Australian Journal of Basic and Applied Sciences, 9(28) Special 2015, Pages: 230-234

ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com

Antimicrobial Activity of Lactic Acid Bacteria Isolated from different Stages of Soybean Tempe Production

[1]Balqis Pisol, [1]Noriham Abdullah, [1]Khalilah Khalil and [2]Lilis Nuraida

[1] *Faculty of Applied Science, University Teknologi MARA (UiTM), 40450, Shah Alam, Selangor Malaysia*
[2] *Department of Food Science and Technology, Faculty of Agricultural Technology, and SEAFAST Center, Bogor Agricultural University, IPB Darmaga Campus, PO BOX 220, Bogor, West Java, Indonesia*

ARTICLE INFO

Article history:
Received 23 June 2015
Accepted 25 July 2015
Available online 30 August 2015

Keywords:
Antimicrobial activity, lactic acid bacteria, soybean tempe,

ABSTRACT

Biochemical changes in tempe largely depends on the microorganisms associated with tempe processing and lactic acid bacteria (LAB) is one of the common microorganism involved. LAB in fermented foods is important to prevent the growth of spoilage microorganisms and this is believed due to LAB antimicrobial properties. In this study, the isolation of LAB at different stages in tempe processing was carried out. Subsequently, the inhibitory activity of LAB isolates against common food spoilage pathogens was determined. Based on the result obtained, it shows that all twelve identified LAB isolates exhibited varying degrees of inhibitory activity against three pathogens namely, *E.coli, S. typhimurium* and *S. aureus*. However, none of the isolates were observed against *Candida albicans* and *Aspergillus niger*. Isolates A, B, H, I and J showed convincing antibacterial activity with inhibition ranged from 14 to 15.7 ± 1.2 mm when tested against *E. coli* while others showed medium tolerance. As for the antimicrobial activity against *S. typhimurium*, all LAB isolates appeared to have medium tolerance with diameter of inhibition zone (DIZ) > 12-15 mm but showed high tolerance when tested against *S. aureus* with DIZ > 15 mm. This study showed that gram positive bacteria were more sensitive than gram negative bacteria when tested against the LAB isolates. Both tested mould and yeast however showed to be resistance to all LAB isolates. In conclusion, all LAB isolates in this study were able to suppress the growth of food pathogens except for mould and yeast.

To Cite This Article: Baqis P., Noriham A., Khalilah, A. K and Lilis N., Antimicrobial Activity of Lactic Acid Bacteria Isolated from different Stages of Soybean Tempe Production. ***Aust. J. Basic & Appl. Sci.,*** *9(28): 230-234, 2015*

INTRODUCTION

Soybean tempe is a world well known fermented food especially in Indonesia and Malaysia. Tempe is simply define as fungal fermented food being fully covered by mould mainly *Rhizopus sp*. This food was served as side dish and snack. Aside from soybean, other types of legumes were also made into tempe such as barley (Feng, 2006), mucuna (Handajani, 2001), chickpea (Abu-salem and Abou-arab, 2011), red kidney bean (Srapinkornburee *et al*., 2009) and cowpea (Kiers *et al*., 2000). Tempe has been studied extensively over the past few decades by many researchers and excellent up to date reviews on this particular food was reported by Nout and Kiers, (2005). Having numerous advantages from all aspect including improved nutritional value and towards health benefits, tempe is no longer considered as food for poor but as food for life. Consuming tempe was proven to treat diarrhea and improve feed efficiency of enterotoxigenic *Escherichia coli* (ETEC)-challenged weaned piglet (Kiers *et al*., 2003).

Biochemical changes in fermented food were always related to the presense of microorganisms. The type of microorganism involved however varies according to the type and condition of the food itself. In tempe processing, lactic acid bacteria (LAB) is the common microorganisms associate which upon consumption can confer benefit towards the consumers. Presence of LAB in fermented food product was well known and recognized as most LAB involved was generally regarded as safe (GRAS status). Some LAB was purposely introduced into the food processing while some naturally occur during the fermentation process. LAB play an important role starting at the soaking stage of soybean by acidifying the water until the fermentation stage, thereby preventing the growth of spoilage microorganisms (Ashenafi and Busse, 1991; Nout *et al*., 1987). Study done by Moreno *et al*., (2002) and Balqis *et al*., (2013), clearly shows presence of LAB from different stages of soybean tempe production.

