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Isolation and Characterization of *Lactobacillus* Sp. from Different Dairy Products and Fermented Foods for their Application on Gastrointestinal Disorder

Sreerupa Bedi[1], Manisha Maity[2*], Bidyut Bandyopadhyay[3], Tarun Kumar Kar[4], Shibam Pahari[5] and Tumpa Panda[6]

[1]Research Scholar, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore
[2]Assistant Professor and Head, Department of Food and Nutrition, Swami Vivekananda University, Barrackpore*
[3]Professor and Principal, PG Department of Biotechnology and Biochemistry, Oriental Institute of Science and Technology, West Bengal, India
[4]Research Scholar, Department of Biomedical Laboratory Science and Management, Vidyasagar University, Midnapore
[5]Student of Master Degree, Department of Nutrition & Dietetics, Vidyasagar Institute of Health affiliated by Vidyasagar University, Midnapore
[6]Student of Master Degree, Department of Nutrition & Dietetics, Vidyasagar Institute of Health affiliated by Vidyasagar University, Midnapore

***Corresponding Author:** Manisha Maity*
**Email ID- manisham@svu.ac.in*

Abstract

Probiotics are live, nonpathogenic bacteria that promote the health of their hosts. Probiotics and fermented foods are the two most significant and often-used functional food components. Functional foods have a variety of nutritive advantages. Idli, dhokla, pickles, and other traditional Indian dishes that benefit the host include dairy products with probiotics. They increase the live microbial supplements' capacity for survival and implantation. In our study, the detection was done of probiotics that were isolated from fermented sources such as fermented rice water, Whey water, Cow milk, and Idli batter collected from different places in Rangamati, West Midnapore, West Bengal.

The isolation of probiotics was carried out by the standard serial dilution methods in Nutrient media. The confirmatory test was done on De Man, Rogosa and Sharpe broth. Suspected lactic acid bacteria were further purified. Morphological and biochemical characterization of isolated organisms was also done. The isolated bacteria were identified by comparing the investigated characters of the isolates to Bergey's manual of determinative bacteriology. The isolated *Lactobacillus* sp. satisfy the criteria required for a probiotic, such as pH (tolerance to harsh conditions low), and optimum temperature can produce bacteriocin extra-cellularly which may inhibit gastrointestinal malfunctions such as Colitis. These isolates may have the potential to be used as probiotics.

Keywords: Probiotic, Isolation, Fermented food, De Man, Rogosa and Sharpe, Lactobacillus sp., Gastrointestinal malfunctions.

1. Introduction

The term "human microbiome" describes the totality of bacteria that exist on or in human tissues, such as the ovaries, follicles, lungs, saliva, seminal fluids, skin, placenta, and oral mucosa. Probiotics are good microorganisms for human health with the capacity to alter a host's environment's numerous internal and external elements. These are the microorganisms that regularly show a close association with the normal flora on human body surfaces both internally and externally. However, the probiotic bacteria that dominate colonization are typically located in the gastrointestinal tract (Ducatelle et al., 2015).

"Probiotic" is a term that implies "for life." They are categorized as live, non-pathogenic bacteria that interact with the host body and are extremely beneficial to the digestive system. Vergin (1954) used the phrase in his research on gut microbes and their antagonistic mechanism on anti-microbial components (Kavita et al 2015). Later, probiotics were redefined by Lily and Stillwell (1965) (Seppo et al., 1999; Amarna, 2016) as a product that may encourage the growth of other microorganisms. Furthermore, Fuller (1992) regarded this probiotic as non-pathogenic. Finally, they were identified by FDA and WHO as a bacterium that, when given to a host, had some positive effects on that host (Hardy et al., 2013; He and Shi, 2017). These bacteria often mediate critical immunological and physiological processes.

The substances that the upper GI tract is unable to absorb are referred to as prebiotics. Following the transformation of the carbohydrates by specific specialized enzymes, they are broken down and absorbed by the beneficial bacteria in the colon (Kuo, 2013). In various researches, prebiotics like pectin and insulin have been found to be able to reduce the prevalence of some gastrointestinal ailments such as bowel problems, intestinal inflammation, colon infections, etc. (Pena, 2007). Dragon fruit, yacon root, and other plants are currently regarded as some of the best sources of prebiotics (Saulnier et al., 2009).

