PJN
ISSN 1680-5194
ansinet.com/pjn

PAKISTAN JOURNAL OF NUTRITION

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2021.108.111

Research Article
The Efficiency of Lactic Acid Bacteria Isolated from Pickled Local Vegetables to Inhibit Pathogenic Bacteria

N. Hayeeyusoh, A.D. Dalee, K. Sali and Z. Hajiwangoh

Program of Microbiology, Faculty of Science Technology and Agricultural, Yala Rajabhat University, 95000 Yala, Southern, Thailand

Abstract

Background and Objective: Sustaining the balance of good and bad microflora in our gut to maintain a healthy digestive system has been an ongoing challenge. Conversely, there has been an increase in the reliance on functional foods fortified with beneficial microorganisms; pickled vegetables are an alternative source of natural probiotics. This study investigated the types and quantities of lactic acid bacteria (LAB) present in pickled local vegetables and their antimicrobial capabilities. **Mehtodology:** Thirteen colonies of LAB from three local pickled vegetables were tested against three strains of pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli and Bacillus cereus*) using agar well diffusion and the minimum inhibitory tests. **Results:** The results revealed four LAB isolated from the pickled tare and Look-Niang (S1, S2, N4 and N5) inhibited the growth of *S. aureus* while one isolate from pickled tare (S2) successfully inhibited *E. coli*. There were no inhibitions recorded for *B. cereus*. Isolate S1 exhibited the highest antimicrobial property against *S. aureus* (20.87 ± 0.2 mm) and a minimum inhibitory value of 200 AU mL^{-1}, where AU was defined as an arbitrary unit. The biochemical tests further revealed that isolate S1 was likely to be in the genus *Lactobacillus*. **Conclusion:** LAB isolated from pickled tare is a viable food source to be reproduced as a probiotic starter culture to help protect the digestive system from harmful microorganisms like *S. aureus*.

Key words: Lactic acid bacteria (LAB), pickled vegetable, pathogenic bacteria, probiotics, gut microflora, natural probiotics

Citation: N. Hayeeyusoh, A.D. Dalee, K. Sali and Z. Hajiwangoh, 2021. The efficiency of lactic acid bacteria isolated from pickled local vegetables to inhibit pathogenic bacteria. Pak. J. Nutr., 20: 108-111.

Corresponding Author: N. Hayeeyusoh, Program of Microbiology, Faculty of Science Technology and Agricultural, Yala Rajabhat University, 95000 Yala, Southern, Thailand

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The consumption of food containing live bacteria is the oldest and common way to increase the concentration of good bacteria called "probiotics" in the intestinal tract[1,2]. Many probiotic-rich foods originate from fermented vegetables as well as cultured milk[3]. Currently, the functional food market is dominated by food products that promote and improve good microflora activity in the intestines[4].

Probiotics are known to maintain the balance between good and harmful bacteria in the gut to maintain a healthy digestive system[5]. The health benefits of probiotics have been thoroughly researched in terms of viability, effectiveness and consumption safety[6,7]. Probiotics containing bioactive compounds inhibit pathogenic microorganisms by producing bacteriocins[8], lowering the pH in the intestine[9] and producing active enzymes[10].

Fermented local vegetables can become a viable alternative to functional foods fortified with probiotics to help maintain a healthy digestive system. This study was conducted to identify and quantify the quantity of LAB found in three types of pickled vegetables in Thailand and how it can be used to inhibit three pathogenic bacteria commonly found in the intestine.

MATERIALS AND METHODS

Sampling and screening of LAB: Three types of pickled local vegetables (Look-Niang, tare and white turmeric) were obtained from markets in Pattani and Yala province. Meanwhile, LAB was cultured using De Man, Rogosa and Sharpe (MRS) agar, by adding 0.04% of bromocresol purple to the media (Hi Media Chemicals Ltd., Mumbai, India). LAB isolated from the samples using the microbial pour plate method was diluted in normal saline (0.85% NaCl), subsequently poured into MRS+ Bromocresol purple 0.04% medium. The microbial pore plates were incubated at 37±2°C, for 24 h. Single LAB colonies that differed in morphology and phenotypic appearance were selected and inoculated in agar slants. LAB colonies were purified and isolated into their respective media by re-streaking on plates until only a single type of colony was present. Pure cultures were subsequently characterized and stained using Gram staining. Gram-positive and rod-shaped bacteria that met similar phenotypical characteristics with *Lactobacillus* species were used in further experiments.

