Probiotic research as highest antimicrobial *Listeria monocytogenes* from virgin coconut oil [VCO] Padang West Sumatra

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Abstract. Virgin Coconut Oil [VCO] is a natural oil, made from fresh coconut milk fermentation without adding chemicals and other additives. Several Lactic acids Bacteria [LAB], have been isolate and characterization as probiotic. Several 5 different VCO sample products in local Padang Market have been determined. Specifics MRS medium has been used for LAB isolation and morphological characterization. The samples code are A, B, C, D, and E for 5 VCO samples. The highest total colony of LAB is from Samples A, which is about 1377 x10⁷ CFU/mL. 18 isolates have been identified as Gram-positive bacteria while 8 bacteria's are coccus and others form basil. All 18 LAB were grown at pH 2, and showing a resistance of about 49 %. Only 50 % of LAB resistant 0,3% oxgall for 4 hours. 3 isolate namely isolate A-1, A-3, and D-1 were showed the highest antimicrobial activities against *Listeria monocytogenes* with holozone averages of 26 mm. The hydrophobicity against xylene was also determined. The potential A-3 colonies are the highest percentages grown about 19% and resistant to several antibiotics such as Ampicillin, Chloramphenicol, Erythromycin, and Tetracycline. Molecular identification for potential A-3 using 16S RNA, showing phylogenetics species as *Lactobacillus fermentum* strain KF7.

Keywords: Probiotic, VCO, antimicrobial, *Listeria monocytogenes*
1. Introduction

The are several methods to produce Virgin Coconut Oil [VCO], by fermentation and other by mechanic machine without adding chemical and additive. The VCO product will results in different in quality as active compounds and oil contain such as Lauric Acids [LA] and other essential oil [1]. The natural fermentation methods involved some Lactic Acids Bacteria [LAB] and Saccharomyces. VCO fermentation contains more high active compounds such as antioxidants, vitamins, and lauric acid [2]. Not all VCO products from Padang local Market will find probiotic bacteria, especially when the process of making other than fermentation techniques [2]. VCO fermentation contains more high active compounds such as antioxidants, vitamins, and lauric acid [1][2]. The variety of plant species such as hybrid, Mutant, and geography where plants grow [1][2][3]. Coconut [Cocos Nucifera] has been using among local people for a long time for cooking and medicinal herbs because of the bioactive compounds, healthy oil, mineral, and vitamins [4][6]. VCO is also known to lower cholesterol levels in the blood serum, higher HDL [High Density Liporotein], improve several metabolisms, anti-obesity, anti-cancer cell, anti-apoptosis in vitro, antidiabetic type 2 gene resistant insulin, also an immunomodulator [4][5][6][7][8]. However, in this paper, we are interested in finding probiotic potential among VCO products in the local Padang market by random sampling. This paper aims to determine potential probiotics of several VCO Products, to have strong antimicrobial against food bond Listeria monocytogenes and other indicator bacteria. There is no report so far concerning potential probiotics and antimicrobial against Listeria monocytogenes observe from VCO local market in Padang.

2. Methods

2.1. Total colonies of lactic acid bacteria in VCO

VCO is taken using micropipette as much as 1 ml, then dissolved in the reaction tube of 9 ml of De Man Ragosa and Sharpe [MRS] Broth Merck solution, then vortex to homogeneous. Dilution was performed according to standard microbiology procedures [1].

2.2. Isolation of lactic acid bacteria

Each of five sample VCO products [1 mL] was inoculated in 9 mL MRS broth and incubated anaerobically for 24 hours at a temperature of 37ºC. Then, the culture was diluted with serial dilution methods [1], and inoculated on the MRS agar medium, and incubated for 48 hours at 37degree. Every single colony then was characterized by their morphology [2].

2.3. Isolation of potentially probiotic

LAB isolates resistance test against low pH was performed using MRS broth medium which is regulated at pH 2 by adding 5N HCL [4].

2.4. Isolation of resistance bile salts

Resistance test against bile salts was performed growth of LAB using MRS broth medium, and added Oxgall [0.3%]. MRS broth without the addition of Oxgall used as a control medium. The isolates culture was incubated anaerobic at a temperature of 37 degree for 18-24 hours. The procedure use as established[1].

2.5. Antibacterials of LAB isolates against pathogenic bacteria

Antibacterials test of the LAB isolates conducted against the test bacteria Escherichia coli, Staphylococcus aureus, and Listeria monocytogene collected from microbiology lab Veteriner University
of Andalas. The LAB cultures were grown in MRS broth for 18-22 hours. The antibacterial procedures use as established[1][2].

