Special Issue, 2022, 1, 8 - 15: International Conference On Industry - Academia Initiatives In Biotechnology and Chemistry 2021

MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)

http://mjbmb.org

PRELIMINARY ASSESSMENT OF ANTIFUNGAL ACTIVITY OF LACTIC ACID BACTERIA FROM SELECTED MALAYSIAN TRADITIONAL FERMENTED FOOD

Sakina Shahabudin[1], Mohd Faizulnazrie Masri[1], Mohd Nizam Lani[2] and Nina Suhaity Azmi[1]*

[1]*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Gambang, Kuantan, Pahang, Malaysia*

[2]*Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia*

*Corresponding Author: nina@ump.edu.my

History

Received: 30 April 2022

Accepted: 15 November 2022

Keywords:

Candida albicans; Antifungal agents; Fermented food; Lactic acid bacteria; Antifungal activity

Abstract

Candida albicans, naturally a part of a healthy gut and oral microflora, is an opportunistic pathogen that commonly infects humans. However, current antifungal agents are made of synthetic drugs that cause varied adverse effects in patients. This drives efforts to look for natural and safer alternatives to antifungal agents. Fermented food has been studied for its ability to produce lactic acid bacteria with antimicrobial properties. This study examined the antifungal activity of lactic acid bacteria isolated from four traditional Malaysian fermented foods. Pickled chilies, pickled garlic, pickled *maman*, and *tapai* were prepared by using homemade recipes and fermented at 4°C. Lactic acid bacteria were isolated from the fermented food during a two-week fermentation on three selective growth agars. During fermentation, bacterial cells were counted at intervals. Growth trends for pickled garlic and pickled chilies presented an exponential phase between days 0 – 4, followed by stationary and death phases between days 8 – 14. Meanwhile, pickled *maman* and *tapai* showed continuous growth throughout the 14 days. Antifungal potential of the lactic acid bacteria was assessed via dual agar diffusion assay by spotting pickled water of each fermented food on De Man, Rogosa, Sharpe agar placed above a layer of Sabouraud Dextrose agar with pre-inoculated *C. albicans*, followed by measurement of the zone of inhibition. Pickled *maman* produced 1.2 ± 0.05 mm inhibition. In conclusion, pickled *maman* and *tapai* produced lactic acid bacteria throughout the two-week fermentation. Based on the dual agar diffusion, pickled *maman* showed an antifungal potential against *C. albicans*.

INTRODUCTION

Candida albicans is a ubiquitous fungus that asymptomatically lives in the gastrointestinal flora, oral or vaginal mucosa of healthy persons. During circumstances that compromise the host immune defenses, this fungal species may undergo a phase transition to become pathogenic. *C. albicans* is the most prevalent cause of both superficial and systemic candidiasis. *Candida* infections such as oral candidiasis and vulvovaginal candidiasis are mainly superficial, but they can be severe and life-threatening among immunocompromised individuals such as HIV/AIDS and cancer patients, neonates, and the elderly [1]. *C. albicans* is the most common *Candida* species causing invasive candidiasis [2]. *C. albicans* also caused 54% of cases of nosocomial bloodstream infections, the highest percentage among *Candida* species [3]. *Candida* species are major pathogenic fungi that raise clinical concerns due to their serious effects and high mortality rate in humans.

There have been many studies done to develop antifungal agents that can be used to treat *Candida* infections. Commonly used antifungal agents are classified into five groups (azoles, polyenes, echinocandins, pyrimidine analogues and allylamines) with different spectrum of activity and mechanism of actions [4–5]. However, these drugs can cause both mild and severe adverse effects in treated patients [6–7]. Severe side effects of amphotericin B such as hepatotoxicity and nephrotoxicity, may increase the risk of death [8]. Therefore, more efforts are carried out to explore natural resources that are safer to use, inexpensive, and possess antifungal activity against *Candida* species.

