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Probiotic Attributes of Lactic Acid Bacterial Isolates of Sweet Corn (*Zea mays* L. *saccharata*)

M.S. Yashaswini[1]*, Suvarna, V.C.[2], Banu Deshpande[3], Shashank S.[4] and Arpitha T.[4]

[1]Ph.D. Scholar, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru (Karnataka), India.
[2]Professor, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru (Karnataka), India.
[3]Professor, Department of Food Technology, College of Agriculture, Hassan (Karnataka), India.
[4]M.Sc. Scholar, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru (Karnataka), India.

(Corresponding author: Yashaswini, M.S.)*
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ABSTRACT: Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The focus of this research is to explore the probiotic capabilities of lactic acid (LA) bacteria of sweet corn, with the prospect of creating functional foods and supplements that enhance gut health. The LA bacterial isolates that exhibited significant biopreservation activity were further evaluated for their probiotic attributes. The two best LA bacterial strains, *Lactiplantibacillus pentosus* UASBMIC_18, *Lactiplantibacillus plantarum* UASBMIC_22 and a reference culture, *Lactobacillus acidophilus* NCIM 2908 were assessed for their probiotic potential. The viability of LA bacteria is crucial to deliver significant health benefits to the host by withstanding the conditions prevailing in the gastrointestinal tract. All the LA bacterial strains were evaluated for tolerance to diverse pH levels, temperature variations, bile salts, NaCl and phenol. Additionally, evaluations included adhesive properties such as auto-aggregation, cell hydrophobicity and safety assessments through haemolytic and antibiotic susceptibility tests. *Lactiplantibacillus plantarum* UASBMIC_22 strain demonstrated significant results, outperforming the reference *Lactobacillus acidophilus* strain NCIM 2903 in terms of all the evaluated probiotic properties. Allthe LA bacterial strains were negative for haemolytic activity, signifying their safety for consumption.

Keywords: Probiotics, Lactic acid bacteria, Sweet corn, *Lactiplantibacillus plantarum* UASBMIC_22, *Lactobacillus acidophilus* NCIM 2908.

INTRODUCTION

Probiotic foods hold a major part of the functional food market, making up 60-70% of total sales. This reflects their popularity and the growing variety of options for consumers (Mohammadi *et al*., 2011). Probiotics are live microorganisms which when administered in adequate amounts, confer a health benefit on the host (FAO /WHO, 2002). These beneficial effects are primarily associated with the support of a well-balanced gut microbiota, its enhanced durability and the ability to influence factors like lactose intolerance, digestive health, gastrointestinal comfort, diarrhea prevention, cholesterol reduction, blood pressure management as well as regulating immune responses (Marteau *et al*., 2001). Among the probiotic microorganisms, lactic acid (LA) bacteria are Gram positive, catalase variable, microaerophilic to anaerobic bacteria and produce lactic acid as the main organic acid. They play a crucial role in human health as their antimicrobial properties make them effective to inhibit food borne pathogens such as bacteria, yeasts and molds (Bharath *et al*., 2023). They regarded as a major group of probiotic bacteria. The widely used probiotics belong to the genera of Lactobacillus and Bifidobacterium, but other LA bacteria such as the Lactococcus, Streptococcus and Enterococcus genera and certain yeast strains are also used as probiotics (Ohland and MacNaughton 2010). The effectiveness of a probiotic strain relies on its ability to fulfill various criteria, such as surviving the acidic environment of the stomach, navigating through the presence of bile salts at the starting of the intestinal tract, preserving its viability and effectively adheringto mucosal surfaces (Goldin and Gorbach 1992). These strains should be non-pathogenic and possess "Generally Regarded As Safe" (GRAS) designation. They should demonstrate ease of cultivation and reasonably robust survival capacity (Holzapfel *et al*., 1998).

Generally probiotic cultures are employed to restore the body's innate gut microbiota following a course of antibiotics. They mitigate the risk of gastrointestinal infections caused by harmful bacteria and provide therapeutic relief for conditions such as diarrhoea and urogenital diseases. Research reports have indicated that probiotics enhance the immune system's capacity to combat allergies, stress, excessive alcohol consumption and various diseases (Sanders, 2003). Moreover,

probiotics are under investigation as a live delivery vehicle for transporting vaccines, antimicrobials or enzymes to specific locations within the gastrointestinal tract or mucosal surfaces.

The research studies on microbial association with sweet corn remain scarce, with most of the studies focusing on the plant growth promoting microorganisms of sweet corn (Pande *et al*., 2020). Nevertheless, there are no reports on probiotic attributes of lactic acid bacteria from sweet corn available to date. Therefore, the LA bacterial strains isolated from sweet corn that exhibited significant biopreservation activity were evaluated and further screened for their probiotic attributes. The aim of this paper was to assess the probiotic potential of LA bacteria isolated from sweet corn. The selected LA bacterial strains were subjected to a series of *in vitro* analyses to evaluate their probiotic properties.