Corresponding Author: Noriham, A., Faculty of Applied Science, University Teknologi MARA (UiTM), 40450, Shah Alam, Selangor, Malaysia
Tel.: + 012-3317037; Fax: +603-55444562; E-mail: noriham985@salam.uitm.edu.my

Lactic acid bacteria (LAB) are gram positive, non motile, non spore forming rods and cocci, catalase negative, anaerobic but aerotolerant, fastidious and can either be homofermentative or heterofermentative. The LAB was first acknowledged to compromise of four genera which are *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus.* Recent taxonomic study proposed new genera which consist of the following: *Aerococcus, Alloicoccus, Carnobacterium, Dolosigranulum, Entercoccus, Globicatella, Lactococcus, Tetragenococcus, Vagococcus, Oenococcus* and *Weissella* (Khalid, 2011).

Main by-products of LAB are lactic acid and acetic acid aside from other organic acid which were produced in much smaller amount (De Vuyst and Vandamme, 1994). These organisms also produce antimicrobial compounds including hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, D-isomers of amino acids, reuterin and bacteriocins (Cintas *et al*., 2001). Darsanaki *et al*., (2012) reported that *Lb. plantarum, Lb. casei* and *Lb. brevis* isolated from fresh vegetables had shown good antimicrobial activity for inhibiting *S. aureus, S, typhimurium* and *E.coli.*

Over the past years, an increased drive has existed for LAB producing bacteriocin, since this compound is capable to inhibit the close related microorganisms. Moreno *et al*., (2002), successfully isolate two bacteriocinogenic *Enterococcus faecium (E. faecium)* having the ability to inhibit various types of gram positive bacteria, including strains of *E. faecium, E. faecalis, Carnobacterium divergens, C. piscicola, Lb. brevis, L. pentosus* and *Paralactobacillu selangorensis.*

Although there are a lot of research on isolation and characterization of LAB from the end product of soybean tempe, not many focused on isolation and characterization of LAB from different stages of tempe processing. Therefore the aim of this study is to determine LAB isolated from different stages of tempe processing for their inhibitory effect against common foodborne spoilage bacteria.

MATERIALS AND METHODS

Twelve LAB strains were isolated from different stages of soybean tempe production. The stages where the sample was taken is shown in Figure 1. All isolates were grown on de Man Rogosa Sharpe (MRS) agar at anaerobic incubation of 37°C for 48 h. The selectivity of LAB isolates were confirmed by the gram stain, morphology observation, catalase test and identification at molecular level using 27f and 1429r primers. Isolates A, B, C, D, E, F and I were identified as *Enterococcus faecium*, isolates G, M and N as *Leuconostoc lactis* and isolates H and J as *Leuconostoc sp*. (Balqis *et al*., 2015).

Test microorganisms:

A total of five common human and food pathogenic strains was selected for antimicrobial test. These microorganisms were *Escherichia coli (E.coli*) ATCC 11229, *Salmonella typhimurium (S. typhimurium)* ATCC 27592, *Staphylococcus aureus (S. aureus)* ATCC 43300, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. All microorganisms were obtained from Microbiology Culture laboratory, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia. Each microorganism represents Gram positive and negative bacteria while yeast and mould and were grown in their respective medium (nutrient broth and potato dextrose broth) overnight at 37°C.

Standardisation of test microorganisms:

The test microorganisms were standardized by using 0.5 Mc Farland standard which is equivalent to cell density of 1.5 x 10^8 CFU/ml. Mc Farland Standard was used as reference to adjust the turbidity of microbial suspension. The turbidity was adjusted using steriled distilled water.

Preparation of cell-free supernatant:

LAB isolates were sub-cultured in steriled test tube containing MRS broth and incubated for 24 h at 37°C prior to usage. Bacterial cell were removed by centrifuging the culture at 10,000 rpm for 10 min (Pundir *et al*., 2013). The supernatant was filtered using Millpore, 0.45 µl.

Screening of antimicrobial activity by agar well diffusion method:

Blank plates containing 20 ml nutrient agar and potato dextrose agar were prepared in triplicate for each test microorganisms. The entire surface of the agar was swabbed with the test microorganisms, twice to ensure even distribution of the inoculums. The plates were allowed to dry and wells (7 mm in diameter) were made using steriled cork borer. Each well was filled with 50 µl LAB culture free supernatant, (Lelise *et al*. 2014), steriled distilled water (control negative), 0.04 mg/ml chloramphenicol (control positive for bacteria) and 0.1% cycloheximide (control positive for yeast and mould). After wells were dried, plates were incubated for 24 h (bacteria)/48 h (yeast and mould) at 37°C. Appearance of clear zone around the well was measured as diameter of inhibition zone (DIZ).