2.6. Molecular characterization of 16S rRNA
16S rRNA gene amplification was determined by PCR [Polymerase Chain Reaction][1]. The pure bacterial culture is used as a template DNA [10]. The primer used, namely the Forward 1369 [5 ' CGG TGA ATA CGT TCC CGG3 '] and Reverse 1492 [5 ' TAC GGC TAC CTT GTT ACG ACT T3 '].

2.7. Nucleotide sequencing
Data sequence analysis was conducted by using MEGA6 software program[10]. The sequence alignment analysis was conducted by using BLAST [Basic Local Alignment Search Tool] [http//www.NCBI,NLM,NIH.gov]

2.8. Data analysis techniques
Quantitative data obtained has been analyzed using ANOVA one-way analysis and Tukey test [9][11]. All statistical analyses were conducted with SPSS 16.

3. Results and Discussion

| Products | Colour                  | Aroma     | Total Colony LAB[CFU/mL] |
|----------|-------------------------|-----------|-------------------------|
| A        | broken white and clear  | smell coconut | 1377 × 10^7             |
| B        | broken white clear      | slight coconut | 434 × 10^5              |
| C        | Broken white clear      | slight rancid | 1700 × 10^4             |
| D        | not clear               | rancid     | 682 × 10^4              |
| E        | not clear               | rancid     | 857 × 10^4              |

The total colony of LAB was shown in Table 1, resulting in a variation of 5 products sample of VCO, the highest total colony is sample A, is about 1377 x 10^7 and further used for screening as a potential probiotic test. The total colony was found to depend on lactic acids and environmental conditions [1].

![Figure 1. Colonies of LAB growing in MRS agar for products A, B, C, D, and E. A= Isolate A-3; B= isolate B-1; C= Isolate C-1; D= Isolate D3; E= Isolate E-1](image)

The colonies of 5 samples grown in petridish with MRS agar medium shows the variation in total colonies, while sample A shows much bigger and more.
3.1. Resistant against oxgall

Resistant against oxgall can be explained in Table 2.

| Isolate LAB | Percentage of Resistant [%] |
|-------------|----------------------------|
| A-1         | 20.83 ± 0.36 b              |
| A-2         | 13.91 ± 0.80 a              |
| A-3         | 21.33 ± 0.50 b              |
| A-4         | 17.08 ± 0.37 ab             |
| B-1         | 16.96 ± 0.93 ab             |
| C-1         | 19.72 ± 1.33 b              |
| C-2         | 19.26 ± 2.41 ab             |
| C-3         | 17.16 ± 0.52 ab             |
| D-3         | 20.84 ± 1.52 b              |

P < 0.05

The percentage of resistance 20-40% was showed to survive the bile salts and a good candidate for probiotic [1].LAB can withstand in a condition of bile salts, we found most of the LAB strains have good Bile Salt Hydrolase [BSH] Enzymes, [2] [10] [11].

3.2. Antibacterial activity

| Isolate LAB | Inhibition diameter zone [mm] |
|-------------|-------------------------------|
|             | E.coli | S.aureus | L.monocytogenes |
| A-1         | 12,00 ± 1,00a         | 11.67 ± 0.67b | 26.00 ± 0.58c |
| A-3         | 12.33 ± 0.33a         | 12.67 ± 0.33b | 25.67 ± 0.33c |
| D-1         | 12.17 ± 1.09a         | 12.00 ± 0.57b | 26.33 ± 1.67c |

[p > 0.05], not significant differences

The inhibition activity of LAB against other microorganisms is because LAB produces a product of antimicrobial metabolites, among others, diacetyl, hydrogen peroxide, organic acids, and bacteriocin [7] [10][11]. LAB that was isolated from some of the Dadih curds and VCO from West Sumatra has an inhibition zone diameter of about 10 of mm-21m [2].
3.3. The electrophoresis of the PCR product

In Figure 2, the PCR product of isolates A-3 was obtained at 100-200 bp, range of 144-149 bp. It was to be continued for the analysis of nucleotide sequences. Based on NCBI data [Primer-BLAST, 2017], the results of the PCR product for *Lactobacillus sp.* in the range of 144-149 bp. Thus, it can be assumed that A-3 isolates belong to the genus *Lactobacillus sp.*

3.4. Analysis Nucleotide Sequence of potential probiotic Isolat A-3

In Figure 3, it is confirmed that the A-3 isolates are almost identical to the *Lactobacillus fermentum* strain KF7 at 99%.
4. Conclusion

In this study, we find the potential probiotic of VCO fermentation from Padang, West Sumatra, that have been isolate and characterized biochemically and molecular DNA, conformed was isolates A-3 strain *Lactobacillus fermentum* KF7.

**Competing interest statement**

The author declares no conflict of interest.

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