Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Antagonistic Activity of Probiotics against Gram Negative Bacteria

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Abstract

Probiotics are live cells with various beneficial properties that have been thoroughly researched and investigated for use in a wide range of products on the global market. Numerous scientific researchers have demonstrated their benefits for both human and animal health. The current study set out to isolate probiotic bacteria that could be hostile from a variety of curd samples in order to isolate them. After a preliminary screening process, 39 bacterial strains were identified as promising probiotics from the samples. The probiotic qualities and antagonistic activity of each of the chosen isolates against clinical stool samples obtained from patients and utilized for the isolation of bacterial pathogens were then assessed in vitro. Pathogens and aggregation tests using automated identification systems (VITEK) were used to identify the pure bacterial isolates. The results demonstrated that the most efficient strains for preventing the growth of all test pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteous vulgaris*, and *Enterobacter* sp., were the prospective probiotic isolates Lact, S2, M3, F1, Y1, Y3, and Y4. The isolates were identified as excellent, promising in vitro antibacterial probiotic isolates against pathogens based on the data obtained; further in vivo evaluation and human health benefits in their actual environments are required.

Keywords: Probiotics, Antagonistic Activity, Gram Negative Bacteria, pathogens.

1. Introduction

These probiotic bacteria are necessary to have a positive impact on the health of a certain organism and to provide nourishment to the host for a healthy digestive system. "Live microorganisms when administered in adequate amounts; confer a health benefit on the host" is the definition of probiotics [1-3]. This definition admits that probiotics may have a place in medicine, but it makes no mention of the possibility that they may, on rare occasions, cause illness. The administration of these living microorganisms to cure or prevent disease has gained more attention from the scientific community and general public throughout the past ten years.

There are 1012 bacteria for every gram of big intestine contents in the colon, which makes up the great majority of all cells in the body. There is no need for a bacterial supplement because a protective gut microflora naturally arises. However, our changing lifestyles and eating habits compel us to consume sterile, processed food, which influences the kind of bacteria that can colonize us and our ability to access them [4-6].

The Lactobacillus genus is a member of the animal and human normal mucosal microbiota [7]. This particular group of bacteria is critical for preserving the integrity of the digestive system, guarding against infections, and promoting overall intestinal health [8]. Many lactobacilli species are thought to be harmless, and some of them have the ability to interact with intestinal epithelial cells. The Lactobacillus bacteria, which are primarily isolated (43.48%) from locally produced domestic products and sold commercially in milk parlors, are an important category. Lactobacilli often have strict anaerobic growth requirements and are facultative organisms. They create lactic and other acids because they prefer an acidic environment. As a whole, lactobacilli are thought to be non-pathogenic and have not been linked to any illnesses. Isolates have even been shown to be able to withstand an acidic environment, NaCl concentration and resistance to bile.

Because they produce bactericidal bioactive compounds that can inhibit the growth of the pathogens, strains of lactic acid bacteria (LAB) hold promise. Benefits of Lactobacilli include the suppression of both positive and gram-negative pathogenic bacteria, as reported by [9] and [10]. Maintaining probiotics' antimicrobial properties will support their application in the creation of functional meals that improve

the health of those who consume them [11, 12]. Antimicrobial substances such as bacteriocins, hydrogen peroxide, and organic acids were produced by Lactobacillus isolates [13–15].

The current search's objectives are to identify and screen for a probiotic strain with antibacterial properties, as well as to investigate the potential of probiotics as biotherapeutic agents. Additionally, we highlight this probiotic's encouraging antagonistic action against Gram-negative bacteria.

2. Materials and methods

2.1. Isolation of probiotic bacteria

Various curd samples are selected at random from commercially available milk, cheeses, veggies, pickles, and baby feces at the local market. These samples were gathered in sterile, clean, wide-mouthed containers with tight-fitting, leak-proof lids that were free of detergent or disinfectant residue. In order to prevent contamination and deterioration, the samples were aseptically stored in a low temperature (4˚ C) refrigerator as soon as they were collected and taken to the laboratory for microbiological investigation.

