

Article

***Lactobacillus xylosus* isolated from butter showed potentiality to be used as probiotic and biopreservative**

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Abstract: Today, in an era of antibiotic-resistant pathogens and other looming microbial threats, the value of prevention of infection is recognized. To circumvent the indiscriminate use of antibiotics and emerging resistance to them, to reduce the use of chemical preservatives and to abate abdominal, gastrointestinal and urogenital disorders- probiotics and bacteriocins are getting paramount priority in recent times. We investigated the probiotic and bacteriocinogenic potentiality of *Lactobacillus* isolated from milk products. 15 lactic acid bacteria (LAB) were isolated from milk product samples using De Man Rogosa Sharpe (MRS) medium. Among them, only one (6%) isolate showed potential antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis* in agar well diffusion method. Following conventional methods, genus and species of the isolate were confirmed as *Lactobacillus xylosus*. The isolate exhibited growth competency at wide range of temperatures (27-45°C), pH (2-9), NaCl (1-7%), bile salt (0.5-2%) and could produce bacteriocin or BLS, thus implying its potential probiotic nature. Bacteriocins or BLS produced by *Lactobacillus xylosus* inhibited *E. coli* and *S. aureus* and could retain their antibacterial activity at wide range of temperatures (37°C to 100°C) and pH (2–9) treatments. These crude bacteriocins or BLS of 5% concentration reduced the initial bacterial load of cheese and milk up to 41% and 43% respectively, after 48 h of preservation at room temperature. The experimental data revealed that our study isolate *Lactobacillus xylosus* could be used as probiotics and their bacteriocin or BLS could be used as bio-preservatives.

Keywords: probiotics; *Lactobacillus xylosus*; bacteriocin; lactic acid bacteria; bio-preservatives; antibacterial activity

1. Introduction

Modern world is becoming more and more conscious about the correlation between proper nutrition and good health. This tendency has prompted huge research on the discovery and identification of food items and food adjuncts that confer special benefits upon consuming (Agrawal, 2005). Probiotics are one of the optimistic additions to this quest. Several individual researchers and organizations have so far tried to define “probiotics” of them; most widely accepted definition is that of Fuller (1989). According to him, “probiotics are live microbial food supplements that beneficially affect the host animal by improving the intestinal microbial balance” (Fuller, 1989). The Food and Agriculture Organization of the United Nation (FAO) and the World Health Organization (WHO) define probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). Probiotics modulate our gut microbiota by

both direct and indirect biological effects. Direct effects include adhesion to specific areas of gastrointestinal tract and exclusion of pathogens through nutrients competition. Indirect effects include production of lactic acid which decreases the pH, production of H₂O₂ that interacts with the toxins produced by pathogens, synthesis of bacteriocins and mucins (Hu *et al.*, 2017; Islam *et al.*, 2016; Dinkçi *et al.*, 2006). Upon ingestion, probiotics exert some beneficiary effects on human body, such as (i) reducing the duration of infectious and/or antibiotic associated diarrhea through influencing enteric nervous system and/or immune system to produce neuropeptides, cytokines or hormones that minimize secretion of water and electrolytes across the intestinal epithelium (Rahman *et al.*, 2018; Andersson *et al.*, 2001), (ii) control of lactose intolerance through delivery of microbial lactase to small intestine (Sanders, 2003), (iii) control of irritable bowel syndrome through alteration of population of intestinal microflora (Rahman *et al.*, 2018; Sanders *et al.*, 2003), (iv) prevention of ulcers associated with *Helicobacter pylori* infection through antipathogen activity (Sanders, 2003; Nami *et al.*, 2019), (v) prevention of urinary tract infection, kidney stones and flare-ups of Chron's disease (Reid, 2005), (vi) prevention of inflammatory bowel disease through down regulation of inflammatory response (Sanders, 2003), (vii) exhibition of anti-mutagenic and anti-carcinogenic features through mutagen absorption and inhibition of carcinogen producing microflora (Sanders, 2003; Nami, 2019), (viii) increased intestinal mucosal barrier (Ahl *et al.*, 2016).

Lactic acid bacteria (LAB) are a major broad group of probiotic bacteria. LAB are gram positive, non-sporing, catalase negative, cocci-shaped, usually non-motile organisms that are devoid of cytochrome C and are non-aerobic, but are aerotolerant and acid tolerant which produce lactic acid as the major end product (Agrawal, 2005; Nami *et al.*, 2019; Chowdhury *et al.*, 2012). These microbes are ubiquitous in nature and are indigenous inhabitants of the human gastrointestinal tract, vagina and human skin, but are also found in soil, water, vegetables products, meats, fermented and cooked meat and dairy products (Andersson *et al.*, 2001; Li *et al.*, 2018). LAB deploy strong antagonistic activity against pathogenic, non-pathogenic and spoilage organisms in fermented milk, foods and beverages (Gilliland *et al.*, 1975) and demonstrate antimutagenic properties through reducing the activity of β -glucuronidase, nitro reductase and azoreductase which convert precarcinogen into its active form (Guarner *et al.*, 2003).