MATERIAL AND METHODS

Lactic acid (LA) bacterial strains. The two best LA bacterial strains that exhibited significant biopreservation activity against spoilage microorganisms of sweet corn, *Lactiplantibacillus pentosus* UASBMIC_18 and *Lactiplantibacillus plantarum* UASBMIC_22 obtained from Department of Agricultural Microbiology, GKVK were further evaluated for their probiotic potential. *Lactobacillus acidophilus* NCIM 2908 procured from National Collection of Industrial Microorganisms, Pune was used as a reference culture.

Tolerance to varying pH. The pH tolerance of LA bacterial strains was assessed using the methodology outlined by Meena *et al*. (2022), with slight modifications. The LA bacterial cultures were grown overnight (24 h) in de Man, Rogosa and Sharpe(MRS) broth by incubating at 37ºC.Subsequently, the activated cultures were centrifuged at 5,000 rpm at 4 ºC for 10 min, the cell pellets were washed in phosphate-buffered saline (PBS) solution with a pH of 7.2. The cells were resuspended in test tubes, each containing 10 mL of sterile MRS broth adjusted to various pH values (2.0, 3.5, 5.0, and 7.0) and a control sample had the pH of 6.5. The test tubes were then incubated at 37ºC for different time intervals. The survivability and growth of LA bacterial isolates was recorded by measuring the optical density (OD) at 600 nm at 3, 6 and 24 h intervals.

Tolerance to varying temperature. The overnight (24 h) grown LA bacterial strains were inoculated to 10 mL sterile MRS broth and incubatedat varying temperatures, *i.e.* 25, 30, 37 and 40 ºC. The growth was determined by reading absorbance at 3, 6 and 24 h intervals at 600 nm (Tambekar and Bhutada 2010).

Tolerance to different concentrations of bile salts. The bile salts tolerance of LA bacterial strains was assessed using bile salts, as described by Yadav *et al*. (2016). The pellets were obtained as described above and suspended in 5 mL MRS broth containing varying concentrations of bile salts (0.5, 1.0, 1.5 and 2.0 %) and control sample was without bile salts. The samples were incubated at 37 ºC and the observations were recorded by taking OD values at 3, 6 and 24 h intervals at 600 nm.

Tolerance to different concentrations of NaCl. The salt tolerance of LA bacterial strains was evaluated by following Meena *et al*. (2022), with some modifications. The overnight (24 h) LA bacterial strains were inoculated to MRS broth with different NaCl concentrations (4, 5 and 6 %) and incubated at37 ºC. The growth of bacteria was measured using the spectrophotometer by considering the absorbance values at 3, 6 and 24 h intervals at 600 nm.

Resistance to phenol. The phenol resistance of the selected LA bacterial strains were assessed according to the protocol reported by Yadav *et al*. (2016). The ability to withstand phenol is a crucial assessment criterion for potential probiotic strains due to phenol's potential to inhibit LA bacteria in the gastrointestinal tract. The sterilized MRS broth test tubes supplemented with 0.4% (v/v) phenol were prepared. The overnight (24 h) cultures were inoculated to the broth tubes and incubated at 37 ºC. The optical density was recorded after incubation by recording OD values at 0 and 24 h intervals at 600 nm.

Auto-aggregation ability. The autoaggregation abilities (cell adhesion properties) of LA bacterial cells in the gut are important properties for colonization of LA bacterial populations in the gut. The cell auto aggregation abilities were measured according to the method of Ahire *et al*. (2021), with slight modifications. The 24 h old culture of LA bacterial strains were centrifuged at 8,000 rpm for 10 min at 4 ºC. The harvested cells were then washed twice with phosphate buffered saline (PBS) and suspended in the same PBS buffer, adjusted the initial optical density of the culture suspension to 0.5 at 600 nm. Subsequently, 5 mL of diluted mixture was placed in a 15 mL Falcon tube and incubated at 37 ºC for 1 hour after gentle vortexing for 10 seconds. The sample was allowed to stand for a while, by incubating an aerobically at 37 ºC. The supernatant was checked for the absorbance at 600 nm at time intervals of 0, 1, 2, 3, 4 and 5 h. The auto aggregation was measured (in per cent) using the formula, Auto aggregation (%) = $[1 - (A_{time}/A_0) \times 100]$, where A_{time} - absorbance at a particular time and A_0 - absorbance at time zero.

Cell surface hydrophobicity (Meena *et al*., 2022). The 24 h old cultures of LA bacterial strains in MRS broth were centrifuged at 8,000 rpm for 10 min at 4ºC. The harvested cells were washed twice with PBS and resuspended in PBS buffer followed by measuring the absorbance (A_0) at 600 nm. A cell suspension of 3 mL was blended with 1 mL of hydrocarbon (xylene) and incubated at 37 ºC without shaking for 1 h for separation of the aqueous and organic phases. The aqueous phase (1 mL) was removed carefully and the absorbance (A_1) was measured at 600 nm. The per cent hydrophobicity was measured by a decrease in absorbance and calculated using the following formula: Cell surface hydrophobicity (%) = $(1 - A_1/A_0) \times 100$,

where A_1 represents the absorbance after 1 hr of incubation and A_0 represents the absorbance at time 0.

