Chemical and Biomolecular Engineering
2016; 1(2): 49-54
http://www.sciencepublishinggroup.com/j/cbe
doi: 10.11648/j.cbe.20160102.14

Antifungal Activity of Lactic Acid Bacteria Isolated from *Nem Chua*

Huynh Xuan Phong, Tran Hong Van, Nguyen Ngoc Thanh, Bui Hoang Dang Long, Ngo Thi Phuong Dung

Biotechnology Research and Development Institute, Can Tho University, Can Tho City, Vietnam

Email address:
hxphong@ctu.edu.vn (H. X. Phong)

To cite this article:
Huynh Xuan Phong, Tran Hong Van, Nguyen Ngoc Thanh, Bui Hoang Dang Long, Ngo Thi Phuong Dung. Antifungal Activity of Lactic Acid Bacteria Isolated from *Nem Chua*. *Chemical and Biomolecular Engineering*. Vol. 1, No. 2, 2016, pp. 49-54.
doi: 10.11648/j.cbe.20160102.14

Received: December 15, 2016; **Accepted**: January 5, 2017; **Published**: January 24, 2017

Abstract: Lactic acid bacteria (LAB) play an important role in fermented food preservation thank to their antifungal ability as well as bacteriocin activity. Fermented meat products including *Nem chua* are usually placed under negative effects due to typical manual production process, uncontrolled ambient preservation condition, and consuming without cooking. In this study, the antifungal ability of lactic acid bacteria isolated from *Nem chua* was examined. Five *Nem chua* samples were collected at the local markets in Mekong Delta, Vietnam. They had good sensory evaluation (typical odor of lactic acid fermentation product like sour, meat and leaf odor) with the total score over 7/10. Average pH for 5 samples was found at 4.7 and average lactic acid content was 1.6 g/100 g of sample. The results of enumeration of total plate count and mould were found to be acceptable (3.0×10^7 CFU/g and 3.3×10^3 CFU/g, respectively) and average LAB count was 2.2×10^7 CFU/g. From 5 samples of *Nem chua*, 19 isolates of LAB and 9 isolates of mould were collected. Antifungal activity was found in most LAB isolates with various degrees. Only 9 LAB isolates, 47% (P32B, P41A, V13A, P21B, P31B, R11B, R14B, R22B and K34B) exhibited strong activity against 7/9 mould isolates (over 10+ degree of activity). Isolates P32B, P41A and V13A were chosen to be sequenced due to their strong inhibitory activity and were identified as *Lactobacillus plantarum* (P32B and V13A) and *Pediococcus pentosaceus* (P41A).

Keywords: Antifugal, Lactic Acid Bacteria, *Lactobacillus plantarum*, *Nem chua*, *Pediococcus pentosaceus*

1. Introduction

Meat fermentation became popular as adding sugar and salt to get nutritious and tasty food. *Nem chua* is one of typical traditional foods of Vietnam made of pork, pork skin and other materials. Frequently, *Nem chua* is produced manually and locally with natural fermentation process. Besides, this kind of food is usually consumed after 3 to 4 days fermentation without cooking. Therefore, food safety is in concern for producer and consumers. Mould contamination is commonly detected in fermented meat products [1]. Nowadays, chemical preservation has been denied in food industry because of some negative effects on health. Thus, it is necessary to screen for microorganisms have potential on fermenting, stabilizing, improving food quality as well as preserving food.

Lactic acid bacteria (LAB) have been widely applied in food fermentation and preservation. The use of LAB is aim to produce organic acids such as lactic acid. Beside the widely known bacteriocin activity, LAB were found to have antifungal activity [2]. They produce antifungal compounds like organic acids, hydrogen peroxide [3], cyclic dipeptide, hydroxyl fatty acid [2], etc.

Hence, isolation and selection of antifungal lactic acid bacteria become necessary to contribute to the improvement of LAB fermentation and preservation.