MRS medium containing (g/l) peptone, 10.0; meat extract, 8.0; yeast extract, 4.0; D(+) glucose, 20.0; dipotassium hydrogen phosphate, 2.0; sodium acetate trihydrate, 5.0; triammonium citrate, 2.0; magnesium sulfate heptahydrate, 0.2; and magnesium sulfate tetrahydrate, 0.05, with a pH of 6.2 was used in this study to isolate bacteria from curd samples [16]. For the primary isolation of probiotic Lactobacillus bacteria, 10 grams of each collected sample were diluted with sterilized phosphate-buffered saline and then transferred to 100 milliliters of MRS broth at pH 6.5. This was done using MRS [16] medium. After six hours of incubation, these solutions were added to the MRS broth and streaked over the MRS agar plates. The plates were aerobically incubated at 37 °C for 24-48 h. Cells were grown under a cool-white light. After incubation, white colonies that formed were selected for single colony isolation and to isolate different strains of Lactobacillus species.

2.2. Culture characteristic and Gram's staining

Create a smear of the isolated culture on a freshly cleaned slide, and then use mild warming to fix it. Next, apply a crystal violet dye gradually over the smear. Hold it for a minute. Next, use distilled water or clean tap water to remove the discoloration.

The smear should then be covered with a drop of Gram's iodine and left for a minute. After that, wash with water and absolute alcohol, a decolorizing agent, and then wash with water right away again. Finally, dry the smear and examine it under a microscope, first with a 10x objective to confirm staining, and then with a 100x objective submerged in oil. Note the outcome. Rod-shaped, gram-positive bacilli cells were seen. For facultative and aerobic anaerobes, 35–37 °C is the ideal growing temperature.

2.3. Isolation of intestinal Gram negative bacteria

The clinical stool specimens were taken from the patient and centrifuged together with sterile saline to isolate infectious bacteria. The mixture was spread out onto blood agar, nutrients, Salmonella-Shigela, and McKanky (Merck, Germany), and it was then incubated at 37˚C for 24 hours. Gram stain was used for differentiation and colonies. Automated identification systems (VITEK) were used to identify the pure bacterial isolates [17].

2.4. Antagonistic activity against intestinal Gram negative Bacteria

Using the agar well diffusion method, the antibacterial activity was measured as follows: Poured into 20 cm diameter Petri dishes, 40.0 ml of nutritional agar medium incubated at 55–60°C was injected with 100 µl of the pathogenic bacteria cell suspensions under test individually, mixed thoroughly, and allowed to harden. Using a sterile cork borer, holes with a diameter of 5.0 mm were created in the agar plate following solidification. An automatic micropipette was used to pour 100 µl of the probiotic isolate cultures into the holes that had been drilled for each sample. The Petri dishes were kept in the refrigerator for one hour at 5°C to permit homogenous diffusion of the samples before growth of the tested pathogens, and then the plates were incubated at 37°C for 24 h. The antagonistic activities of the isolates under study were determined by measuring the diameter of inhibition zone [12].

3. Results and Discussion

3.1. Isolation and Characteristics of probiotic and test pathogens

Seven samples of curd were gathered from the surrounding area and brought into the city. After culturing for 48 hours, 39 strains were chosen as forming wide, white colonies on the MRS agar plates. Of the isolates of lactic acid bacteria, Lactobacillus spp. were the most common and

predominant strains based on observations of their colony morphology, physiological traits, and certain biochemical features (Table 1). Under a microscope, they were rod-shaped, non-motile, Gram-positive, catalase-negative, and endospore-free. 36 bacterial isolates were found in stool diarrheal samples, according to the results of the bacterial isolation process. However, the automatic identification systems (VITEK) showed that there were five distinct bacterial genera: *Proteous vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli.* and *Enterobacter sp*.