Tropical countries such as Malaysia are rich with natural ingredients, ranging from cereals, edible plants, marine and dairy products. Fresh food such as fruits and vegetables are available in abundance and accessible, but they are also easily perishable. Humans have been preparing and consuming fermented foods for centuries. Fermented foods are originally produced to preserve food during seasonal changes, but they also render other desirable qualities [9]. They have unique tastes, appearances, textures, and functions as compared to their raw, fresh ingredients. Lactic acid fermentation prolongs shelf life of perishable food and enhances their nutritional values. Many lactic acid bacteria including *Lactobacillus plantarum, L. pentosus, L. casei*, and *L. brevis* that are potential sources of probiotics can be found in fermented foods [10]. Widely studied for their probiotic potential, *Lactobacillus* species have also been found to show antifungal activity against pathogenic *Candida* species [11–13]. Probiotic foods are generally consumed to improve human natural microbiome and to be used in treating some medical conditions such as diarrhea [14]. Consumption of probiotic bacteria is believed to provide protection against pathogenic bacteria and yeasts. In this study, we examined four types of Malaysian traditional fermented food for their ability to produce lactic acid bacteria that have antifungal potential against human fungal pathogen *C. albicans*.

MATERIALS AND METHODS

Materials

De Man, Rogosa, Sharpe (MRS) broth was purchased from Formedium (Norfolk, United Kingdom). Sabouraud Dextrose Agar (SDA) was purchased from Merck KGaA (Darmstadt, Germany). Peptone bacteriological and dextrose bacteriological grade were purchased from Oxoid (United Kingdom). Agar-agar powder and calcium carbonate were purchased from QReC and Bendosen, respectively.

The ingredients to prepare fermented food were purchased at a supermarket in Kuantan, Pahang, Malaysia. The *maman* plants were purchased from a local wet market in Kuantan. The *C. albicans* strain used in this study was obtained from the microbial collections of Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang.

Preparation of Fermented Food Sources

Four different traditional food sources were prepared and fermented in homemade style by using standard recipes obtained from local sellers. The selected fermented foods were pickled *maman*, fermented glutinous rice (*tapai*), pickled garlics and pickled chilies.

Preparation of Pickled Maman

Pickled *maman* was prepared by using fresh ingredients including young leaves, stem, and shoots of *maman* plant (scientific name: *Cleome gynandra*), salt, filtered water, and cold cooked rice. First, the young *maman* shoots were cut into small parts, washed, and drained. The *maman* shoots were hand massaged with coarse salt until the leaves softened. Then, the softened *maman* shoots were put into a tight-lidded container and added with filtered water until the water covered all shoots. Fine salt and a small amount of cold cooked rice were added to the container and properly mixed. The container was tightly covered and kept at a cool place for 24 h. For the following three days, the concoction was thoroughly mixed and checked for sourness and saltiness for each day. After the third day, the concoction was continued to ferment at 4°C.

Preparation of Tapai

In preparing *tapai*, glutinous rice was washed and soaked for 12 h, then steamed until cooked. The steamed glutinous rice was mixed with a half glass of water. The glutinous rice was steamed again until the added water was thoroughly absorbed into rice, followed by cooling down the glutinous rice. A starter culture cake (also known as *ragi tapai*) was crumbled into small pieces and sieved through coffee filter. The *ragi* was thoroughly mixed into the glutinous rice. Then, the rice mixture was put into container and allowed to ferment at room temperature for two days. Afterwards, the fermentation process was continued at 4°C.

Preparation of Pickled Garlics

Pickled garlics were prepared by washing garlic bulbs without removing the outer peels, and steamed for 20 min. Water, salt, sugar, and vinegar were mixed in a separate pot and cooked until boiling. The steamed garlic bulbs were added to the boiling mixture and left to boil for 5 min. The bulbs were cooled down at room temperature and put into a tightly closed container. The pickled garlics were kept fermented at 4°C.

Preparation of Pickled Chilies

Pickled chilies were prepared by washing chilies, tossed dry, and cut into small pieces. The chilies were put into a glass jar and added with sugar and salt. Vinegar was added until all chili pieces were fully submerged. Afterwards, the jar was tightly closed and continued to ferment at 4°C.

Isolation of Lactic Acid Bacteria from Fermented Food Sources

Lactic acid bacteria were isolated from different fermented food sources by adapting techniques by Bulgasem *et al*. [11].

Approximately 10 g of each fermented food was mixed in 90 ml peptone water (0.1% w/v) in sterile tubes, and they were manually agitated. Then, 1 ml of the mixtures was added into 9 ml of MRS broth and incubated at 30°C for 24–48 h, followed by serial dilution with peptone water (0.1% w/v). Afterwards, 0.1 ml of the dilutions was separately spread plated on MRS agar, MRS agar with 0.8% calcium carbonate ($CaCO_3$), and MRS agar with 1% glucose. The plates were incubated anaerobically at 37°C for 24–48 h until the colonies grew sizably.

Culturing of *Candida* Strain

The *Candida* strain was grown and maintained on SDA agar at 4°C until further usage.