2. Experimental

2.1. Materials

Five samples of *Nem chua* were collected from difference sources in Can Tho province, Vietnam. Medium: MRS agar (De Man, Rogosa and Sharpe), MRS broth, MEA (Malt extract agar), PCA (Plate count agar), SDA (Sabouraud dextrose agar).

2.2. Collection of Samples

Five samples of *Nem chua* were bought from Xuan Khanh market and local stores in Can Tho City, Vietnam. These samples were used to evaluate quality, pH value, lactic acid

amount; to enumerate total plate count, mould and LAB; to isolate LAB and mould.

2.3. Sensory Evaluation, Determination of pH Value and Lactic Acid Content

Sensory of *Nem chua* was determined by evaluating color, flavor, viscosity, odor and mould presence. pH value was determined by pH meter. Lactic acid content was analyzed by titrate method with NaOH 0.1N and also determined through Therner value.

2.4. Enumeration of Total Plate Count, LAB and Moulds

Ten grams of sample was homogenized with 90 mL SPW and diluted. Culture medium (15-20 mL) was poured into each dish which content 1 mL of sample solution. The culture medium varied for each criterion, PCA used for total plate count, MRS used for LAB and SDA used for mould. Inoculated dishes were incubated at 30°C for 72 h and counted all the typical colonies.

2.5. Isolation and General Identification of LAB from Nem Chua

The mix of 10 g sample and 90 mL peptone water was homogenized for 30 seconds and of which 20 mL solution was transferred to a flask containing 100 mL MRS broth. After incubation at 37°C for 48 h, each suspension was spread on MRS agar and anaerobically incubated at 37°C for 48 hrs. Typical colonies were sub-cultured several times by streaking on new MRS dishes until the purity of the isolate was obtained.

Pure culture was defined by observing colony morphology on dish and cell morphology in microscope. Bacterial isolates were characterized by Gram staining, catalase test, oxidase test and disintegration of $CaCO_3$.

2.6. Isolation of Moulds from Nem Chua

Ten grams sample was homogenized with 90 mL peptone water. 1 mL of this solution was spread on SDA dish. Culture dishes were incubated at 25°C for 48-72 hrs. The colonies were detected and sub-cultured to new SDA dishes. This subculture was continued until pure isolates of mould were obtained. Pure isolates were determined by observing colony morphology on dishes and cell morphology in microscopes.

2.7. Determination of Antifungal Activity of LAB Isolates

Mould isolates were transferred to malt extract agar to grow at 25°C for 5-7 days. Then, mould spores were collected by adding peptone water into culture dish and withdrawing that solution after shaking. Spore concentration was determined and adjusted to 10^5 spores/mL peptone water.

Dual culture overlay assay [4] was applied to detect the inhibitory activity of LAB against mould. LAB isolates were inoculated in two 2 cm lines on MRS agar plates and allowed to grow at 30°C for 48 hrs. The plates were then overlaid with 10 mL of malt extract soft agar (0.05% malt extract, 1% agar) containing 10^5 spores/mL of mould (the ratio of 1 mL mould inoculum with 10 mL malt extract agar). After incubation at 30°C for 48 h, the inhibition zone was measured.

2.8. Identification of the Selected LAB Isolates

The target LAB isolates were identified based on molecular technique. Bacterial 16S rDNA was amplified by PCR using the primers 1492R (5'-TACGGTTACCTTGTTACGACT-3') and 27F (5'-AGAGTTTGATCCTGGCTC-3'). The resulting PCR product was purified and sequenced using automated sequence analyser. Nucleotide sequence was aligned and compared with the data obtained from Gene Bank (www.ncbi.nlm.nih.gov/).

3. Results and Discussion

3.1. Sensory Evaluation, pH Value and Lactic Acid Amount

Sensory evaluation

Mould growth was not detected in all samples by vision. Most samples had sour flavor, good taste. However, there were differences for other criteria. For color, good product was supposed to be reddish. Only three samples from Cai Rang met this requirement while Tu Kien sample had deep red color and Xuan Khanh sample was light pink. For odor, *Nem chua* usually has typical odor of lactic acid fermentation product like sour, meat odor, leaf odor. The smell of Tu Kien sample was too strong while Xuan Khanh sample had slight odor.

