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Characterization of Lactic Acid Bacteria (LAB) Isolated from Homemade Fermented Kimchi in Bangladesh

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Abstract

The purpose of this present study was to explore lactic acid bacteria (LAB) from homemade kimchi. Traditionally, kimchi was made in this study at home using fresh vegetables such as cabbage, carrots, radish, green onion leaf, red pepper, garlic, ginger, salt and rice flour, and fermented for seven days. LAB were isolated using selective media and identified by macroscopic and microscopic morphological analysis and short biochemical tests such as catalase, oxidase and Kliger's iron agar (KIA) test. Probiotic potential of LAB was investigated by acid, salt, temperature tolerance and sugar including glucose, fructose, sucrose, xylose and lactose fermentation test. Four distinct single colonies were isolated from four different kimchi preparations. All isolated bacteria were identified as lactic acid bacteria and found gram positive, non-spore forming, survive in both aerobic and anaerobic condition, catalase and oxidase negative. Isolated LAB survive in both highly acidic (pH 2) and alkaline (pH 8) medium, up to 4% NaCl concentrations and at temperatures ranging from 4°C to 55°C. The isolated LAB have also ability to ferment sugars. These findings reveal that isolated LAB found suitable to survive in the environment of human gastrointestinal tract and have probiotic potential.

Key word: LAB, kimchi, probiotic.

Introduction

LAB are found in various fermented foods, including yogurt, kimchi, cheese and kefir, which have long been consumed by humans (Kohinur *et al*., 2017; McSweeney, 2004; Park *et al*., 2014 and Simova *et al*., 2002). LAB is the main focus of international research for their beneficial role in most fermented foods. Kimchi, a traditional fermented food in Korea, Japan and China, is prepared through the fermentation of vegetables (Jung *et al.,* 2014). Vegetables have been preserved via lactic acid fermentation for over 1500 years (Surh *et al*., 2008). Kimchi is a gut-friendly food and also well known as probiotics (Kim *et al*., 1997 and Wu *et al*., 2000). It is used in different parts of the world for specific health benefits such as anti-inflammatory, antioxidant, antiobesity, antibacterial, anticancer, probiotic, antiaging and cholesterol-reducing properties (Patra *et al.*, 2016). Now a days, the use of kimchi has been increased and developed as a global cultural product after being registered with codex in 2001 (Kim *et al.*, 2006).

Recently, there is a great challenge for research of microecosystem for most fermented foods and health benefits. In addition to being a significant source of nutrients, fermented foods also offer flavor, texture and reduce toxicity, and promoting health and disease prevention (Kabak and Dobson, 2011). Fermented foods help to boost immunity by fostering the growth of beneficial microorganisms. Addition of fermented foods to the diet also helps to promote intestinal health. Now a days, commercial product of kimchi are available in grocery shop or super market in worldwide. However, kimchi is not available in

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commercial products in Bangladesh and also not popular for homemade kimchi. There is no published data available about LAB from homemade or commercial kimchi in Bangladesh. This present study aims to isolate and characterize LAB from homemade kimchi using local ingredients based on morphological, biochemical and probiotic potential characteristics.

Materials and Methods

Preparation of kimchi: Traditionally kimchi was prepared at home using fresh cabbage, rice flour, garlic, fresh ginger root, red pepper powder, radish, green onions, carrot and non-iodized salt (NaCl) (Figure 1). Briefly, cabbage was cut into small pieces and then rinse with water. 3% salt solution was used to dip cabbage pieces for 4 hours. The cabbage pieces were then rinsed three time with water. Then the rice flour paste and other ingredients were mixed with cabbage thoroughly. Finally, the kimchi was packed tightly into the container to prevent air exposure and promote brine formation. The container was kept in relatively constant room temperature (about 30°C) for 37 days, tasting it daily until it reaches preferred tangy taste and desired texture. Stored fermented kimchi was covered tightly in the refrigerator.

Figure 1. Homemade fermented kimchi.

Enrichment and isolation of bacteria from kimchi samples: A total of four handmade kimchi samples were tested for this study. 1 gm samples of each preparation of kimchi were enriched in 99 ml of MRS broth (Hi-Media, India) in conical flask and incubated for 24 hours at 37°C and kept in shaker at 80 rpm. One loopful of broth culture was streaked on MRS agar plates and incubated at 37°C for 48 hours. Single discrete characteristic colony was isolated and tested for identification of LAB by short biochemical tests, gram staining and spore staining (MacFaddin, 2000; Bergey *et al.*, 1994). A single colony was stored in an MRS agar slant for further study.