RESULTS AND DISCUSSION

All LAB isolates were tested for antimicrobial activity against common pathogenic microorganisms. The interpretation of the results were as according to Shahidi Bonjar, (2004) where the activities were rated into three classes as: poor (DIZ< 12 mm), medium (DIZ 12 to <15 mm) and high (DIZ > 15

mm). The results are shown in Table 1. Figure 2 illustrates the zones of inhibition against some of the pathogenic bacteria under study.

The antimicrobial activity of all twelve LAB isolates was tested against *E. coli, S. typhimurium, S. aureus, Candida albicans* and *Aspergillus niger* using the agar well-diffusion method and the diameter of inhibition was recorded. Supernatant obtained from all isolates exhibited varying degrees of inhibitory activity towards bacteria only. No antimicrobial activity was observed against *Candida albicans* and *Aspergillus niger*. According to Darsanaki *et al*., (2012) bacteria with an average inhibition of 7.91 mm indicate good capacity of inhibiting pathogenic bacteria.

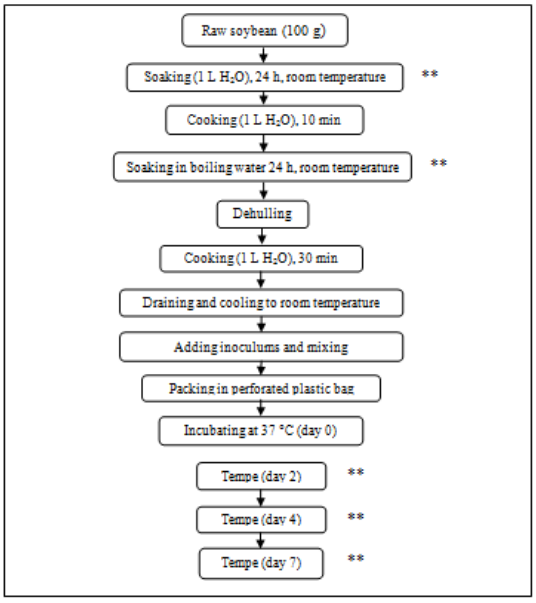

Fig. 1: Flowchart of tempe processing ****** indicate stages where samples were taken for LAB isolation.

Five LAB isolates and chloramphenicol showed superior antibacterial activity with DIZ >15 mm when tested against *E. coli*. The five isolates were labelled as isolate A, B, H, I and J. Other isolates show medium tolerance with DIZ in the range of 12 - 15 mm. Study by Lelise *et al*., (2014) reported two LAB isolates have the antibacterial activity in the range of 14.3 ± 0.6 mm to 16 ± 1.7 mm when tested against standard *E.coli* ATCC 2592, while Darsanaki *et al*., (2012) reported much smaller DIZ in the range of 6.8 - 9 mm. Our results were between 14 to 15.7 ± 1.2 mm which were better than the value reported by Darsanaki *et al*., (2012) and in agreement with Lelise *et al*., (2014). Pundir *et al*., (2013) on the other hand reported that 12 out of 26 LAB isolates from various food samples showed very high antibacterial activity against *E.coli,* followed by 2 LAB isolates showed medium tolerance.

Table 1: Antimicrobial activity of LAB isolates.

LAB strain	Diameter of inhibition zone (DIZ), mm				
	Gram negative		Gram positive	Yeast	Mould
	E.coli	*S. typhimurium*	*S. aureus*	*C. albicans*	*A. niger*
A	+++	++	+++	NA	NA
B	+++	++	+++	NA	NA
C	++	++	+++	NA	NA
D	++	++	+++	NA	NA
E	++	++	+++	NA	NA
F	++	++	+++	NA	NA
G	++	++	+++	NA	NA
H	+++	++	+++	NA	NA
I	+++	++	+++	NA	NA
J	+++	++	+++	NA	NA
M	++	++	+++	NA	NA
N	++	++	+++	NA	NA
Chloramphenicol	+++	+++	+++	-	-
Cycloheximide	-	-	-	+++	+++

Note: + = poor tolerance with DIZ < 12 mm,
++ = medium tolerance with DIZ 12 to <15 mm,
+++ = high tolerance DIZ > 15 mm
NA = No activity

As for antimicrobial activity against *S. typhimurium*, all LAB isolates appeared to have medium tolerance with DIZ > 12-15 mm and only chloramphenicol has a high tolerance towards this gram negative bacteria. Among all LAB isolates, isolate N showed the highest DIZ with 14.33 mm while isolate I showed the smallest DIZ with only 12 mm.