After fermentation, lactic acid was produced making meat material become sticky and packed together but if products are too viscous, the flavor and appearance might be affected. Good viscosity of Tu Kien and Trang samples made up good construction and taste for products. Xuan Khanh sample did not meet the requirement for this since the materials did not stick tightly together.

Generally, 3 samples from Cai Rang had better results of sensory evaluation when they got above 9/10. Tu Kien and Xuan Khanh still had acceptable results with 7.34/10.

Determination of pH value

The results indicated that there were no statistically significant differences between pH values of Co Phuc and Tu Kien. Xuan Khanh sample had the lowest pH (4.23) while that value of Thu Oanh and Trang were over 5.0. This meant CP, TK and XK were more acidic than TO and NT (Table 1). After fermentation for 3 to 5 days, pH value was supposed to be 4.0 or 5.0. In fact, 3 samples had pH value belong to this range (XK, CP, TK). However, these were pH of the fourth day fermentation so that these values may drop in the following days. Samples with pH over 5.0 also might continue reducing to less than 5.0 to reach the expected value.

Table 1. *pH value at the fourth day fermentation.*

No	Sample	Abbreviation	pH
1	Tu Kien	TK	4.64^{c}
2	Thu Oanh	TO	5.35^{a}
3	Co Phuc	CP	4.74^{c}
4	Trang	NT	5.19^{b}
5	Xuan Khanh	XK	4.23^{d}
CV			9.2%

Note: Value in the table was average value of triplication; values with the same letter were not significantly different at 95% confidence level.

Compared to the pH values of *Nem chua* from Tran [6] at the fourth day fermentation, this result showed more differences between samples with higher values. Five

samples of Tran Thi Thanh Thao collected from Ho Chi Minh city had similar pH, around 4.15 to 4.41. Generally, samples had average pH about 4.7. At this pH value, unexpected organisms might be inhibited apart from moulds (moulds are inhibited at pH < 2) [5].

Determination of lactic acid content

As shown in Table 2, XK sample had the highest lactic acid content with 2.46 g/100 g sample and 273.33°T. Following XK, TK samples had 2.09 g acid/100 sample and 231.67°T. Three samples from Cai Rang stayed at the bottom. In fact, TO and NT samples did not show statistically significant difference with 1.11 g and 1.15 g acid/100 g sample. This also meant Therner value of these two was lowest over all. In addition, CP sample had significant acid content with 1.35 g and 150°T.

Table 2. *Therner value and lactic acid content.*

No	Sample	Therner value (°T)	Lactic acid (% w/v)
1	TK – Lai Vung	231.67 [b]	2.09 [b]
2	TO – Cai Rang	123.33 [d]	1.11 [d]
3	CP – Cai Rang	150.00 [c]	1.35 [c]
4	T – Cai Rang	127.50 [d]	1.15 [d]
5	XK – Ninh Kieu	273.33 [a]	2.46 [a]
CV		37.3%	37.3%

Note: Value in the table was average value of triplication; values with the same letter were not significantly different at 95% confidence level.

In fermentation of liquid products, LAB ferment materials to get 300°T, approximately 2.7 g lactic acid in 100 g sample. For the situation of *Nem chua*, the results were rational.

3.2. Enumeration of Total Plate Count, Mould and LAB

Low pH, a factor could inhibit other bacteria but favor the growth of LAB. Therefore, LAB was mostly dominant in the microorganism population of products. However, moulds were found to be able to resist this condition because pH was not low enough to inhibit completely these creatures. Furthermore, mould growth was difficult to prevent because *Nem chua* was produced manually, normal preservation condition and raw consumption.

