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Characterization of Probiotic Features Isolated From Fruits and Vegetables

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Abstract

The most beneficial bacteria to society are probiotics, which are utilized in the manufacturing of numerous fermented foods that improve immunity and digestion. In order to identify the possible probiotic qualities of certain fruits and vegetables, the objective study set out to separate and describe the probiotics' members. Probiotic strains were isolated and chosen for this investigation based on their morphology and biochemistry. The next step is to ascertain their probiotic characteristics, which include coagulase action, hemolytic activity, antibiotic sensitivity, bile-salt tolerance, and acid resistance. After 16 bacterial isolates were separated and purified from fruits and vegetables, they all demonstrated the highest levels of bile tolerance and resistance to acidic pH 2.0. All isolates were sensitive to tetracycline, and the majority of probiotics shown sensitivity to the investigated antimicrobial drugs. Every isolate was thought to be streptomycin-resistant. According to our findings, isolates show promise as probiotics that could be used further in the production of probiotic products.

Keywords: Probiotics, features, Fruits, vegetables, biochemical characterization, safety.

1. Introduction

The goal of probiotic microbe research has been to develop food supplements. The isolation, identification, and assessment of lactic acid bacteria (LAB), which are naturally occurring in food and have probiotic qualities, are

therefore of tremendous interest to the food and pharmaceutical sectors (1-3).

Probiotics are specific living bacteria that help the host's health when given in sufficient quantities. However, the probiotic needs to fulfill a number of requirements in order to be included in food (4-6). While the majority of commercially available probiotic LAB come from the human gastrointestinal system, they can also be isolated from food, particularly dairy items. Fruits, however, have been extensively researched as a potential substitute source for these microbes' isolation. These LAB isolates demonstrated promising outcomes in *in vitro* investigations, which will be followed up with *in vivo* studies to confirm their positive health effects (7,8).

Since people who are lactose intolerant, vegan, or allergic to milk protein have limited consumption of dairy matrices, it is crucial to take into account that the development of probiotic products made from fruit-based food matrices is a feasible alternative to diversify the population's consumption of these microorganisms (9–11).

In LAB research, the local fruits can be valuable matrices to investigate. Therefore, studies to find species with probiotic potential are crucial due to the significance of probiotics and the lack of knowledge on the presence of this microbe as a component of the native fruit microbiota (12).

Functional foods are a class of foods that, in addition to being nutrient-dense, have been shown to have physiological effects or lower the risk of chronic illnesses. They also give the body components that help cure illnesses or lower the risk of contracting them, as well as metabolic and physiological effects that help maintain both physical and mental well-being. These foods can keep the body's natural balance of vitamins and electrolytes and avoid chronic disease. Beyond the typical nutritional impact that enhances health and lowers the risk of disease, functional foods have been shown to have positive effects on one or more bodily processes, according to the International Life Science Institute (13).

In order to exploit non-dairy products, such as fruits and vegetables, as prospective sources of probiotics, this study set out to extract, describe, and assess the probiotic properties in the native sources.

2. Material and methods

2.1. Collection of samples

Five samples of fruits and vegetables (guava, kiwi, tomato, broccoli, and cabbage) were gathered from the local market and thoroughly cleaned with tap water after being rinsed twice with double

distilled water and 2.0% sodium hypochlorite for five minutes or thirty seconds. The produce was hand chopped using a sterile cutter while maintaining aseptic conditions, and it was then placed in sterile, empty petri dishes for storage.

2.2. Isolation of probiotic bacteria

Nine ml of sterile MRS broth were inoculated with one gram of fruit sample, and the mixture was then incubated for 48 hours at 37 °C in a rotary shaker running at 120 rpm (14). Probiotic bacteria were grown using the Agar plate technique, following the methodology outlined by Pundir et al. (15). Each dilution's 0.1 ml of inoculum was applied to the MRS agar plate. The plates were incubated at 37 °C for 48 hours while inverted. Following incubation, colonies were chosen at random, removed, and purified for additional characterization using the streak plate technique on MRS agar. The colonies were then kept in a refrigerator at 4 °C for subsequent study and cultured for 24 hours at 37 °C (14).

2.3. Characterization of probiotic isolates features, potential and properties

Only isolates that tested negative for catalase were subjected to Gram stain examination after being grown to measure catalase activity (3% v/v H_2O_2). Gram-positive isolates were examined under an microscope to assess their morphology. Gram-positive, catalase-negative, cocci, or bacilli isolates that were most likely identified as probiotics were chosen, injected in MRS broth, and then incubated at 37 °C for 48 hours in an aerobic environment. Following turbidity of the culture media, 1 mL of the sample was taken and put into 80%, v/v glycerol solution in eppendorf micro-tubes, which were then stored at -80 °C. Each bacterial culture was sub-cultured in MRS broth under aerobic conditions at 37 °C every 24 hours prior to the commencement of the studies.

2.4. Acid tolerance

The isolates were cultured in MRS broth at 37°C for a full day. 10 ml of MRS broth with a pH of 2 was inoculated with an aliquot of 0.1 ml from various cultures that had been incubated for 24 hours, and the mixture was then incubated for three hours at 37°C. Using the plate-count approach, the cultures' pH tolerance was measured on MRS agar plates [16].