Haemolytic activity. Bacteria can make enzymes that break red blood cell membranes, a crucial factor in making diseases worse by helping pathogens get nutrients like iron, leading to anemia. Therefore, the evaluation of bacterial haemolytic activity is crucial for assessing *in vitro* safety (Vesterlund *et al*., 2007). The haemolytic activity of LA bacterial strains was tested using agar well diffusion method on Sheep blood agar medium, prepared with 5 % sheep blood in Trypticase Soy Agar (TSA). The wells of 7 mm diameter were inoculated with 50 µL of LA bacterial strains. The sterile distilled water was taken as control. The observations were recorded after three days of incubation at 30 °C.

Antibiotic susceptibility. The antibiotic susceptibility of LA bacterial strains was tested using Kirby-Bauer disk diffusion test (Adetoye *et al*., 2018). The antibiotics employed in this study were ampicillin (10 µg), azithromycin (15 µg), chloramphenicol (30 µg), gentamycin (10 µg), amphotericin B (20 µg) and nystatin (50 µg). The MRS agar medium was seeded with 3 % of LA bacterial strains (10^8 CFU /mL). The plates were incubated at 37 ºC for 48 h and diameter of inhibition zone was recorded.

RESULTS AND DISCUSSION

pH tolerance. The tolerance to growth inhibitory conditions like low pH is very important characteristic to be fulfilled by probiotic strains. A probiotic strain should be able to withstand the transit through the stomach, where the pH can be as low as 2. They should survive through low pH gastric acid environment in upper small intestine to exert its beneficial effects in gut. Therefore, tolerance to highly acidic conditions is another crucial characteristic of a probiotic strain (Dunne *et al*., 2001; Guo *et al*., 2009). The LA bacterial strains were evaluated for their viability at pH 2, 3.5, 5, 6.5 and 8, all the strains had better survivability at 6.5 pH. *Lactiplantibacillus plantarum* UASBMIC_22 recorded the highest tolerance to varying pH followed by *Lactiplantibacillus plantarum* UASBMIC_18 and *Lactobacillus acidophilus* NCIM 2908 (Fig. 1).

Our results align closely with Balcazar *et al.* (2008), studied the ability of *Lactococcus lactis* CLFP 101, *Lactobacillus plantarum* CLFP 238 and *Lactobacillus fermentum* CLFP 242 obtained from fish to survive under low pH levels. Chen *et al*. (2020) reported similar findings, demonstrating that isolate HSM-1, HSM-10, HSM-14 and HSM-18 exhibited their optimal growth at pH 4.0 in MRS conditions, with respective growth rates of 27.33, 24.27, 25.22 and 23.75 %. However, the survival rates showed a noticeable decline ranging from 8-9 % at pH 3.0 and further decreased to 6-7 % at pH 2.0.

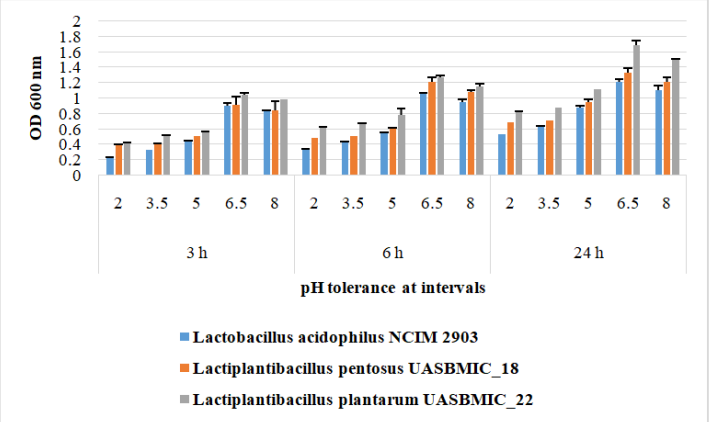


Fig. 1. Tolerance of LA bacterial strains topH levels at intervals.