As shown in Table 3, average total plate count of *Nem chua* was 3.0 x 10^7 CFU/g. Specifically, NT sample had the highest total plate count with 3.9 x 10^7 CFU/g while TO sample had the lowest count with 2.2 x 10^7 CFU/g. In comparison to the limitation [7] and WQA standard with 10^7 CFU/g, these results seem to be quite high but still acceptable. In addition, total plate count somehow shows the risk of unexpected microorganisms and food damage. Therefore, fermented meat products can consider to be easily infected by unexpected microorganisms, which lead to the need of effective treatment methods.

Table 3. *Enumeration of total plate count, mould, and LAB (CFU/g).*

No	Sample	Total plate count	Mould	LAB
1	Tu Kien	$2.8x10^7$	$1.9x10^3$	$1.7x10^7$
2	Thu Oanh	$2.2x10^7$	$2.0x10^3$	$1.4x10^7$
3	Co Phuc	$2.3x10^7$	$1.5x10^3$	$1.6x10^7$
4	Trang	$3.9x10^7$	$7.4x10^3$	$2.7x10^7$
5	Xuan Khanh	$3.3x10^7$	$3.8x10^3$	$3.5x10^7$

Note: Value in the table was average value of triplication.

Mould is one of the main causes of food poison and deterioration. The defined mould count from *Nem chua* was quite high with 3.3 x 10^3 CFU/g in average. NT sample was detected with the highest mould density 7.4 x 10^3 CFU/g, followed by XK sample with 3.8 x 10^3 CFU/g. CP sample had the least present of mould, 1.5 x 10^3 CFU/g. However, compared to the WQA standard with 10^4 CFU/g, these figures of mould could be considered acceptable. Furthermore, this result was recorded at the fourth day of fermentation so that the mould density might rise in the following days. Once LAB growth decreases, the extension of mould might rise instantly.

LAB count for *Nem chua* was 2.2 x 10^7 CFU/g in average. The highest LAB density belonged to XK sample with 3.5 x 10^7 CFU/g while the lowest count was found in TO sample, 1.4 x 10^7 CFU/g. In comparison to the LAB density counted by Tran [6] with 10^8 CFU/g, those figures of *Nem chua* from this study were lower. Generally, 5 *Nem chua* samples collected from Can Tho had close values to each other.

3.3. Isolation of Lactic Acid Bacteria from Nem Chua

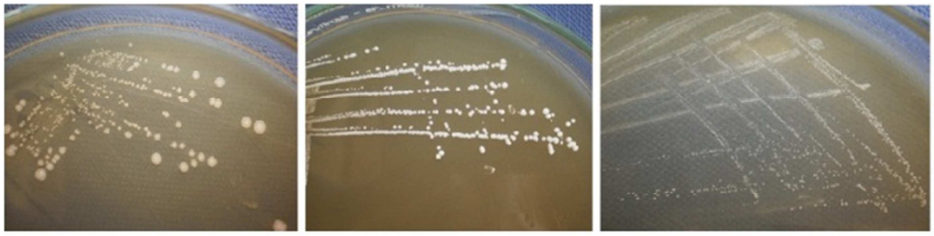
Totally, 19 isolates of LAB were collected throughout the process. Sample sources and code of 19 LAB were shown in Table 4. The coding for LAB isolate was formed by following the principle, the first letter was taken from source's name, the number was the order of isolate, and the final letter was added defined as the replicate, to differentiate from mould isolate (mould isolate code just include 3 letters).

Table 4. *Sample sources and codes of LAB isolates.*

No	Source	Isolate code
1	Tu Kien	V11B, V13A, V21B, V31B
2	Thu Oanh	O22A, O32A, O33A
3	Co Phuc	P21B, P31B, P32B, P41A
4	Trang	R11B, R13A, R14B, R22B, R33B
5	Xuan Khanh	K21A, K32A, K34B

All bacterial colonies had circular shape, round edge or lobe edge, opaque or milky color (Figure 1). There were 5 isolates with low bulgy surface. 11 isolates were found to have medium colony size (58%), 1-2 mm in diameter, 6 over 19 isolates had small colony (>1 mm in diameter), only 2 isolates had colony's diameter more than 2 mm. Ten isolates had short rod shape (53%) and 9 isolates had cocci shape. Although cell shape might be various among LAB isolates, still there was size differences. All the variety of LAB morphology showed the diversity of this kind of bacteria in the natural environment.