2.5. Resistance to bile salts

The investigated isolates' overnight cultures were injected into MRS broth for three hours at 37°C using 0.5 % bile salt. Following plating onto MRS agar plates, they were incubated at 37°C for 24 hours. As a control, the MRS without bile salt

was employed. The plate count for each was used to calculate the viable count [16].

2.6. Antibiotic resistance

The disk diffusion method was used to assess the isolates' sensitivity to several antibiotics (17). Novobiocin NV (30), Piperacillin/Tazobactam TZP (110), Imipenem IPM (10), Azithromycin AZM (15), (30) Tetracycline TE, TOB (tobramycin) (10), CTX cefotaxime (30), CFR (30), Cefadroxil and Streptomycin S (10) were applied to the plate surface and incubated at 37 °C for 24 hours. A ruler was used to evaluate the growth inhibition zones, and the isolates were classified as susceptible (S), or resistant (R) for each antibiotic (18).

2.7. Hemolytic activity

Isolates were cultivated on MRS agar supplemented with 5% freshly sheep blood to confirm their ability to produce hemolysin. Following 48 hours of aerobic incubation at 37 °C, the outcomes were assessed (19). The β-hemolysis (bright zones surrounding colonies), α-hemolysis (green zones surrounding colonies), and γ-hemolysis (no zone surrounding colonies) reactions were used to confirm hemolytic activity.

2.8. Activity of coagulase

0.3 mL of each isolate was put into sterile tubes with 0.3 mL of rabbit plasma and incubated for 6 hours at 37 °C for the coagulase test. A full clotting or a significant clot formation was regarded as a positive test result (20).

3. Results and discussion

3.1. Probiotic bacteria isolation and selection

A total of 16 bacterial isolates were found in samples of two vegetables (broccoli and cabbage) and three fruits (guava, kiwi, and tomato). Typical colonies were white and off-white when the isolates were plated on MRS agar media. Two cocci and bacilli cell morphology made up the total of 14 isolated colonies. One crucial factor in the selection of probiotic bacteria is catalase activity. Every isolate had a pH of 4.0 to 4.5 due to acid production, was Gram positive, and was catalase negative (Table 1).

3.2. Evaluation of probiotic isolates features

When choosing probiotic bacteria, acid tolerance is a crucial factor. Probiotic bacteria must be able to endure in the acidic stomach environment and are mostly supplied by the food system. Resistance to low pH is crucial for survival during transit through the gastrointestinal tract. Because they must pass through the harsh environment of the stomach in order to reach the small intestine. The food most

likely remains in the stomach for three hours or longer. However, the pH of the stomach can drop as low as 1.5, and at pH 2.0, there was no discernible drop in strain survival. At the lowest pH of 2, all of the isolates were able to survive (Table 2).

Table 2 shows the development of a few chosen isolates at various bile salt concentrations. The most important factor in choosing probiotics, which are the primary constituents of bile, is bile salt tolerance (21). Bacterial cell membranes are destroyed by bile fluid released in the small intestine, which also affects the bacteria's viability and cell permeability. Bile salt concentrations of 0.15% to 0.3% are advised for human use in order to select probiotic bacteria (22). The growth of bacterial isolates at a 0.5% bile salt concentration for three hours was used in the current investigation to evaluate the isolates' capacity to withstand bile. A tolerance of 0.5% sodium thioglycocholate was demonstrated by 16 isolates.

Table 1: Isolation of probiotics from fruits and vegetables with microscopic and biochemical characterization

Source	Isolate code	Colony color	Catalase test	Gram reaction	Cell microscopy	Final culture pH
Guava	G1	White	Negative	G+	Bacilli	4.0
	G2	Off-white	Negative	G+	Bacilli	4.0
	G3	White	Negative	G+	Bacilli	4.0
Kiwi	K1	White	Negative	G+	Bacilli	4.0
	K2	White	Negative	G+	Bacilli	4.0
	K3	Off-white	Negative	G+	Bacilli	4.0
Tomato	T1	White	Negative	G+	Bacilli	4.0
	T2	Off-white	Negative	G+	Bacilli	4.0
	T3	White	Negative	G+	Bacilli	4.0
Broccoli	B1	White	Negative	G+	Bacilli	4.0
	B2	White	Negative	G+	Bacilli	4.0
	B3	White	Negative	G+	Cocci	4.5
	B4	White	Negative	G+	Bacilli	4.0
Cabbage	C1	White	Negative	G+	Bacilli	4.0
	C2	White	Negative	G+	Bacilli	4.0
	C3	White	Negative	G+	Cocci	4.5

3.3. Evaluation of the safety of probiotics

Hemolytic activity and coagulase levels of probiotics were also used to assess their safety. Hemolytic and coagulase activity values were negative (Table 2). There was no virulence activities observed in the fresh fruit isolates (23). Selection criteria for possibly probiotic microbes include strong

antibiotic susceptibility and the absence of hemolysis, DNAse, gelatinase, and coagulase activities, which indicate non-virulence and safety. Since environmental factors affect gene expression, this result does not imply that the gene responsible for these actions is absent; rather, it indicates that the gene was not detected in the assay conditions. Therefore, testing to confirm the profile of genetic determinants should be the main focus of future study, with an emphasis on the genetic mechanisms driving virulence and resistance to phenotypic antibiotics (24).