A major group of LAB, known as *Lactobacillus* species, are most widely utilized group of microorganisms as probiotics due to their "Generally recognized as safe" (GRAS) status (Islam *et al.*, 2016). *Lactobacillus* spp. are omnipresent in nature, especially in the human gut, particularly in the small intestine as this portion of GIT provides anaerobic condition, sugars as a carbon source, a range of minor nutrients to support their growth and sustainability (Rahman *et al.*, 2018). *Lactobacillus* spp. have been proved to be effective in allergic children (Martínez-Cañavate *et al.*, 2009), preventing vasculopathy in obesity (Toral *et al.*, 2014), reducing the risk of necrotizing enterocolitis in low birth weight premature babies (Hoyos *et al.*, 1999).

Having a moderate pH (6.4-6.6), adequate nutrients, high water contents, milk provides an excellent environment for microbial growth and fermented milk could be a valuable source of autochthonous Lactic Acid Bacteria (LAB) (El Soda *et al.*, 2003). The dietary and therapeutic values of milk products are determined by presence of probiotic microbes (Boor *et al.*, 2001). *Lactobacillus* and *Streptococcus* spp. are most commonly used probiotics in commercial fermented and non-fermented milk product now a days (Chowdhury *et al.*, 2012). Some strains even contribute to the preservation of fermented milk and foods by producing bacteriocins (Ten Brink *et al.*, 1994). Bacteriocins are ribosomally synthesized antimicrobial peptides that exhibit bactericidal activity against closely related organisms (Bromberg *et al.*, 2004). Since bacteriocin producing LAB are mostly isolated from dairy foods and meat products, these proteinaceous substances thus have been consumed for a long time. So, considering all the facts discussed above, our present study focused on isolation of *Lactobacillus* spp. from dairy products and in *in-vitro* evaluation of their probiotic and bacteriocinogenic potentiality.

2. Methods and Materials

2.1. Sample collection, isolation and purification of *Lactobacillus*

Multiple sets of milk products samples (Butter and cheese) were collected from local shops of Chittagong area in Bangladesh. For isolation of *Lactobacillus*, serial dilution (10⁻¹ to 10⁻⁶) agar plate technique was used. *Lactobacillus* was purified by streak plate method on De Man Rogosa Sharpe (MRS) agar.

2.2. Test microorganisms

The target pathogenic organisms used in this study were *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* IFSTIM22, *Pseudomonas aeruginosa* CRL (ICDDR, B), *E. coli* ATCC25922, *Salmonella typhi* AE14296. The test microorganisms were standardized by using 0.5 McFarland standard. A 0.5 McFarland gives approximate

cell density of 1.5×10^8 CFU/mL, having absorbance of 0.132 at wavelength of 600nm (Andrews *et al.*, 2001). We used this standardization technique for all the necessary steps of this study.

2.3. Screening of isolated *Lactobacillus* for antibacterial activity

MRS broth was used for antimicrobial metabolite production from *Lactobacillus*. 200 mL of MRS broth was autoclaved at 121 °C for 15 minutes and inoculated with the colonies of a *Lactobacillus* isolate and incubated at 37 °C for 2-3 days under stationary condition. Then it was centrifuged (Model 6930, Kubota, Japan) at 9000 rpm for 15 minutes at 4 °C. The supernatant was then filtered through Whatman No. 1 filter paper to remove residual cells. Petri-plates were prepared by pouring sterile molten Mueller Hinton medium and allowed it to solidify. A hundred microliters of each standardized test microorganisms were spread on agar plates. Two wells (each 7 mm in diameter) made into agar plates with sterile borer. The wells were loaded with 100 µL of filtered LAB culture supernatant and 100 µL sterile broth. Plates were incubated at 37 °C for 24 hours. After incubation, diameter of zone of inhibition was observed and measured (Pundir *et al.*, 2013).

2.4. Identification of *Lactobacillus* isolates

Lactobacillus species were identified based on their morphological characteristics including size and shape of the organism, arrangement of the cells, presence or absence of the spores, regular or irregular forms, acid fastness, gram reaction etc.; cultural and physiological characteristics including H₂S production, nitrate reduction, deep glucose agar test, fermentation of different carbohydrates etc. All these characteristics were then compared with the standard description of ‘‘Bergey’s Manual of Determinative Bacteriology’’, 8th edition (Buchanan and Gibbons, 1974).