Dual Agar Overlay Assay against *C. albicans*

C. albicans (10^7 CFU/mL) was first inoculated on the surface of SDA and incubated at 25°C for 24 h. A layer of MRS agar (15% agar) was prepared, left until slightly warm and poured gently on top of the SDA layer with the pre-inoculated *C. albicans.* After the MRS agar layer had set, ten microliters of pickled water from each of the four fermented food were spotted onto the overlaid MRS agar. For negative control, sterile peptone water was used.

After 48-h aerobic incubation at 25°C, the inhibition zone diameter (IZD) around the colony diameter of the spots of pickled waters (CD) was observed and measured by using a ruler. The GIA (growth inhibitory activity) was calculated as the following equation:

$$\text{GIA (in mm)} = \frac{(\text{IZD} - \text{CD})}{2}$$

The GIA was recorded based on a GIA grading system [13] as shown in Table 1. All tests were repeated in duplicate, and the data were represented as mean ± standard deviation.

Table 1. GIA grading system

Growth inhibitory activity (mm)	Grade	Symbol
Less than 0.5	Negative	–
Between 0.5 and 2	Weak positive	+
Between 2 and 3.5	Intermediate positive	++
More than 3.5	Strong positive	+++

RESULTS AND DISCUSSION

Growth Trends of Fermentation

Four types of Malaysian traditional fermented food sources were made by using homemade recipes. The fermented foods were kept for storage at 4°C after fermentation started because fermented foods are usually kept in chiller for longer freshness and safe for human consumption. In this testing, the fermented foods were fermented over two weeks. Then, they were processed, serial diluted, and spread plated on three types of modified agar media. All agars contained nutrients needed for the growth and survival of lactic acid bacteria, with slight differences in each agar. Glucose was added as a primary source of carbon to promote growth of lactic acid bacteria. The addition of calcium carbonate is helpful to buffer the medium and maintain the pH [15]. The bacteria that grew well on MRS agars added with carbon carbonate and showed halo zones surrounding the colonies were presumed to be lactic acid bacteria. This is agreed by studies by Bulgasem *et al.* [11] and Jannah *et al*. [16]. The bacteria growing on MRS agars supplemented with glucose and MRS only agars showed typical morphological appearance as shiny, rounded, and milky white colonies (Table 2).

Table 2. Appearance of bacterial colonies growing on three types of agar media

	MRS + 0.8% $CaCO_3$	MRS + 1.0% glucose	MRS
Appearance of bacteria	Opaque white, circular colonies with surrounding clear zones	Opaque white, circular colonies	

Colony counts were taken at intervals of 4–6 days and recorded in the unit of colony forming unit per gram (CFU/g) that represented the total bacterial counts in each dilution (Table 3). The bacterial counts were ranging from 3.0 x10^7 to 7.0 x10^{11} CFU/g. Pickled *maman* and *tapai* produced comparably similar bacterial counts for all three types of agars during the two-week fermentation. From the start until the end of fermentation, the numbers of bacteria growing on each agar kept increasing.

In contrast, bacteria in pickled chilies only started to grow after Day 0. No bacterial count for pickled chilies was recorded for MRS agar for the whole fermentation period. After fermented for eight days, pickled chilies did not grow any bacteria. As for pickled garlics, bacterial counts were considerably high for all three agars since the start of fermentation period. However, no bacterial colonies were growing on MRS with added glucose and MRS agars on the eighth day of fermentation, followed by lack of growth on MRS agar with added $CaCO_3$ by the fourteenth day.

Table 3. Total bacterial counts (CFU/g) of microbial growth on three types of agar media

Fermented food source	Day of fermentation	Bacterial counts (CFU/g)		
		MRS + 0.8% $CaCO_3$	MRS + 1.0% glucose	MRS
Pickled chilies	0	0	0	0
	4	2.5×10^{10}	0	0
	8	1.7×10^{8}	6.0×10^{8}	0
	14	0	0	0
Pickled garlics	0	3.8×10^{10}	2.0×10^{9}	1.4×10^{10}
	4	2.2×10^{10}	3.0×10^{10}	8.6×10^{10}
	8	7.2×10^{9}	0	0
	14	0	0	0
Pickled *maman*	0	3.9×10^{10}	1.1×10^{10}	2.5×10^{10}
	4	1.8×10^{9}	2.8×10^{10}	2.5×10^{10}
	8	1.4×10^{10}	3.1×10^{10}	2.4×10^{10}
	14	2.3×10^{11}	6.9×10^{11}	6.7×10^{11}
Tapai	0	1.5×10^{10}	2.0×10^{10}	7.8×10^{9}
	4	1.8×10^{10}	7.8×10^{10}	4.6×10^{10}
	8	2.6×10^{9}	2.0×10^{10}	1.2×10^{10}
	14	1.3×10^{11}	1.7×10^{11}	1.9×10^{11}