Gibson first coined the term "symbiotic." According to his theory, the presence of prebiotics and symbiotic (probiotic + prebiotic) organisms will boost the probiotic's ability to modulate health (Vrese and Schrezenmeir, 2008). The symbiosis affects the microbial environment of the gastrointestinal tract by specifically promoting the growth of probiotics (Cencic and Chingwaru, 2010). Numerous elements, including the misuse of antibiotics, a stressful lifestyle, an unmaintained diet, and various pathogenic diseases, can result in dysbiosis, which again impairs immune function and may even produce low-grade chronic inflammation (Vieira et al., 2013). Probiotics have a variety of significant and early benefits that have so far been well-researched. According to Harish and Varghese (2006), they include benefits that are anti-oxidant, anti-bacterial, anti-inflammatory, and lessen cholesterol assimilation. They also include the protection of different gastrointestinal illnesses.

Probiotic species often fall under the LAB group. The probiotics *Lactobacillus*, *Streptococcus,* and *Bifidobacterium* have been the subject of the most research for their potential to promote health. The various strains of Lactobacillus, such as *L. rhamnosus*, *L. bulgaricus*, *L. fermentum*, *L. casei*, and *L. reuteri*, among others, are used as probiotics. *Propionibacterium sp., Escherichia coli*, *Enterococcus, Bacillus* and *Streptococcus* are among the other groups of bacteria that are not included in the LAB (Rastogi et al., 2011).

Using *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, "bio yogurts"—gently acidified yogurts—were created in Germany in the late 1960s (Goktepe et al., 2006). Probiotic items, such as fermented milk drinks, yogurt, cheese, ice cream, sausages, etc. with specific starting cultures, are highly demanded in the markets because probiotic bacteria may have therapeutic or preventative effects. Probiotic industrial uses, though, might be difficult. Numerous strategies were employed by healthy bacteria to control illness prevention, gut immunity, and the environment in which they exist. It was well acknowledged that this diet offered the right nutrients. These meals were also well known for their relaxing qualities. The fermented rice-milk beverage products produced when *L. acidophilus* and *Streptococcus thermophillus* are utilized as starter cultures with rice steep liquor-milk exhibit a particular flavor, a pleasant taste, and a milky color (Yong-Jin et al., 2010). The key preservative in food fermentation, according to Jagadeeswari et al. (2010), is the creation of the primary metabolite, lactic acid, and the accompanying decrease in pH. By supplying probiotics, bran fiber, and other bioactive components, cereal ingredients, such as products made from wheat bran and fermented with probiotics, boost consumer health, claim Lamsal and Faubion (2009). It is known that stress, a number of diseases, bad eating habits, chlorinated water use, alcohol consumption, and the use of antibiotics can alter the composition and functionality of gut flora. These consequences, which are comparable to what occurs following systemic antibiotic therapy on such occasions, lead to the proliferation of opportunistic and undesirable bacteria in the gut. The quantity of beneficial bacteria is declining, which results in the production of too much gas, bloating, constipation, intestinal toxicity, and inadequate nutrition absorption. Probiotics must be enriched in the human stomach in order to maintain the probiotic balance through regular eating of probiotic meals. In addition to low pH resistance, bile tolerance, adhesiveness, antibacterial activity, and antibiotic

susceptibility, many species of lactobacilli also possess these probiotic traits. According to research by Sandholm et al. (2002), different species from different origins have different probiotic characteristics.
Genetically modified (GM) probiotics run the risk of recombination and horizontal gene transfer resulting in the development of harmful pathogens (Cummins and Ho, 2006). The creation and application of GM probiotics are restricted. As a result, many scientists are looking into new strains with improved probiotic potential from various sources. In nations where rice is a staple, one of the underappreciated foods is fermented rice. There is also a dearth of knowledge regarding fermented rice and the probiotic bacteria that are present in it, despite the large number of probiotic bacteria strains that have been isolated from many sources, including traditional fermented foods. The current study's goal was to extract and identify possible probiotic bacteria from fermented rice against this background.

2. MATERIALS AND METHODOLOGY

2.1 Raw materials: The various fermented sources, including fermented rice water (FRW), fermented whey water (FWW), cow milk (CM), and fermented idli batter (IB), were acquired from a local market, and the rice water and whey water were made at home, for identification purposes. The samples are stored at room temperature for two weeks to ferment.

