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Antimicrobial and Antifungal Activity of Lactic Acid Bacteria Isolated from Coconut Milk Fermentation.

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ABSTRACT

Virgin Coconut Oil (VCO) is known as nutritious and healthy oil and is consumed by many people to maintain their health. The purpose of this study is to investigate the antimicrobial and antifungal activity of lactic acid bacteria isolated from coconut milk fermentation from the process of VCO production.In this study, we isolated LAB from fermented coconut milk in the manufacturing process of VCO, and the antibacterial and antifungal activity was assayed. We had collected 187 isolates of Lactid Acid Bacteria (LAB) by De Man Rogosa Sharp (MRS) agar containing 0.5% $CaCO_3$ and GTA + 0,5 % $CaCO_3$ media. The antibacterial activity was tested for Gram-positive and Gram-negative pathogenic bacteria consisting of *Escherichia coli, Bacillus substiliss, Staphilococcus aureus, Listeria monocitogenes* and *Salmonella typhyphosa*.The tests were conducted by paper disk method. Antifungal analysis for *Candida sp., Aspergillus niger*, and *Rhizopus sp*. was also performed for pathogenic fungi with a disk plate method.The result showed that the LAB could inhibit five pathogenic bacteria and three pathogenic fungi. The isolated LAB which identified by morphological, physiological, and biochemical tests were seven species including *Lactobacillus plantarum, Corineaebacterium bovis, Corineaebacterium xerosis, Micrococcus luteus,*and *Lactobacillus thermobacterium.* Among 187 isolates selected from the MRS agar, therrewe 102 strains (55.1%) showed Gram-positive and catalase-negative results. Of 187 isolates, twenty five isolates were tested forits antimicrobial and antifungal. The best isolate was M0 (inhibition zone average, 14-19 mm). Molecular identification result showed that the isolate was *Lacobacillus plantarum* NM178-5 (1463 bp). Registered in the genebank DDBJ with acession number AB890143,version AB890143.1

Key words: *antimicrobial, LAB, antimicrobial activity, antifungal activity, fermentation.*

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INTRODUCTION

Lactic acid bacteria (LAB) include in group of 'good' bacteria and known as GRAS (Generally Recognized As Save). LAB is a group of Gram-Positive bacteria which produce no spores, and they ferment carbohydrates to produce lactic acid. LAB from immemorial times was used in processes of fermentation for preservation of nutritious properties of various product (Hakobyan et al, 2008). In the last decade, the interest in natural preservatives is increasing, which is in accordance with the consumer's demand for healthy, safe, and fresh food (Smith, 1993; Hufner, 2007). There are many substances synthesized by LAB with antibacterial activity such as hydrogen peroxide, carbondioxide, diacetyl, organic acids, fatty acids (antifungal components), and finally bacteriocins (Jay, 1982).

Bacteriocins are synthesized in ribosomes of Gram-positive bacteria. They are extracellularly released bioactives peptides or peptide complexes (usually composed of 30-60 amino acids), which have bactericidal or bacteriostatic effect on other (usually closely related) species (Garneau, 2002).

Currently there are several criteria, according to which bacteriocins are divided into 4 classes (Klaenhammer, 1993):

- The antibiotics containing unusual, post-translationally modified amino acids, such as dehydroalanine, dehydrobutyrine, lantionine or β-metyl-lantionine – lantibiotics. They have small weight, thermostable. Nisin is widely known representative of the given group.
- Small, not modified peptides. This class is divided into two subclasses: class 2a, or antilisterial peptides, representatives of which are recently described as lactococcin MMF2 and sakacine G; class 2b embraces antibiotics, which for full functionality have to include two different peptides, such as lactococcin G or lacticin F.
- Large thermolabile bacteriocines. This class is not studied as well as the previous one. Only a few greater bacteriocins, such as helveticin J, produced by *Lactobacillus helveticus* 481, or *Enterolysin A*, produced by *Enterococcus faecalis* LMG 2333, are described at a molecular level. However, some are not completely identified antibacterial components as helveticin V-1829 from *L. helveticus* 1829, an antimicrobial substance from strain L. helveticus CNRZ 450, or recently described enterocin R69 from E. faecalis P69 can be referred to the third class also.
- The fourth class includes uncertain mixtures of proteins, lipids, and carbohydrates