Temperature tolerance. Probiotics must exhibit resilience to endure challenging environmental conditions and maintain their viability throughout transportation and storage. Temperature is one of the critical factors that can have a profound impact on bacterial growth. The ability of LA bacterial strains and reference culture *Lactobacillus acidophilus* NCIM 2903 to survive and grow at selected temperature range (25-40 ºC) was evaluated. They should be capable of surviving in both animals and humans considering the variations in normal body temperatures (37 °C for humans and 40 °C for animals). This temperature range was selected with the intention of assessing the survivability of LA bacterial strains to thrive within the typical range of human body temperatures. This choice was crucial because if the isolates do not survive within this temperature range, then they will not be able to survive in the human gut, which is a fundamental requirement for probiotics to demonstrate their efficacy. *Lactiplantibacillus plantarum* UASBMIC_22 recorded the highest tolerance to varying temperatures followed by *Lactiplantibacillus plantarum* UASBMIC_18 and *Lactobacillus acidophilus* NCIM 2908 (Fig. 2). Our findings indicated that the LA bacterial strains exhibited the highest growth at 37 °C, simulating the typical human body temperature and the lowest growth at 25 °C. The reduction in growth was observed as the temperature increased to 40 °C compared to the optimal of 37 °C. Our results confirmed that the isolates indeed exhibited excellent growth within the specified temperature range.

Ayo-Omogie and Okorie, (2016) reported results in line with our findings, indicating that LA bacterial isolates such as *Leuconostoc cremoris*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus cellobiosus*, *Lactobacillus jensenii* and *Leuconostoc mesenteroides* exhibited tolerance and growth across a temperature range of 25-40 ºC. Similarly, Reuben *et al.* (2019) observed that LA bacterial isolates could survive within the temperature range of 25 to 40 °C, with an inability to thrive under extreme temperatures. These consistent outcomes are also supported by the studies conducted by Divyashree *et al.* (2021); Banik *et al.* (2023).

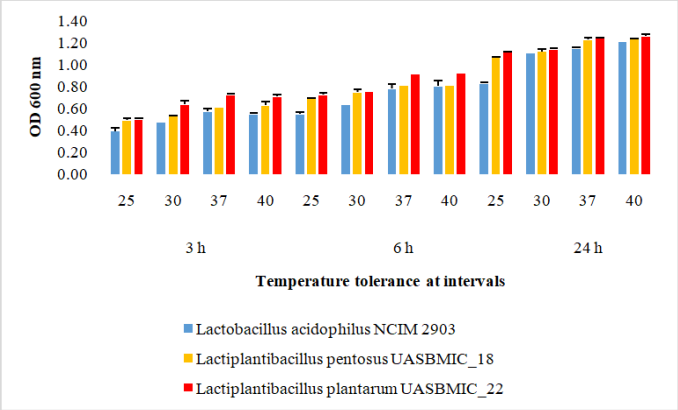


Fig. 2. Tolerance of LA bacterial strains to different temperatures at intervals.

Bile salt tolerance. One of the crucial requirements for a LA bacteria to be considered as a probiotic is its ability to withstand the impact of bile salts in the upper small intestine to exert its beneficial effects in the gut (Lee and Salminen 1995). However, currently there are no reports regarding the precise concentration for a selected strain to exhibit tolerance. The physiological concentration of bile salts in the small intestine typically falls within the range of 0.2 to 2.0 % as elucidated by Gunn (2000). Therefore, in our study all the LA bacterial strains were subjected to a bile salt concentration up to 2 %. This represents the highest concentration typically encountered in intestines of both animals and humans during the digestive process (Gotcheva *et al*., 2002).

The survival ability of LA bacterial strains at different bile salts concentration (0.5, 1.0, 1.5 and 2.0 %) was examined at 3, 6 and 24 h incubation. All the LA bacterial strains exhibited good resistance to 2 % bile salt, even after being exposed for 24 h. *Lactiplantibacillus plantarum* UASBMIC_22 recorded better bile tolerance followed by *Lactiplantibacillus pentosus* UASBMIC_18 and the lowest tolerance was observed with *Lactobacillus acidophilus* NCIM 2903 (Fig. 3). This resistance to bile is closely associated with the presence of bile salt hydrolase (BSH), an enzyme responsible for hydrolyzing conjugated bile, thereby mitigating its potential harmful effects (Du Toit *et al*., 1998). The extent of resistance varies significantly among different LA bacterial species and their respective strains. Since our LA bacterial strains were members of the *Lactobacillus* genus, exhibited a uniform resistance pattern.

Similar results were reported by Vanniyasingam *et al.* (2019), they evaluated survival ability of LA bacterial isolates at various bile salt concentrations (0.2, 0.3, 0.4 and 0.5 %) over 24 h incubation. *Lactobacillus plantarum* strain M6 exhibited the superior bile tolerance. Kuppusamy *et al.* (2020) evaluated the bile salt tolerance of the LA bacterial strains. *Lactobacillus plantarum* RJ1 and *Pediococcus pentosaceus* S22 exhibited different levels of bile salt resistance after 12 h of exposure especially RJ1 strain was able to resist a higher bile concentration and *P. pentosaceus* S22 had a slightly lower resistance to bile salt.

