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Isolation Of Nitrogen-Fixing Bacteria From Digestive Sludge Of Palm Oil Mill Waste On Ultisols Soil

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ABSTRACT

The anaerobic digestion of the biogas process results in Biogas Sludge palm oil liquid waste, which contains nitrogen-fixing bacteria that can increase nitrogen nutrients in the soil.The purpose of this study is to determine the characteristics and potential of nitrogen-fixing bacteria (NFB) in biogas sludge from anaerobic digestion of palm oil mill effluent in increasing nitrogen nutrient availability in ultisol soil.These stages of research would include (1) isolating NFB and obtaining morphological characteristics at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, (2) ability assessment for the potential isolate of NFB in producing nitrogenase enzyme activity at the Soil Research Institute, Bogor, West Java, (3) ability to provide nitrogen nutrients using the Complete Randomized Design (RAL) within three replications. This research was observed for eight months in the field. Provide nitrogen nutrients using a Complete Randomized Design (RAL) over three replications. This study was carried out in the area for eight months.The analysis indicated that seven nitrogen-fixing bacteria isolates had flat elevations, smooth edges, white color, Gram- negatives, and coccus cell shapes. Isolate N3 has the highest nitrogenase enzyme activity of 2.2 nmol/hour compared to other isolates and can increase the highest levels of ultisol soil nitrogen nutrients by 65% compared to controls. These findings indicate that isolating NFB from biogas sludge can increase nitrogen nutrient availability in ultisol soils.

Keywords: Nitrogen Fixing Bacteria, Palm Oil Mill Effluent, Ultisol, Biofertilizer

INTRODUCTION

Sludge biogas is a waste product from biogas installations by-product of an anaerobic composting system. Anaerobic digestion is used to minimize solid waste and produce biogas with the help of microorganisms,using a variety of strategies [1][2].This can also be a form of renewal for energy development in rural areas[3]. To increase biogas production, large anaerobic granules should be preferred in grain-based reactors[4]. This processing requires only low cost and low energy

consumption [5][6].The toxic oily-biological sludge that results is a potential source of biogas energy recovery.[7]. During decomposition, chemical changes and the microbiome are interdependent [8]. In addition, biogas sludge is a by-product of an anaerobic decomposition system that is free of pathogens and has a reasonably high nutrient content. These nutrients can be used as organic fertilizers to maintain soil fertility and increase crop production[9].

The increase in crop production is influenced by the nutrient content of biogas sludge which can be used as a source of plant organic matter to support photosynthesis. However, the nutrient source of biogas sludge can also threaten humans and the environment due to organic pollutants and heavy metals[10], [11]. In addition, seasons affect the life of plant organisms, helping to provide nutrients[12].

Palm oil liquid waste is a biogas raw material and contains several microorganisms such as acid-forming and hydrocarbon-degrading[13]–[16]. Bacteria associated with palm oil waste can produce antibiotics, biofertilizers, biohydrogen, bioinsecticides, organic acids, and enzymes. The bacteria contained in the biogas sludge have the potential as a biofertilizer.Further treated wastewater exhibits low biodegradability [17].For hydrolysis to increase, treatment before anaerobic digestion is required [18]. It implies a 15-day feeding strategy of 0.2 to 1 m3 d1 at three-day intervals[19].Maximizing the recovery of NH 3 gas during the composting process has the potential to provide economic benefits by producing high-value biomass without compromising the digestate quality[20], [21].

One of the nutrients contained in the biogas sludge is nitrogen. According to, nitrogen is a macronutrient essential for plant growth and development and the main protein and nucleic acids[22].The addition of nitrogen is an important factor that can change the dynamics of soil viruses and become better than water because of its nutritional capacity [23][24]. Fertilization using inorganic fertilizers can reduce soil health;using a biofertilizer containing nitrogen-fixing bacteria[25].Clostridium, Azomonas,Azotobacter, Klebsiella, Azospirillum,blue-green algae, and some Bacillus and Pseudomonas can convert nitrogen in the atmosphere into ammonia, which can grow freely in the rhizosphere (non-symbiotic) or symbiosis with plants.[26]–[28].M. vaginalus also acts as a spatial regulator of the cyanosphere because its composition focuses on nitrogen-fixing functions [29]. Plant size or age affects soil microbial diversity [30].Nitrogen quality impacts topsoil harvest in ultisols [31].

