



EXPLORATION OF PROBIOTIC BACTERIA FROM PLANT BASED MILK

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Abstract

Probiotics are most beneficial microorganisms that support the balance of intestinal micro-flora and contribute in maintaining health. Probiotics are mainly obtained from dairy products. Conversely, some people are lactose intolerant and have allergies because of dairy products. Hence, it is necessary to choose a non-dairy alternative as a probiotic supplement as which Plant based milk alternatives or PBMs can be utilized.

In accordance to this, the present research deals with the exploration of probiotic bacteria from the Plant Based Milk viz. Almond milk, Coconut milk, Soya milk. The study also deals with the antimicrobial activity of probiotic bacteria against MDR human pathogens viz. *E.coli*, *Shigella*, *Salmonella*, *S.aureus*, antibiotic resistance pattern of probiotic isolates and enzyme assay viz. protease, amylase, phosphate, and lipase respectively. The results depicts the presence of different *Lactobacillus* species, such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactococcus lactis* and *Bifidobacterium longum* in plant based milk. The probiotic isolates showed antimicrobial activity against most of the pathogens indicating its health benefits as immune boosters. The results of Antibiotic resistance showed that only *Lactobacillus plantarum* showed resistance against Azithromycin and Penicillin. However, maximum *Lactobacillus species* showed sensitivity against tested antibiotics. Most of the probiotic isolates showed positive results for enzyme assay indicating that probiotics are beneficial for digestive system. Hence, plant based milk could be consume for health benefits.

Keywords: Plant-based milk, Antimicrobial activity, Lactic acid bacteria, Pathogenic microorganism, Lactose intolerance.

1. Introduction

Probiotic are live microorganisms are associated with the beneficial health effects and contribute to intestinal micro floral balance and digestive system. The most popular probiotic strains are represented by the species such as Lactobacillus, Streptococcus, enterococcus and Bifidobacterium.

Probiotics are present in different types of milk and dairy products such as yoghurt, curd, buttermilk, etc. Lactic acid bacteria (LAB) are predominantly present in dairy products which are mostly used as probiotics. But on contrary some people have allergies to dairy products and are lactose intolerant. Therefore such people have deficiencies of vitamins and lack of nutritious elements obtained from dairy products.

Lactose intolerance is a digestive disorder characterized by the body's inability to effectively metabolize lactose, a sugar found in milk and dairy products. This condition arises due to insufficient production of lactase, the enzyme responsible for breaking down lactose into simpler sugars, glucose, and galactose. As a result, individuals with lactose intolerance may experience symptoms such as bloating, abdominal pain, gas, and diarrhoea after consuming lactose-containing foods. [1-2] It is essential to note that lactose intolerance is distinct from a milk allergy, which involves an immune system response to proteins in milk rather than the inability to digest lactose. [3] Consequently it is necessary to choose a non-dairy alternative as a probiotic supplement. Thus, the need for Plant based milk alternatives or PBMs has grown in recent years as a result of shifting lifestyles, allergies, and lactose intolerance. [4] Almond milk is a popular Plant-based milk alternative being Dairy-free, Lactose-intolerant and vegan individuals can safely consume it. Additionally, it is Low in calories and saturated fat as compared to cow's milk also rich in calcium and vitamins. [5-8] Another Plant-based milk is Coconut milk is a good source of vitamins and minerals.[9] These are some beneficial properties of coconut milk it may enhance the metabolism, improve digestion, protect against viral and fungal infections, and fight against bacteria. It may have antioxidant properties, and protect against cancer. [10-13] Soy milk is a good source of plant-based probiotic and could be a good alternative for people who are lactose intolerant and vegan. It is also low in saturated fat and cholesterol-free. Soy milk is a good source of calcium, high protein levels and vitamins. Soy milk may help to lower cholesterol levels. [14-15] The studies show the presence and impact of micro-nutrients and bioactive compounds in plant materials. [16] Thus, such plant based milk could be used as probiotic source to maintain overall health and especially intestinal and gut associated diseases. The current study "Studies of probiotic bacteria present in plant-based milk" aims to further investigate the potential of Plant Based Milk as a viable source of probiotic bacteria as an alternative. The microbial composition of the Plant Based Milk viz. Almond milk, Coconut milk, Soya milk studied to identify and characterize specific strains of probiotic bacteria that contribute to health benefits.