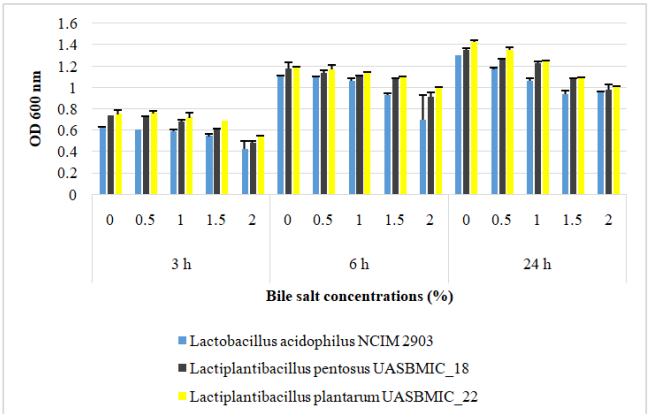


Fig. 3. Tolerance of LA bacterial strains tobile salts at intervals.

NaCl tolerance. NaCl is an inhibitory agent capable of impeding the proliferation of specific bacterial strains. Probiotic organisms must exhibit resilience to elevated salt levels within the human intestine. The LA bacteria generally exhibit a high tolerance to elevated salt concentrations that help in initiating metabolism. This metabolic activity results in acid production, further inhibiting the proliferation of undesirable microorganisms. The ability to withstand NaCl is indicative of the strains' osmotolerance. As cells produce lactic acid, an alkaline substance is introduced to the fermentation broth to prevent excessive pH reduction in industrial fermentation processes. Consequently, the free acid is transformed into its salt form, leading to an increase in osmotic pressure on the cells. Therefore, it is essential for a probiotic to maintain its viability even under high NaCl concentrations both during production and consumption.

All the LA bacterial strains exhibited the highest growth at 4 % and the lowest growth at 6 % concentration, during incubation periods of 3, 6 and 24 h. *Lactiplantibacillus plantarum* UASBMIC_22 consistently demonstrated the highest growth, significantly outperforming all other strains and *Lactobacillus acidophilus* NCIM 2908 displayed the lowest growth across all time intervals (Fig. 4).

Similarly, our findings align in line with reports of Divya *et al*. (2012), they noted that all five LA bacterial isolates demonstrated the highest growth in the presence of NaCl concentrations up to 6 %. However, as the NaCl concentration increased beyond this threshold, the growth and viability of the LA bacterial isolates B6, C9 and G1 reduced noticeably. The LA bacterial isolates P8 and G4 exhibited a higher tolerance, capable of thriving in environments having NaCl concentration up to 8 % with isolate P8 displayed the highest level of tolerance. Elzeini *et al*. (2021) conducted a study revealing that LA bacterial strains exhibited strong tolerance to a range of NaCl concentrations spanning from 1 to 6 %. However, there were exceptions to this trend. *Enterococcus faecalis* displayed a total plate count of 6.200 ± 0.04 at 6 % NaCl concentration and *Lactococcus garvieae* showed a total plate count of 5.874 ± 0.03 at 1 % NaCl concentration.

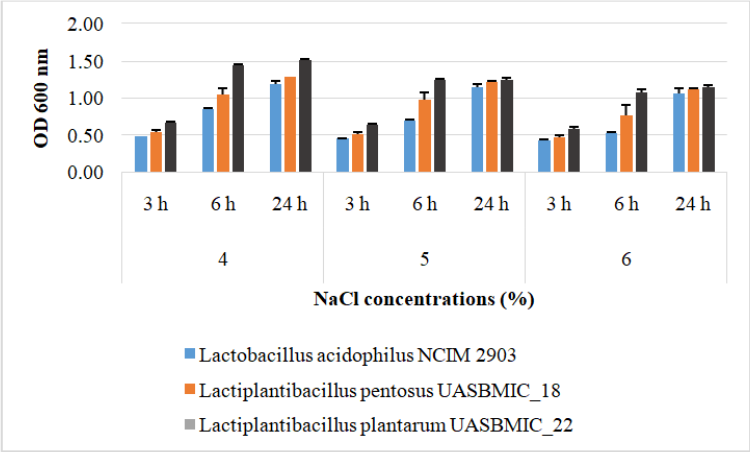


Fig. 4. NaCl tolerance of LA bacterial strains at intervals.

Phenol tolerance. The ability to withstand phenol is a significant probiotic trait, as phenol can arise from the deamination of specific aromatic amino acids by certain bacteria and can exert a bacteriostatic effect (Suskovic *et al*., 1997). An effective probiotic bacteria should effectively navigate and establish themselves in the intestine despite this bacteriostatic effect. *Lactobacillus brevis*, *Lactobacillus plantarum* and *Pediococcus* reported to decarboxylate the phenolic carboxylic acids, ferulic and p-coumaric acids (Cavin *et al*., 1993).