All LAB isolates had deep blue color of violet-iodine complex so that they were concluded as Gram (+) bacteria. Catalase test with H_2O_2 agent was applied and all isolates showed negative results (no gas formation). Oxidase test was applied and negative results were obtained by most isolates without changing color of the paper. All isolates had ability to disintegrate $CaCO_3$ due to the formation of lactic acid. Positive results were obtained with the formation of clear zone surrounding bacterial colonies in MRS medium containing 1.5% of $CaCO_3$.



Note: (a): K21A, milky white, large; (b): P41A, opaque, mediume; (c): O32A, milky white, small size.

Figure 1. *Colony morphology of isolated LAB.*

Generally, collected bacterial isolates had round, smooth, white colonies, cocci or rods, producing typical smell of acid. They were Gram (+), negative catalase, negative oxidase and have ability to disintegrate $CaCO_3$. These characteristics were identical to description about LAB [8].

3.4. Isolation of Moulds from Nem Chua

Nem chua samples were used to isolate mould by subculture in SDA medium until colony uniform and cell uniform were obtained. As result, 9 isolates were collected from different samples (TK: L41 and L42; TO: A11 and A24; CP: C21 and C23; NT: T22 and T71; and XK: X20).

All isolates had spores and 8 isolates (89%) had dark spore while L41 had white spores. The length of mycelia was different among isolates so that the height of colony was also various. Moreover, colony color was typical for each isolate due to the color of spores and mycelia. Mould colony grew quickly in the medium and mycelia spread widely so that morphology of colony might change constantly. However, the original shape was circular for all isolates.

All isolates had spores, gray or black, branching mycelia, cell wall or no, sphere or pear vesicle, single layer seriation. Different morphologies of mould were shown in Figure 2. Isolate T22 had dark green color, X20 had sphere vesicle and dark green spores. In addition, L41 had white spores and C21 had sphere vesicle with black spores.

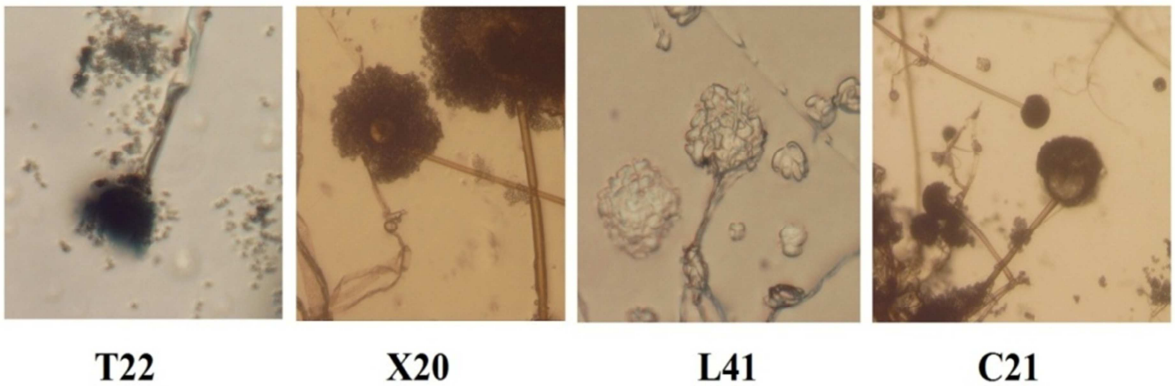


Figure 2. *Mould morphology under microscope in the magnitude of 400 times.*

3.5. Determination of Antifungal Activity of LAB Isolates

Effects antifungal activity was represented by the clear zone (inhibitory zone) which was measured lately. The measurement of inhibitory zone was the average distance (d) from the edge of LAB colony line to the edge of clear zone. Then, d value would be transformed to degree of inhibitory activity (- or +, ++, +++). Figure 3 illustrates inhibitory degrees of anti-mould activity performed by LAB against moulds collected in this assay.