One of the processed coconut oil products without heating and chemicals is known as Virgin Coconut Oil (VCO). Virgin coconut oil (VCO) is one of refined oil products which began widely known by the public. Production of VCO can be done through three ways: mechanical, provocation, and enzymatic. One of the manufacturing process of virgin coconut oil widely used is enzymatic (fermentation). The process of making pure coconut oil by fermentation did not undergo a process of heating and no addition of chemical substances, so that pure coconut oil produced has good quality.VCO is herbal products, such as medium chain fatty acids and essential oils either used as nutritional supplements or as food preservatives are known to possess antimicrobial properties. Lauric acid is a major component of virgin coconut oil. Similarly, many coconut oils, used as a food-flavoring agents, have been postulated to possess a broadspectrum of antimicrobial activity due to their high content of lauric acid that can be converted into monolaurin. Eventhough it is generally accepted that coconut oils contain high concentrations of lauric acid possess potential antimicrobial effects, little actual investigation has been performed using the natural product to treat superficial or systemicinfections due to bacteria, viruses, or fungi.,(Manohar et al., 2013).

Many studies about VCO have been done, but study on antimicrobial and anti fungal that contained in LAB which resulting bacteriocin has not been performed yet. We investigate whether bacteriocin and Lactic Acid Bacteria (LAB) contribute to human health.

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MATERIAL AND METHODS

Isolation of LAB from Fermented Coconut Milk.

Five strains of lactid acid bacteria, namely *Lactobacillus plantarum* (isolate M.0, A19.22, A20, B19.5, B29), *Lactobacillus thermobacterium* (isolate M8, B19.6, A20, B1, A22), *Corineaebacterium bovis* (isolate M16.1, M16.2, A3, A5, A14), *Corineaebacterium xerosis* (isolate A9, A18, A6, A8, A2), and *Micrococcus luteus* (isolate M16.3, A24, A37, M16.4, M16.16.2) were isolated from fermented coconut milk by one kg of shredded coconut added with 2 L of water, or the ratio of shredded coconut and water was 1:2 . The mixture was squeezed and filtered. The waste was discarded and the coconut milk was fermented overnight.

Lactic Acid Bacteria was isolated by using several media (MRSA (Merck),MRSA+CaCO3 0,5%, and GTA+CaCO3 0,5%). LAB was extracted by using MRSA medium, from coconut milk which fermented overnight. This fermentation resulted three layers; oil layer (M), skim layer (B), and water (A). Extraction was proceeded by dilution method, thus, each layer was pipetted 1 ml and diluted up to 10 ml by adding physiological saline (NaCl 10 %, sterilized). Dilution was done until 10^{-7} reached. We performed an extraction process for every dilution by using pour plate method with MRSA, MRSA+CaCO3, and GTA+CaCO3 as cultured media. The solution was incubated overnight, until the colonies of LAB were rose, with the 'halo' zone.

Each colony of LAB struck onto MRSA, incubated overnight for growing colonies. We cultured 2-3 times a colony on different media to get homogen colonies on a plate. Finally, the pure colony re-cultured onto MRS Agar, stored in the refrigerator temperature. The isolates were coded and labeled for further identification.

Identification of LAB

LAB isolates were morphologically characterized from the shape and color of the colony and physiologically characterized by gram staining, catalase test, sucrose test, and maltose based on standard procedure.The results of tests were compared with identification key (Kandler and Weiss, 1984), and using Standard Method "*Manual for the Identification of Medical Bacteria*" (Cowan and Steels, 1975).

Antimicrobial Activity Test

Antimicrobial activity of LAB isolate was determined by using modified paper disk method from Savadogo et al (2004) and Girum et al (2005). Purified single colony from five isolate was transferred into sterilized MRS broth (Merck), incubated at 37^{o}C for 48 hours. The culture was centrifuged at 10.000 rpm, 4^{o}C, for 20 minutes. Supernatant was obtained. Microorganisms used as indicator for antimicrobial activity were (*Escherichia coli* NBRC 14237, *Bacillus substiliss* NBRC 13276, *Staphilococcus aureus, Listeria monocitogenes* and *Salmonella typhyphossa*). These bacteria had grown before in NA media overnight at 37^{o}C. These pathogenic bacteria were inoculated to sterilized aquadest, rotated several times, and the number of cell was compared to the Standard of Mac Farland. The culture was transferred to sterilized petridish with NA media by using a sterile cotton bud. A few sterilized filter paper which used as disk was put into isolate culture for a while. Paper disk was placed onto petridish with NA. Petridish was incubated overnight at 37^{o}C. Each petridish was observed, diameter of clear zone was measured as indicator that there was growing inhibition of pathogenic bacteria in that area because of the isolate (Assefa et al, 2008).