The phenol tolerance of LA bacterial strains significantly differed ranging (0.83 -0.91) at 0.4 % phenol after 24 h at 600 nm. *Lactiplantibacillus plantarum* UASBMIC_22 recorded the highest tolerance (0.91) followed by *Lactiplantibacillus plantarum* UASBMIC_18 and *Lactobacillus acidophilus* NCIM 2908 at 0.4 % phenol after 24 h at 600 nm (Fig. 5). The phenol tolerance of LA bacterial strains up to 0.5 % has been reported in several studies (Divya *et al*., 2012; Somashekaraiah *et al*., 2019; Meena *et al*., 2022). The survival rate of the LA bacterial isolates decreased with increase in phenol concentration. Our findings are in conformation with Pinto *et al*. (2006), observed *Lactobacillus plantarum* strains being less sensitive to phenol and four of the six strains tolerated phenol up to 0.4 % for 24 h. Reuben *et al*. (2019) reported only six LA bacterial isolates out of 63, were able to tolerate 0.4 % phenol with OD values > 1.00. The viability of all the LA bacterial isolates examined differed significantly with respect to phenol concentrations.

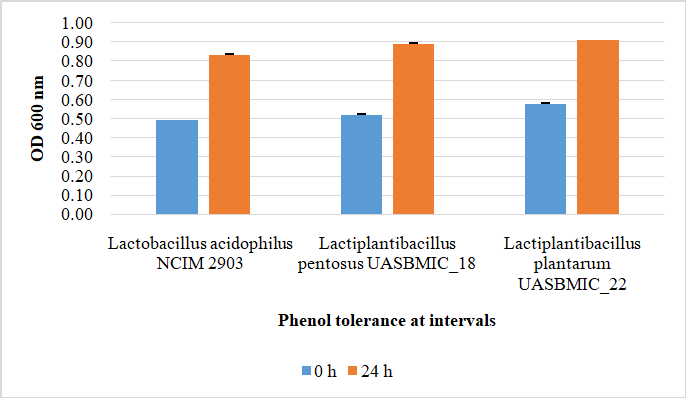


Fig. 5. Phenol tolerance of LA bacterial strains.

Auto-aggregation of LA bacterial isolates. Auto-aggregation is the intrinsic capacity of probiotic bacteria to aggregate together, allowing them to sustain an optimal cell density that is essential for delivering a range of health benefits to the host. The interaction of diverse LA bacteria with the mucosal lining of the gastrointestinal tract is responsible for their colonization and potential to modulate the immune system. All the LA bacterial isolates exhibited varying levels of auto-aggregation capacity after 5 h of incubation period. Among all the strains screened, *Lactiplantibacillus plantarum* UASBMIC_22 strain demonstrated the highest auto-aggregation ability (81.66 %), while *Lactobacillus acidophilus* NCIM 2908 exhibited the lowest auto-aggregation potential measuring 73.39 %. *Lactiplantibacillus pentosus* UASBMIC_18 displayed an auto-aggregation ability of 78.13 % (Table 1). There was a clear correlation between the per cent auto-aggregation ability and duration of incubation, indicating that auto-aggregation increased with an increase in incubation duration (0-5 h).

Our observations concur with Cai *et al*. (2022), they evaluated auto-aggregation ability of *Lactococcus lactis* (S1 and S2) *Enterococcus faecalis* (F3 and F7) strains. The strains S1 and S2 exhibited similar auto-aggregation abilities of 66.30 % and 69.20 % respectively, were higher than those of strains F3 (54.00 %) and F7 (50.00 %) after 10 hours of incubation. Similarly, *Lactobacillus plantarum* NCU001563 and *Streptococcus thermophilus* NCU074001 strains also expressed a higher auto-aggregation rate after 4 h incubation (Madjirebaye *et al*., 2022).

Cell surface hydrophobicity of LA bacterial isolates. The cell surface hydrophobicity refers to the capacity of bacterial cells to adhere to the hydrophobic surfaces. This quality holds significant importance for probiotics as it signifies their ability to adhere to the gut's epithelial cells. Probiotics get easily washed out and eliminated from the gastrointestinal tract, if the cells are devoid of this property. However, it is desirable for probiotics to remain in the gut for an extended period to confer health benefits to the host. Therefore, probiotic candidates should exhibit high level hydrophobicity. The Bacterial Adhesion to Hydrocarbon (BATH) test aids in assessing the various forces, either physical or chemical involved in the adhesion of probiotic strains to the human gastrointestinal epithelial layers. This test provides insights into the physico-chemical characteristics of the microbial cell surface. This adhesive property increases the capacity of probiotic microorganisms to obstruct pathogen entry through steric interactions or by specifically blocking cell receptors (Otero *et al*., 2004).

