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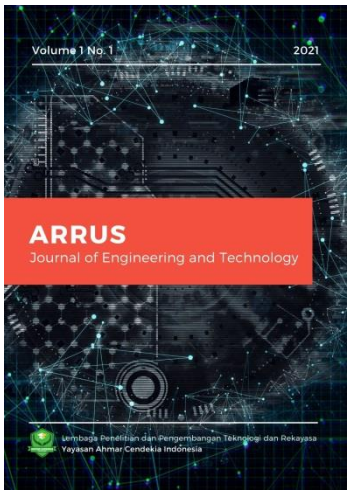

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RESEARCH ARTICLE

New Probiotic Isolation of Coconut Water's Helpful Lactic Acid Bacteria Cure Covid-19 Patients

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Abstract: Probiotics from coconut water lactic acid bacteria have not isolated nor identified them, and no one has studied that these probiotics can help cure Covid-19 patients. This study aimed to isolate and identify probiotic lactic acid bacteria in coconut water, both morphologically, physiologically, and biochemically. Further studied his ability to help cure Covid-19 patients. Using coconut water samples, probiotics are isolated by isolating lactic acid bacteria through dilution methods and pour plate methods. The medium used is MRSA+ CaCO3 0.3%. Physiological and morphological identification. The following identification is to perform biochemical tests such as the Gram test. The results showed that there were 73 bacterial isolates and 24 fungal isolates, consisting of 4 species of bacteria, *Pediococcus sp, Lactobacillus paracasei, and Lactobacillus plantarum*, as well as *candida sp* and *rhizopus* fungi. In comparison, the results of the antimicrobial analysis showed that *Lactobacillus plantarum* has better antimicrobial ability against the pathogen bacteria *Pseudomonas aureginosa*. *Lactobacillus plantarum*'s antimicrobial ability is less good against *S. aureus* bacteria. These findings suggest that probiotics from coconut water can be isolated and may aid the healing of Covid-19 patients. This study has implications that in the future Covid-19 patients can be helped with coconut water.

Keywords: Probiotics, Virus, Covid -19, coconut water, isolation.

1. Introduction

Covid-19 is an acute infection of the respiratory tissue caused by the virus (Abdullah et al., 2019)(Wölfel et al., 2020), especially for comorbid sufferers (Berretta et al., 2020; Middleton et al., 2020; Shen et al., 2020). such as diabetes, heart disease, and hypertension. So many of them are exposed to covid-19 starting from Wuhan (Chan et al., 2020; Holshue et al., 2020; Wu et al., 2020; Zhu et al., 2020), to millions around the world, who suffer from acute respiratory distress syndrome and require ventilators. Respiratory failure will cause death. More dangerously, trials of drugs such as oral or intravenous dexamethasone cause death as well after 28 days (Peter HorbyF.R.C.P., Wei Shen LimThe members of the writing committee (Peter Horby, F.R.C.P., Wei Shen Lim, F.R.C.P., Jonathan R. Emberson, 2021). There are also drugs like. Lopinavir/Ritonavir, Remdesivir, Nucleoside Analogues,

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Neuraminidase inhibitors, arbidol, RNA synthesis inhibitors (such as TDF, 3TC), anti-inflammatory drugs, and others (Brain et al., 2021; Fajgenbaum & Rader, 2020; Lu, 2020).

Efforts to defend themselves from the covid-19 virus, carried out using chemical drugs are also carried out using herbs such as ShuFengJieDu Capsules and Lianhuaqingwen Capsules of traditional Chinese medicine (Lu, 2020). In addition to natural ingredients containing probiotics, such as *Lactobacillus casei, Lactobacillus reuteri, Lactobacillus rhamnosus* GG, and *Saccharomyces boulardii*, (Chuah et al., 2019; Keikha & Karbalaei, 2021). Lactobacillus fermentum strain, and L. sakei. Because the virus mutates very quickly the possibility of resistance of antiviral agents can occur, the control can be done using probiotics derived from lactic acid bacteria that complain of bacteriocin. This bacteriolyine is the antiviral agent (Muharram et al., 2020).