Based on the results, (-) did not have inhibitory activity, (+) showed weak activity with $d \leq 2$ mm, (++) showed medium activity with $d \leq 8$ mm, and (+++) was considered as strong activity with $d > 8$ mm. The overall summary of inhibitory test of 19 LAB isolates against 9 mould isolates was illustrated in Table 5.

The results showed that P32B performed the strongest activity over all with total 22+against all 9 mould isolates. This result was outstanding with strong inhibitory activity (+++) against 4 mould isolates L42, C21, T22 and T71 (44%), moderate activity (++) against other five, no (-) result was recorded. Following P32B, two isolates V13A and P41A also gave good results. V13A was found to be able to inhibit 9 mould isolates but its inhibitory activity was not as good as P32B's and got 18+in total.

It was noticeable that V13A showed strong activity (+++) to T22 and T71 while A24 and X20 were weakly inhibited by this isolate. In addition, P41A stayed at the third place with total 17+against 8 over 9 mould isolates. Strong inhibition was performed by this isolate to L42 and T22. However, antifungal ability was not effective to C21. Beside three LAB isolates above, there were 6 other isolates with good inhibitory activity against 7 over 9 moulds and over 10+degree of inhibition. They included P21B, P31B, R11B, R14B, R22B and K34B. Thus, there were generally 9 over 19 LAB isolates showed good inhibitory activity (47%). Having opposite pattern, V31B and O33A could inhibit only 1 mould T71 with moderate activity 3+and 2+, respectively.

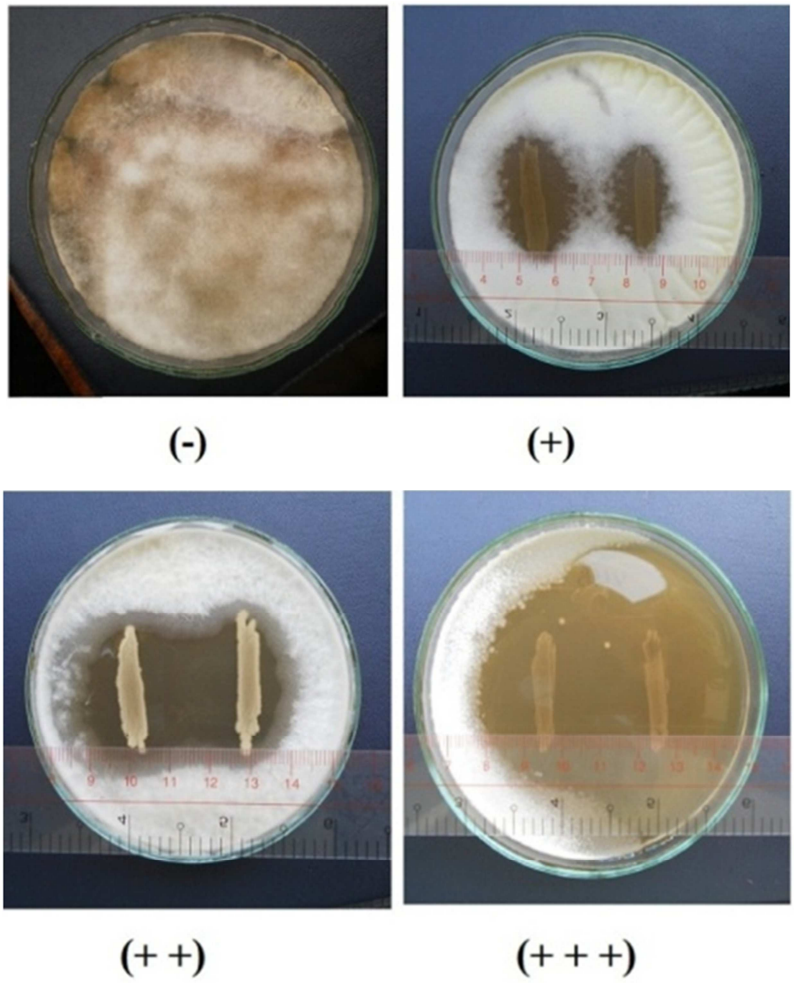


Figure 3. *Degrees of antifungal activity of LAB. Levels of appearance of antifungal activity ranging from - (nothing) to +++ (very much).*

