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Identification Of Potential Indigenous Microbe From Local Fermented Vegetables With Antimicrobial Activity

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ABSTRACT

The objectives of this study were to identify and determine the antimicrobial activity of the indigenous microbes isolated from local fermented vegetables. Ninety nine isolates were obtained from fifteen samples of local fermented vegetables including jeruk lobak putih (pickled radish), jeruk lobak (pickled carrot), jeruk petai (pickled bitter bean), jeruk sawi sie park choy (pickled mustard), jeruk rebung (pickled bamboo shoot), jeruk maman (pickled maman), jeruk bawang putih (pickled garlic) and jeruk kobis (pickled cabbage). The antagonistic act of the isolates was assayed using well diffusion assay against five common pathogens namely, Bacillus cereus, Escherichia coli, Listeria monocytogens, Salmonella typhi and Staphylococcus aureus. Fifty three isolates showed inhibitory zones in the range of 10-18 mm under well diffusion assay. B. cereus was inhibited predominantly by the isolates (62 isolates) followed by L...monocytogens (58 isolates), E.coli (57 isolates), S.typhi (51 isolates) and S.aureus (44 isolates). A total of 19 potential isolates exhibited strong antimicrobial activity (≥10 mm) and inhibited more than two types of pathogens were selected and identified by16sRNA sequencing. Lactobacillus plantarum genera, dominates the local fermented vegetable product with 14 isolates followed by Lactobacillus brevis (3 isolates) and Pediococcus pentosaceus (2 isolates). It was acknowledge that fermented vegetable are high in salt and strongly acidic however some lactic acid bacteria are capable to withstand such growth conditions and showed antagonistic activity towards pathogens. This is a positive remark that these strains are promising candidates for development of a starter culture and to further study their potential as probiotics.

1. INTRODUCTION

Malaysia produces numerous fermented foods from seafood, vegetables, fruits, cereals and many more. Fermented vegetables or also known as pickles are a home-made indigenous product consumed by many races in Malaysia. Several types of the fermented vegetables are commonly prepared by households or cottage industries for sale in retail stores and open markets. These pickles are eaten raw or cooked. The vegetable pickles are often made when the raw materials are excess during certain season. Many varieties of vegetables are pickled depending on the season and availability. Among the most common are radish, carrot, bitter beans, mustard, bamboo shot, cucumber, onion and garlic. Simple brining is commonly used to pickle vegetables and this technique results in fermentation by lactic acid bacteria. Shelf life of perishable foods such as vegetables can be improved by fermentation, which is an old and cheap technology compared to canning or freezing [1] [17].

Lactic acid bacteria (LAB) is regarded as a major group of probiotic bacteria. In general, it is believed that probiotics help keep the balance between harmful and beneficial bacteria in the gut thus maintaining a healthy digestive system [2]. LAB occur naturally in several raw materials to perform both acidification and production of flavour compounds [16][17]. Moreover, LAB protects foods from spoilage and pathogenic microorganisms due to the production of lactic and acetic acids, hydrogen peroxide, diacetyl, fatty acids, phenyllactic acid and bacteriocins [15].

The production of these antimicrobial substances promote antagonistic properties of LAB allied to their safe history of use in traditional fermented food and make them very attractive as bio preservatives [4][5]. In addition, some LAB exhibit potent antimicrobial activities in the form of small, heat stable, antimicrobial peptides called bacteriocins [6][7]. Bacteriocins are extra-cellularly released peptides with a bactericidal or bacteriostatic mode of action against closely related species. Several types of bacteriocins from LAB that have been identified and characterized are Nisin, Diplococcin, Acidophilin, Bulgarican, Helveticins, Lactacins and Plantaricins [8]. The aim of this study was to identify the potential LAB from the fermented vegetable with antimicrobial properties.