The cultures were stored and maintained at 4°C on MRS agar slants until further use.

Preparation of LAB: The selected LAB isolates were inoculated into 9 mL MRS broth and incubated at 35°C±2°C for 18 h, subsequently the broth turbidity was adjusted to match McFarland standard No. 03.

Growth of pathogenic bacteria: Pure cultures of pathogenic bacteria *E. coli*, *S. aureus* and *B. cereus* were inoculated from slants to Muller Hinton broth (MHB) and incubated at 37°C±2°C for 24 h in 9 mL saline. The broth cultures were adjusted to match McFarland standard No. 05.

Agar well diffusion method: The antimicrobial activity of LAB isolates was determined using the agar well diffusion method. Only 1 mL of cell suspension was spread over the surface of nutrient agar plates using a sterile cotton swab. Upon drying, uniform wells were cut into the agar using a sterile cork borer (8 mm). Then, 80 mL of LAB culture were filled into respective wells and incubated at 37°C for 24 h. Antimicrobial activity was determined by observing the zone of inhibition (ZOI) around the wells. Results were considered positive if the diameter (mm) of the ZOI was greater than 1 mm. The experiment was carried out in triplicates and reported as ZOI±SD. Nutrient Broth (NB) was used as a negative control and Chloramphenicol disc (30 micrograms) as a positive control.

Spot-on-lawn method: The bacteriocin production of LAB cultures was determined using the Spot-on-lawn method[4]. The *E. coli*, *S. aureus* and *B. cereus* cultures were subculture twice and adjusted according to McFarland standard No. 05. Then, the LAB cultures were centrifuged at 4000 rpm for 20 min before the supernatant was serially diluted (two-fold) into a test tube. The cell-free supernatant (CFS) was neutralized to pH 6 using 0.5 M of Sodium hydroxide (NaOH), then sterilized by filtration. The agar plate contained 5 mL of soft agar (0.7-1% w/v) seeded with 10 µL of freshly-grown indicator strain. The antibacterial activity was determined by pipetting 10 µL of neutralized CFS onto the surface of the agar plate and determined by the presence of inhibition zones after overnight incubation. The antibacterial activity was expressed in arbitrary unit (AU) using the formula (1000/10) D where D was the dilution factor.

RESULTS

Thirteen colonies of LAB cultures were successfully isolated from three local pickled vegetables. Table 1 shows the morphological characteristics of the LAB cultures isolated from three local pickled vegetables.

Five isolates that had similar phenotypic characteristics with *Lactobacillus* spp. such as being Gram-positive and rod-shaped were selected for further biochemical tests

Table 1: Morphology of LAB cultures isolated from three local pickled vegetables

		Morphology of colony					
Sample	Isolates	Size (mm)	Margin	Elevation	Pigmentation	Gram stain	Cell shape
Pickled Look-Niang	N1	0.5	Entire	Convex	White opaque yellow	+	Cocci
	N2,N3	2.0	Entire	Convex	White opaque yellow	+	Cocci
	N4	1.5	Entire	Convex	White opaque	+	Rod
	N5	1.0	Entire	Convex	White opaque	+	Rod
	N6	1.0	Entire	Convex	White opaque yellow	+	Cocci
Pickled tare	S1, S4	1.0	Entire	Convex	White opaque yellow	+	Rod
	S2	1.5	Entire	Convex	White opaque yellow	+	Rod
	S3	0.5	Entire	Convex	Cream	+	Rod
	S5	1.0	Entire	Convex	Cream	+	Cocci
	S6	2.0	Entire	Convex	White opaque yellow	+	Cocci
Pickled white turmeric	K	0.5	Entire	Convex	Cream	+	Cocci