All LAB isolates and chloramphenicol possessed high tolerance when tested against *S. aureus* with DIZ > 15 mm. Hence this showed that all isolates exhibited superior antibacterial activity towards this particular gram positive microorganism. The results were in agreement with results reported by Lelise *et al*., (2014) where both LAB isolates tested exhibited DIZ in the range of 11.3 ± 1.5 to 15.7 ± 1.2 mm when tested against standard *S. aureus*. According to Gilliland and Speck, (1977), lactobacilli showed stronger antibacterial properties against Gram positive (*S. aureus*) than gram negative bacteria (*E. coli* and *S. typhi*). Our result complies with those reported by Pundir *et al*., (2013) and Ali *et al*., (2013), who showed that gram positive bacteria were more sensitive than gram negative bacteria.

Both mould and yeast tested however showed to be resistance to all LAB isolates. Research by Pundir *et al*., (2013) reported 14 out of 26 LAB isolates have no antimicrobial activity against *Candida albicans* while 11 LAB isolates showed no activity against *Aspergillus sp*.

Sumathi and Reetha, (2012) concluded that *Lb. acidophilus, Lactococcus lactis sub sp. lactis* and *Pediococcus acidilactici* isolated from various types of food products show strong DIZ when tested against *E.coli, S. typhi, S. aureus, Enterobacter* and *Listeria monocytogenes*. Meanwhile, Darsanaki *et al*., (2012) reported that *Lb. plantarum, Lb. casei* and *Lb. brevis* isolated from fresh vegetables shown good antimicrobial activity for inhibiting *S. aureus, S, typhimurium* and *E.coli.*

The antibacterial activity might be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compounds (Bezkorovainy, (2001); Tambekar *et al*., (2009).

Production of lactic acid and acetic acid were the main inhibitory substances of LAB aside from other organic acids, which include formic acid, free fatty acids, ammonia, ethanol, hydrogen peroxide, diacetyl, acetaldehyde, benzoate, acetoin, 2,3-butanediol, bacteriolytic enzymes, bacteriocins and antibiotics (De Vuyst and Vandamme, 1994; (Tambekar *et al*., 2009)). Study done by Lelise *et al*., (2014) showed that two LAB isolated from Ethiopian traditional fermented drinks having good antimicrobial activity, produce high potential of lactic acid and low production of hydrogen peroxide in the range of 720.64 to 1396.24 mg and 1.93 to 5.35 mg respectively.

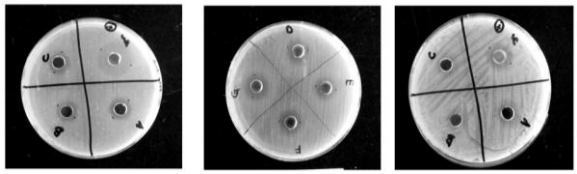

Fig. 2: Inhibition zones of LAB against pathogenic bacteria by agar well diffusion method: a) *S. typhimurium*, b) *E.coli* and c) *S. aureus.*

Conclusion:

All twelve LAB isolates (*Enterococcus faecium*, *Leuconostoc lactis* and *Leuconostoc sp*) showed good antibacterial activity against all tested pathogens, but no activity was observed when tested against mould and yeast. Therefore, it can be concluded that LAB can produce different types of antibacterial compounds in different types of samples. The production of these compounds is largely influenced by the physical and chemical parameter under different environmental conditions which in this study is the difference in processing conditions of the soybean tempe.

ACKNOWLEDGEMENTS

This study was supported by the collaboration of Universiti Teknologi MARA (UiTM), Malaysia with Institut Pertanian Bogor (IPB), Indonesia and funded by the Research Cluster Grant (600-RMI/ DANA 5/3/CG (18/2012)) and Fundamental Research Grant Scheme (FRGS) (600-RMI/ FRGS 5/3 (147/2013)).

REFERENCES

Abu-salem, F.M. and E.A. Abou-arab, 2011. Physico-chemical properties of tempeh produced from chickpea seeds. Journal of American Science, 7(7): 107–118.

Ali, F.S., O.A.O. Saad and A.H. Salwa, 2013. Antimicrobial activity of probiotic bacteria. Egyptian Academic Journal of Biological Sciences, 5(2): 21–34.

Ashenafi, M. and M. Busse, 1991. The microflora of soak water during tempeh production from various beans. Journal of Applied Bacteriology, 70: 334-338.

Balqis, P., A. Noriham, A.K. Khalilah and N. Lilis, 2015. Natural population of lactic acid bacteria from different stages of traditional Malaysian tempe

production: Isolation and identification. Manuscript submitted for publication.

Balqis, P., N. Lilis, A. Noriham and A.K. Khalila, 2013. Isolation and Characterization of Lactic Acid Bacteria from Indonesian Soybean Tempe. In the 2013 4th International Conference on Biology, Environment and Chemistry IPCBEE, pp: 32–36).