2. EXPERIMENTAL

2.1 Sampling and Isolation of the indigenous microbes

A total of 15 fermented vegetables were collected from local markets all around Malaysia (Table 1). The pH of the samples was recorded according to the pH of 10 mL Ringer solution containing 1 g of sample prior to the isolation procedure. Approximately 10g of the fermented vegetable samples were homogenized in 100mL of Ringers solution. Ten percent of the homogenized samples were inoculated into de Man, Rogosa and Sharpe (MRS) broth and incubated at 30° C, 48h for enrichment of lactic acid bacteria (LAB). LAB strains were screened and isolated by spread plating of appropriate dilutions of the enrich cultures onto selective media, i.e. MRS agar containing 0.3% $CaCO3$ (MRS-$CaCO3$) for screening of acid producer.

2.2 Screening of acid producer.

Isolates from the vegetables samples (Table 1) were preliminary screened for acid producer by halozone around the colonies after 48h incubation (Figure 1)[9]. Dilution streaking was performed to obtain a pure single colony of the isolates. The isolated cultures were kept for storage in 30% glycerol+70% MRS broth at-80°C for further investigations.

2.3 Antimicrobial activity test by agar well diffusion method.

Each pure culture was thawed and subcultured onto MRS agar and incubated at 30°C for 24-48 hours. Then, a single pure colony from each isolates was selected and transferred to 10ml MRS broth incubated at 30° C for 48 hours shaking at 150 rpm. Indicator food pathogens namely, Bacillus cereus, Escherichia coli, Listeria monocytogens, Salmonella typhimurium and Staphylococcus aureus were subcultured in nutrient broth at 37°C for 24 hours [8]. Then, these broth cultures were mixed by vortexing and 100 µl of the culture fluid was diluted to 10 ml with sterilized saline. This suspension was used to evaluate antimicrobial activity of the isolates using agar well diffusion method on Mueller Hinton (MH) agar [3].

Antimicrobial activity of isolates against all indicator pathogens was determined under aerobic conditions at 37°C. Agar plates were inoculated with diluted 100 µl suspension of each indicator microorganisms. Wells (5 mm in diameter) were cut in MH agar plate and 100 µl of crude cell suspension of the isolated strains was loaded into each well. After incubation at 37°C for 18-24 hours the diameter (mm) of the inhibition zone around the wells were measured. A negative control consisting sterilized distilled water was used (Figure 2)[3].

2.4 Genomic DNA purification, PCR amplification and 16S rRNA Sequencing

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used for the amplification of the 16SrRNA gene. The PCR amplification was conducted in a reaction mixture containing 10mM dNTP mix, 10μM each primer, 5μL %X PCR Buffer, 2U/μL Taq DNA polymerase, 1μL DNA template (genomic DNA) and 18.5μL nuclease free water. The thermal program for amplification was set for initial denaturation at 94°C for 2 mins, 1 cycle. Then it was followed by further denaturation at 98°C for 15s, annealing process was done at 51°C and 30s and extension was set at 72°C for 10 mins, 1cycyle [20]. The purified PCR products were analyzed in a 1% TAE agarose gel and then were outsourced to sequencing service at 1st Base Sdn. Bhd. Malaysia. The DNA sequence were aligned by Plasmid editor proggrame and used for the similarity search against NCBI GenBank database using the BLAST program available at website http://blastncbi.nlm.nih.Gov/blast.cgi.

3. RESULTS AND DISCUSSION

A total of 99 isolates were positively screened as acid producers from 15 various types of fermented vegetables product. The acid producers were determined from the halozone produced around the colonies on MRS agar containing 0.3% CaCO3 (MRS-CaCO3) (Figure 1). The number of isolates obtained from different types of samples is shown in Table 1. Most of the samples such as pickled garlic, pickled bitter bean, pickled carrot, pickled radish, pickled cabbage and pickled bamboo shoot exhibited high acidity consequently the acid producers were able to retain in such conditions. Among all the samples pickled garlic showed the lowest pH value about 3.32-3.58. Owing to the high acidity microbes were not able to grow and cultured on the selective media (Table 1). In contrast some pickled mustard samples exhibit the highest pH value compare to other samples about 6.13-6.66, although there are some pickled mustard samples from different locations showed lower pH value about 3.58-4.32 which is maybe due to the different fermentation method being practiced. Mostly all colonies on the media were small, white and creamy able to grow under aerobic conditions furthermore producing acid on (MRS-CaCO3)[18]. Thus, it can be presumed that LAB constitutes the predominant element of the microbial flora in fermented vegetable products due to the high acidity of the product [19]. The major role of LAB in fermented product is to produce organic acid and reduce pH in order to inhibit spoilage and pathogenic bacteria and thereby preserve the food products [20].