Based on Figure 1, the data of pickled chilies was generated by using 10^{-5} dilution, which showed zero CFU/g. However, these results may not be accurate because the lower dilutions (10^{-1} to 10^{-4} dilutions) were not tested simultaneously. As for pickled garlics, the bacterial colonies were also inoculated from 10^{-5} dilution, showing high bacterial counts that reached 1.0×10^{10} CFU/g as early as Day 0. The high number of bacteria might be originated from the short period of fermentation possibly occurring at higher temperature when the boiled mixture of garlic bulbs was allowed to cool down. The growth trends for both pickled chilies and pickled garlics showed an exponential phase occurred within four days of fermentation and decreased to zero after two weeks of fermentation. The presence of vinegar in both pickled chiles and pickled garlics may have contributed to the low pH of both fermented foods, hence, creating an unfavorable condition for lactic acid bacteria to grow in a long period of time. Pickled chilies and pickled garlics both had pH level of 3 on Day 8 of fermentation. The pH values of different types of vinegars are ranging from 2.52 to 3.15 [17]. Meanwhile, most lactic acid bacteria generally grow at neutral pH levels of 4.5–7.0 [18]. At low pH, overacidification of the cultures does not only cause lactic acid bacteria to grow slower, but also cause cell damage and decrease cell viability [18]. Therefore, accumulation of acids over time may have affected the growth and survival of lactic acid bacteria in these two fermented foods.

In the meantime, pickled *maman* also showed high bacterial counts on Day 0. This is because pickled *maman* were left to ferment at room temperature for three days before plating was done on Day 0. As for *tapai*, the high bacterial counts on Day 0 were due to *tapai* being left to ferment at room temperature for two days before the first plating. The growth trends for pickled *maman* and *tapai* showed an exponential phase occurring between 8–14 days of fermentation. For this study, the number of bacterial counts remained high at the end of two-week fermentation, indicating that lactic acid bacteria in pickled *maman* and *tapai* remained viable over an extended period.