2.2. Isolation of lactic acid bacteria from different samples: All the samples were taken and stored at 4ºC for further use. Rice was mashed and added water to it to prepare a rice water solution. Then the water solution was allowed to ferment for 7 days. FRW was taken for organism isolation. Whey water (WW) was also allowed to ferment for 7 days. Other samples (CM and IB) were taken directly for organism isolation. 1 ml of each sample was added with 9 ml of sterile saline solution.

2.3. Inoculum preparation:
Samples from 10^{-7},10^{-4} dilution were taken under aseptic conditions and 1 ml of samples were added to 50 ml of MRS broth (10 g peptone, 5 g yeast extract, 10 g beef extract, 20 g glucose, 2 g potassium phosphate, 5 g sodium acetate, 0.2 g magnesium sulfate, 0.05 g manganese sulfate, 1.08 g tween 80, 2 g Ammonium citrate per liter, pH 7.5) to prepare inoculums.

2.4. Culture preparation: All of the sample inoculums (10 µl) were spread out on the MRS agar and left for overnight anaerobic incubation at room temperature. Incubated plates of FRW (10^{-5}), IB (10^{-7}), CM (10^{-4}) and WW (10^{-4}) dilutions were selected for organism collection. Single colonies were collected and streaked on MRS agar plates. Plates were incubated for 24 h at room temperature.

2.3 Pure Culture and colony morphology: Selected colonies from MRS agar plates were collected and streaked on MRS agar plates to prepare pure culture and incubated at 37º C. Every pure culture was subcultured separately to maintain the purity at three weeks' regular intervals.

2.4. Characterization (morphological and biochemical) of isolated organisms
Isolated pure organisms were subjected to the characterization process. Colony morphologies (shape, color, elevation, margin, gram character) were evaluated according to Chettri et al. (2016). Various biochemical tests (KOH, Catalase, methyl red, indole, starch hydrolysis, casein hydrolysis, citrate reduction, and growth in the presence of NaCl) were performed to identify the genus of the species. Different sugars such as galactose, lactose, and dextrose (1% solution) were used to assess the acid production capacity of isolated organisms. Finally, all the characterization was verified in accordance with Bergey‘s Manual of Systematic Bacteriology (Holt et al., 1994).

2.5. Growth at different pH: Four distinct pH values, ranging from 5 to 9, were utilized to check the growth of the bacterial isolates at various pH levels. 1N HCL w/v and 1N NaOH w/v were used to prepare the solution with desired pH. Samples were incubated in MRS broth (5ml) with various pH for 48 h at 37ºC.

2.6. Growth at different pH: Three distinct temperature ranges, including 20°C, 37°C, and 50°C, were set up to test the growth of the bacterial isolate at various temperatures according to the procedure described by Bao et al., 2016.

3. RESULTS AND DISCUSSION

3.1. Screening and isolation: Different colonies were isolated from FRW, FWW, CM and IB sources on MRS medium. The morphological, biochemical and staining characteristics of isolated microorganisms were evaluated and presented in Table 1.

Table 1: Morphological, staining and biochemical characteristics of isolated organisms from FRW, FWW, CM and FIB

Parameters	Characterization			
Morphological Characteristics	**FRW**	**FWW**	**CM**	**FIB**
Shape	Round/oval	Round	Round	Round
Margine	Undulate	Undulate	Undulate	Undulate
Elevation	Raised	Flat	Raised	Raised
Color	Creamy	White/creamy	White	White
Staining Characteristics				
Gram reaction	+	+	+	+
Cell shape	Rod	Rod	Rod	Rod
Biochemical Characteristics				
KOH	+	+	+	+
Catalase	-	-	-	-
Methyl-red	+	-	+	-
Indole	-	-	+	-
Starch hydrolysis	+	+	+	+
Casein hydrolysis	+	+	+	+
Citrate reduction	+	+	+	+

(FRW- Fermented Rice Water, FWW- Fermented Whey Water, CM-Cow Milk and FIB- Fermented Idli Batter)

The effect of different NaCl (%) concentrations on the growth of isolated organisms was also evaluated and represented in Table 2.