1.1. Lactid Acid Bacterial (LAB)

LAB probiotics through bacteriocins help the covid-19 virus inhibit the development of the virus by (1) direct interaction between probiotics and viruses; (2) bacteriolyine virus-inhibiting metabolites; and/or (3) through immune system stimulation (Menni et al., 2020). At this time probiotics can even be added to foods that can last for a year (Fenster et al., 2019). Lactic acid bacteria inhibit microbial growth in various ways, but generally in the presence of bioactive compounds that can kill viruses or pathogenic bacteria (Siedler et al., 2019), (Fauzan, Marganof,, Suryani, 2020), (Suryani et al., 2017), as can be explained as follows the active compounds produced by lactic acid bacteria that can inhibit the development of viruses and other microorganisms are,

a. Acid derivatives of amino acids namely, 2-Hydroxy-3 methylbutanoic acid and indolelatic acid, Phenyllactic acid 2-Hydroxy-4-, 2-Hydroxy-4- methylpentanoic acid and 2-Hydroxy-(4-methylthio) butanoic acid.
b. Organic acid compounds namely, Lactic acid, acetic acid, succinic acid, propionic acid, succinic acid, benzoate acid, formic acid, p-kumaric acid, hydroxydoneic acid, and vanic acid.
c. Fatty acids such as, 3-Hydroxydodecanoic acid and 3-Hydroxy-5-cis-dodecanoic acid
d. Cyclic dipeptide compounds such as Cyclo(L-Phe-L-Pro) Reutericyclin, and Cyclo(L-Phe-4-OH-L-Pro)
e. Other compounds such as Mevalonolactone, δ-Dodecalactone and Methylhydantoin, and H_2O_2.

Bacteriocin is a peptide produced by lactic acid bacteria, can be active in nanomolar amounts. It is not toxic and found in fermented food products (Emmanuel E.I. & Solomon E., 2021), there are several bacteriocins produced by the following lactic acid bacteria, L. plantarum, L. brevis, and L. sake (Scano et al., 2021), (Suryani et al., 2020), (Suryani, 2020).

1.2. Coconut Water

Coconut water, and since time immemorial used as a medicine, now even with the presence of covid-19 is also used as a supplement to help inhibit the development of the virus (Anaya et al., 2020). It turns out that after being studied, coconut water has 4 peptides that have the ability as antibacterial, antifungal and antiviral, AMP, CnAMP1, CnAMP2 and CnAMP3. Among the 4 peptides, cnamp1 can inhibit the cell growth of microorganisms. At the time of toxicity examination, there are no signs that coconut water is toxic or in other words safe to consume. With the discovery of cnamp1 peptide, which has the potential to be a new antimicrobial, drinking coconut water can help cure covid and be safe.

But until now no one has isolated the lactic acid bacteria present in coconut water and can be used as a new probiotic. Furthermore, no one has identified acidic bacteria from coconut water. So also no one has isolated the bacteriolyine in the lactic acid bacteria.

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To learn the potential of coconut water can be used as a new probiotic to survive the covid-19 virus, it is necessary to isolate lactic acid bacteria, as well as identification of both morphologies, physical and molecular identification. So also need to do antimicrobial analysis such as antibacterial analysis, antifungal, let alone antiviral. So coconut water can be beneficial for humans as a probiotic which is a supplement that can help cure covid-19.

2. Research Method and Materials

2.1. Sample

Coconut water for isolation and identification of lactic acid bacteria and patients with Covid-19 as a sample to find out the ability of coconut water can help grow. The mediums used are MRS Broth, MRSA, and MRSA+ 0.3% CaCO3, which are selective media for lactic acid bacteria, the medium for isolating bacteriocin is MRS broth as well.

2.2. Instrument

Instruments used for this study are glass tools commonly used such as test tubes, Erlenmeyer, Petri dishes, measuring cups, cup glasses that are all pyrex output. In addition, Laminar Flow, an Autoclaff sterilization device with Dayhan WACS-1045 specifications, and a centrifuge with General Purpose Centrifuge CE 25340-GSL Trends Ventures specifications.used to format your manuscript.