Table 5. *Antifungal activity of LAB isolates.*

LAB	MOULDS									Antifugal activity [1]	Number of inhibited mould isolates
	L41	L42	A11	A24	C21	C32	T22	T71	X20		
V11B	- [2]	-	-	++	+	+	++	+++	-	9+	5/9
V13A	++	++	++	+	++	++	+++	+++	+	18+	9/9
V21B	-	+	-	+	-	-	-	+	-	3+	3/9
V31B	-	-	-	-	-	-	-	+++	-	3+	1/9
O22A	-	-	-	-	+	-	-	+++	-	4+	2/9
O32A	-	-	-	-	+	-	+	+++	-	5+	4/9
O33A	-	-	-	-	-	-	-	++	-	2+	1/9
P21B	++	-	+	+	+	+	++	+++	-	11+	7/9
P31B	++	-	++	++	++	++	++	+++	-	15+	7/9
P32B	++	+++	++	++	+++	++	+++	+++	++	22+	9/9
P41A	++	+++	+	++	-	++	+++	++	++	17+	8/9
R11B	++	-	-	++	++	++	+++	++	+	14+	7/9
R13A	+	-	+	-	++	+	++	-	-	7+	5/9
R14B	++	-	+	-	+	++	+	+++	+	11+	7/9
R22B	+	-	+	+++	+	+	++	+++	-	12+	7/9
R33B	-	-	-	++	+	-	-	+++	-	6+	3/9
K21A	+	-	+	-	-	+	+	+++	+	8+	5/9
K32A	-	-	-	+	-	+	+	++	-	5+	4/9
K34B	++	+	-	++	+	+	++	++	-	11+	7/9

Note: [1] Total antifugal activity of LAB isolate; [2] Ranging of antifungal activity: (-) d = 0; (+) d ≤ 2 mm, (++) d ≤ 8 mm; (+++) d > 8 mm.

Compared to all isolated moulds, T71 was the most widely inhibited by LAB, 95% of all isolates. R13A was the only one could not inhibit T71. Moreover, weak activity was only recorded for V21B, other bacterial isolates had good performance. Beside of T71, there were 5 other mould isolates (L41, A24, C21, C32 and T22) inhibited by over 50% of LAB isolates. Meanwhile, L42 and X20 were less likely to be inhibited by LAB.

Thus, most LAB isolates show inhibitory activity against isolated moulds. 47% of LAB isolates with strong inhibition

was a high percentage, which confirmed that antifungal activity was generally effective. Furthermore, number of inhibited mould was significant, 67%. It meant mould reduction and elimination were possible and effective with biotechnology methods. LAB had antifungal activity since these bacteria produce chemical compounds inhibiting mould growth such as short polypeptides, organic acids, hydroxyl fatty acids, hydrogen peroxide [3], cyclic dipeptide [9, 10].

The results of Magnusson et al. [9] showed that 48 LAB isolates had inhibitory ability against indicator *A. fumigatus* and 37 from 48 isolates performed strong activity against other 4 moulds. Most of isolates were identified as *L. plantarum*, *L. coryniformis* and *P. pentosaceus*. Strong activity (+++) was also measured with 8% area without mould growth (approximately d >7.2 mm).