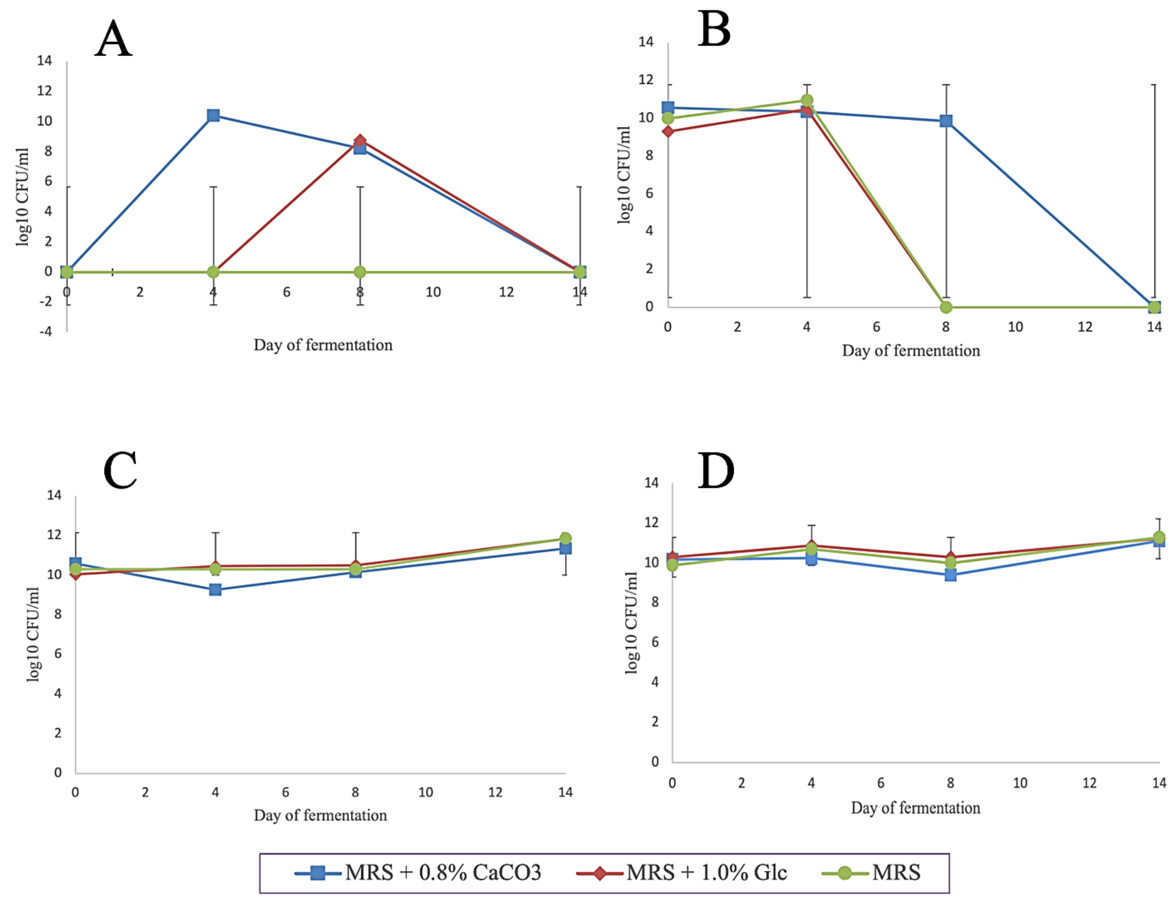

Figure 1. Growth trend of microbial growth of fermented food on three types of agar media. A: pickled chilies; B: pickled garlics; C: pickled *maman*; D: *tapai*

On the eighth day of fermentation, the pH values of *tapai* and pickled *maman* were 4 and 5, respectively. This is supported by Yusmarini *et al.* [19] that reported glutinous rice *tapai* that was fermented at room temperature for two days had a pH value at approximately 3.8. Sabah's *tapai* that was prepared as beverage, contrary to food, showed a slow decline of pH from 6.6 to 3.4 in fourteen days, followed by an increase to 4.0 [20]. On the other hand, there is not much information available on pickled *maman*. One study reported that after three-day fermentation, the pH values of *maman* leaves and stems decreased from 5.46 ± 0.02 to 4.70 ± 0.56, and from 5.38 ± 0.04 to 5.29 ± 0.01, respectively [21].

Yang *et al.* [22] found that the optimal pH for bacteriocinogenic lactic acid bacteria specifically *Lactobacillus curvatus*, *Lactobacillus paracasei* subsp. *Paracasei*, *Enterococcus faecium*, and *Streptococcus thermophilus* ranged from 6.2 to 8.5, demonstrating that neutral pH levels are crucial for bacteriocin production. However, they tested only the bacteriocin activity against food-borne pathogenic bacteria, but not *Candida* species [23]. It is possible that bacteriocins specific to target fungal pathogens may be able to grow at lower pH.

Antifungal Potential of Pickled Water from Fermented Food

During the preliminary testing, the antifungal potential of the fermented food was tested by dual agar diffusion method. This was done by growing *C. albicans* on the bottom SDA agar layer, followed by spotting approximately 10 µl of pickled water of each fermented food on the top MRS agar layer. Based on Table 4, out of the four fermented foods, only pickled *maman* formed zones of inhibition surrounding the spots of pickled water. Pickled *maman* produced 1.2 ± 0.05 mm inhibition zone. This is a weak positive GIA result and not significant enough as relative to previously reported zones of inhibition for antifungal activity. Other publications that reported significant zones of inhibition for antifungal activity used isolated lactic acid bacteria [11,13], meanwhile this study used unprocessed pickled water from the fermented food sources. The difference in the tested samples themselves may have led to the low significance level in this study.

Even though the spreading growth of fungi surrounding the agar surface was uneven, circular shapes were visible around the spots of pickled water of pickled *maman* on the fourth day of fermentation (Figure 2). On the other hand, several ambiguous shapes resembling spots on the plates of pickled chilies were not accepted as positive GIA and considered as negative inhibition.