2.5. Determination of probiotic efficiency of *Lactobacillus xylosum*

2.5.1. pH tolerance and temperature sensitivity

The *Lactobacillus* cultures were inoculated into sterile MRS broth tubes of varying pH, i.e., 2, 4, 7 and 9 and incubated at 37 °C for 24-48 hours. Another set of inoculated MRS broth was grown at varying temperatures, i.e., 27, 37, and 45 °C for 24-48 hours. The absorbance of MRS broths were taken at 600 nm by a spectrophotometer (Model T60U, pg instruments, UK) to measure microbial load (Rahman *et al.*, 2019).

2.5.2. Bile salt and NaCl tolerance

The MRS broth media with varying concentrations of bile salt (0.5, 1.0 and 2.0%) and NaCl (1, 3 and 7%) were inoculated separately with each *Lactobacillus* culture and incubated at 37 °C for 48 hours. Then the absorbance of MRS broths were taken at 600 nm by a spectrophotometer for measuring microbial load (Rahman *et al.*, 2018).

2.5.3. Lactose utilization

The acid production by *Lactobacillus* cultures was detected by observing the change in color of the medium. Sterilized fermentation medium (Peptone 10 g, NaCl 15 g, phenol red 0.018 g, lactose 5 g, for 1 L distilled water and final pH 7.0) was inoculated with *Lactobacillus* cultures and incubated at 37 °C for 24-48 h. Change in color from red to yellow indicates the production of acid (Ahmed and Kanwal, 2004).

2.5.4. Antibiotic susceptibility test

The antibiotic susceptibility of *Lactobacillus* was assessed by using Kirby-Bauer discs diffusion method on MRS agar plates. The used antibiotics were penicillin G (10 IU), chloramphenicol (30 µg), erythromycin (15 µg), cefixime (5 µg), cephradine (30 µg), streptomycin (10 µg) and rifamycin (5 µg) (Rahman *et al.*, 2019).

2.5.5. Determination of bacteriocin production capability of the *Lactobacillus xylosum*

This experiment has been carried out according to the method described by Yang *et al.*, 2012 (Yang *et al.*, 2012). One milliliter of frozen *Lactobacillus* isolate was cultured 24 hours in 20 mL MRS broth. Then 1 mL culture was sub-cultured 24 h in 20 mL MRS broth. Cells were removed by centrifuging at 9000 rpm for 15 minutes. The supernatant was filtered through a sterile Whatman No. 1 filter paper and 100 µL of the pH unadjusted aliquot of cell free supernatant (CFS) was added to the first well. The remaining CFS was adjusted to pH 6.0 with 1M/IN NaOH in order to rule out possible inhibitory effects due to organic acids. 100 µL of the pH adjusted CFS was filtered and added to the second well. The neutralized CFS was then treated with 1mg/mL of catalase (Merck KGa A, Germany) at 25 °C for 30 min to eliminate the possible inhibitory action of H₂O₂ and filtered. Then 10 µL catalase treated CFS was placed in the third well. If inhibition zone were found in the

third well, the isolates were able to produce bacteriocin or BLS. To confirm the production of a proteinaceous compound, CFS displaying antimicrobial production after acid neutralization and H₂O₂ elimination were treated with 1 mg/mL of proteolytic enzymes including papain and trypsin (Sigma-Aldrich Corporation, USA). 5ml of bacteriocin was taken in test-tubes and treated with papain/trypsin (1 mg/mL) at pH 7. The test tubes with and without the enzyme (control) were incubated at 37 °C for 2 h and then heated at 100 °C for 3 min to denature the enzyme. Both the control and samples were assayed for antimicrobial activity by using agar well diffusion method (Rahman *et al.*, 2019).

2.5.6. Heat stability and effect of pH on the crude Bacteriocin or BLS

Five millilitres of crude bacteriocin in different test-tubes was taken and then heated at 37, 45, 60, and 100°C for 15 minutes respectively. In another set, 5 mL of crude bacteriocin or BLS was taken in test-tubes and the pH of the contents were adjusted to pH 2, 4, 7 and 9 separately, using either diluted NaOH or HCl and allowed to stand at room temperature for 2 hours. The heat and pH treated crude bacteriocin or BLS samples were then assayed for antimicrobial activity. Agar well diffusion method was used and 100 µL of sample was added in each well (Nithya *et al.*, 2012).

2.5.7. Bio-preservative efficiency of the crude Bacteriocin or BLS

Milk and cheese samples were added with 5% of crude bacteriocin or BLS of *L. xylosus* MB1 separately and kept at room temperature for 48 hours. The controls were maintained without adding crude bacteriocin or BLS. After 24 hours of incubation, the samples (both test and control) were serially diluted up to 10⁻⁶ and the plates were incubated at 37 °C for 24 hours. The colony count was recorded and compared with the control (Joshi *et al.*, 2006).