Biochemical tests

Gram staining: Gram staining procedure was performed for all isolated LAB according to the standard procedure. Microscopic examination was done to observe the color and shape of the bacteria by an oil immersion method (100X). The positive and negative controls were used as *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, respectively.

Spore staining: Spore staining was done according to the procedure of Leboffe and Pierce (2015). Briefly, bacterial smear was prepared by air dried heat fixed on glass slide. The slide was then flooded with Malachite Green (primary stain) and steamed the slide for 5 to 7 minutes in a water bath and then gently rinsed with distilled water. After that the slide was counter stained with safranin and was viewed with the microscope under the oil immersion objective (100X). *Bacillus subtilis* was used as positive control.

Catalase test: A fresh single colony was placed on a sterile glass slide, then a drop of 3% hydrogen peroxide was added and thoroughly mixed. The presence of bubbles or froth indicated a positive catalase reaction, whereas the absence of bubbles or froth indicated a negative catalase reaction. The positive and negative controls were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, respectively.

Oxidase test: Oxidase test was performed with 1% solution of N_1N_1-Tetramethyl-phenyldiamine-dihydrochloride which was soaked in a piece of filter paper. A portion of the colony of the test organism was picked up with a sterile tooth pick and touched on to the paper with impregnated reagent. A dark purple color development within 5-10 second was considered positive and no change of color was interpreted as a negative result for oxidase. *Pseudomonas aeruginosa* and *E. coli* ATCC 25922 were used as positive and negative control, respectively.

*Kliger's iron agar (KIA) test:*All isolates were subjected to the Kligler iron agar (KIA) test to determine entrobacteriaceae based on sugar fermentation and H_2S production. Isolates were inoculated in the KIA agar slant for 24 hours incubation at 37°C, results were interpreted by observing color changes in the butt and slant, and H_2S or gas formation. *S. aureus* ATCC 25923 and the medium without inoculation were used as the positive control and negative control, respectively.

pH tolerance test: MRS broth was prepared and pH was adjusted to 2, 3, 4 and 8 by using 1N HCl and 1N NaOH. Fresh bacterial isolates were inoculated and incubated for 48 hours at 37°C. Only the medium used as a negative control. After 24 and 48 hours, the turbidity of the culture media was used to evaluate the positive results and the negative control showed no signs of growth.

NaCl tolerance test: The NaCl tolerance of the isolated LAB was assessed using MRS broth with 2%, 4% and 8% NaCl concentrations. A fresh bacterial culture was inoculated into each broth and incubated at 37°C for 48 hours. Results were determined by observing the turbidity of the cultures after 24 and 48 hours. The presence of bacterial growth indicated positive result and absence of bacterial growth indicated negative results. The medium without bacterial inoculation, served as the negative control.

Temperature tolerance test: The temperature tolerance test was performed for the isolated LAB by inoculating bacteria into MRS broth and incubated at temperatures of 4°C, 45°C and 55°C. Additionally, a control set was incubated at the standard temperature of 37°C for 48 hours. Results were evaluated by observing the turbidity of bacterial growth after 24 and 48 hours. Subsequently, streaking was performed on MRS agar plates and incubated for 48 hours. The presence or absence of growth in the media ensured the survival or non-survival of LAB in different temperature. Only the medium, without bacterial inoculation, served as the negative control.

Sugar fermentation test: The sugar fermentation test was carried out by using 1% (w/v) sugar in MRS broth, incorporating glucose, fructose, sucrose, xylose and lactose. Phenol red solution served as the indicator. In each test tube, 10 ml of the media was dispensed and a Durham's tube was inserted invertedly. Fresh bacterial cultures were then inoculated into the test tubes, which were incubated at 37°C for 24 hours. As a negative control, only the medium without bacterial inoculation was used. Results were assessed by observing color changes in the media and the presence of gas formation in the Durham's tube.

Results and Discussion

LAB is predominant bacteria for fermentation of foods such as cheese, yogurt, sauerkraut, kefir, kimchi. *Lactobacillus* exhibits numerous beneficial effects on health, such as enhancing the immune system and supporting the proliferation and establishment of beneficial gut bacteria. Kimchi is a raw, lactic acid-fermented vegetable product and considered to be a potential source of advantageous LAB. The primary objective of this study was to identify and characterize LAB isolates from homemade kimchi (Figure 1). In this study, only four distinct bacteria were identified and designated as KM1, KM2, KM3 and KM4 by specific morphological features, catalase negativity and their gram-positive rod shape (Tables 1 & 2 and Figures 2 & 3). Previous study reported presence of *Lactobacillus* spp. through gram-positive and catalase-negative results from yogurt, sorghum-based traditional fermented food (Kohinur *et al.*, 2017; Rao *et al.*, 2015; Salvetti *et al.*, 2012). *Lactobacilli* bacteria genetically and physiologically diverse group of rod-shaped, gram-positive, catalase-negative bacteria (Hoque *et al*., 2010).