2. Materials and Methods:

2.1.Isolation of probiotic bacterial strains from PBM'S:-

This study is conducted in the Microbiology Department at R.A College, Washim. The study involved preparing plant-based milk, including almond, coconut, and soy milk. The milk was prepared by soaking almonds overnight in water, blending them with fresh water, and storing them in a bottle. Coconut milk is

prepared by grated coconut and added some water for blending and stored them in a bottle. Soya milk is purchased from the market and carries to laboratory for further studies. The samples were then diluted in test tubes and examine for probiotic bacteria. The isolates were then observed on MRS media plates, which were then incubated at 37°C for 24-48 hours.

2.2. Identification

Isolated colonies were identified based on morphology, colony characteristics, IMViC classification, biochemical and enzyme assay. Observations included colour, shape, size, and nature of the colony. Gram staining and motility were used to observe morphological characteristics. Biochemical assay was used to characterize the isolates, and all cultures were maintained as pure cultures. [17]

2.3. Antimicrobial activity of Probiotic bacterial isolates

The study investigated the antibacterial activity of probiotic bacterial isolates against four MDR human pathogens: E. coli, Shigella, Salmonella, and S. aureus. The antibacterial activity of LAB supernatants was determined using the Kirby Bauer disc diffusion technique. [18] The zone of inhibition was observed and measured using the Hi Antibiotic Zone Scale.

2.4. Antibiotic Resistance

Bacterial isolates were tested for antibiotic resistance using the Disc diffusion method. The antibiotic resistance against four antibiotics including Tetracycline, Azithromycin, Penicillin, Cephalexin. The turbidity was compared with McFarland standards. [19]

2.5. Enzymes Assay: -

Bacterial culture is a crucial tool for determining various enzymes. It can produce protease, amylase, phosphate, and lipase. Protease activity is determined by inoculating skim milk agar plates and incubating them at 37°C for 48 hours. Amylase activity is determined by inoculating starch agar plates and incubating them at 37°C for 48 hours. Phosphate solubilization is evaluated using Pikovskaya's agar medium containing calcium phosphate. Lipase activity is determined by inoculating Tributyrin agar plates and incubating them at 37°C for 48 hours. These assays help to determine the bacterial culture's ability to produce specific enzymes.

3. Result and Discussion:

I. Isolation and Identification of bacterial strain from plant -based milk:-

The result on isolation and identification are presented in (table 1). from the table it is observed that the bacteria present in plant -based milk (Almond milk, Coconut milk, Soya milk) belongs to Lactobacillus species which is classified as belonging to lactic acid bacteria (LAB) group.

Characteristics of isolated bacteria Samples						
Colony Characters	A1	A2	A3	C1	C2	S2
Size	0.7-1.1 x 2.0-4.0 µm	1.9-3.2 µm	0.8-1.0 µm	0.9-1.2 µm	0.5-1.5 µm	2-10 µm
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth	Smooth	Liquid	Smooth

Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Convex	Raised	Raised	Convex	Raised	Convex
Morphological Charactaterics:						
Colour	Pink tone	White creamy	White	White	White	Gray
Shape	Rod	Rod	Rod	Rod	Cocci	Rod
Gram Staining	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
Motility	Non- motile	Non- motile	Non-motile	Non-motile	Non-motile	Non-motile
Biochemical Characteristics:						
Indole	- Ve	+ Ve	-Ve	-Ve	-Ve	- Ve
Citrate	+ Ve	+ Ve	-Ve	-Ve	+ Ve	- Ve
MR	-Ve	+ Ve	- Ve	- Ve	- Ve	- Ve
VP	-Ve	+ Ve	-Ve	-Ve	- Ve	- Ve
Glucose	+ Ve	+ Ve	- Ve	-Ve	+ Ve	+ Ve
Mannitol	+ Ve	+ Ve	+ Ve	-Ve	+ Ve	- Ve
Sucrose	+ Ve	+ Ve	- Ve	-Ve	-Ve	+ Ve
Maltose	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
Gas	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	-Ve
Possible Species	<i>Lactobacillus casei</i>	<i>Bifidobacterium Longum</i>	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus plantarum</i>	<i>Lactococcus lactis</i>	<i>Lactobacillus Acidophilus</i>