2.3. Procedure

To isolate lactic acid bacteria from coconut water begins with a dilution of coconut water samples using sterile aquades, up to 10-7 dilutions. Furthermore, colonies of bacteria that grow in the "Halo" area, are scratched to be up to 3 times to get their isolate. Identification is carried out by morphologically and biochemical tests. After that, the bacteriocin is isolated. Bacteriocine insulation is done by deposition method using ammonium sulfate. To find out its antimicrobial capabilities, antimicrobial analysis is carried out with good methods so that they have been modified. Antimicrobial analysis using test bacteria E.coli, Staphylococcus aureus, Listeriamonocytogenes, Salmonella typhosa, and Pseudomonas aureginosa Last trialed the ability of coconut water to help cure in covid-19 patients with the provision of coconut water for a week

3. Results and Discussion

Isolation of lactic acid bacteria from coconut water with dilution up to 10^{-7}, obtained results at dilution of 10^{-1} to 10^{-4} using MRSA media, still obtained very tight colonies, and can not be calculated. At a dilution of 10^{-4} to 10^{-7} can be seen the growth of colonies that can already be calculated and are single colonies. As can be seen in Figure 1.

Figure 1 shows a single colony of lactic acid bacteria growing on MRSA media without the addition of $CaCO_3$. From the results of the colony count obtained isolate lactic acid bacteria at dilution 10-4 to 10-7, there are 73 bacterial isolates and 24 fungal isolates. The growth of colonies on MRSA media without the addition of CaCO3 because this medium is suitable for the growth of lactic acid bacteria. But the colony grew one-on-one, not within the "Hallo" area. As done by (Abdullah et al., 2019; Bartkiene et al., 2020; Syukur et al., 2018)

Figure 2 shows colonies of lactic acid bacteria grown on the selective medium MRSA + 0.3% CaCO3. Colonies of lactic acid bacteria grow in the middle of the "Halo" area. Where in the halo area, there is a neutralizing reaction between acids produced by lactic acid bacteria and CaCO3 bases. So that the area becomes clear, because this medium is a selective medium for these lactic acid bacteria, by using this medium, it can be ascertained that the one that grows in the Halo area is lactic acid bacteria, so it can be directly identified. As well as done by (Abdullah et al., 2019; Franco et al., 2020).

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Figure 1. Colony of lactic acid bacteria on MRSA media. Dilution 10^{-4}

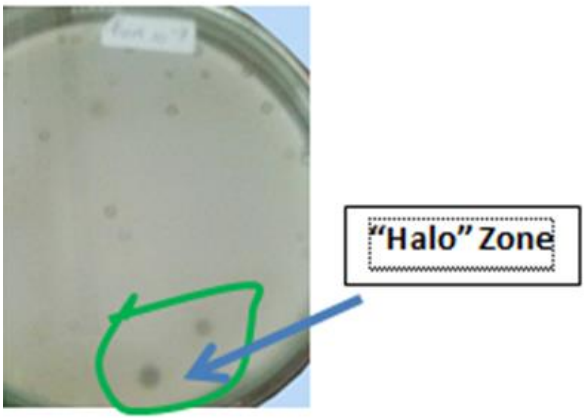

Figure 2. Colonies of lactic acid bacteria on MRSA + 0.3% CaCO3 media. Dilution 10-7

When it wants to be propagated and wants to be scratched on the Petri or to tilt, then the colony is taken in the middle.like figure 3. Figure 3. Showing the results of scratches of colonies of lactic acid bacteria taken from the "Halo" area in the middle and using MRSA media. It grows into a single colony for further identification of this single colony.

Isolation of lactic acid bacteria using 2 types of media namely MRSA media only and MRSA + CaCO3 give different results because, in MRSA + CaCO3 media, there is a neutralization reaction between lactic acid produced by BAL and CaCO3 base, which forms the Halo region. These results follow those conducted in the isolation study of lactic acid bacteria (Suryani, Dharma A, Manjang Y, Arief S, 2016).

Next, the colony is grown on the inesing to tilt the test tube, identified, to determine the type of microorganisms that grow. It turns out that Table 1 suggests that the results of isolation from lactic acid bacteria derived from coconut water, obtained as many as 73 bacterial isolates consisting of 17 isolates are *pediococcus* then *pediococcus sp* 2 as many as 2 isolates, and so on. This is by what is done by (Dowarah et al., 2018; Suryani et al., 2014; Yang et al., 2018).

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Figure 3. Colonies of lactic acid bacteria on the MRSA medium are scratched to tilt.