Table 1: Isolation and classification of probiotic bacteria according to sources

No	Isolates Code	Source	Final pH
1	A5	pickles	4.0
2	A4	pickles	4.0
3	2	pickles	4.0
4	A6	pickles	4.0
5	17	pickles	4.0
6	42	pickles	4.0
7	11	pickles	4.0
8	35	pickles	4.0
9	A2	pickles	4.0
10	22	pickles	4.0
11	H4	Sheep Milk	4.5
12	13	Sheep Milk	4.5
13	5	Sheep Milk	4.5
14	34	Sheep Milk	4.5
15	19	Sheep Milk	4.5
16	R	Cow Milk	4.5
17	7	Cow Milk	4.5
18	ATP	Cow Milk	4.5
19	14	Cow Milk	4.5
20	IVF	Cow Milk	4.5
21	Lact	Cow Milk	4.5
22	Z	Cow Milk	4.5
23	3	Cow Milk	4.5
24	10	Cow Milk	4.5
25	S1	infants stool	4.5
26	S2	infants stool	4.5
27	M1	chees	4.5
28	M2	chees	4.5
29	M3	chees	4.5
30	F1	vegetables	5.0
31	F2	vegetables	5.0

32	F3	vegetables	5.0
33	F4	vegetables	5.0
34	Y1	Yogurt	4.0
35	Y2	Yogurt	4.0
36	Y3	Yogurt	4.0
37	Y4	Yogurt	4.0
38	Y5	Yogurt	4.0
39	Y6	Yogurt	4.0

3.2. The screening of antagonistic activity

By using a modified agar-well diffusion method, it was possible to observe the antibacterial activity of the chosen probiotic isolates. At this phase, the probiotic strains' antagonistic activities were tested against indicator bacteria like *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Proteous vulgaris*, and *Enterobacter sp.* When tested against all indicator bacteria, all thirty-nine probiotic strains and the reference strain exhibited antagonistic effects; however, the probiotic strains' levels of antagonism differed.

The probiotic strains Lact, S2, M3, F1, Y1, Y3, and Y4 were the most efficient strains in suppressing the development of all test pathogens, according to Table 2 and Figure 1. The isolated strains showed an average inhibition (9–20 mm) on the growth of test pathogens.

One of the most important selection criteria for successful and innovative probiotics is antimicrobial activity. All Lactobacillus isolates maintain their antimicrobial activity by generating bacteriocins, hydrogen peroxide, organic acids (lactic, acetic, propionic, and succinic acids, among others), and low molecular weight antimicrobial compounds [18].

It is well established that probiotics, such as *Lactobacillus, Bifidobacterium,* and *Streptococcus* species, prevent the growth of a variety of intestinal infections in humans. Apart from their beneficial effects on illnesses brought on by an imbalance in the gut microbiota, probiotic bacteria may also prevent the growth of colon tumors, according to a number of experimental findings [19].

Probiotics are known to suppress the growth of a variety of intestinal infections in humans, according to reported evidence. Several experimental findings have demonstrated a possible preventive impact of probiotic bacteria against the formation of colon cancers, in addition to their beneficial

benefits against diseases brought on by an imbalance of the gut microbiota [20].

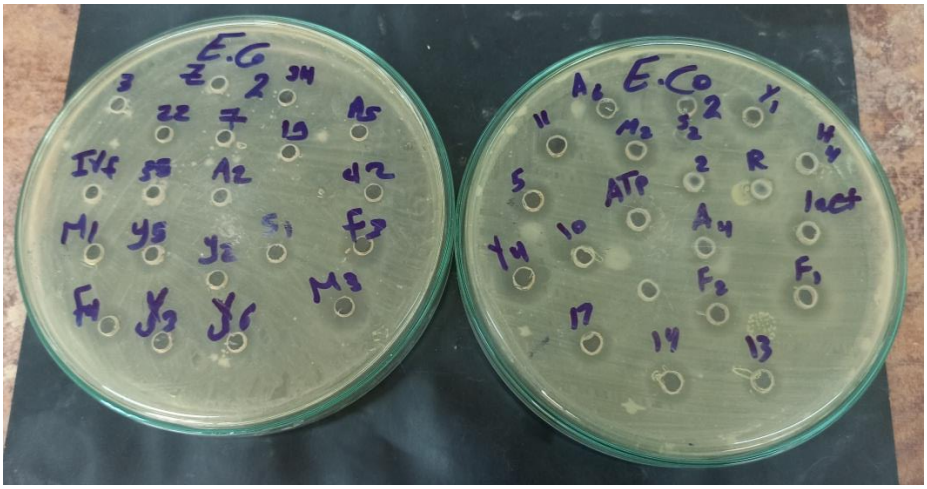
Lactobacillus species isolated from fermented dairy products demonstrated antibacterial activity against several clinically significant pathogens, including *Salmonella typhimurium* (4.3 mm), Enterotoxigenic *E. coli* (4.2 mm), and *L. monocytogenes* (5.0 mm), according to a study by Osuntoki et al. [20]. Compared to these Lactobacillus spp. isolates, the isolates from our investigation have superior antibacterial properties.