3. Results and Discussion

The present study aimed to isolate *Lactobacillus* spp. from milk products samples (butter and cheese), evaluation of their probiotic potentiality and assessment of their bacteriocin or bacteriocin-like substance (BLS) production capability. The *Lactobacillus* isolates were primarily screened for their antibacterial activity as probiotic *Lactobacillus* should be good antimicrobial producer.

3.1. Isolation, screening based on their antibacterial activity and identification of *Lactobacillus*

A total of 15 *Lactobacillus* were isolated from the milk products samples using MRS medium. Among them, only 3 isolates (20%) showed potential antibacterial activity against at least three test organisms and only one (6%) isolate displayed antibacterial potentiality against all the five test microorganisms. This isolate, labelled as MB1, showed antibacterial activity against *S. aureus* (15 mm), *E. coli* (11 mm), *S. Typhi* (13 mm), *P. aeruginosa* (15 mm) and *B. subtilis* (14 mm). The genus and species of the isolate (MB1) were confirmed by conventional methods using cultural, morphological characteristics and biochemical reactions as described in ‘‘Bergey’s Manual of Determinative Bacteriology’’, 8th edition (Buchanan *et al.*, 1974) (Table 1). Finally, having identified the isolate as *Lactobacillus xylosus*, we approached for further experiments.

3.2. Evaluation of probiotic potentiality of *L. xylosus* MB1

3.2.1. pH tolerance, temperature sensitivity, bile salt and NaCl tolerance

The growth of *L. xylosus* MB1 accelerated with the gradual increase of pH (2-7) and reached its optimum level at pH 7 and then again dropped with an increased pH (Figure 1a). Lactic acid bacteria must pass through the gastric juice of stomach having an acidic environment (lower pH), which destroys most of the microorganisms ingested (Charteris *et al.*, 1998). Therefore, resistance to low pH is an important selection criterion for the probiotic microorganisms. Other studies also ensured that, although the growth rate of *Lactobacillus* strains dropped after being exposed to pH values of 2.5–4.0, yet they can continue to survive. Moreover, their survival in the media resembling the stomach environment (having physiological pH of 2-3) was found to be variable and strain dependent. However, this survival rate was reported to be of approximately 85%, which is very significant for the probiotic bacteria (Belicová *et al.*, 2013).

An efficient probiotic *Lactobacillus* must be capable of surviving at wide range of pH and temperature, tolerating various concentrations of bile salt and NaCl, utilizing lactose and resistance against several antibiotics. The study isolate *Lactobacillus xylosus* MB1 was able to tolerate a wide range of temperatures (27–45 °C), but the best growth was found at 37 °C (Figure 1b). This value was chosen to mimic the internal body temperature and to determine whether the *Lactobacillus* were able to grow and survive within the human gut, an essential character of probiotics to be beneficiary for human health (Rahman *et al.*, 2019).

At the same time the organism should resist the bile salt in the small intestine and should adhere to the intestinal walls for its effectiveness (Agrawal, 2005). This will help *Lactobacilli* to colonize the intestinal environment and balance the intestinal microflora (Tambekar and Bhutada 2010). Our study isolate was able to withstand 0.5-2% concentration of bile salt *in vitro* growth media (Figure 1c) whereas the optimum growth was found at 0.5% concentration.

NaCl acts as an inhibitory compound that may muffle the growth of certain types of bacteria (Hoque *et al.*, 2010). The current *L. xylosus* MB1 strain was able to grow well at 1-7% of NaCl concentrations and its best growth was found with 7% NaCl (Figure 1d). The experimental results of our present study are in accordance with some previous findings (Pundir *et al.*, 2013; Rahman *et al.*, 2019).

3.2.2. Lactose utilization and antibiotic susceptibility

Our present study isolate MB1 could utilize lactose *in vitro* growth medium. Lactose is the predominant carbohydrate in milk. Insufficient β -galactosidase activity in the small bowel mucosa of newborn children perturbs the absorption of lactose from breast milk (Andersson *et al.*, 2001) and causes lactose intolerance. This also happens in adult people who cannot metabolize lactose due to lack of this essential enzyme. In both the cases, through its passage from the small intestine, lactose is converted to gas and acid in the large intestine by the colonial microflora and the respective individuals suffer from symptomatic abdominal pain, cramping and diarrhea (Rahman *et al.*, 2019). In these cases, addition of certain starter cultures with specific lactic acid bacteria to milk products allows the lactose digestibility and absorption in lactose intolerant people without the usual rise of breath hydrogen or associated symptoms (Pundir *et al.*, 2013). So, fermented dairy products are being produced using specially selected strains of *Lactobacillus* as starter cultures which are playing significant role in infant nutrition (Dinkçi *et al.*, 2006).