Table 2: Evaluation of probiotic isolates features Hemolytic activity, Coagulase test, pH tolerance and Bile-salt resistance

Isolate code	Hemolytic activity	Coagulase test	pH Survival (%)	Bile-salt Survival (%)
G1	γ-hemolysis	Negative	93.5	99.0
G2	γ-hemolysis	Negative	97.0	93.8
G3	γ-hemolysis	Negative	96.4	89.0
K1	γ-hemolysis	Negative	95.7	96.5
K2	γ-hemolysis	Negative	98.2	94.2
K3	γ-hemolysis	Negative	94.5	90.5
T1	γ-hemolysis	Negative	99.0	95.0
T2	γ-hemolysis	Negative	93.8	91.0
T3	γ-hemolysis	Negative	95.0	88.9
B1	γ-hemolysis	Negative	93.0	90.5
B2	γ-hemolysis	Negative	95.4	95.4
B3	γ-hemolysis	Negative	92.3	93.5
B4	γ-hemolysis	Negative	91.8	97.0
C1	γ-hemolysis	Negative	99.0	94.8
C2	γ-hemolysis	Negative	96.5	96.4
C3	γ-hemolysis	Negative	94.2	95.7

3.4. Antibiotics sensitivity

Tetracycline sensitivity was demonstrated by all isolates, and the majority of probiotics tested positive for antimicrobial compounds. According to Table 3, every isolate was deemed resistant to streptomycin. Because of the possible risk of resistance gene dissemination, strains with antibiotic resistance genes must be distinguished from other strains in the production of probiotics intended for human consumption. Antibiotic-resistant bacteria are a public health problem because they reduce the efficacy of medications used to treat infectious illnesses (25).

Antibiotic resistance in probiotics is expected, as this trait is thought to be a natural or "intrinsic" feature of these bacteria. Enzymatic inactivation, which

stops these antibiotics from attaching to their particular targets, is one of the mechanisms by which probiotics develop intrinsic resistance to quinolones. On the other hand, the lack of cytochrome-mediated electron transport, which lowers drug absorption, is responsible for intrinsic resistance to aminoglycosides (26).

Therefore, the isolates of *Lactococcus, Levilactobacillus,* and *Enterococcus* from the pomegranate demonstrated resistance to ciprofloxacin (only *Enterococcus*), as well as sensitivity or moderate sensitivity to ampicillin, erythromycin, gentamicin, and tetracycline; in contrast, the majority of isolates in this study demonstrated resistance to cephalothin (27). With the exception of gentamicin, which was discovered to be extremely resistant, isolates from fruits from the Peruvian Amazon, *L. plantarumm, W. cibaria, L. brevis,* and *W. confusa* likewise demonstrated significant susceptibility to the investigated antimicrobials (28).

The susceptibility of the isolates found in the Amazonian açaí (29) has been ascertained by using the disk-diffusion methodology, in addition to the reference used in this study (30), and cutoff points for *Enterococcus* that are standardized for other LAB (CLSI–Clinical and Laboratory Standards Institute Performance standards of antimicrobial susceptibility testing, 2012) (31). On the other hand, *P. acidilactici* would be categorized as gentamicin and tetracycline sensitive when comparing the cutoff points.

Table 3: Antibiotics sensitivity of probiotics isolates

Isolate code	Antibiotics								
	Azithromycin	Imipenem	Piperacillin/Tazobactam	Novobiocin	Tetracycline	Tobramycin	Cefotaxime	Cefadroxil	Streptomycin
G1	S	S	S	S	S	S	S	S	R
G2	S	S	S	R	S	R	R	R	R
G3	R	R	S	R	S	R	R	R	R
K1	S	S	S	R	S	S	S	S	R
K2	S	S	S	R	S	S	S	S	R
K3	S	S	S	R	S	S	S	R	R
T1	R	R	S	R	S	R	R	R	R
T2	R	R	S	R	S	R	R	R	R
T3	S	S	S	S	S	S	S	S	R
B1	R	R	S	R	S	R	R	R	R
B2	S	S	S	R	S	R	R	R	R
B3	S	S	S	R	S	R	R	R	R
B4	S	S	S	R	S	R	R	R	R
C1	R	R	S	R	S	R	R	R	R
C2	R	R	S	R	S	R	R	R	R
C3	R	R	S	R	S	R	R	R	R

4. Conclusion

Probiotics are made to satisfy requirements for shelf life, food safety, technological efficacy, and financial viability. The isolates exhibited probiotic qualities. However, it's possible that no single isolate has every beneficial characteristic of probiotics. In order to utilize their probiotic potential in food products, it is also necessary to thoroughly evaluate it. To further verify the identity of the bacterial isolates, molecular identification of these isolates must be done. Therefore, it is possible to draw the conclusion that probiotic isolates from fruits and vegetables could be used to create probiotic food items.

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