Table 2: Effect of different NaCl salt concentrations (%) on isolated organisms

Salt (NaCl) Concentration (%)	Characterization			
	FRW	**FWW**	**CM**	**FIB**
3	+	+	+	+
5	+	+	+	+
7	-	-	+	-
9	-	-	-	-
11	+	+	+	+

(FRW- Fermented Rice Water, FWW- Fermented Whey Water, CM-Cow Milk and FIB- Fermented Idli Batter)

3.2. Effect of sugar in fermentation medium

Generally, probiotics can produce acids by utilizing different sugars. Here in this present study, isolated organisms from FRW and WW sources showed acid production and viable growth in the presence of galactose, maltose, sucrose and lactose. Isolate from CM also showed a positive result in the presence of maltose, sucrose and lactose, but can't grow in the presence of galactose. Isolates from IB source can grow and produce acids in the presence of galactose, sucrose and lactose (Table 3).

Table 3: Growth characteristics of isolated organisms in presence of various sugars

Sugar Fermentation	Characterization			
	FRW	**FWW**	**CM**	**FIB**
Galactose	+	+	-	+
Maltose	+	+	+	-
Sucrose	+	+	+	+
Lactose	+	+	+	+

(FRW- Fermented Rice Water, FWW- Fermented Whey Water, CM-Cow Milk and FIB- Fermented Idli Batter)

3.3. Effect of different pH of isolated organisms

The viable count of isolated organisms was assayed in the presence of various pH concentrations. The pH tolerance of isolated organisms was assayed in the presence of pH 3,5,7 and 9 (Table 4). All the organism showed their growth tolerance at pH 3 and 5. However, their growth was not observed in pH 7 and 9.

Table 4: Effect of different pH on isolated organisms

pH	Characterization			
	FRW	FWW	CM	FIB
3	+	+	+	+
5	+	+	+	+
7	-	-	-	-
9	-	-	-	-

(FRW- Fermented Rice Water, FWW- Fermented Whey Water, CM-Cow Milk and FIB- Fermented Idli Batter)

3.4. Effect of different temperatures on the growth of isolated organisms

Temperature is a vital parameter for the growth of any microorganism. Without proper temperature, optimum growth will not be possible. The effect of temperature on an isolated organism was evaluated in the presence of 20, 40, and 50º C and shown in Table 5. All the isolated organisms showed positive growth at 20º C. However, only organisms isolated from IB and FRW were able to grow in the presence of 40º C, but isolates from WW and CM were not able to grow at that mentioned temperature. All the isolated isolates were unable to grow at 50º C.

Table 5: Effect of different temperature on isolated organisms

Temperature (º C)	Characterization			
	FRW	FWW	CM	FIB
20	+	+	+	+
37	+	-	-	+
50	-	-	-	-

(FRW- Fermented Rice Water, FWW- Fermented Whey Water, CM-Cow Milk and FIB- Fermented Idli Batter)

Numerous locally sourced samples were used to isolate the lactic acid bacteria. By using a standard microbiological process and inoculating a solid medium, the isolation was carried out. Colonies that showed the typical Lactobacillus characteristics (creamy white hue, soft consistency with undulated edge, flat elevation, and round shape) were chosen for more research. The bacterial isolate was determined to be Gram-positive, rod-shaped, non-spore-forming, and catalase-negative after being stained for Gram and endospores. The isolate allows for bacterial growth between 20°C and 40°C. Think about two different temperatures (20° and 40°), notice the best bacterial growth using the CFU/ml plate counting method, and accept a greater salt content of up to 5%. The isolated bacterial strain can flourish in acidic environments. It was discovered that the bacterial isolate exhibited enzyme activity, including protease and amylase activity. The capacity of the organism to yield favorable outcomes was shown in both of these tests. According to taxonomic criteria and the 8th edition of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), the bacterial isolate was provisionally identified as *Lactobacillus delbreuckii* sub spp. *Bulgaricus* based on morphological and biochemical features. Only procedures influenced by culture were used to produce this result. It was stated that lactobacillus sp. was discovered to be prominent and play a vital role during fermentation in several investigations on fermented idli batter and other fermented foods (Nam et al., 2012; Hwang et al., 2020; Kharnaior and Tamang, 2022; Tamang et al., 2022).

4. CONCLUSION

The *lactobacillus* sp. found in fermented foods can aid in the fermentation process and also create a number of advantageous substances, including those with anti-oxidant, anti-cancer, anti-diabetic, anti-allergic, and immunoregulatory qualities. Another study identified Lactobacillus as the primary microbe responsible for producing the crucial enzyme activity required for the therapy of cancer and many degenerative diseases.

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