Antimicrobial activity by well diffusion assay showed that isolates from fermented vegetables are able to inhibit the indicator pathogens tested. B. cereus was inhibited the most by the isolates (63%) and showed the highest diameter of inhibition zone (8-18 mm) followed by L. monocytogens (59%); (6-15 mm) E. coli(58%); (5-17 mm), S. typhimurium(51%); (5-16 mm) and S.aureus (44%) with(6-14 mm) inhibition zone(Table 2) (Figure 2). These results are similar to previous study that had been done whereby B.cereus was also the most inhibited pathogens reported [12][13][14][15]. There were some isolates from several samples such as pickled mustard and pickled 'maman' with low content of acidity as shown by the higher pH of the samples could exhibit strong antagonistic activity against pathogens. This happened probably due to the presence of other antimicrobial compounds such as hydrogen peroxide, diacetyl, acetoin and bacteriocins [21].

A total of 19 isolates showed strong antimicrobial activity with diameter zone, ≥10mm and inhibited most of the pathogen tested were considered as potential isolates were identified (Table 3). LAB isolates such as L. plantarum, L. brevis and P. pentosaceus possess strong antagonistic activity against the pathogenic strains tested was identified mainly from pickled 'maman', pickled mustard, pickled carrot, and pickled bitter bean identified (Table 3) .

The results of 16s RNA sequences of the 19 isolates show high sequence similarity (at least 98%) with the members from lactic acid bacteria in the NCBI database. The range of LAB strains isolated and identified from the indigenous fermented vegetable are similar to previous studies by [9][10][11].

1(a) 1(b)

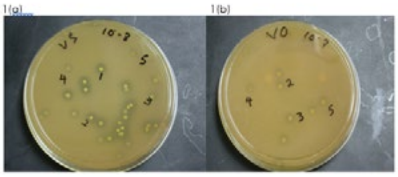

Figure 1 A total of 99 isolates were preliminary screened for acid producers by halo zone around the colonies on de Man Rogosa Sharpe (MRS) agar containing 0.3% Ca;CO₃.
Figure 1(a) VS (Pickled bitter bean) from Pasar Beranang, **Figure1 (b)** VO (**Pickled** mustard,'Siew park choy') Pasar Tawau.

Table 1 List of indigenous fermented vegetables, sampling locations, pH of samples and total number of acid producers.

Bil	Codes	Samples	Identified bacteria
1	VN1	Pickled maman	*Pediococcus pentosaceus* strain MRSD2
2	VN2		*Lactobacillus plantarum* strain X3-5B
3	VN4		*Lactobacillus futsaii* strain YM 0188
4	VN5		*Lactobacillus plantarum* strain MCC 2156
5	VN6		*Pediococcus pentosaceus* isolate:qz-201
6	VN7		*Lactobacillus paralimentarius* strain Akhavan a-Q2
7	VO1	Pickled mustard	*Lactobacillus plantarum* strain DJ-04
8	VO2		*Lactobacillus plantarum* strain 49
9	VO3		*Lactobacillus plantarum* strain B4 3.1
10	VO5		*Lactobacillus plantarum* strain Akhavan-Q3
11	VO6		*Lactobacillus plantarum* strain isolate 11
12	VO7		*Lactobacillus plantarum* strain SM71
13	VP1	Pickled carrot	*Lactobacillus brevis* strain Lb14F1
14	VP2		*Lactobacillus plantarum* strain TN635
15	VP4		*Lactobacillus brevis* strain PQ25
16	VS1	Pickled bitter bean	*Lactobacillus plantarum* strain HT-W104-B1
17	VS2		*Lactobacillus pentosus* strain SM35
18	VS5		*Lactobacillus brevis* strain SD2S7L1
19	VS6		*Lactobacillus plantarum* strain SKT109