+Ve: Positive , -Ve: Negative

II. Antimicrobial activity of Probiotic bacterial isolates:-

The antimicrobial activity of probiotic bacterial isolates was studied. The results were presented in table 2 and fig.1. It is found that Lactobacillus species show increased in zone of inhibition against all the four test pathogens as compare to uninoculated control. Hence, Lactobacillus species isolated from plant-based milk (almond milk, coconut milk, soy milk) is found to possess antimicrobial activity against intestinal pathogens and can be consider as probiotics.

Pathogens	Zone of inhibition Exhibited by LAB					
	A1	A2	A3	C1	C2	S2
E. coli	12mm	15mm	14mm	12mm	14mm	12mm
Salmonella	12mm	10mm	12mm	14mm	12mm	14mm
Shigella	15mm	14mm	15mm	15mm	13mm	13mm
S. aureus	14mm	13mm	16mm	10mm	17mm	15mm
Identified Species	<i>Lactobacillus casei</i>	<i>Bifidobacterium Longum</i>	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus plantarum</i>	<i>Lactococcus lactis</i>	<i>Lactobacillus Acidophilus</i>

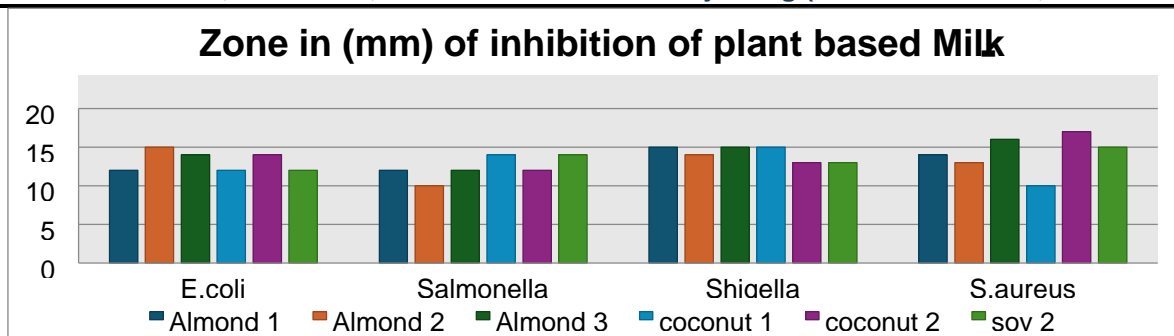


Figure 1:- Antimicrobial activity of plant-based milk

III. Antibiotic Resistance of PBM's:-

Table 3. fig.2. represents the findings on the antibiotic resistance of isolated Lactobacillus species. From the table it is observed that all the Lactobacillus showed resistance against the tested antibiotics viz. Tetracycline (30µg), penicillin (10µg), Azithromycin (15µg), and Cephalexin (30µg).

L. casei showed the zone of inhibition which showed sensitivity against all of the four antibiotics tested. Similarly, *Bifidobacterium longum*, *L. Rhamnosus*, *Lactococcus lactis*, *L. Acidophilus*. Only *L. plantarum* showed resistance against penicillin and azithromycin. In Tetracycline, the minimum zone of diameter (28mm) was exhibited by Lactobacillus species. In case of Penicillin, the maximum zone of inhibition (25mm) was exhibited Lactobacillus species. *L. plantarum* showed the minimum zone of inhibition (12mm) which is resistant against penicillin. In case of Azithromycin, the minimum diameter of zone of inhibition was (22mm) shown by Lactobacillus species. Similarly, in Cephalexin minimum zone was (20mm) shown by Lactobacillus. From the above results, it is concluded that the Lactobacillus species present in plant-based milk was affected by antibiotics. However, if this probiotic bacterium is consumed in day-to-day life it may increase the gut immunity and may not be affected by antibiotics. The results on present studies are in accordance with the experimental findings of most of the research workers enlighten same line of research.