Antifungal Activity Test

This procedure was similar to antimicrobial method, but the microorganism indicator for antifungal activity analysis was *Candida* sp., *Aspergillus niger*, and *Rhizopus* sp. The results were also obtained by measuring diameter of clear zone. This indicated that pathogenic fungi were inhibited by the isolates (Assefa et al., 2008).

Screening LAB for Antibacterial and Antifungal Activity

Of 187 isolates identified with morphological, physiological, and biochemical test, 5 species were found (revealed) (Table 1). The further analysis for five isolates was conducted by using antimicrobial and

antifungal activity test. Five isolates were analyzed for each strain (Total 25 isolates from 5 strains). Four isolates showed good activities and among the four isolate there was one isolate which show the best activity, namely (M0). Then, M0 was identified with molecular method.

Table 1: Grouping of identification result by using morphological, physiological, and biochemical test of LAB

No.	Sub genus	Isolate code
1.	*Lactobacillus plantarum*	**M.0**; A19.22; A20; B19.5; B29; and so on (97 isolates)
2.	*Lactobacillus thermobacterium*	M8; B19.6 ; A20; B1; A22.
3.	*Corineaebacteriumbovis*	M16.1; M16.2; A3; **A5**; A14 and so on (23 isolates)
4.	*Corineaebacteriumxerosis*	A9; A18; **A6;** A8; A2.
5.	*Micrococcusluteus*	M16.3 ; A24; A37 ;**M16.4;** M16.16.2

Molecular Identification with PCR

Identification of isolate bacteria was performed with molecular method, based on partial genetic analysis ***16S rDNA***. DNA extraction was done by using GES method (Pitcher *et al,* 1989. Modified)**.** PCR amplification of ***16S rDNA*** used **9F: 5`-- AAG GAG GTG ATC CAG CC – 3`** and **1541R: 5`--GAG TTT GAT CCT GGC TCA G –-3`primers** (White *et al.,* 1990; O`Donnell, 1993). Purification of *PCR product* was performed with *PEG precipitation method* (Hiraishi *et al.,* 1995), the result then analyzed for reading the sequence of DNA. Sequence of DNA was re-purified with *Ethanol purification method*. Analysis of nitrogen base sequence reading used *automated DNA sequencer* (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). Raw data of sequencing result was trimmed and assembled by using BioEdit program (http://www.mbio.ncsu.edu/BioEdit/biodet.html). Assembled sequence data analyzed with BLAST by comparing with genome data which enrolled in DDBJ/ DNA Data Bank of Japan (http://blast.ddbj.nig.ac.jp/) or NCBI/ National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) to determine taxon/species which has highest and closest *homology/ similarity* with molecular analysis.

RESULT AND DISCUSSION

LAB Isolation

Fermented coconut milk resulted three layers; the upper layer was oil (M), middle layer was skim (B) and the lower layer was water (A). Each layer was pipetted and isolated by using dilution method until 10^{-7}. Isolates growed in MRSA + $CaCO_3$ 0,5% , and GTA+ $CaCO_3$ 0,5% by using pour plate method. Clear zone or 'halo' was resulted after incubated at 37^{o}C. Halo took place because of addition of $CaCO_3$ 0.5% as indicator, where $CaCO_3$ reacted with organic acid resulting from LAB and finally formed clear zone. Single colony which placed in the center of 'halo' zone was transferred onto petridish with MRS agar, incubated overnight, done 2 – 3 times until pure colony was reached. Single colony was transferred onto test-tube contained agar media, stored at 4^{o}C. There were 187 totals of LAB isolates. Seventy seven of isolates were derived from oil layer encoded with (M), 46 isolates from skim layer encoded with (B), and 64 isolates were derived from water layer encoded with (A) and it was obtained from dilution 10^{-5} - $10^{-}7$. All of isolates then were identified.