The potential of biofertilizer from biogas sludge is huge. However, the characteristics and magnitude of the potential in oil palm liquid waste sludge have not been found. Sterilized oil palm leaf tissue can be isolated to show nitrogen fixation ability [32]. Thus, fundamental research is required to determine the characteristics and potential of nitrogen-fixing bacteria isolated from

biogas sludge derived from palm oil waste for increasing nitrogen availability in ultisol soils.The purpose of this study is to determine the characteristics and potential of nitrogen-fixing bacteria (NFB) in biogas sludge from anaerobic digestion of palm oil mill effluent in increasing nitrogen nutrient availability in ultisol soil.

RESEARCH METHODS

Research Location

The research was carried out at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, and the Research and Technology Laboratory of PT. Socfindo, Bangun Bandar, and the Soil Research Institute, Cimanggu, Bogor, West Java. The source of the isolates was obtained from the palm oil mill biogas sludge (PKS) PT. Nubika Jaya, Pinang City, Labuhanbatu District.

Preparation of JNFB Media (James Nitrogen Free Malate Bromthymol Blue)

JNFB Media was made with 5 gr malic acid, 0.6 gr k2hpo4, 1.8 gr kh2po4, 0.2 gr mgso4.7h2o, 0.1 gr NaCl, CaCl2.2H2O 0,2 g, tritiplex 0.066 g, KOH 4.5 g, Bromthymol Blue 2.0 ml, and micronutrients (peptone) 2.0 ml., 15.0 g agar, 1000 ml distilled water with an analytical balance and dissolved with distilled water until dissolved, then heated the media solution on a hotplate until homogeneous.

Isolation of Nitrogen-fixing Bacteria

A total of 1 ml of the isolated bacterial suspension obtained is put into a test tube containing 9 ml of sterile distilled water and homogenized. Serially, 1 ml of the suspension from the previous dilution was added to 9 ml of new sterile distilled water. Then, the dilution was made to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over the JNFB medium aseptically. The culture medium was incubated for 2-3 days at room temperature. A positive test of nitrogen-fixing microbes was indicated by the presence of colonies growing on JNFB media. Bacteria that grew on JNFB media were selected to be purified and further tested.

Nitrogen-Fixing Bacterial Isolates' Morphological Characteristics

Morphological Characteristics of Nitrogen-Fixing Bacteria Isolate 1 ml was taken using a micropipette and then inoculated into a petri dish. The petri dish used contains jelly and is placed using the pour method. The biogas sludge suspension was incubated at 350C for 48 hours until colonies grew. The selection of purified bacterial colonies was based on differences in the appearance of colony morphology, including shape, elevation, margin, color, gram and cell shape, to obtain pure isolates (Table 1). The gram properties and shape of bacterial cells were determined by staining with crystal violet, iodine, 95% alcohol, and safranin and then observed using a stereo microscope with 400x

magnification. Gram-positive bacteria are shown as purple cells, while gram-negative bacteria are shown in red.

Nitrogen-fixing Bacteria Potential Test

The purified bacterial isolates were then retested for their ability to fix nitrogen based on the size of the pellicle formed on semi-solid JNFB media. A total of 1 bacterial culture was inoculated on semi-solid JNFB media aseptically and incubated for tendays at room temperature. The surface pellicle formed on the semi-solid JNFB media was measured and recorded.

Nitrogen-Fixing Bacteria's Resistance to N-total Ultisol Soil

The soil was taken from Simalingkar area, Medan Tuntungan District, Medan City with ultisol soil type, and several soil Physico-chemical characteristics were analyzed. The analysis results showed that the dominant ultisol soil characteristics were classified as very acidic for soil pH, low for Ca-dd, Na-dd, Al-dd, C-organic, N-total, K-dd, and Mg-dd (Table 1). Purified isolates were cultured in Nutrient Broth (NB) media for two days. Ultisol soil was autoclave sterilized and weighed 100 g before being placed in an Erlenmeyer flask. One milliliter of the isolate was inoculated and incubated for fourteen days (previously tested for total N content). The culture was shaken at 100 rpm periodically. This experiment was conducted using a completely randomized design (CRD) with three replications. At the end of incubation, the total N was determined by the Kjedahl method and compared with the control.Data on the ability of phosphate solubilizing bacteria to increase available P were analyzed usingvariance at the 5% level using SPSS software version 21. If the treatment had an effect, proceed to Duncan's Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

Physico-chemical characteristics of ultisol soils

In the study, preliminary data on the Physico-chemical characteristics of the soil were analyzed. The Physico-chemical characteristics of the ultisol soil in the study can be seen in table 1.