A small-scale antibiotic susceptibility test revealed that *Lactobacillus xylosus* MB1 is sensitive to penicillin G, chloramphenicol, erythromycin, cefixime, chephradine, streptomycin and rifamycin. Such kind of susceptibility to a wide range of antibiotics suggests that introduction of this *Lactobacillus* spp. in order to replenish the microbial flora in patients undergoing antibiotic therapy would not be feasible. Rather it could be used in fermented milk products as a form of preventive therapeutic approach to maintain a good balance of commensal gut microbiota as we have earlier proved its antibacterial potentiality.

3.2.3. Determination of bacteriocin or BLS production capability of *L. xylosus* MB1

To determine the bacteriocin or BLS production capability of *L. xylosus* MB1, its cell free supernatant (CFS) has been neutralized with NaOH to rule out the possible antimicrobial activity of organic acids and subsequently treated with catalase enzyme for reducing antimicrobial effect of H₂O₂ (Table 2; Figure 2).

If the CFS of *Lactobacillus xylosus* MB1 can exert the antimicrobial effects on selected test organisms after being acid neutralized and catalase treated, then the isolate may be considered as bacteriocin or BLS producer (Yang *et al.*, 2012). Same test pathogens, which were previously used to determine the antibacterial potentiality of the current study isolate, are also employed here to ascertain whether it could produce bacteriocin or BLS. We found that our study isolate produces crude bacteriocin or BLS which has antibacterial activity against *E. coli* and *S. aureus* (Table 2). After treating with proteolytic enzymes papain and trypsin, the crude bacteriocin or BLS of our study isolate lost its antibacterial activity, demonstrating the proteinaceous nature of this crude substance (Figure 3). One previous study implied that many of the bactericidal agents, synthesized by lactic acid bacteria (LAB) and varying in their spectra of activity, are bacteriocins and proteinaceous in nature (Fricourt *et al.*, 1994). Other studies also displayed that *Lactobacillus* spp. could produce bacteriocins which are active against *E. coli* and *S. aureus*, thus further supporting the findings of our present study (Rahman *et al.*, 2018; Rahman *et al.*, 2019).

3.2.4. Effect of temperature and pH on the antibacterial activity of the crude bacteriocin or BLS produced by *L. xylosus* MB1

The crude bacteriocin or BLS of *L. xylosus* MB1 retained its antibacterial activity at wide range of temperature (37°C to 100°C for 15 minutes) and pH (2–9 for 2 hours) (Figure 4). Using both parameters, we found the crude bacteriocin or BLS was active against *E. coli* and *S. aureus* among the five test organisms that we have employed earlier in the current study. Maximum antibacterial activity was observed against both *E. coli* and *S. aureus* after 37°C heat treatment. But in case of pH treatment, the results differed in *E. coli* and *S. aureus*. Maximum antibacterial activity was noted against *E. coli* at pH 4 and *S. aureus* at pH 2 respectively. Rahman *et al.*, (2019) investigated the antibacterial properties of *L. plantarum* and *L. delbrueckii* isolated from vegetables. These strains produced bacteriocins or BLS against *E. coli*, *S. aureus* and *S. typhi* and were found to tolerate the

same range of temperature (37°C-100°C) and pH (2-9) like this present study. In addition, bacteriocins or BLS produced by both *Lactobacillus* spp. exhibited highest antibacterial activity at 37°C temperature, whereas antibacterial effects against *E. coli* and *S. aureus* from bacteriocins produced by *L. plantarum* and *L. delbrueckii* were found to be maximum at pH 4 and pH 2 respectively (Rahman *et al.*, 2019). These findings are exactly in accordance with the results of our current study. Other studies also revealed that bacteriocins produced by *Lactobacillus* spp. were proteinaceous in nature and could endure pH 3-9 and 37-100°C heat treatment (Jayachitra and Ramanathan, 2012; Udhayashree *et al.*, 2012). Another study reported that bacteriocin produced by *Lactobacillus* spp. could even stand 100°C for 30 minutes, indicating that the bacteriocin is relatively resistant to heat, though inactivation occurred after 60 min at 100°C or after autoclaving at 121°C for 5 min (Boris *et al.*, 2001). The heat stability of the crude bacteriocin or BLS pledges an advantage for potential use as bio-preservatives in combination with thermal processing to preserve food products (Rahman, 2019).

3.2.5. Bio-preservative efficiency of the crude bacteriocin or BLS

We observed that 5% crude bacteriocin or BLS of our study isolate could decrease microbial load remarkably in the milk and cheese samples after 48 hours of preservation at room temperature (Table 3). The 5% crude bacteriocin or BLS of *Lactobacillus xylosus* MB1 decreased 41% of initial microbial load in cheese after treatment at room temperatures for 48 hours. In case of milk, crude bacteriocin or BLS of *Lactobacillus xylosus* MB1 decreased 43% of initial microbial load.