By using MRSA media plus $CaCO_3$, it would strengthen the belief that it was indeed Lactid Acid Bacteria growing in there. The same thing was done on the research. Lactic acid bacteria were isolaste and purified from *Nem chua.* LAB were isolated and purified by using pour-plating and streak-plating methods on MRS agar containing 1% CaCO3. The plates were incubated in candle jars at 30 °C for 24 hours. Acid-formers were identified by the presence of clearing zones around the colonies. Representative colonies of acid-formers were picked up and sub-cultured in MRS agar with 1% CaCO3. Purification of the isolates was done by repeated plating and streaking using the same agar medium(*H.T.H. Nguyen* et al.(*2010)*

Identification of Lactic Acid Bacteria

LAB was identified with morphological, physiological, and biochemical test. Investigated from the shape of colony, the shape of cell, color of colony, Gram staining test, Sucrose and Maltose test, and Catalase

test. A total number of 187 isolates, 102 isolates (55,1%) were Gram-positive with catalase-negative. Identification result showed that LAB divided into five groups of LAB sub-genus as shown at Table 1:

From table 1 it can be seen that there were 5 species of LAB isolated from coconut milk fermentation, namely *Lactobacillus plantarum, Corineaebacterium bovis, Corineaebacterium xerosis, Microccus luteus,* and *Lactobacillus thermobacterium.* Lactic acid bacteria were isolated from fermented cummingcordia (pobuzihi), a traditional food in Taiwan produced strains of *Lactobacillus acidipiscis* (Yi-Sheng Chen, 2010). Meanwhile, BAL isolated from Nem Chua was also done. *Nem chua* is a traditional lactic acid fermented meat of Vietnam that is consumed raw. These isolates were identified as *Lactobacillus plantarum* using an API 50 CHL i.d. kit. Amplified gene were sequenced by primers 1101F (5'-AACGAGCGCAACCC-3') the partial 16S rRNA and 1407R (5'-GACGGGCGGTGTGTAC-3') showed 98% homology to *Lactobacillus plantarum* WCFS1. Monika (2011), also has isolated a sporulated lactic acid bacterium (LAB) from cider that produce *Sporolactobacillus sp*. The best result of antimicrobial and antifungal activity analysis was M0, the obtained of data of identification after compared with "Manual for The Identification of Medical Bacteria" (Cowan and steels) showed that the bacteria included into *Lactobacillusplantarum* as shown in Table 2.

Table 2: The result of identification of LAB isolate include *Lactobacillusplantarum*

Characterization		Isolate code
Morphological test • Shape of colony • Color of colony	 Bacill Clear	isolate **M.0**, A19.22, A20, B19.5, B29, (represented 97 isolate)
• Gram staining test • Microscopic observation	+ Stalk	
Biochemical test • Galactose • Lactose • Glucose • Sucrose • Maltose	 + + + + +	
Nitrate Reduction	-	
Hidrolitic Arginin	-	
H_2S	-	
Catalase OF TSIA Aerob/Anaerob	+ + K/K A	

According to Table 2, among 5 species that can be isolated from fermented coconut milk there was one species which showed the best result after antimicrobbial and antifungal tests were conducted. It was Lactobacillus palntarum. The LAB was Gram-positive, lactic positive and able to grow both in the presence and absence of oxygen. Generally, lactic acid bacteria are Gram-positive bacteria that do not form spores and which are able to grow both in the presence and absence of oxygen. According to Monika(2011), similar LAB characteristic also can be seen in Sporolactobacillus sp., namely Gram-positive, endospore-forming. Lactic acid bacterium was isolated from spoiled orange juice., grew microaerobically or and produced acid from various sugars. D-Lactic acid was produced Rieko Fujita, Kaoru Mochida, Yuko Kato and Keiichi Goto(2010)

Analysis of antimicrobial activity

There were 187 isolates found which selected from identification process, finally the results showed there were 5 sub-genus. Five isolates were taken from each sub-genus and futhermore antimicrobial activity was analyzed by using paper disc modified from Savadogo et al. (2004) and Girum et,al (2005). The result showed at Table 3.