In this present investigation, a survivability study was conducted across a pH range from 2 to 8. The isolates demonstrated resilience in both highly acidic conditions (pH 2) and alkaline environments (pH 8). A prior study by Pundir *et al.* (2013) reported that the survival of *Lactobacillus* isolated from fresh vegetables, fruits and curds found within the pH range of 3.5 to 7.0. Similarly, Hoque *et al.* (2010) observed *Lactobacillus* growth in pH 2.5 to pH 8.5. The chosen pH range aimed to assess whether the isolated strains could withstand both acidic and alkaline conditions

Table 1. Macroscopic and microscopic observations of LAB isolated from kimchi.

Isolate no.	Macroscopic observation		Microscopic observation	
	Morphology of colony	Color of colony	Gram staining	Spore staining
KM1	Large, circular, regular	Creamy	Bacilli	Negative
KM2	Large, circular, regular	Creamy	Cocci	Negative
KM3	Small, circular, regular	Creamy	Bacilli	Negative
KM4	Small, circular, regular	Creamy	Bacilli	Negative

Table 2. Results of biochemical tests of isolated LAB.

Isolate no.	Catalase	Oxidase	KIA
KM1	-	-	Acid/alkaline
KM2	-	-	Acid/alkaline
KM3	-	-	Acid/acid
KM4	-	-	Acid/acid

+ = positive; - = negative; KIA: Slant/Butt.

Table 3. Results of NaCl, pH and temperature tolerance test of isolated LAB.

Isolate	pH 2		pH 3		pH 4		pH 8		NaCl concentration						Growth temperature(^oC)					
	24h	48h	24h	48h	24h	48h	24h	48h	2%		4%		8%		4^{o}C		45^{o}C		55^{o}C	
									24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
KM1	++	++	++	++	+	+	+	+	+++	+++	+++	+++	-	-	+	+	+	+	-	-
KM2	++	++	++	++	+	+	+	+	+++	+++	+++	+++	-	-	+	+	+	+	-	-
KM3	++	++	++	++	+	+	+	+	+++	+++	+++	+++	-	-	+	+	+	+	-	-
KM4	++	++	++	++	+	+	+	+	+++	+++	+++	+++	-	-	+	+	+	+	-	-

- = no growth (negative); + = slight growth (positive); ++ = moderate growth (positive); +++ = dense growth (positive); +/- = ambiguous/undecided.

Table 4. Results of sugar fermentation tests of isolated LAB.

Isolate no.	D-Dextrose	Fructose	Sucrose	Xylose	Lactose	Mannitol
KM1	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+
KM2	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+
KM3	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+
KM4	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+	A^+,G^+

A+ = Positive acid; A- = Negative acid; G+ = Positive gas; G- = Negative gas; + = positive.

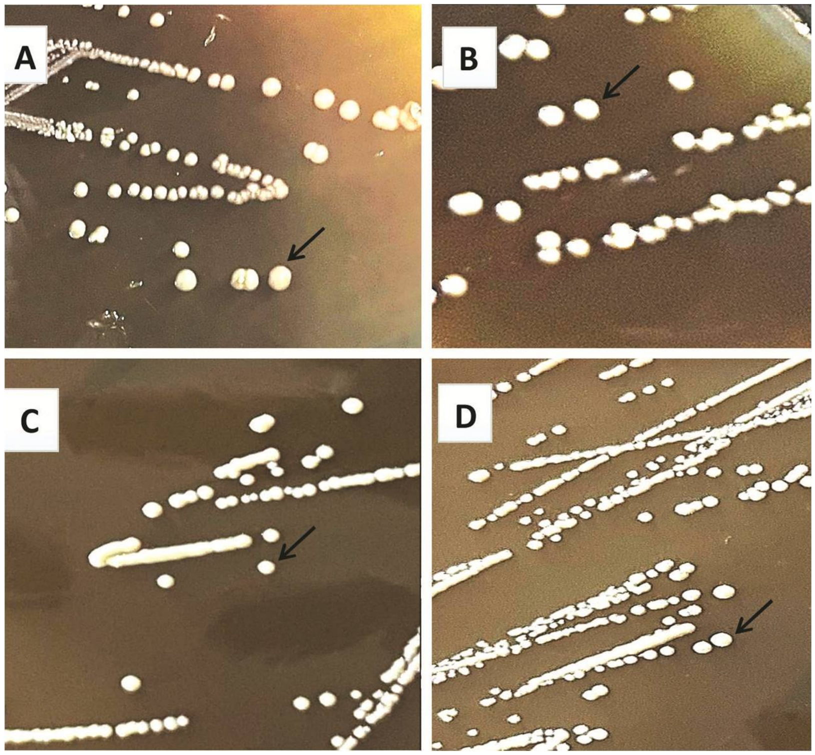

Figure 2. Colonies of bacteria on MRS agar plate (A) KM1 (B) KM2 (C) KM3 and (D) KM4.