3.1. Identify lactic acid bacteria from coconut water

3.1.1. Morphological Identification

Table 2. Explaining that the morphological identification results of lactic acid bacteria isolated from coconut water form round bacteria in groups and tones also that break the stem. The color is grayish and the tone is clear and yellowish.

Table 2. Morphological identification of lactic acid bacteria.

No.	Types of Bacteria	shape	Color
1.	*Pediococcus 1*	Round group	gray white
2.	*Pediococcus 2*	Round group	gray white
3.	*Pediococcus 3*	Round group	gray white
4.	*Pediococcus 4*	Round group	gray white
5.	*Lactobacillus paracasei*	Basil	Clear yellow
6.	*Lactobaciillus plantarum*	Basil	Clear
7.	*Candida sp*	hyphae	White
8.	*Rizhopus*	hyphae	White blur

3.1.2. Identification with biochemical tests

To determine the type of lactic acid bacteria conducted a gram test that produces grams that are gram-positive, the test is a positive sugar test against Galactose, sucrose, maltose, and Lactose. This sugar test was also conducted by other researchers whose results bore similarities to this research, (Monika et al., 2017), (Husmaini et al., 2011) and show n by Table 3.

3.1.3. Isolation of bacteriocins from lactic acid bacteria

Lactic acid bacterial isolates, from *Lactobacillus plantarum*, *Lactobacillus para casei*, and *Pediococcus* in their bacteriocine insulation, and in deposition with ammonium sulfate deposit, which indicates the present or positive of the bacteriocin as figure 4. With deposits at the ends of the Eppendorf, it proves to us that our lactic acid contains bacteriocin according to what was studied (Emmanuel E.I. & Solomon E., 2021; Pingitore et al., 2009; Suryani, 2016; Zhang et al., 2013)

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Figure 4. Bacteriocinl precipitate

Table 3. Results of biochemical tests on isolate lactic acid bacteria from coconut water

Characterization	*L.plantarum*	*L.paracasei*	*Pediococcus*
Gram staining Test	+	+	+
Microscopic observation	Stalk	Stalk	group
Biochemical test			
Galactose	+	+	+
Lactose	+	+	+
Glucose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Nitrate Reduction	-	-	-
Hydrolytic Arginin	-	-	-
H2S	-	-	-
Catalase	+	+	+
OF	+	-	-
TSIA	K/K	K/K	-
Aerob/Anaerob	A	A	A

3.2. Antimicrobial/ antibacterial analysis

Antimicrobial or antibacterial analysis was conducted using 5 test bacteria, namely, *E.coli*, *Staphilococcus aureus, Listeria monocytogenes, Salmonella typhosa*, and *Pseudomonas aureginosa*. The result is a "Halo" area at the site of inhibition of the growth of pathogenic bacteria by lactic acid bacteria from coconut water, as in figure 5. The presence of lactic acid bacteria that can destroy pathogenic bacteria causes the area around the lactic acid tray colony that is sucked with pathogen bacteria to become "clear". Because the pathogenic bacteria cannot grow and die by antimicrobial analysis of lactic acid bacteria contained in coconut water in accordance with what is done (Ben Lagha et al., 2017; Emmanuel E.I. & Solomon E., 2021; Nahariah et al., 2020; Tenea & Lara, 2019).

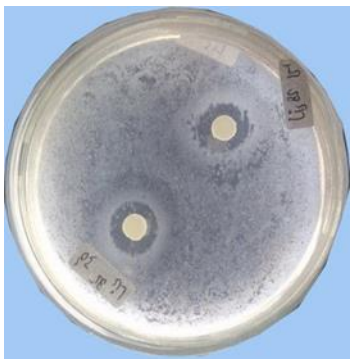

Figure 5. The "Halo" area inhibited the growth of Listeria monocytogenes by BAL from coconut water

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3.3. Results of antimicrobial analysis of BAL coconut water

The results of the antimicrobial analysis can be seen in table 5.