Table 3 showed that there were 4 isolates with maximum result entirely (M0, A5, M16.4, A6). Clear zone area on *E.coli* coloni was small, but it was larger on other pathogenic bacteria. The size of clear zone indicated the activity of antimicrobial. From table 3, it also can be seen that there were 5 isolates in which its clear zone against Escherichia coli was larger than other isolates, namely M0 (17 mm), M16.3 (17 mm),

B19.6(20 mm) dan M16.4(15 mm). However, if we compared its ability to isolate other pathogenetic bacteria such as Listeria monocytogenes, the it turn out that M0 remained to have the largest clear zone. But for M16.3, only 14 mm diameter zone that it can isolate. In addition, M16.3 even did not have clear zone, it means that it cannot isolate bacterial activity of Staphilococcus aureus. From the comparison of magnitude of the diameter of the clear zone of each isolate (25 isolates) against each of 5 pathogenetic bacteria, it is found that there were 4 isolates with good ability to isolate bacteria, namely M0, A5, M16.4,A6.

Table 3: Anti microbial activity analysis of LAB on the form ofclear zone diameter (mm)

No.	Isolate	Species	*Escherichia coli* (mm)	*Lactobacillus Monocitogenes* (mm)	*Bacillus Substiliss* (mm)	*Salmonella Typhyphosa* (mm)	*Staphillococcus Aureus* (mm)
1.	M16.1		16	16	15	14	16
2.	**M0**	Lactobacillus	**17**	**18**	**15**	**14**	**16**
3.	M16.4	*plantarum*	**15**	**11**	**11**	**15**	**14**
4.	M16		12	11	15	9	15
5.	B29		11	11	15	9	16
6.	M16.3		17	14	11	11	-
7.	**M16.16.2**	*Lactobacillus*	**13**	**12**	**11**	**11**	**17**
8.	M.8	*thermobacter*	13	12	13	14	12
9.	B19.5		14	13	12	10	14
10.	B19.6		20	15	12	14	8,5
11.	A1922.		12	12	11	12	10
12.	A20	*Corineae bac*	13	11	11	14	11
13.	A8	*terium bovis*	13	12	10	12	12
14.	A22		16	14	12	12	9
15.	B1		15	15	11	10	14
16.	A2		14	14	11	11	12
17.	A24		16	11	12	15	9
18.	**A5**	*Corineae bac*	**14**	**13**	**16**	**12**	**9**
19.	A.9	*terium xerosi*	12	12	11	16	16
20.	B2		13	12	12	14	8,5
21.	A18		11	14	11	15	11
22.	A25		12	16	11	15	20
23.	A.37	*Micrococcus*	12	16	10	14	14
24.	**A6**	*luteus*	**13**	**20**	**16**	**15**	**15**
25.	A14		-	19	20	16	15

Clear zone was formed because of reaction of bacteriocins which attack wall cell of pathogenic bacteria; the wall was broken and caused the death of cell. The clear zone showed the area of the death bacteria. In other words, bacteria could be able to grow. We repeated analysis of antimicrobial activity for four isolates. Evidently, M0 showed the best result, and then this isolate was identified for molecular analysis with PCR.

Molecular Identification with PCR by Using 16sRNA

Molecular identification result showed that the isolate was *Lacobacillus plantarum* NM178-5 (1463 bp),as shown in Figure 1.

Analysis of Antifungal Activity

Procedure for analysis of antifungal activity was similar to analysis of antimicrobial activity, in which pathogenic fungi were used as an indicator. They were *Candida* sp., *Aspergillus niger*, and *Rhizopus* sp. The result of analysis was showed at Table 4 .

At table 4 , if compared every isolate (25 isolate) against clear zone produced by each isolate on 3 fungi, i,e *Fussarium, Candida*, dan*Aspergillus,* it is found that M0 clear zone against Fussarium was 18 mm, the largest of all analyzed isolates. So is Candida, its clear zone was 19 mm. Also it was the largest of 25 analyzed

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isolates. Similarly, the clear zone of Aspergillus was 15 mm, which is also the largest. For isolate A5, M16, A6 their clear zones were large. Thus, it is found that isolates with antifungal ability were M0, A5, M16, A6.

Table 4: The analysis result of antifungal activity of LAB on the form of clear zone diameter (mm)