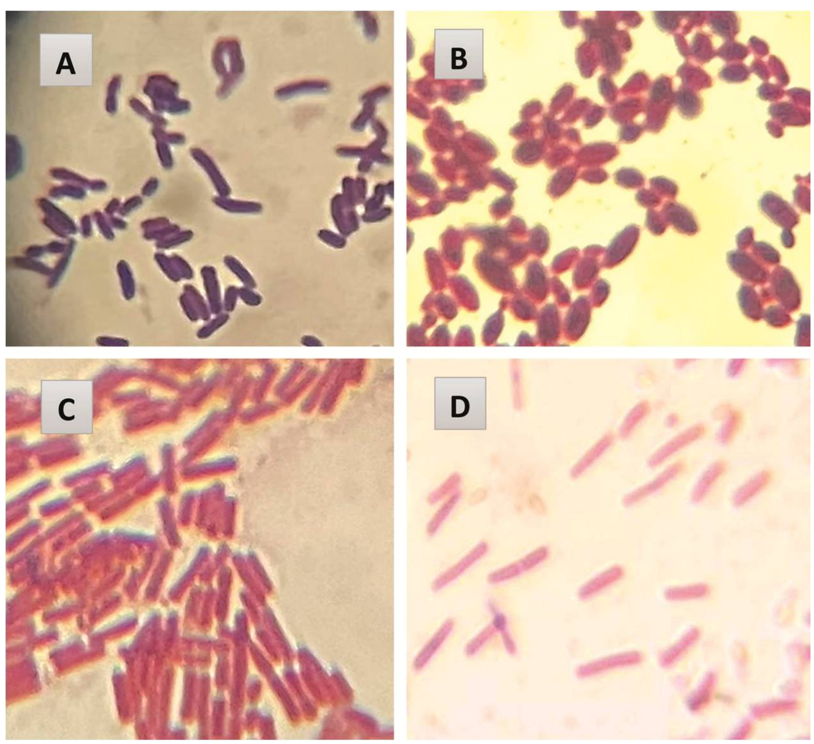

Figure 3. Observation of bacterial cell morphology for isolates using the gram staining method magnification of 100X (A) KM1 (B) KM2 (C) KM3 (D) KM4.

within the gastrointestinal tract. Another test was performed evaluating the NaCl tolerance of LAB. The NaCl tolerance test encompassed concentrations of 2%, 4% and 8% NaCl. NaCl, known to inhibit the growth of certain bacteria (Hoque *et al*., 2010) was tested for its impact. All the isolates exhibited growth in high NaCl concentrations (4%) (Table 3). This finding aligns with Farhad *et al*. (2015), who reported that *Lactobacillus* species were unable to grow in elevated NaCl concentrations. Said *et al.*, (2018) reported that *Lactobacillus* can grow at temperature ranging from 10°C to 45°C. In the present study, temperature tolerance of isolated LAB was observed at temperatures 4°C, 45°C and 55°C (Table 3). The result indicated that the isolates can survive at temperature ranging from 4°C to 45°C but not at 55°C**.** The results of the sugars fermentation test for LAB revealed positive outcomes along with gas formation (Table 4). The isolated LAB have shown fermentation activity for D-dextrose, fructose, sucrose, xylose, lactose and mannitol. Klaenhammer and de Vos (2011) reported previously that the isolates exhibited fermentation activity for glucose, lactose, xylose, fructose and sucrose. Present study of LAB found consistency with the results of the previous study.

Conclusion

Fermented vegetable foods kimchi comprises both traditional and functional significance in worldwide. In Bangladesh, most of the people lack awareness regarding the probiotics presents in fermented foods and their associat ed health advantages. Despite the abundance of seasonal and favorable conditions for food fermentation, there exists a vast opportunity for the development of fermented foods in Bangladesh. Unfortunately, the widespread recommendation for the consumption of probiotics in fermented foods is hindered in our country due to a scarcity of research and scientific exploration on this subject. These findings indicate that the isolated LAB are well-suited to survive within the human gastrointestinal tract. In the future, it is essential to undertake research focused on isolating and identifying specific species of LAB from kimchi, assessing their probiotic potential and exploring their health benefits.