Table 2: Biochemical tests carried out on the selected isolates

Samples	Isolates	Morphology	Gram stain	Catalase test	Acid and gas from glucose	Fermented glucose	Growth at 10°C	Growth at 45°C	Growth at 6.5% NaCl	Growth at 18% NaCl	Growth at pH 4.5	Growth at pH 5.5
Pickled tare	S1	Rod	+	-	-	+	+	-	+	-	+	+
	S2	Rod	+	-	+	-	+	+	-	-	+	-
	S4	Rod	+	W	-	-	+	-	+	-	-	-
Pickled Look-Niang	N4	Rod	+	-	-	-	+	+	-	-	+	-
	N5	Rod	+	-	+	-	+	-	+	-	+	-

+: Positive, -: Negative, w: Weak

Table 3: Antimicrobial activity of four LAB isolates against three selected pathogenic bacteria

	Diameter of ZOI (mm±S.D)		
Isolates	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*
N4	-	15.50±0.3	-
N5	-	20.10±1.1	-
S1	-	20.87±0.2	
S2	9.0±1.2	10.20±2.3	-
Chloramphenicol (positive control)	29.8±2.1	22.10±1.9	22.7±2.7

(Table 2). Based on the biochemical tests carried out, four cultures that showed the highest level of antimicrobial property were identified as *Lactobacillus* spp. Conversely, isolate S4 was found to be weak catalase-positive and was subjected to further biochemical testing.

The antimicrobial properties of four selected LAB isolates were tested against three pathogenic bacteria (*E. coli*, *S. aureus* and *B. cereus*) using the agar well diffusion method (Table 3). Based on the results obtained, the degree of antimicrobial property among the isolates was ranked as S1>N5>N4>S2 based on the assumption that the greater the diameter of the ZOI, the stronger the antimicrobial activity of the isolate. Looking closer, isolate S1 recorded the highest level of antimicrobial activity against *S. aureus* (20.87±0.2 mm) while only isolate S2 showed antimicrobial activity against *E. coli* (9.00±1.2 mm). LAB isolates showed no antimicrobial activity against *B. cereus.* In general, the LAB isolates exhibited lower antimicrobial activity compared to the positive control, Chloramphenicol.

The inhibitory activities of pathogenic bacteria *S. aureus* and *E. coli* were carried out using the Spot-on-lawn method. Based on the results, isolate S1 inhibited *S. aureus* with a bacteriocin activity of 200 AU mL^{-1} (Fig. 1).

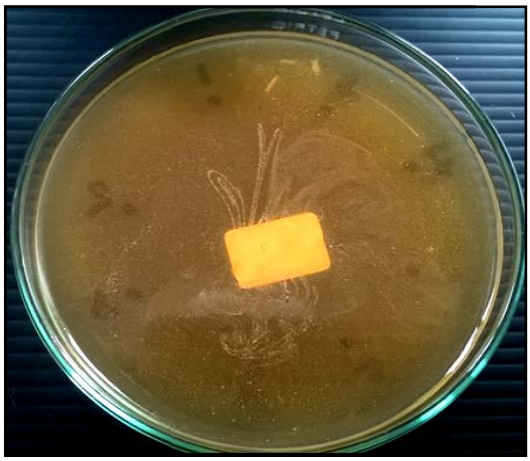

Fig. 1: *Staphylococcus aureus* inhibited by isolate S1 using Spot-on-lawn method

DISCUSSION

Isolate S1 exhibited the highest degree of antimicrobial inhibition at 20.87±0.2 mm. Similar results were seen in isolate TO against *S. aureus*, where the largest inhibitory zone

(19.3 mm) was determined to exhibit the highest antimicrobial property[11]. However, it is difficult to ascertain the reason for the variability in the antimicrobial properties among the isolates due to the uniqueness of each isolate. Interestingly, isolate S2 was equally antagonistic against both Gram-positive *S. aureus* as well as Gram-negative E. *coli*. The accumulation of primary metabolites like lactic acid, bacteriocins production and the production of antimicrobial compounds like formic acid, hydrogen peroxide and benzoic acids may have contributed to the LAB isolates ability to inhibit[11] *B. cereus*. However, the LAB isolates were not successful in inhibiting *B. cereus*. The inhibitory activities of LAB against Gram-positive pathogens were contributed by protease-sensitive bacteriocins[12]. However, the antagonistic effects of LAB toward Gram-negative pathogens could be related to the production of organic acids and hydrogen peroxide[13].