No.	isolate (mm)	Species	*Fussarium* (mm)	*Candida* (mm)	*Aspergillus* (mm)
1.	M16.1		14	11	14
2.	**M0**	*Lactobacillus*	**18**	**19**	**15**
3.	M16.4	*plantarum*	**13**	**11**	**11**
4.	M16.2		11	10	15
5.	B29		11	11	15
6.	M16.3		13	14	11
7.	**M16.16.2**	*Lactobacillus*	10	12	9
8.	M.8	*thermobacter*	10	10	10
9.	B19.5		-	11	12
10.	B19.6		12	10	10
11.	A19.22		10	11	10
12.	A20	*Corineae bac*	10	11	11
13.	A8	*terium bovis*	10	12	10
14.	A22		10	11	11
15.	B1		10	12	11
16.	A2		10	12	10
17.	A24		12	11	12
18.	**A5**	*Corineae bac*	13	13	12
19.	A.9	*terium xerosi*	11	11	11
20.	B2		12	10	9
21.	A18		11	12	11
22.	A25		10	10	11
23.	A.37	*Micrococcus*	10	11	11
24.	**A6**	*luteus*	12	13	12
25.	A14		-	10	12

Figure 1

>Contig0 (Lactobacillus plantarum strain NM178-5, 2699 bits, 1463 bp, 99%, 0.0, acc. no: HM218736.1)
CCTAATACGATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATT
TGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAA
CAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGAGGTAACGGCTCACCATGGCAA
TGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACT
CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACAAGTCTGATGGAGCAACGCCGC
GTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTTTAAAGAAGAACATATCTGAGAGT
AACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGSAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCG
GTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGA
AACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATAT
GGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGT
ATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGT
GTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGT
ACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAG
AGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCATT
AAGTTGGGCACTCTGGTGAGACTGCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGC
GAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAAC
TCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTCGGTGG
GGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGGACAGATGA

Figure 2. Clear Zone from Antimicrobial Activity Analysis of LAB .

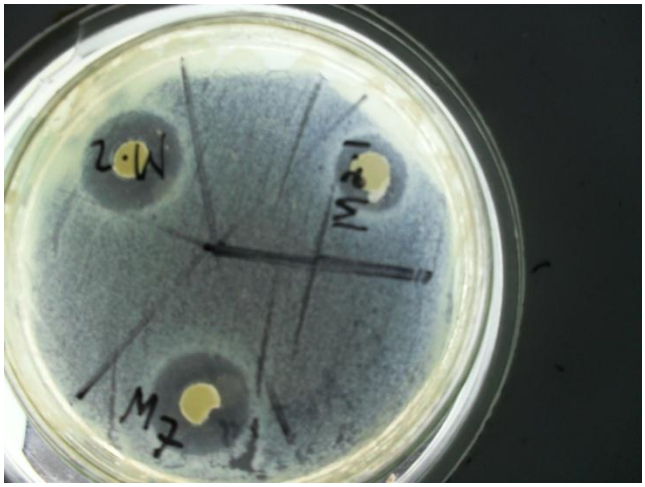
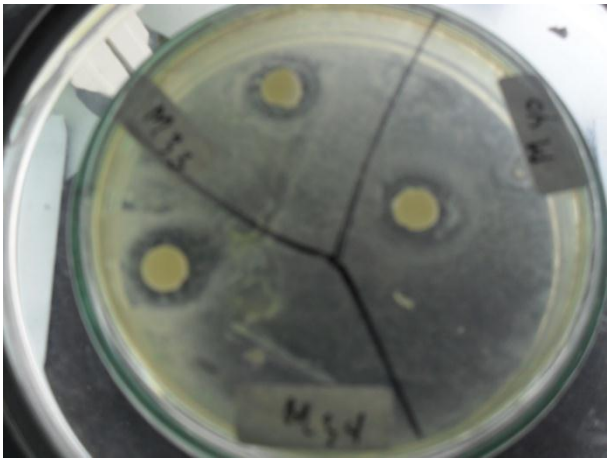


Figure 3. Clear Zone from Antifungal Activity Analysis of LAB



Five groups of strains LAB were found which derived from fermented coconut milk into VCO; *Lactobacillus plantarum, Corineaebacterium bovis, Corineaebacterium xerosis, Micrococcus luteus,* and*Lactobacillus thermobacterium,*. They have an ability to inhibit growing of several patoghenic bacteria (*Escherichia coli, Bacillus substiliss, Staphilococcus aureus, Listeria monocitogenes* and *Salmonella typhyphosa*). LAB was also against the development of pathogenic fungi (*Candida* sp.*, Aspergillus niger*, and *Rhizopus* sp). The result showed that the best isolate activity from analysis of antimicrobial and antifungal activity was demonstrated by M0, and the identification of molecular analysis with PCR/16sRNA showed that the species of bacterium was *Lacobacillus plantarum* NM178-5 (1463 bp).

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