Figure 2 Antimicrobial activity of the isolates from sample VJ, VM, and VI and by well diffusion assay on indicator pathogens. *E. coli* (EC.) *S. typhi* (ST). *B.cereus* (BC). *L. monocytogens (LM) and*

Table 2 Number of isolates that inhibit the indicator pathogens and the range of inhibition zone

No.	Pathogens	Number of isolates capable of inhibiting	Inhibition zone (mm)
1.	*Staphylococcus aureus*	44(45%)	6-14
2.	*Bacillus cereus*	62 (63%)	8-18
3.	*Escherichia coli*	57 (58%)	5-17
4.	*Listeria monocytogens*	58 (59%)	6-15
5.	*Salmonella typhimurium*	51 (52%)	5-16

Total number of

Table 3 List of 19 potential isolates which exhibited strong antimicrobial activity (≥10mm) and inhibited all types of pathogens were selected and identified by16sRNA sequencing

2	VI	Pickled mustard('Siew park choy')	Pasar Kuantan	6.13	10
3	VJ	Pickled bitter bean	Pasar Kuantan	4.09	10
4	VK	Pickled garlic	Pasar Kemaman	3.32	0
5	VL	Pickled carrot	Pasar Kemaman	4.97	10
6	VM	Pickled bamboo shoot	Pasar Kemaman	4.44	10
7	VN	Pickled'maman'	Pasar Kemaman	5.87	7
8	VO	Pickled mustard('Siew park choy)	Pasar Tawau	3.58	7
9	VP	Pickled carrot	Pasar Tawau	4.41	5
10	VQ	Pickled raddish	Pasar Semenyih	4.63	4
11	VR	Pickled mustard	Pasar Semenyih	6.66	0
12	VS	Pickled bitter bean	Pasar Beranang	4.41	6
13	VT	Pickled garlic	Pasar Beranang	3.58	0
14	VU	Pickled mustard	Pasar Kajang	4.32	10
15	VV	Pickled cabbage	Pasar Kajang	3.91	10
			Total		99

4. CONCLUSION

It can be concluded that our local fermented vegetable products consist of microbes with inhibitory effect towards common food borne pathogens tested in this study. It was assumed that the stronger the antimicrobial activity of the isolates, it will exhibit greater zone of inhibition towards pathogens. Lactobacillus plantarum dominates most of the samples and showed strong antimicrobial activity.

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REFERENCE

[1] Merican.1977.Indigeous Fermented Food Involving an Acid Fernetations, Preserving and Enhancing Organoleptic and Nutrional Quality of Fresh Foods In Keith H. Steinkraus(ed)Handbook of Indigenous Fermented Foods Second Edition,Revised and Expanded. Marcel Dekker, Inc, New York.

[2] Hugo A.A, Antoni G.L. de and P.P.F. 2006.Lactobacillus delbrueckii subsp lactis strain CIDCA 133 inhibits nitrates reductase activity of Escherichia coli. Int. J Food Microb.111:p.191-196.

[3] Diop,M.B.E.Dubois Dauphin A. Tine,J. Ngom, Destain and P. Thonart. 2007, Bacteriocin producers from Traditional food products. Biotechnol.Agron Soc.Environt.11:275-281.

[4] Parada JL. 1984. Use of Lactic acid bacteria as industrial microorganism. Int j.Microbiol., 146:93-102

[5] Caplice E. and Fitzegerald GF. 1999. Food fermentation: Role of microorganism Iin food production and preservation. Int. J. Food Microbiol, 50:131-149.

[6] Riley MA and Wertz JE. 2002. Bacteriocins :Evolution,Ecology and application. Annu. Rev.Microbiol., 56:117-137.