This very experimental data indicates that crude bacteriocin or BLS of *Lactobacillus xylosus* MB1 could possibly be used in the preservation of milk and milk products after further purification and ensuring that it would not be toxic, cause hypersensitivity or any other side effects to the human health. Previous researchers used 5% crude bacteriocin of *L. fermentum* to preserve milk and mushroom and got satisfactory results (Nithya *et al.*, 2012). Another study showed that BLS of *Lactobacillus* spp. isolated from ovine cheese exhibited antimicrobial activity against *Listeria monocytogenes* (Nespolo and Brandelli, 2010). Likewise, our examined bacteriocin or BLS also substantially reduced bacterial colony count when applied on cheese samples. Bio-preservation of cheese is very important from commercial and therapeutic perspective because cheese has a higher pH than yogurt and fermented milk and hence can support survival of probiotic bacteria for a longer duration (Gardiner *et al.*, 1998). However, bio-preservation of milk is never less valuable because recently specific starter cultures using specially selected strains of *Lactobacillus* are being used in the production of fermented dairy products (Dinkçi *et al.*, 2006). Bacteriocin or BLS produced by our study isolate significantly mitigated bacterial colony count when applied on milk samples too, thus strengthening its candidature as an efficient bio-preservative in dairy industry. Bio-preservatives can be an effective alternative to chemical preservatives because excessive use of chemical preservatives may have deteriorative effects on human health (Rahman *et al.*, 2019). Here, bacteriocins can play a major role. Most of the bacteriocins kill the susceptible bacteria by inducing permeabilization and pore formation on the cytoplasmic membrane or by interactions with essential enzymes. Because bacteriocins are degraded by the proteolytic enzymes of the gastrointestinal tract and seem to be nontoxic and non-antigenic to animals and humans, they can be utilized to improve the safety and shelf-life of many food products (Dinev *et al.*, 2018).

Our study isolate *Lactobacillus xylosus* MB1 passed the *in-vitro* assessments for probiotic potentiality and showed tolerance to a wide range of pH, temperature, bile salt and NaCl concentration. This isolate is also capable of producing bacteriocins or bacteriocin like substances (BLS) which are competent to sustain at different temperature and pH levels and exert their antibacterial activity. The bacteriocins or BLS also proved their efficiency in bio-preservation of milk and cheese samples. All these evidences obviously suggest *Lactobacillus xylosus* MB1 to be a prospective candidate for probiotic bacteria. Probiotic lactic acid bacteria (LAB) have protective effects on human health including boosting of the immune system, inhibition of the growth of pathogens, prevention of diarrhea from various causes, prevention of colorectal cancer, reduction of the risk of inflammatory bowel movements, improvement of digestion of proteins and fats, synthesis of vitamins and detoxification and protection from toxins (Chowdhury and Ferdouse, 2012). Based on the beneficiary effects and more secured aspects of probiotic therapy than pharmaceutical agents (Bengmark, 2002) current study isolate *Lactobacillus xylosus* MB1 can be used as probiotics because there are historical data supporting the safety of lactobacilli for human use (Adams and Marteau, 1995).

Table 1. Morphological, cultural and biochemical characteristics of *L. xylosus* MB1.

Parameters	<i>L. xylosus</i> MB1
Colony characteristics	
<i>Form</i>	Circular
<i>Elevation</i>	Raised
<i>Margin</i>	Entire
<i>Surface</i>	Rough (Dry)
<i>Color</i>	Off-white
Slant Character	Filiform
Broth Character	Turbid growth with sediment
Microscopic Observation (vegetative cell)	Short rod, single or diplobacilli, 2.93–3.51 µm in length and 1.56–1.95 µm in width
Gram staining	Gram positive
Spore staining	Non spore former
Acid fast staining	Non-acid fast
Motility test	Non motile
Indole test	Negative
Methyl Red (M.R) test	Negative
Voges Proskauer (V.P) test	Negative
Deep glucose agar test	Strict aerobes
Glucose broth	Turbid
Growth in synthetic media	Turbid
Growth in inorganic salt	No growth
H ₂ S production test	Positive
Urease test	Negative
Nitrate reduction test	Positive
Citrate utilization test	Turbid with flocculent
Catalase activity	Negative
Starch hydrolysis	Negative
Casein hydrolysis	Negative
Egg albumin test	Negative
Gelatin liquefaction	Negative
Fermentation test	
<i>Acid production without gas formation</i>	Galactose, Sucrose and Inulin
<i>Alkali production without gas formation</i>	Maltose, Mannitol and Xylose
<i>No change</i>	Glucose, Fructose, Rhamnose, Raffinose and Starch

Table 2. Determination of Bacteriocin/BLS production by *L. xylosus* MB1 after eliminating antibacterial effect of acids and H₂O₂ (diameter of zone of inhibition in mm).