Table 4. Formation of zone of inhibition around the spots of pickled water of fermented food on agar dual diffusion assays. Diameter of zone of inhibition is presented in the unit of mm. GIA symbol is presented in the parentheses

Day of fermentation	Negative control	Pickled chilies	Pickled garlics	Pickled *maman*	*Tapai*
0	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)
4	0 ± 0 (-)	0.20 ± 0.05 (-)	0 ± 0 (-)	1.20 ± 0.05 (+)	0 ± 0 (-)
8	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)
14	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)	0 ± 0 (-)

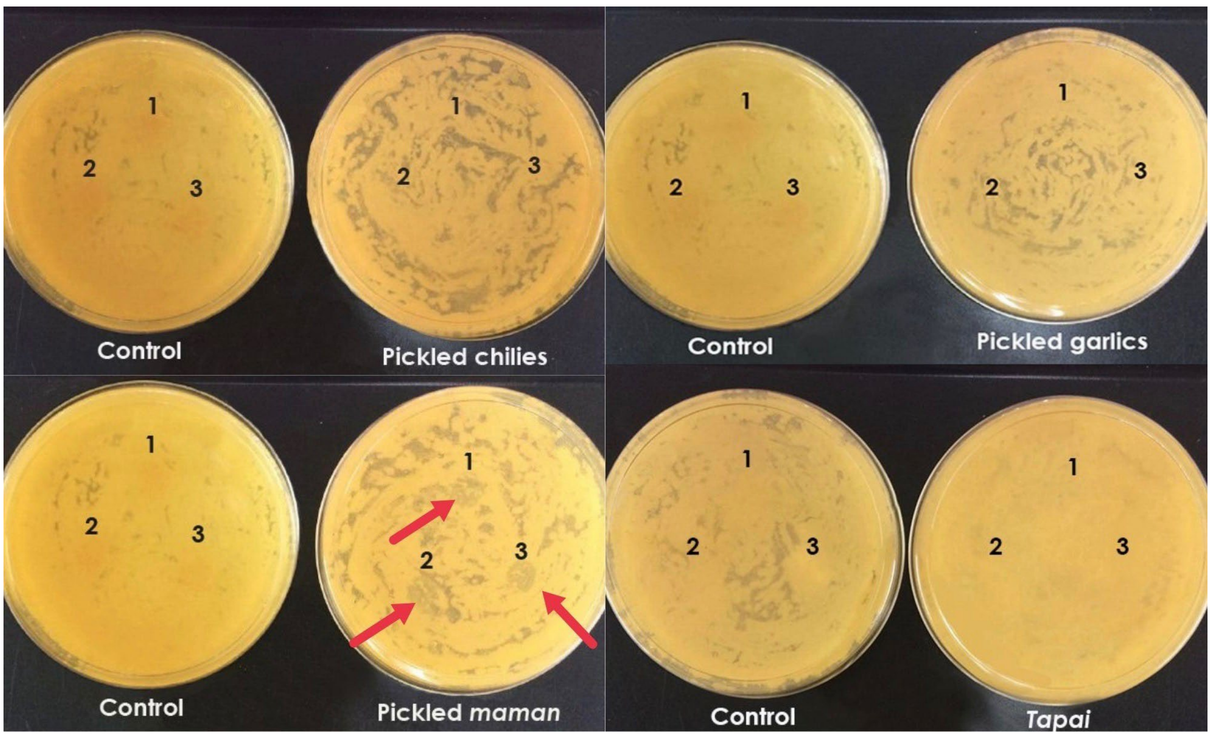

Figure 2. Formation of zone of inhibition surrounding spots of pickled water from four fermented food by dual agar diffusion assay as observed on the Day 4 of fermentation. The numbers 1, 2 and 3 represented the quantities and locations of the spots. Red arrows are showing the clear zones of inhibition around spotted pickled water samples

In this study, there is a possibility that within four days of fermentation, the lactic acid bacteria strains from pickled *maman* produced functional metabolites that can inhibit *C. albicans* growth. No similar inhibition effect was seen on other days of fermentation. After the fermentation went beyond a week, the affluent growth of *C. albicans* had dominated the area on agar. *C. albicans* grows best in aerobic conditions, while lactic acid bacteria thrive in anaerobic conditions. The dual agar plates were incubated aerobically, encouraging the growth of *C. albicans* instead of lactic acid bacteria. This might have caused lactic acid bacteria to be overwhelmed by fungi and ceased to survive in the plates after more than 7 days. Therefore, the lack of production of the metabolites with antifungal potential produced negative GIA for all samples on the Days 8 and 14 of fermentation.