The same result was achieved by Jeong-Dong [11] when 5 LAB isolates collected from kimchi showed strong antifungal activity with no mould growth over 8% dish area. Indicator moulds involved *A. fumigatus, A. flavus, F. moniliforme, P. commune* and *R. oryzae*. Recently in the research of Belat and Zaiton [12], 17 LAB isolates were obtained from tempeh and fruit products which had strong inhibitory activity against *A. oryzae* (3-8 mm and over 8 mm inhibitory zone). Three bacterial isolates with outstanding results were identified as *L. brevis* G004, *L. fermentum* Te007 and *P. pentosaceus* Te010.

It was obvious that antifungal activity of LAB from this assay was similar with the results of previous studies. Hence, the results were rational. P32B, V13A and P41A isolates with the best performances were chosen to be identified to species level.

3.6. Identification of the Selected LAB Isolates

The defined sequences of bacterial 16S rDNA were aligned to the database on NCBI Genebank. The sequences of isolates P32B and V13A showed high homology (99%) to that of *L. plantarum* with the accession number FJ751793.1 and HF562938.1, respectively. The bacterial isolate P41A showed high homology (99%) to the sequence of the species *P. pentosaceus* with the accession number JX314608.1.

4. Conclusions

From five *Nem chua* samples, 19 LAB isolates and 9 mould isolates were obtained. Generally LAB isolates performed good inhibitory activity against moulds. Nine LAB isolates (P32B, P41A, V13A, P21B, P31B, R11B, R14B, R22B and K34B) (47% bacterial isolates), showed good inhibitory activity against 7 over 9 mould isolates. Three LAB isolates P32B, P41A, V13A were selected to be sequenced due to their highest antifungal activity and were identified as *L. plantarum* and *P. pentosaceus*.

Acknowledgements

This research was jointly sponsored by the Ministry of Science and Technology of Vietnam (contract nr. 09/2014/HĐ-NĐT); the Advanced Program in Biotechnology, Can Tho University; and the Core-to-Core Program (2014-2019).

References

[1] Mižáková A, Pipová M, Turek P (2002). The occurrence of moulds in fermented raw meat products. *Czech J. Food Sci.*, 20: 89-94.

[2] Magnusson, J (2003). Antifungal activity of lactic acid bacteria. Dissertation (ISBN 91-576-6405-6). Acta Universitati Agriculturae Sueciae, Agraria.

[3] Dalié DKD, Deschamps AM, Richard-Forget F (2010). Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control.,* 21: 370-380.

[4] Mayh-Harting A, Hedges AJ, Befikley F (1972). Methods for Studying Bactoriocins. In *Methods in Microbiology*. Noris, JB and Ribbons NW, 7A: 315-442.

[5] Pitt JJ, Hocking AD (1997). *Fungi and Food Spoilage*, 2nd ed. Gaithersburg, MD: Aspen Publications.

[6] Tran Thi Thanh Thao (2010). Study the bacteriocin producing activity of lactic acid bacteria from *Nem chua*. Thesis of Food Technology. HCM Nong Lam University, Ho Chi Minh City, Vietnam.

[7] Wilson NRP, Dyertt EJ, Hughes BR, Jones CRV (1981). Meat and meat products, factors affecting quality control, 5th ed. Applied Science Publishers Ltd., England.

[8] Kandler O, Weiss N (1986). In: *Bergey's Manual of Systematic Bacteriology*, Sneath PHA, Mair NS, Sharpe ME, Holt JG (Eds), Vol. 2: 1209-1234.

[9] Magnusson J, Strom K, Roos S, Sjogren J and Schnurer J (2003). Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiology Letters*, 219: 129-135.

[10] Ström K, Sjögren J, Broberg A, Schnürer J (2002). *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo (l-Phe-l-Pro) and cyclo (l-Phe-*trans*-4-OH-l-Pro) and 3-phenyllactic acid. *Appl. Environ. Microbiol.*, 68 (9): 4322-4327.

[11] Jeong-Dong K (2005). Antifungal activity of lactic acid bacteria isolated from Kimchi against *Aspergillus fumigates*. *Mycobiology*, 33 (4): 210-214.