Test organisms	Raw CFS	CFS adjusted at pH 6	pH adjusted CFS after catalase treatment
<i>E. coli</i>	26	24	24
<i>S. typhii</i>	16	-	-
<i>B. subtilis</i>	23	7	-
<i>P. aeruginosa</i>	17	-	-
<i>S. aureus</i>	23	22	22

Note: - = Do not show antimicrobial activity against respected pathogen

Table 3. Bio-preservative efficiency test of the crude bacteriocin or BLS on milk and cheese samples.

Samples	Total count of bacteria (CFU/mL)	
	Control (without bacteriocin/BLS)	Test (with crude bacteriocin/ BLS produced by <i>L. xylosus</i>)
Cheese	11.6×10 ⁶	4.8×10 ⁶
Milk	12.1×10 ⁶	5.3×10 ⁶

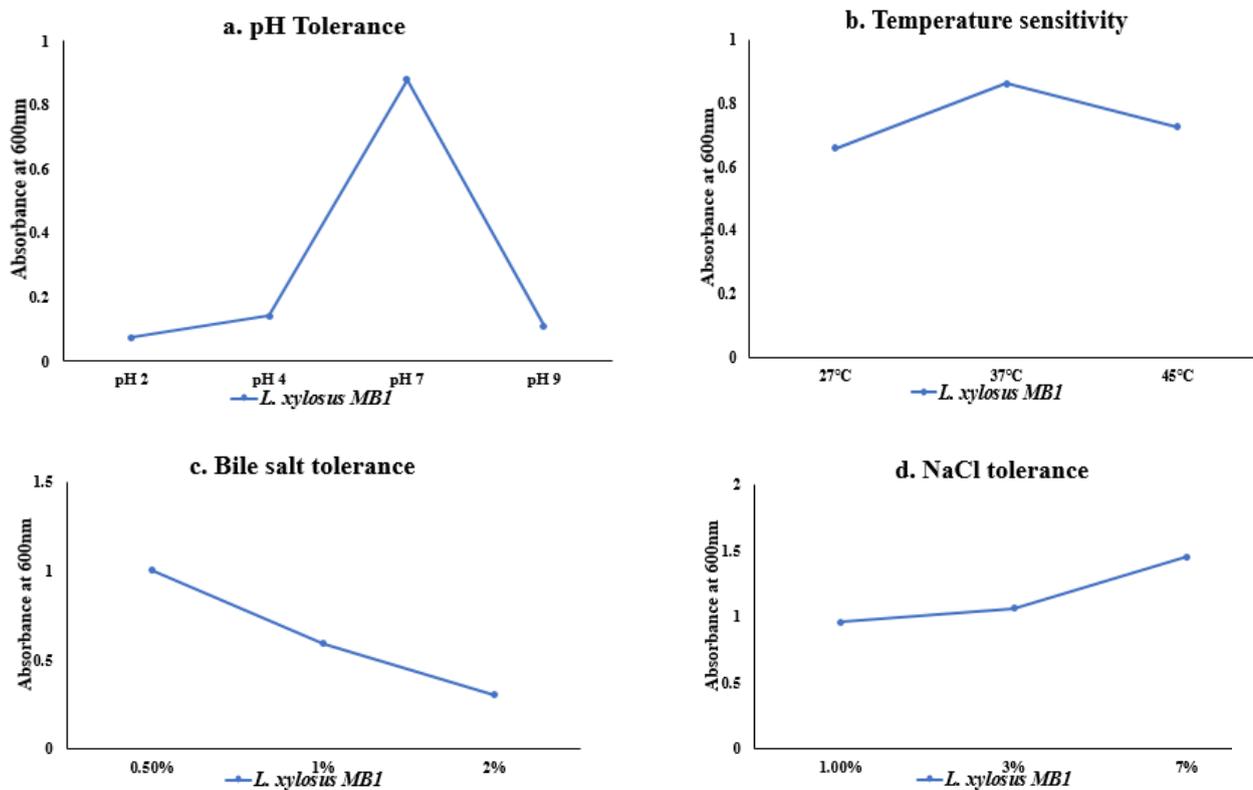


Figure 1. *Lactobacillus xylosum* MB1 can tolerate a wide range of (a) pH (2-9), (b) temperature (27-45°C), (c) bile salt (0.5-2%) and (d) NaCl (1-7%).

