10.1080/19490976.2018.1491265. Epub 2018 Aug 17. PMID: 30118379; PMCID: PMC6363062.
5. Somashekaraiah, Rakesh, B. Shruthi, B. V. Deepthi, and M. Y. Sreenivasa. "Probiotic properties of lactic acid bacteria isolated from neera: A naturally fermenting coconut palm nectar." Frontiers in Microbiology, 2019; 10.
6. Tulini FL, Winkelströter LK, De Martinis ECP Identification and evaluation of the probiotic potential of *Lactobacillus paraplantarum* FT259, a bacteriocinogenic strain isolated from Brazilian semi-hard artisanal cheese. Anaerobe, 2013; 22: 57–63
7. Fernandez MF, Boris S, Barbes C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol, 2003; 94: 449–455
8. Solieri L, Bianchi A, Mottolese G, Lemmetti F, Giudici P. Tailoring the probiotic potential of non-starter *Lactobacillus* strains from ripened Parmigiano Reggiano cheese by in vitro screening and principal component analysis. Food Microbiol, 2014; 38: 240–249.
9. Experimental microbiology by Rakesh Patel. https://berrima.net.in.tum.de/textual?rackid=A77b146&FilesData=Experimental_Microbiology_By_Rakesh_Patel.pdf