The cell surface hydrophobicity of LA bacterial strains were evaluated using hydrocarbon, xylene. All the three strains exhibited high adhesion capability. *Lactiplantibacillus plantarum* UASBMIC_22 exhibited the highest cell surface hydrophobicity of 71.97 % followed by *Lactiplantibacillus pentosus* UASBMIC_18 with 64.33 %. However, the reference culture *Lactobacillus acidophilus* NCIM 2908, displayed 70.08 % hydrophobicity (Table 1). These findings provide an additional validation for considering these strains as potential probiotic candidates, consistent with earlier studies (Palaniswamy and Govindaswamy 2016; Wang *et al*., 2021; Sakoui *et al*. 2022). Similar results were reported by Meena *et al*. (2022), they evaluated the cell-surface hydrophobicity of the bacterial cells using hydrocarbon xylene. *Lactobacillus delbrueckii* subsp. *bulgaricus* KMUDR1 displayed the highest cell surface hydrophobicity of 75.30 %. *Lactobacillus plantarum* NCU001563 strain had a lower hydrophobicity (54.09 %) than *Streptococcus thermophilus* NCU074001 (70.36 %) at 2 h incubation. Further, after exposing for 4 h, *L. plantarum* NCU001563 and *S. thermophilus* NCU074001 exhibited stronger hydrophobicity ability of 79.33 and 73.68 % respectively (Bangotra *et al*., 2023).

Table 1: Per cent auto aggregation and cell hydrophobicity of LA bacterial strains at intervals.

Treatments	Auto aggregation (%)					Cell hydrophobicity (%)
	1 h	2 h	3 h	4 h	5 h	24 h
Control	0.00	00.00	00.00	00.00	00.00	00.00
Lactobacillus acidophilus NCIM 2903	0.81	32.26	48.39	60.48	73.39	70.08
Lactiplantibacillus pentosus UASBMIC_18	5.63	24.38	36.88	58.13	78.13	64.33
Lactiplantibacillus plantarum UASBMIC_22	6.99	24.89	48.91	64.19	81.66	71.97

Table 2: Safety assessment of LA bacterial strains.

Treatments	Antibiotic sensitivity					
	Antibacterial				Antifungal	
	Gentamycin (EC 10 µg /disc)	Chloramphenicol (EC 30 µg /disc)	Azithromycin (EC 15 µg /disc)	Ampicillin (EC 10 µg /disc)	Nystatin (EC 50 µg /disc)	Amphotericin - B (EC 20 µg /disc)
Lactobacillus acidophilus NCIM 2903	R	R	R	R	R	R
Lactiplantibacillus pentosus UASBMIC_18	R	R	R	R	R	R
*Lactiplantibacillus plantarum*UASBMIC_22	R	S	S	S	R	R

Note: 'R'- Resistance, 'S'- Susceptible

Haemolytic activity of LA bacterial isolates. The selected LA bacterial strains were evaluated for their haemolytic activity. The exotoxins produced by haemolytic bacteria lyse red blood cells (RBCs) and haemoglobin, resulting in three kinds of haemolysis: α-haemolysis, β-haemolysis and γ-haemolysis. α-haemolysis is a form of partial haemolysis recognized by a greenish discoloration surrounding the colonies. β-haemolysis represents complete haemolysis of both RBCs and haemoglobin leading to a clear zone around colonies, while γ-haemolysis in contrast is non-haemolytic, characterized by colony expansion without any haemolysis (Georgieva *et al*., 2015).

The probiotic bacteria should exhibit non-haemolytic characteristics signifying their non-pathogenic nature and safe for consumption. The LA bacterial strains, *Lactiplantibacillus pentosus* UASBMIC_18 and*Lactiplantibacillus plantarum*UASBMIC_22 along with reference culture *Lactobacillus acidophilus* NCIM 2908 were tested for their haemolytic activity on sheep blood agar medium (Plate 1). All the strains demonstrated γ-haemolysis, confirming non-haemolytic nature and thus safe for human consumption. Several reports are in line with our results, Kowsalya *et al*. (2022) reported that *Lactobacillus plantarum* M2 and *Lactobacillus plantarum* KO9 strains exhibited γ-haemolytic activity with no clear zone formation on blood agar plates. *Lactococcus lactis* (S1 and S2) and *Enterococcus faecalis* (F3 and F7) capable of producing high quantity of acid were screened for haemolytic activity. All the strains showed negative results for haemolytic activity indicating their safety for use as potential probiotics (Cai *et al*., 2022).

Antibiotic sensitivity of LA bacterial isolates. Probiotic strains intended for both human and animal consumption need to be sensitive to antibiotics. Probiotics strains if found resistant to antibiotics, the possibility of the genes responsible for antibiotic resistance may be transmitted to the intestinal microbiome or potential pathogens *via* horizontal gene transfer mechanisms. Hence, the antibiotic susceptibility test was carried out as per Bauer *et al*. (1966) method for evaluating the quality and safety of LA bacterial isolates as probiotics in food processing. The LA bacterial isolates were screened against four antibacterial antibiotics *viz*., gentamycin, chloramphenicol, azithromycin, ampicillin and two antifungal antibiotics *viz*., nystatin and amphotericin-B. *Lactiplantibacillus pentosus* UASBMIC_18 and reference culture *Lactobacillus acidophilus* NCIM 2908 were found to be resistant to all the tested antibacterial and antifungal antibiotics. *Lactiplantibacillus plantarum* UASBMIC_22 exhibited resistance to gentamycin (antibacterial antibiotic), nystatin, amphotericin - B (antifungal antibiotics) and susceptibility to antibacterial antibiotics *viz*., chloramphenicol, azithromycin and ampicillin (Table 2, Plate 2).