Antifungal activity of lactic acid bacteria against fungal pathogen *C. albicans* has been previously reported by several studies. Crowley *et al.* [24] mentioned about active metabolites produced by lactic acid bacteria including reuterin, fatty acids, and bacteriocins that exhibit antifungal effects against *C. albicans*. Axelsson *et al.* [25] had discovered reuterin, an antimicrobial compound produced by *Lactobacillus reuteri* and demonstrated broad-spectrum ability to inhibit growth of many bacterial pathogens. Two *L. reuteri* strains showed antifungal properties against five common oral *Candida* species, including *C. albicans, C. glabrata, C. tropicalis, C. dubliniensis*, and *C. parapsilosis* [26]. Bergsson *et al.* [27] reported that medium-chain fatty acids such as lauric (C_{12}) and capric (C_{10}) acids were potent in killing the cells of *C. albicans.* Adebayo and Aderiye [28] investigated the effects of a bacteriocin called brevicin SG1 produced by *Lactobacillus brevis* SG1 against *C. albicans* cells, and observed that it reduced yeast hyphal branching at lower concentration, while completely arresting hyphal development at higher concentration. Based on the results of this preliminary testing involving Malaysian fermented food, further investigation of possible functional metabolites produced by lactic acid bacteria isolated from pickled *maman* could be carried out to discover their interaction or mechanism in affecting the fungal growth.

The pervasive presence of *C. albicans* and other kinds of fungi on human skin and mucous membranes can become pathogenic and cause severe implications if they invaded systemic organs. The currently limited number of safe antifungal treatments has encouraged efforts among researchers to discover better alternatives of antifungal drugs. This study provided preliminary results of antifungal potentials of lactic acid bacteria isolated from selected Malaysian fermented foods by dual agar diffusion assay. Pickled *maman* and *tapai* showed ability to sustain the growth of lactic acid bacteria over prolonged fermentation period at low temperature. The pH level of fermented foods played an important role in supporting the growth and viability of lactic acid bacteria. Pickled *maman* showed weak antifungal activity against *C. albicans* among all four fermented foods. However, this testing needs to be improved to obtain a better GIA score.

In future works, pure bacterial colonies will be isolated from these fermented foods for further characterization of probiotic properties. Finally, the alcohol content in these fermented foods should be determined over a prolonged fermentation period. This will be valuable insight to determine the Halal status of fermented food in Malaysia.

ACKNOWLEDGEMENTS

This study acknowledges grant supports from the Universiti Malaysia Pahang (UMP-RDU180304) and the Postgraduate Grant Research Scheme (PGRS190352).

CONFLICT OF INTEREST

The authors declare that no conflict of interest is involved in the preparation of this manuscript.

REFERENCES

1. Lescher, P.J. (2011) in Pathology for the Physical Therapist Assistant. F. A. Davis Company, Philadelphia, pp. 357.
2. Pfaller, M.A. and Diekema, D.J. (2007) Epidemiology of invasive candidiasis: A persistent public health problem. Clin. Microbiol. Rev. 20 (1), 133–163.
3. Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P. and Edmond, M.B. (2004) Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39 (3), 309–317.
4. Deoghare, A. (2014) in Antifungal drugs, in Textbook of Oral Medicine (Ghom A.G. and Ghom, S.A., eds.) Jaypee Brothers Medical Publishers, New Delhi, pp. 977–982.
5. Scorzoni, L., de Paula e Silva, A.C.A., Marcos, C.M., Assato, P.A., de Melo, W.C.M.A., de Oliveira, H.C., Costa-Orlandi, C.B., Mendes-Giannini, M.J.S. and Fusco-Almeida, A.M. (2017) Antifungal therapy: New advances in the understanding and treatment of mycosis. Front. Microbiol. 8, 36.
6. Workman, M.L. and LaCharity, L.A. (2016) in Anti-infectives: Antitubercular and antifungal drugs. In Understanding Pharmacology - E-Book: Essentials for Medication Safety. Elsevier, St Louis, MI, pp. 157–169.
7. McManus, D.S. (2017) in Antifungal drugs. In Side Effects of Drugs Annual: A Worldwide Yearly Survey of New Data in Adverse Drug Reactions (Ray, S.D., ed.) Elsevier, Amsterdam, Netherlands, vol. 39, pp. 245–258.
8. Hamill, R.J. (2013) Amphotericin B formulations: A comparative review of efficacy and toxicity. Drugs 73 (9), 919–934.
9. Tamang, J.P., Cotter, P.D., Endo, A., Han, N.S., Kort, R., Liu, S.Q., Mayo, B., Westerik, N. and Hutkins, R. (2020) Fermented foods in a global age: East meets West. Compr. Rev. Food Sci. Food Saf. 19, 184–217.
10. Swain, M.R., Anandharaj, M., Ray, R.C. and Rani, R.P. (2014) Fermented fruits and vegetables of Asia: A potential source of probiotics. Biotechnol. Res. Int. 2014, article ID 250424.
11. Bulgasem, B.Y., Lani, M.N., Hassan, Z., Wan Yusoff, W.M. and Fnaish, S.G. (2016) Antifungal activity of lactic acid bacteria strains isolated from natural honey against pathogenic *Candida* species. Mycobiology 44 (4), 302–309.
12. Matsubara, V.H., Wang, Y., Bandara, H.M.H.N., Mayer, M.P.A. and Samaranayake, L.P. (2016) Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Appl. Microbiol. Biotechnol. 100, 6415–6426.
13. Wang, S., Wang, Q., Yang, E., Yan, L., Li, T. and Zhuang, H. (2017) Antimicrobial compounds produced by vaginal *Lactobacillus crispatus* are able to strongly inhibit *Candida albicans* growth, hyphal formation and regulate virulence-related gene expressions. Front. Microbiol. 8, 564.
14. Matussin, N.B.A., Chin, Y.Y., Ishan, I. and Shivanand, P. (2016) Fermented food in Asia as a source of potential probiotics: Properties and beneficial effects. Scientia Bruneiana – Special Issue 15.
15. Marković, M., Markov, S., Pejin, D., Mojović, L., Vukašinović, M., Pejin, J. and Joković, N. (2011) The possibility of lactic acid fermentation in the triticale stillage. Chem. Ind. Chem. Eng. 17 (2), 153−162.
16. Jannah, S.N., Saraswati, T.R., Handayani, D. and Pujiyanto, S. (2018) Antibacterial activity of lactic acid bacteria isolated from