Table 4. Antimicrobial activity analysis of LAB in the form of clear zone diameter (mm)

Lactic Acid Bacteria	Code sample	E. coli (mm)	Listeria (mm)	S.typhy (mm)	S.aureus (mm)	P. aureginosa
L.plantarum	Pl 2.1	17	16	14	13	18
	Pl 2.5	16	15	15	12	19
	Pl .2.2	17	13	13	14	15
L.paracasei	Pr 1.5	14	17	12	13	17
	Pr. 2.3	14	15	9	14	15
	Pr. 1.1	15	16	14	13	16
Pediococcus 1	P 1.3	16	17	14	14	18
	P 1.2	12	17	13	12	17
	P 1.5	14	14	15	13	19
Pediococcus 2	P 2.3	15	16	12	13	19
	P 2.5	12	17	13	14	17
	P 2.1	14	17	12	12	15
Pediococcus 3	P 3.2	16	18	14	16	18
	P 3.1	15	15	12	17	16
	P 3.4	17	17	13	15	17
Pediococcus 4	P 4.2	15	18	16	16	17
	P 4.1	17	16	17	16	16
	P 4.5	14	18	18	15	19

3.4. The results of the Virgin Coconut Oil Use survey helped cure Covid-19 sufferers

To prove that VCO can help cure covid-19 sufferers, a survey of 50 VCO consumers was conducted. The consumer is asked to answer some questions through Google Form, the results of which are as follows,

Respondents came from several cities in Indonesia, namely Jakarta, Depok, Yogyakarta, Bukittinggi, Payakumbuh, Bandung and others (Figure 6).

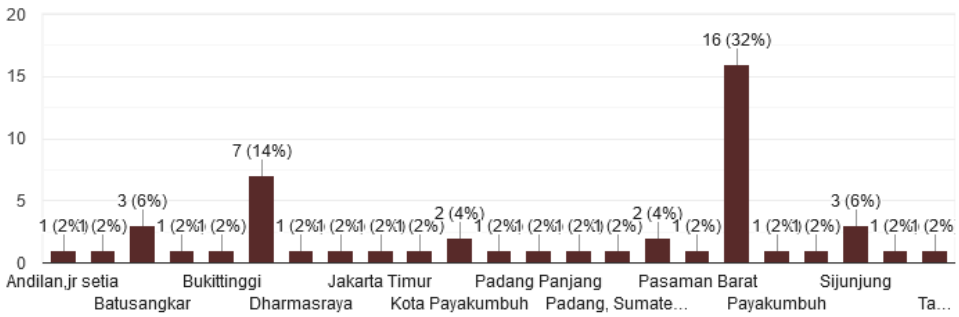

Figure 6. Respondents came from several cities in Indonesia.

Respondents consumed VCO for the purpose of Diet, Healing covid-19, to prevent infection by the covid-19 virus, lower blood pressure, lower blood sugar levels and lower cholesterol levels in the blood. And it turns out that the most is to help cure from covid-19, as in figure 7.

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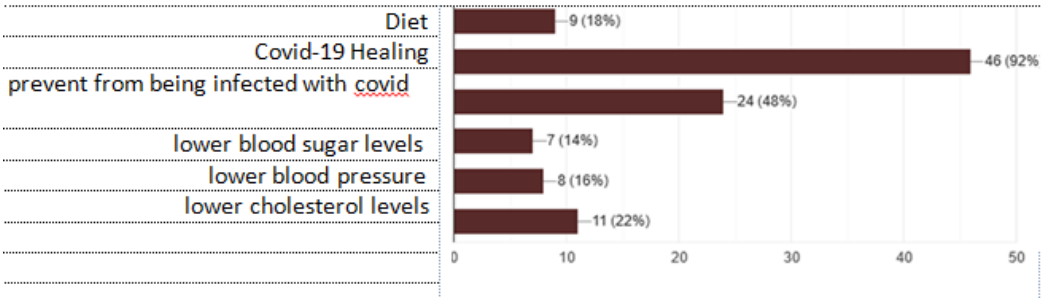

Figure 7. The use of VCO for its consumers.

4. Conclusion

It can be concluded from research that new probiotics can be isolated from coconut water. It turns out that eight new probiotic isolates can be isolated from coconut water. This new probiotic from coconut water has antimicrobial abilities against 5 test bacteria, namely *E.coli*, *Staphilococcus aureus*, *Listeriamonocytogenes*, *Salmonella typhosa*, and *Pseudomonas*. This unique probiotic from coconut water can serve as a supplement to help the treatment of Covid-19 patients. Thus, coconut water is a very large contribution to public health in the future.

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Hopefully this research can contribute to public health and to the progress of the Indonesian nation

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