Zone of inhibition exhibited by LAB						
Antibiotics	A1	A2	A3	C1	C2	S2
Tetracycline	28mm (S)	33mm (S)	40mm (S)	37mm (S)	40mm (S)	32mm (S)
Penicillin	19mm (S)	20mm (S)	20mm (S)	12 mm (R)	25mm (S)	18mm (S)
Azithromycin	23mm (S)	22mm (S)	26mm (S)	11mm (R)	27mm (S)	22mm (S)
Cephalexin	21mm (S)	23mm (S)	35mm (S)	22mm (S)	23mm (S)	28mm (S)
	<i>Lactobacillus casei</i>	<i>Bifidobacterium Longum</i>	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus plantarum</i>	<i>Lactococcus lactis</i>	<i>Lactobacillus Acidophilus</i>

Table 3: Antibiotic resistance of isolated lactobacillus species

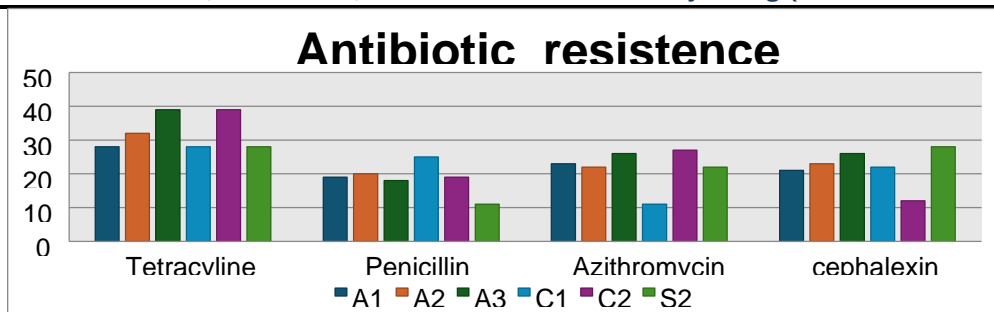


Figure 2:- Antibiotic resistance of isolated lactobacillus species

4. Enzymes Assay

Table 4. represents the Enzyme activity of isolated Lactobacillus species. To detect protease activity the isolated Lactobacillus species was inoculated on skim milk agar plates. After incubation it is observed that the isolated Lactobacillus strains showed Clear Zone of inhibition around the colony indicating the Positive protease activity. For detection of amylase activity, after 48 hrs. of incubation of LAB on starch agar plates, the zone of amylase activity was detected by flooding plates with Gram's iodine. It is observed that all the isolated Lactobacillus species showed Clear Zone around the colony indicative of amylolytic activity. For detection of Phosphatase activity, the isolated Lactobacillus species after 48hrs of incubation, showed the Clear zone around the around the bacterial colony of A1 isolate which indicates the solubilisation of phosphate and remaining LAB isolates showed no zone of inhibition. For the detection of Lipase activity, it is observed that all the isolated Lactobacillus species after 48 hrs incubation showed Clear Zone around the colony positive indicative of Lipase activity.

Enzymes	LAB					
	A1	A2	A3	C1	C2	S2
Protease	+	+	+	+	+	+
Amylase	+	+	+	+	+	+
Phosphatase	+	-	-	-	-	-
Lipases	+	+	+	+	+	+

Table 4: Extracellular enzyme producing ability of the isolated lactobacillus species

Conclusion:

The isolated Probiotic Species from Plant- Based Milk was found to possess antimicrobial activity against the pathogenic microorganisms. Hence, it protects the gut from enteric infections.

The probiotic population was found to possess different enzyme activities viz. Protease, Amylase, lipase and Phosphatase which are very important for maintaining the gut health by restoring the normal microorganisms after antibiotic therapy. This Plant- based Milk if consumed by lactose intolerant patients could help to provide nutrition gained from dairy products and could be used as an alternative. [20] These potential probiotic strains could contribute to the development of novel dairy-free products.

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