Table 2: Antagonistic activity of probiotic isolates against intestinal Gram negative pathogens

Isolates Code	Inhibition zone diameter (mm) against				
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Proteous vulgaris*	*Enterobacter sp.*
A5	0.0	0.0	0.0	0.0	12
A4	10	0.0	0.0	16	10
2	0.0	0.0	0.0	0.0	14
A6	14	12	0.0	15	18
17	12	10	0.0	15	19
42	0.0	0.0	0.0	0.0	0.0
11	13	0.0	0.0	0.0	0.0
35	0.0	0.0	0.0	0.0	0.0
A2	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0
H4	0.0	0.0	0.0	0.0	13
13	13	10	0.0	14	15
5	14	0.0	9	11	12
34	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0
R	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0
ATP	11	11	0.0	0.0	12
14	0.0	9	0.0	12	0.0
IVF	0.0	16	0.0	0.0	0.0
Lact	14	9	10	11	13
Z	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0
10	10	10	0.0	9	0.0
S1	0.0	0.0	0.0	0.0	0.0
S2	15	18	16	19	21
M1	0.0	13	7	0.0	11
M2	14	0.0	0.0	12	17
M3	17	18	15	19	20

F1	15	14	11	15	14
F2	14	11	0.0	15	19
F3	14	14	0.0	15	12
F4	13	14	8	0.0	15
Y1	12	10	9	14	19
Y2	0.0	14	0.0	0.0	10
Y3	12	10	7	13	15
Y4	17	18	10	16	15
Y5	11	0.0	8	12	0.0
Y6	11	12	0.0	10	0.0

A



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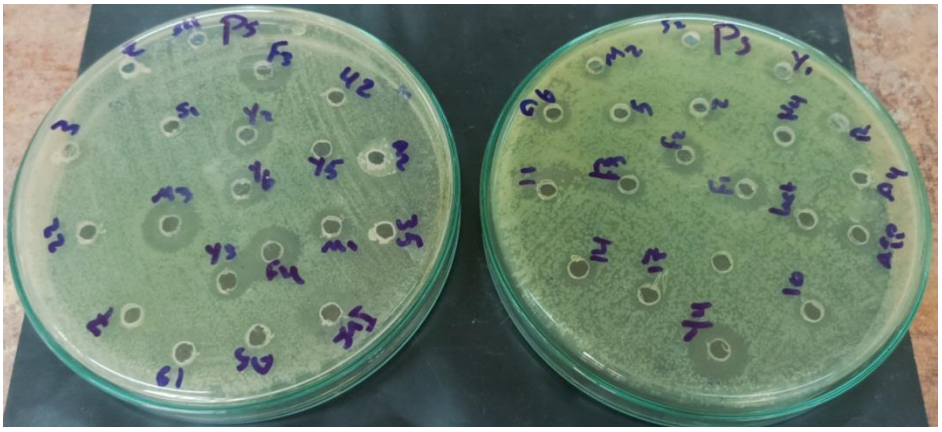

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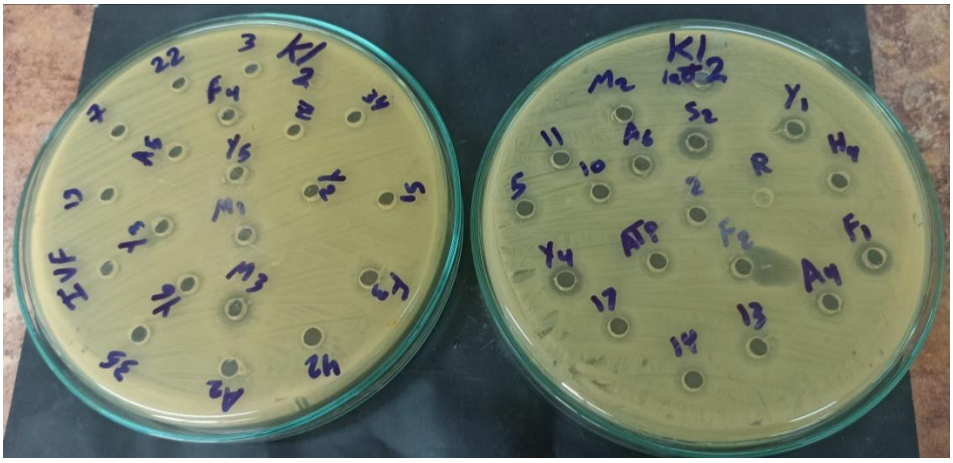