Table 1. The physico-chemical characteristics

No	Soil physico-chemical	Value	Category
1	Soil Texture		Clay Loam
	% sand	43	
	% dust	28.5	
	% clay	28.5	
2	Soil pH		Very Sour Soil

	Actual (H_2O)	4.10	
	Potential (KCl)	3.46	
3	C-organic (%)	1.97	Low
4	N-total (%)	0.17	Low
5	P-Available(mg/kg)	135.90	Very High
6	KTK (me/100 g)	31.06	High
7	K-dd (me/100 g)	0.35	Rendah
8	Ca-dd (me/100 g)	0.46	Very Low
9	Mg-dd (me/100 g)	0.63	Low
10	Na-dd (me/100 g)	0.06	Very Low
11	Al-dd (me/100 g)	0.05	Very Low

Nitrogen-Fixing Isolates' Morphological Characteristics

The isolation and selection of nitrogen-fixing bacteria resulted in seven pure isolates from biogas sludge, each with distinct morphological characteristics, as shown in table 2.

Table 2 shows the Morphology and Gram Traits of Nitrogen-fixing Bacteria.

Isolate Code	**Colony Morphology**				**Gram**	**Cell Morphology**
	Shape	**Tepi**	**Elevation**	**Colour**		
N1	Round	Smooth	Flat	White	-	coccus
N2	Round	Lobate	Flat	White	-	coccus
N3	Irregular	Irregular	Flat	White	-	Basil
N4	Irregular	Smooth	Convex	Clear Yellow	-	Basil
N5	Round	Smooth	Convex	White	-	coccus
N6	Irregular	Lobate	Flat	White	-	coccus
N7	Irregular	Smooth	Flat	Yellow	-	coccus

In Table 2, it can be seen that the shape of the nitrogen-fixing bacteria isolates from biogas sludge was round and irregular. The dominant isolates (N1, N4, N5, and N7) had smooth edges, and the other isolates (N2,N3,and N6) had curved and irregular edges. The dominant isolates (N1,N2,N3, N6, and N7) had a flat elevation, two isolates (N4 and N5) had a convex elevation. There were five types of isolates (N1, N2, N3, N5, and N6) with milky white color, and the remaining isolates N4 and N7 were clear and yellow. Overall, isolates of nitrogen-fixing bacteria have gram-negative and cocci cell shapes. Thus, nitrogen-fixing bacteria isolates from biogas sludge had flat elevation, smooth edges,

white color, were gram-negative, and had a cocci cell shape, indicating that 19 of the 20 nitrogen-fixing isolates isolated from palm roots were Gram-negative.Nitrogen fixers appear to be housed in special cavities and have oxalotrophic properties[33].The results of research by, from the isolates studied, the most dominant colony elevation on nitrogen-fixing bacteria isolates was flat, the color was white and yellow, while the cell shape was round and rods and the nitrogen-fixing bacteria obtained were Gram-negative. Potentially attractive plant organs for fixation and exhibit long-term differences in organ-specific bacterial communities associated with different supplies, mainly formed by plants[34]. Microclimate, soil depth, plant density affect community structure [35].

Test of the Ability of Isolates of Nitrogen

Table 3 shows the test results for nitrogen-fixing bacteria isolates' ability to form pellicles on semi-solid JNFB media, as well as their nitrogenase activity.

Table 3. Nitrogen Isolate Capability Testing

No.	Isolate Code	Isolate Picture	Pellicle Thickness (mm)	Nitrogenase Activity (nmol/hour)
1.	N1		8.64	0
2.	N2		2.50	0
3.	N3		2.90	2.2
4.	N4		2.00	0

5.	N5		3.00	0
6.	N6		3.50	0
7.	N7		2.50	1.04

In Table 3, based on the thickness of the resulting pellicle, it can be seen that nitrogen-fixing bacteria have varied N-fixing abilities and showed good conditions for nitrogenase activity[34]. The absence of excess oxygen in the medium causes bacteria to produce pelliclesin JNFB media. The rate of oxygen diffusion is the same as the rate of respiration of the organism, which is a favorable condition for the nitrogenase enzyme activity, which aids in reducing acetylene to ethylene.However, it was known that pellicle thickness did not affect nitrogenase activity in each isolate. For example,the N3 isolate had a pellicle thickness of 2.90 mm, and the highest nitrogenase activity was 2.20 nmol/hour compared to other isolates. Figure 1 depicts nitrogen-fixing bacteria's ability to form a pellicle on JNFB Semi-Solid media.