Bezkorovainy, A., 2001. Probiotics : determinants of survival and growth in the gut 1-3. The American Journal of Clinical Nutrition, 73: 399–405.

Cintas, L.M., M.P. Casaus, C. Herranz, I.F. Nes and P.E. Hernández, 2001. Review: Bacteriocins of Lactic Acid Bacteria. Food Science and Technology International, 7: 281–305.

Darsanaki, R.K., M.L. Rokhi, M.A. Aliabadi and K. Issazadeh, 2012. Antimicrobial Activities of Lactobacillus Strains Isolated from Fresh Vegetables. Middle-East Journal of Scientific Research, 11(9): 1216–1219.
doi:10.5829/idosi.mejsr.2012.11.09.64152

De Vuyst, L. and E.J. Vandamme, 1994. Antimicrobial Potential of Lactic Acid Bacteria. In Bacteriocins of lactic acid bacteria Microbiology, Genetics and Applications, Eds., De Vuyst, L. and E. J. Vandamme. New York, London. Blackie Acedamic and Professional, 91-142.

Feng, X., 2006. Microbial dynamics during barley tempeh fermentation. Doctoral thesis, Swedish University of Agricultural Sciences Uppsala, Sweden.

Gilliland, S. and M. Speck, 1977. Antagonistic action of Lactobacillus acidophilus towards intestinal and foodborne pathogens in associative cultures.Journal of Food Protection, 40: 820-823.

Handajani, S., 2001. Indigenous mucuna tempe as functional food. Asia Pacific Journal of Clinical Nutrition, 10(3): 222–225.

Khalid, K., 2011. An overview of lactic acid bacteria. International Journal of Biosciences, 1(3): 1–13.

Kiers, J.L., J.C. Meijer, M.J.R. Nout, F.M. Rombouts., M.J.A. Nabuurs and J. van der Meulen, 2003. Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. Journal of Applied Microbiology, 95(3): 545–552. doi:10.1046/j.1365-2672.2003.02011.x

Kiers, J.L., R.M.J. Nout and F.M. Rombouts, 2000. In vitro digestibility of processed and fermented soya bean , cowpea and maize. Journal of the Science of Food and Agriculture, 80: 1325–1331.

Lelise, A., G. Belaynesh, M. Mulubrhan, S. Kedija, B. Endashaw and B. Abebe, 2014. Isolation and screening of antibacterial producing lactic acid bacteria from traditionally fermented drinks (“ Ergo ” and “ Tej ”) in Gondar town , Northwest Ethiopia. Global Research Journal of Public Health and Epidemiology, 1(3): 18–22.

Moreno, M.R.F., J.J. Leisner, L.K. Tee, C. Ley, S. Radu and G. Rusul, 2002. Microbial analysis of Malaysian tempeh , and characterization of two bacteriocins produced by isolates of Enterococcus faecium. Journal of Applied Microbiology, 92: 147–157.

Nout, M.J.R. and J.L. Kiers, 2005. Tempe fermentation, innovation and functionality: update into the third millenium. Journal of Applied Microbiology, 98(4): 789–805. doi:10.1111/j.1365-2672.2004.02471.x

Nout, M.J.R., M.A. De Dreu, A.M. Zuurbier and T.M.G. Bonants-Van Laarhoven, 1987. Ecology of controlled soyabean acidification for tempe manufacture. Food Microbiology, 4: 165-172.

Pundir, R.K., S. Rana, N. Kashyap and A. Kaur, 2013. Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study. Journal of Applied Pharmaceutical Science, 3(03): 85–93. doi:10.7324/JAPS.2013.30317

Shahidi Bonjar, G.H., 2004. Evaluation of antibacterial properties of Iranian medicinal-Plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae and Bordetella bronchoseptica. Asian Journal of Plants Sciences, 3(1): 82–86.

Srapinkornburee, W., U. Tassanaudom and S. Nipornram, 2009. The commercial development of red kidney bean tempeh. In International Conference on the Role of Universities in Hands-On Education, 434–444.

Sumathi, V. and D. Reetha, 2012. Screening of Lactic Acid Bacteria for Their Antimicrobial Activity against Pathogenic Bacteria. International Journal of Pharmaceutical & Biological Archives, 3(4): 802–808.

Tambekar, D.H., S.A. Bhutada, S.D. Choudhary and M.D. Khond, 2009. Assessment of potential probiotic bacteria isolated from milk of domestic animals. Journal of Applied Biosciences, 15(15): 815–819.