The antibiotic resistance genes have exhibited notable stability within *Lactiplantibacillus* spp., previously referred to as *Lactobacillus* spp., suggesting a minimal risk of transferring these resistance genes to pathogenic microorganisms, thereby supporting their safety (Campedelli *et al*., 2019). Our findings are in consistence with Dowarah *et al*. (2018); Sirichoat *et al*. (2020); Jung *et al*. (2021). The LA bacterial strains *Lactobacillus plantarum* NCU001563 and *Streptococcus thermophilus* NCU074001 were resistant to kanamycin, gentamicin and ciprofloxacin and sensitive to amoxicillin, ampicillin and erythromycin (Madjirebaye *et al*., 2022). Similarly, Bangotra *et al*. (2023), evaluated antibiotic sensitivity of LA bacterial isolates using commonly prescribed antibiotics such as erythromycin, kanamycin, rifampicin, streptomycin and chloramphenicol. Among the LA bacterial isolates, BK1, BK2, BK4 and BK5 displayed susceptibility to all

tested antibiotics. Conversely, isolates BK6 and BK3 demonstrated resistance to chloramphenicol, streptomycin and rifamicin.

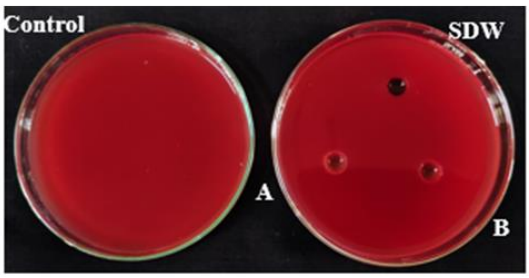
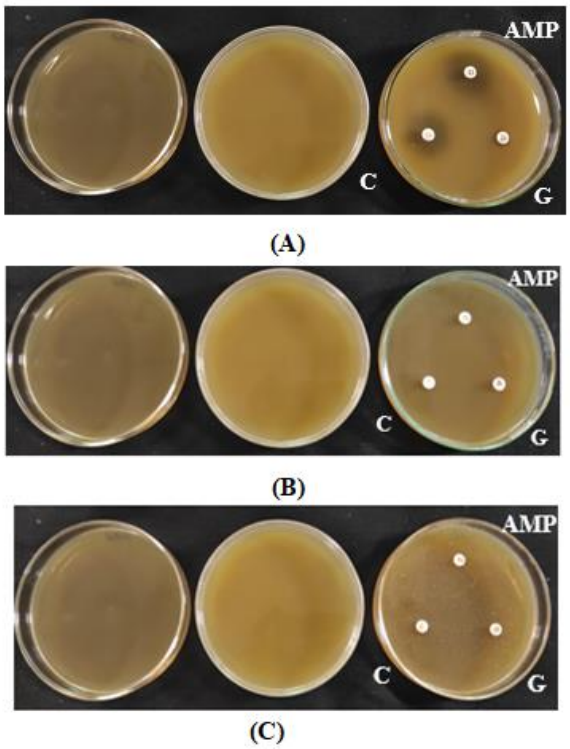


Plate 1. Haemolytic activity of LA bacterial strains (Note: SDW – Sterile distilled water, A- *Lactiplantibacillus plantarum* UASBMIC_22, B- *Lactiplantibacillus pentosus* UASBMIC_18)



Note: AMP – Ampicillin, C – Chloramphenicol and G-Gentamycin

Plate 2. Antibiotic sensitivity of (A) *Lactiplantibacillus plantarum* UASBMIC_22, (B) *Lactiplantibacillus pentosus* UASBMIC_18and (C) *Lactobacillus acidophilus* NCIM 2908.

CONCLUSIONS

Lactic acid bacteria are widely known for their probiotic attributes. The probiotic potential of effective antimicrobial lactic acid bacterial strains, specifically *Lactiplantibacillus pentosus* UASBMIC_18 and *Lactiplantibacillus plantarum* UASBMIC_22 were evaluated. This study marks the first documentation of lactic acid bacterial strains from sweet corn exhibiting probiotic characteristics. The results suggest that these lactic acid bacterial strains isolated from sweet corn possess favourable probiotic properties. They also exhibited non haemolytic activity, indicating their safety in consumption.

FUTURE SCOPE

This study further needs the investigation on the safety of these strains *in vivo* before considering their use in functional foods, ensuring compliance with probiotic property safety regulation.

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Conflict of Interest. None.

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