gastrointestinal tract of "Ayam Kampung" chicken against food pathogens. J. Phys. Conf. Ser. 1025, 012082.

17. Jamaludin, M.A., Amin, A., Fadzlillah, N.A., Kartika, B., Othman, R., Sani, S. and Ramli, A. (2017) Study on physiochemical properties and the halalness of commercially marketed vinegar in Malaysia. Int. Food Res. J. 24, S428–S435.

18. Hutkin, R.W. and Nannen, N.L. (1993) in pH Homeostasis in Lactic Acid Bacteria. Faculty Publications in Food Science and Technology, no. 28.

19. Yusmarini, Johan, V.S., Fitriani, S., Rahmayuni, Artanti, V.F. and Pato, U. (2019) Characteristics of probiotic tapai made by the addition of *Lactobacillus plantarum* 1. Int J. Agric. Technol. 15 (1), 195–206.

20. Chiang, Y.W., Chye, F.Y. and Mohd Ismail, A. (2006) Microbial diversity and proximate composition of tapai, a Sabah's fermented beverage. Malays. J. Microbiol. 2 (1), 1–6.

21. Muhialdin, B.J., Sukor, R., Ismail, N., Ahmad, S.W., Che Me, N. and Meor Hussin, A.S. (2018) The effects of fermentation process on the chemical composition and biological activity of Spider Flower (*Gynandropsis gynandra*). J. Pure Appl. Microbiol. 12 (2), 497–504.

22. Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S. and Waler, B. (2018) Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. AMB Express 8, 10.

23. Yang, E., Fan, L., Jiang, Y., Doucette, C. and Fillmore, S. (2012) Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express 2 (1), 48.

24. Crowley, S., Mahony, J. and van Sinderen, D. (2013) Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. Trends Food Sci Technol. 33 (2), 93–109.

25. Axelsson, L.T., Chung, T.C., Dobrogosz, W.J. and Lindgren, S.E. (1989) Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. 2, 131–136.

26. Jørgensen, M.R., Kragelund, C., Jensen, P.Ø., Keller, M.K. and Twetman, S. (2017) Probiotic *Lactobacillus reuteri* has antifungal effects on oral *Candida* species *in vitro*. J. Oral Microbiol. 9 (1), 1274582.

27. Bergsson, G., Arnfinnsson, J., Steingrimsson, O. and Thormar, H. (2001) In vitro killing of *Candida albicans* by fatty acids and monoglycerides. Antimicrob. Agents Chemother. 45 (11), 3209–3212.

28. Adebayo, C.O. and Aderiye, B.I. (2011) Suspected mode of antimycotic action of brevicin SG1 against *Candida albicans* and *Penicillium citrinum*. Food Control 22 (12), 1814–1820.