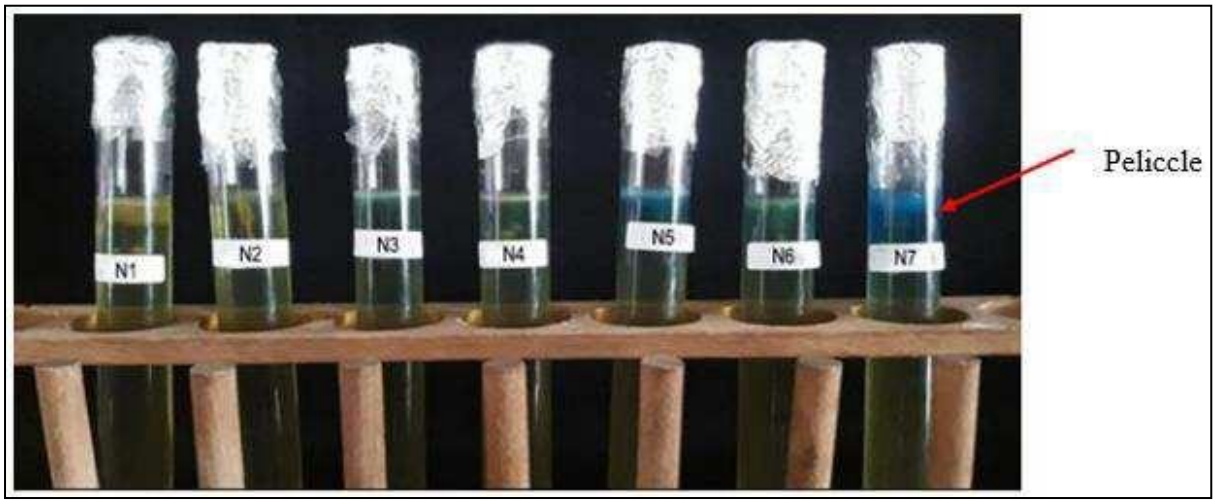

Figure 1. Nitrogen-fixing bacteria's ability to form a pellicle on JNFB Semi-Solid media.

Test the ability of nitrogen-fixing bacteria to increase the total N nutrient of ultisol soil.

A total of seven isolates of nitrogen-fixing bacteria were tested for their ability to increase total N. Table 4 shows the results of the variance test on the effect of nitrogen-fixing bacteria isolates on full N.

Table 4. The Effect of Some Nitrogen Fixing Bacterial Isolates on Total N Nutrients in Ultisols

No	BPN Isolation Treatment	N total (%)
1	N0: Control	0.20 e
2	N1 : Isolate BPN 1 + land100 gr	0.25 c
3	N2 : Isolate BPN 2 + land100 gr	0.28 bc
4	N3 : Isolate BPN 3 + land100 gr	0.33 a
5	N4 : Isolate BPN 4 + land100 gr	0.27 cd
6	N5 : Isolate BPN 5 + land100 gr	0.29 bc
7	N6 : Isolate BPN 6 + land100 gr	0.29 bc
8	N7: Isolate BPN 7 + land100 gr	0.31 ab

Description: In the DMRT test, the mean of treatment in the same column followed by the same letter shows no significant difference at a level of 0.05 percent.

The variance test results in table 4 showed that nitrogen-fixing bacteria isolates had a significant effect on total N in ultisol soils. The N3 isolate showed a very significant increase in total N levels in ultisol soils. This was because the nitrogenase activity of the isolates was highest at 2.20 nmol/hour compared to other isolates. According to him, N-fixing bacteria can help the growth of rice plants better in ultisol soils. N-fixing bacteria can grow well on ultisol soils that are not polluted. In addition to plant growth, Nitrogen-fixing bacteria can also grow on ultisol soils that have been burned, shallow solum, or lack nutrients, and can restore land ecosystems.

CONCLUSION

In seven isolates of dominant nitrogen-fixing bacteria, flat elevation, smooth edges, white color, Gram-negative, and cocci cell shape were discovered. Isolation and testing of potential nitrogen-fixing bacteria from oil palm wastewater biogas sludge yielded seven isolates with different shapes and all of them being gram-negative.The potency test was carried out in the soil, and N3 isolates were able to increase total N-nutrients by 65% compared to the control. This was due to the highest nitrogenase activity of N3 isolates compared to other isolates, which was 2.20 nmol/hour.As a result,

NFB isolates from biogas sludge are recommended for increasing nitrogen nutrient availability in ultisol soils.

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