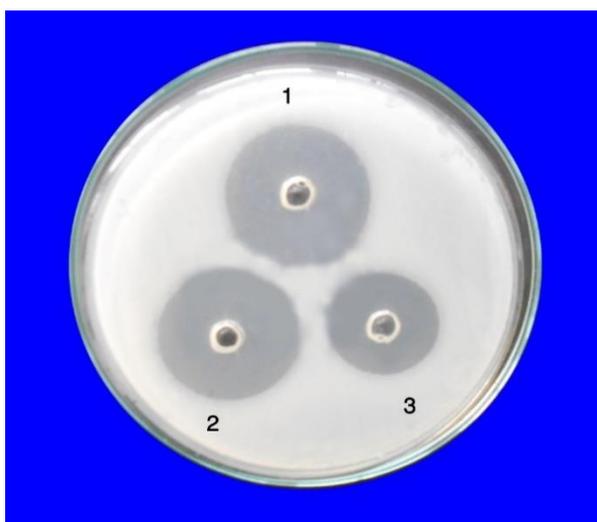


Figure 2. Activity of bacteriocin/BLS produced by the isolate *L. xylosum* MB1 against *S. aureus*.

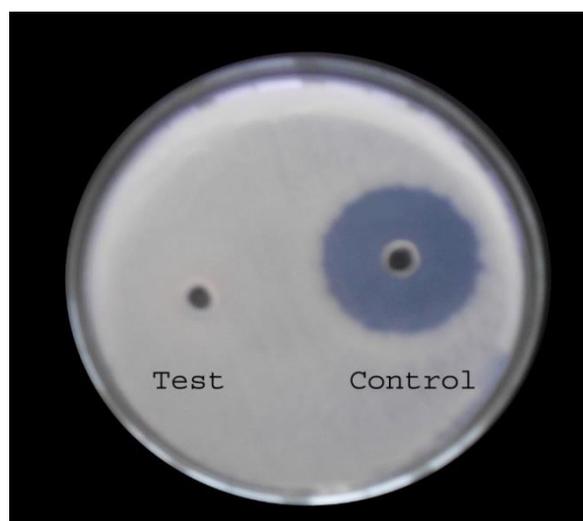


Figure 3. Effect of Trypsin on the antimicrobial activity of the crude bacteriocin/BLS produced by the isolate *L. xylosus* MB1 (Indicator: *S. aureus*).

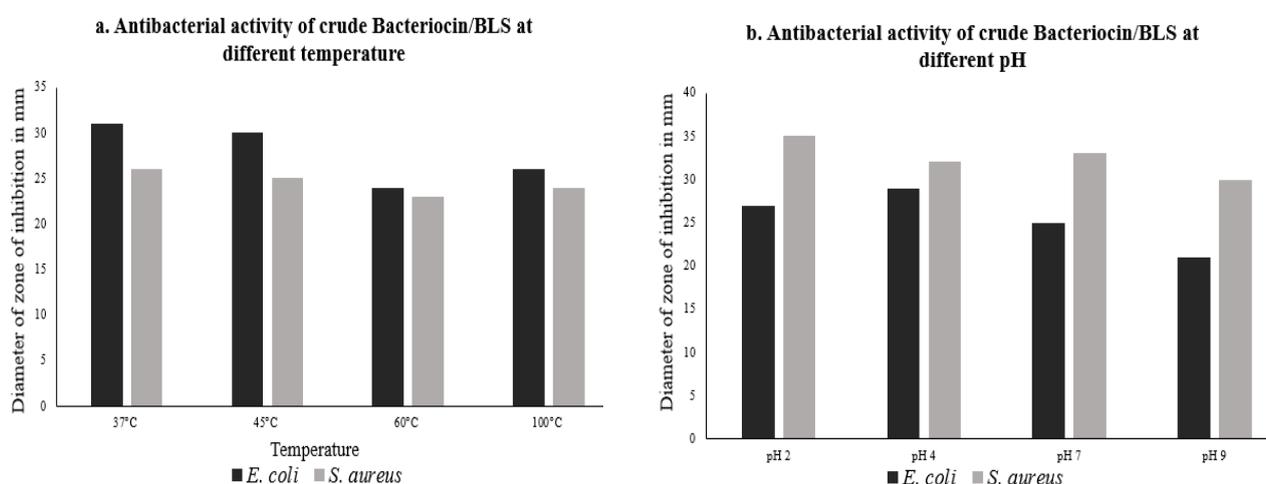


Figure 4. Antibacterial activity of crude bacteriocin or BLS produced by *Lactobacillus xylosus* MB1 after treatment at wide range of (a) temperature (37°C-100°C) and (b) pH (2-9).

4. Conclusions

Based on experimental data and empirical observations from this study, it could be stated that our isolated *Lactobacillus xylosus* MB1 and its crude bacteriocin and BLS has tremendous potentiality to be used as probiotics and bio-preservatives after successfully passing through other relevant *in-vitro* and *in-vivo* tests and experiments.

Conflict of interest

None to declare.

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