Many LAB strains with different bioactive potentials especially antimicrobial properties have been identified from a variety of fermented and pickled vegetables[14]. This study proved that simple, traditional methods of food preservation like fermentation have the potential to improve and decrease the need to depend on chemical antibiotics and artificially enriched functional foods.

CONCLUSION

The findings of this study demonstrated the remarkable antimicrobial attributes of LAB isolated from three local pickled vegetables in Thailand. The results showed that isolates from pickled tare had the best antimicrobial potential against both *S. aureus* and *E. coli*. Moreover, LAB isolated from pickled Look-Niang also exhibited antimicrobial activities against *S. aureus*. Hence, both pickled tare and pickled Look-Niang are suitable candidates to be used as natural probiotics. This study has shown the antimicrobial capabilities of fermented local vegetables and can become a viable alternative to functional foods fortified with probiotics to help maintain a healthy digestive system.

ACKNOWLEDGMENTS

The authors would like to acknowledge Yala Rajabhat University for the research grant (BKS 003/2559).

REFERENCES

1. Saarela, M., L. Lahteenmaki, R. Crittenden, S. Salminen and T. Mattila-Sandholm, 2002. Gut bacteria and health foods-the European perspective. Int. J. Food Microbiol., 78: 99-117.
2. Vinderola, G., A. Ouwehand, S. Salminen and A. von Wright, 2019. Lactic Acid Bacteria: Microbiological and Functional Aspects. 5th Edn., CRC Press, Florida, US, Pages: 764.
3. Tadesse, G., E. Ephraim and M. Ashenafi, 2005. Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. Internet J. Food Saf., 5: 13-20.
4. Ennahar, S., K. Sonomoto and A. Ishizaki, 1999. Class IIa bacteriocins from lactic acid bacteria: Antibacterial activity and food preservation. J. Biosci. Bioeng., 87: 705-716.
5. Kantachote, D., P. Prachyakij, W. Charernjiratrakul, M. Ongsakul and Y. Duangjitcharoen *et al*., 2010. Characterization of the antiyeast compound and probiotic properties of a starter *Lactobacillus plantarum* DW3 for possible use in fermented plant beverages. Electr. J. Biotechnol., Vol. 13. 10.2225/vol13-issue5-fulltext-1
6. Capela, P., T.K.C. Hay and N.P. Shah, 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freezedried yoghurt. Food Res. Int., 39: 203-211.
7. Salvatierra, M., A. Molina, M.G. Mdel and M.L. Arias, 2004. Evaluation of the effect of probiotic cultures on two different yogurt brands over a known population of Staphylococcus aureus and the production of thermonuclease. Arch. Latinoam. Nutr., 54: 298-302.
8. Piard, J.C. and M. Desmazeaud, 1991. Inhibiting factors produced by lactic acid bacteria part 1. Oxygen metabolites and catabolism end-products. Lait, 71: 525-541.
9. Gotcheva, V., E. Hristozova, T. Hristozova, M. Guo, Z. Roshkova and A. Angelov, 2002. Assessment of potential probiotic properties of lactic acid bacteria and yeast strains. Food Biotechnol., 16: 211-225.
10. Goktepe, I., V.K. Juneja and M. Ahmedna, 2019. Probiotics in Food Safety and Human Health. 1st Edn., CRC Press, Florida, US, Pages: 512.
11. Juliyarsi, I., P. Hartini, Yuherman, A. Djamaan and Arief *et al*., 2018. Characterization of lactic acid bacteria and determination of antimicrobial activity in Tempoyak from Padang Pariaman District, West Sumatra, Indonesia. Pak. J. Nutr., 17: 506-511.
12. Karovicova, J. and Z. Kohajdova, 2003. Lactic acid fermented vegetable juices. Hortic. Sci., 30: 152-158.
13. Kimoto, H., M. Nomura, M. Kobayashi, T. Okamoto and S. Ohmomo, 2004. Identification and probiotic characteristics of *Lactococcus* strains from plant materials. Jap. Agric. Res. Q., 38: 111-117.
14. Kazemipoor, M., C.W.J.W.M. Radzi, K. Begum and I. Yaze, 2012. Screening of antibacterial activity of lactic acid bacteria isolated from fermented vegetables against food borne pathogens. Arch. Sci., Vol. 65.