References

Bergey, D.H., 1994. Bergey's manual of determinative bacteriology. Lippincott, Williams & Wilkins.

Farhad, M., Kailasapathy, K. and Tamang, J.P. 2010. Health aspects of fermented foods. *Fermented foods and beverages of the world*. 391-414.

Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M. and Islam, K.M.D. 2010. Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yogurts. *World J. Dairy Food Sci.* **5,** 39-46.

Jung, J.Y., Lee, S.H. and Jeon, C.O. 2014. Kimchi microflora: history, current status and perspectives for industrial kimchi production. *Appl. Microbiol. Biotechnol.* **98**, 2385-2393.

Kabak, B. and Dobson, A. D. 2011. An introduction to the traditional fermented foods and beverages of Turkey. *Crit. Rev. Food Sci. Nutr.* **51**, 248-260.

Kim, M. J., Kwon, M. J., Song, Y. O., Lee, E. K., Yoon, H. J. and Song, Y. S. 1997. The effects of kimchi on hematological and immunological parameters *in vivo* and *in vitro*. *J. Korean Soc. Food Sci. Nutr.* **26**, 1208-1214.

Klaenhammer, T.R. and de Vos, W.M. 2011. An incredible scientific journey. The evolutionary tale of the lactic acid bacteria. In: Ledeboer A, Hugenholtz J, Kok J, Konings W, Wouters J (ed.) The 10th LAB symposium. Thirty years of research on lactic acid bacteria. 24 Media Labs, 1-11.

Leboffe, M.J. and Pierce, B.E. 2015. *Microbiology: laboratory theory and application*. Morton Publishing Company.

Lee, C.W., Ko, C.Y. and Ha, D.M. 1992. Microfloral changes of the lactic acid bacteria during kimchi fermentation and identification of the isolates. *Korean J. Appl. Microbiol. Biotechnol.* (Korea Republic). **20**, 102-109

MacFaddin, J.F., 2000. Biochemical tests for identification of medical bacteria, Williams and Wilkins. Philadelphia, PA, 113.

McSweeney, P.L. 2004. Biochemistry of cheese ripening. *Int. J. Dairy Technol.* **57**, 127-144.

Mannan, S.J., Rezwan, R., Rahman, M.S. and Begum, K. 2017. Isolation and biochemical characterization of *Lactobacillus* species from yogurt and cheese samples in Dhaka metropolitan area. *Bangladesh Pharm. J.* **20**, 27-33.

Patra, J.K., Das, G., Paramithiotis, S. and Shin, H.S. 2016. Kimchi and other widely consumed traditional fermented foods of Korea: a review. *Front. microbiol.* **7**, 1493.

Park, K.Y., Jeong, J.K., Lee, Y.E. and Daily III, J.W. 2014. Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. *J. Med. Food* **17**, 6-20.

Pundir, R.K., Kashyap, S.R.N. and Kaur, A. 2013. Probiotic potential of lactic acid bacteria isolated from food samples: an *in vitro* study. *J. Appl. Pharm. Sci.* **3**, 085-093.

Rao, K.P., Chennappa, G., Suraj, U., Nagaraja, H., Raj, A.P.C. and Sreenivasa, M.Y. 2015. Probiotic potential of *Lactobacillus* strains isolated from Sorghum-based traditional fermented food. *Probiotics and Antimicro. Prot.* **7**, 146-156.

Said, N.S., Fahrodi, D.U., Malaka, R. and Maruddin, F. 2018. The characteristics of lactic acid bacteria isolated from Indonesian commercial kefir grain. *Malays. J. Microbiol*. **14**, 632-639.

Salvetti, E., Torriani, S. and Felis, G.E. 2012. The genus *Lactobacillus*: a taxonomic update. *Probiotics and Antimicro. Prot*. **4**, 217-226.

Simova, E., Beshkova, D., Angelov, A., Hristozova, T.S., Frengova, G. and Spasov, Z., 2002. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *J. Ind. Microbiol. Biotechnol. **28***, 1-6.

Surh, J., Kim, Y. H. L. and Kwon, H. 2008. Korean fermented foods, Kimchi and Doenjang," in Handbook of Fermented Functional Foods, ed E. R. Farnworth (Boca Raton, FL; London: CRC press), 333-351.

Wu, A. H., Yang, D. and Pike, M. C. 2000. A meta-analysis of soyfoods and risk of stomach cancer: the problem of potential confounder. *Cancer Epidemiol. Biomarkers. Prev*. **9**, 1051-1058.