[7] Sablon E, Contreras B. and Vandamme E. 2000. Antimicrobial peptides of Lactic acid bacteria;mode of action, genetics and biosynthesis. Adv.Biochem.Eng.Biotechnol.,68:21-60.

[8] Nur Hasanah. 2004. Bacteriocin production in aeration of level kind and bacteriocin stability of lactic acid bacteria M6-15.Fresh graduate scription.Bogor Agricultural University, Bogor, Indonesia.

[9] Karki T., Okada S.,Baba T., Itoh H. and Kozaki,M.,1983. Studies on the microflora of Nepalese pickles gundruk.Nippon Shokuhin Kogyo Gakkaishi. 30, p, 357-367

[10] Tamang J.P. and Sarkar P.K.1993. Sinki: a traditional lactis acid fermented radish tap root product.Journal of General and Applied Microhiology.39.p 395-408.

[11] M. Tamminen, T. Joutsjoki, M. Sjo¨blom and, M. Joutsen A. Palva3, E.-L. Ryha¨nen1and V. Joutsjoki1 .2004.Screening of lactic acid bacteria from fermented vegetablesby carbohydrate profiling and PCR–ELISA. Letters in Applied Microbiology 2004, 39, 439–444 doi:10.1111/j.1472-765X.2004.01607.x

[12] Nithya K., Senbagam D, Sentilkumar B, Udhayashree N. and Gurusamy R. 2012.Characterization of bacteriocin producing lactic acid bacreia and its application as food preservative.African Journal of Microbiology research. 6(6) p1138-1146.

[13] Corsetti A, Gobetti M, Rossi J and Damiani P.1998.Antimould activity of sourdough lactic acid bacteria:Identification of organic acids produced Lactobacillus sanfranciso CB1. Appl.Microbiol. biotechnol.50:253-256.

[14] Mahnaz Kazemipoor, Che Wan Jasimah Wan Mohamed Radzi, Khyrunnisa Begum and Iman Yaze.2010.Screening of antibacterial activity of lactic acid bacteria isolated from fermented vegetables against food borne pathogens.Arcives des Science.Vol 65.Issue 6.

[15] Bali V, Panesar P.S. and Bera M.B. 2011.Isolation ,Screening & Evaluation of Antimicrobial Activity of Potential Bacteriocin Producing Lactic Acid bacteria isolate.Microbiology Journal(30p113119.DOI:10.3923/mj.2011.113.119.

[16] Desai P. and Sheth T. (1997) Controlled fermentation of vegetables using mixed inoculum of lactic cultures. Journal of Food Science and Technology 34, p155–158.

[17] Ramesh ChandraRay & Rizwana Parveen Rani. 2014. Fermented Fruits and Vegetables of Asia:A Potential Source of Probiotics.Hindawi Publishing Corporation Biotechnology Research International Volume 2014, Article ID 250424,19pages,http://dx.doi.org/10.1155/2014/250424.

[18] Schillinger U., Yousif N.M.K., Sesar L and Franz C.M.A.P., 2003. Use of group-specific and RAPD-PCR analyses for rapid differentation of Lactobacillus strains from probiotic yogurt.

[19] Leisner J.J, Vancanneyt M., Rusul G., Pot B., Lefebrve K., Fresi K. and Tee L.K. 2001. Identification of lactic acid bacteria constituting the predominating microflora in an acid-fermented condiment (tempoyak) popular in Malaysia.Int. Journal of Food Microbiology.63.149-157.

[20] C.Palludan-Muller, C. Madsen, M. Sophanodora P., Gram L. and Møller P.L. 2002. Fermentation and microflora of plaa-som,a Thai fermented fish product prepared with different salt concentrations. Int. Journal of Food Microbiology.73.61-70.

[21] Kaktcham P.M., Zambou N.F., Tchouanguep F.M., El-Soda and M. Choudary M.I. 2011. Antimicrobial and Safety properties of lactobacilli isolated from two Cameroonian traditional fermented foods. Open Access article Scientia Pharmaceutica.80 (1): 189–203.

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