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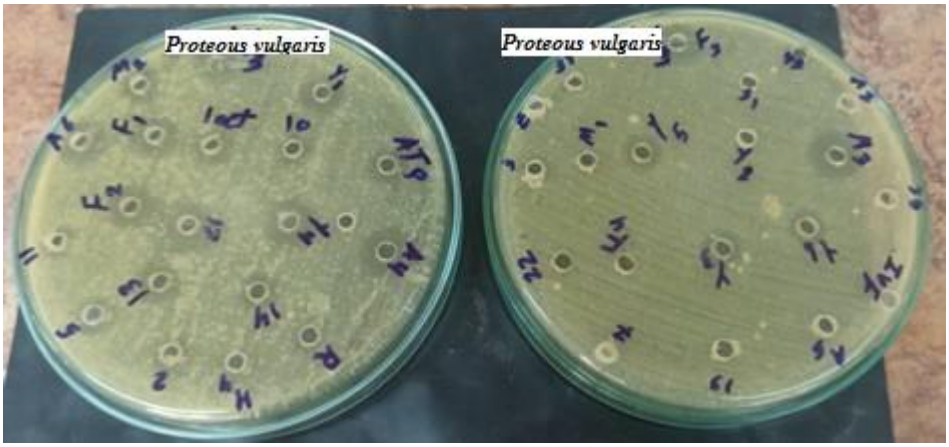

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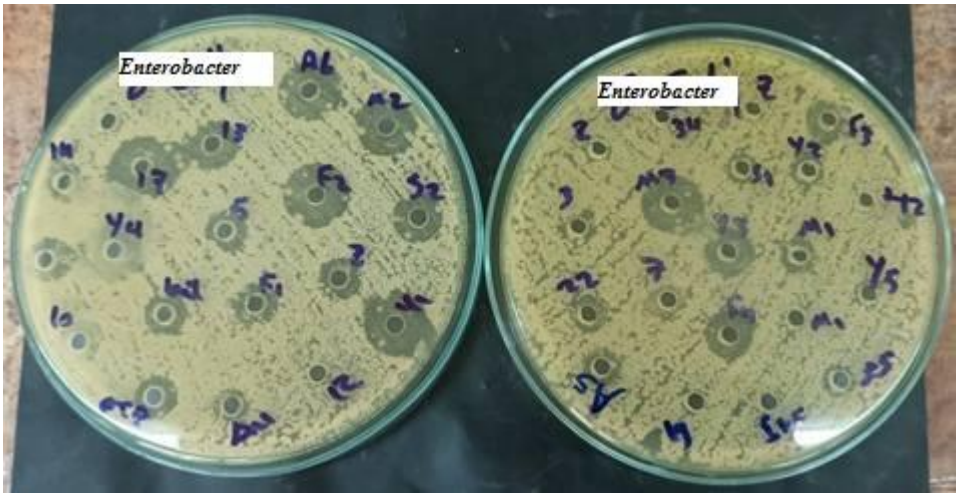

Figure 1: Antagonistic activity of isolates against different test pathogens. A- *Escherichia coli* ; B- *Pseudomonas aeruginosa*; C- *Klebsiella pneumonia; D- Proteous vulgaris* ; E- *Enterobacter sp*.

4. Conclusion

The best antagonistic activity was assessed in bacterial probiotic strains derived from various samples; out of 39 isolates, 7 exhibited the most antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteous vulgaris*, and *Enterobacter sp.* Given its antagonistic spectrum against Gram-negative bacteria and potential as a bio therapeutic agent, the probiotic can be investigated further for potential use in the management of pathogenic bacterial illnesses.

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