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Effect of selected bacteria from biogas sludge on the growth and nutrition of upland rice

Efecto de las bacterias seleccionadas de los lodos de biogás en el crecimiento y la nutrición del arroz de secano

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ABSTRACT

This study evaluated the influence of selected superior bacterial isolates (SBI), biogas sludge, and their interactions on growth and nutrient uptake of upland rice in Ultisols. We used a randomized block design with two factors and seven replicates from October 2020 to April 2021. The first factor used selected SBI (B0 = untreated, B1 = nitrogen-fixing bacteria isolate (N3), B2 = phosphate solubilizing bacteria isolate (P7), B3 = isolate combination (N3+P7)). The second factor was the dosage of biogas sludge (S0 = untreated, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). The parameters were determined by ANOVA and followed by Duncan's multiple range test at $P<0.05$. The results showed that the isolate P7 significantly increased the N uptake by 20.77% and the highest crop growth rate (CGR) of upland rice 2.81 times. Biogas sludge doses from 315 to 630 ml/polybag significantly increased plant height, nutrient uptake of N and P, total fresh and dry weight, and CGR of upland rice. The interaction of N3 with biogas sludge dosage of 630 ml/polybag significantly increased the CGR of upland rice. The application of isolates N3 and P7 and their combination within biogas sludge of 630 ml/polybag has the potential to increase the CGR of upland rice in acidic soils.

Key words: acidic soil, crop growth rate, dosage, sludge potential.

RESUMEN

El presente estudio evaluó la influencia de aislamientos bacterianos superiores seleccionados (ABS), lodos de biogás y sus interacciones sobre el crecimiento y la absorción de nutrientes en el arroz de tierras altas cultivado en ultisoles. Se utilizó un diseño de bloques al azar con dos factores y siete repeticiones desde octubre de 2020 hasta abril de 2021. El primer factor utilizado seleccionó ABS (B0 = sin tratamiento, B1 = aislamiento de bacterias fijadoras de nitrógeno (N3), B2 = aislamiento de bacterias solubilizantes de fosfato (P7), B3 = combinación de aislados (N3+P7)). El segundo factor fue la dosificación del lodo de biogás (S0 = sin tratamiento, S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Los parámetros fueron determinados por análisis de varianza y seguidos de la prueba de rangos múltiples de Duncan a $P<0.05$. Los resultados mostraron que el aislamiento P7 aumentó significativamente la absorción de N en un 20,77% y la mayor tasa de crecimiento del cultivo (TCC) de arroz de tierras altas 2.81-veces. Las dosis de lodos de biogás de 315 a 630 ml/polybag aumentaron significativamente la altura de la planta, la absorción de nutrientes de N y P, el peso fresco y seco total y el TCC de arroz de tierras altas. La interacción de N3 con la dosis de lodos de biogás de 630 ml/polybag aumentó significativamente la TCC del arroz de tierras altas. La aplicación de los aislamientos N3 y P7 y su combinación dentro de lodos de biogás de 630 ml/polybag tiene el potencial de aumentar el TCC de arroz de tierras altas en suelos ácidos.

Palabras clave: suelo ácido, tasa de crecimiento de cultivos, dosis, potencial de lodo,.

Introduction

Biogas sludge is the waste by-product from an anaerobic processing system (FAO, 1977) and has a high nutrient content that can be used as organic fertilizer to increase soil fertility and the plants yield (Adela *et al*., 2014). The following characteristics of the biogas sludge from palm oil waste have been reported: total N of 490 mg L^{-1}, total P of 110 mg L^{-1}, total K of 1.9 mg L^{-1} (Lubis *et al*., 2014), C/N 8; 0.14% N, 1.12% C (Tepsour *et al*., 2019), and NH_3-N of 91 -112 mg L^{-1} (Choorit & Wisarnwan, 2007). The pH may range from 6.8 to 8.3,with the highest bacterial population of 7.21×10^7 cells per ml and the lowest one of 3.15×10^7 cells per ml (Alvionita *et al*., 2019). Additionally, Mustamu and Triyanto (2020) reported that the biogas sludge has nitrogen-fixing and phosphate solubilizing bacteria that have the potential to increase the availability of nitrogen and phosphate in soils.

The diversity of beneficial bacteria such as nitrogen-fixing and phosphate solubilizing bacteria has a greater potential to increase soil fertility and plant growth. Zhang *et al*. (2013) reported that phosphate solubilizing bacteria play an important role in increasing soil fertility, and plant yield, and reducing the use of chemical fertilizers. Sharma *et al*. (2013) described different *Bacillus* species, such as *B. circulans, B. cereus, B. fusiformis, B. pumilus, B. megaterium, B. mycoides, B. coagulans, B. chitinolyticus,* and *B. subtilis* as phosphate solubilizing microorganisms. Ambrosini *et al*. (2016) showed the highest nitrogenase activity in *Bacillus cereus* among 42 different strains of *Bacillus* spp. Lim *et al*. (2018) also reported the dominant bacteria found in the biogas sludge from anaerobic processing using the pyrosequencing and clone library methods, *i.e*., *Proteobacteria, Firmicutes, Bacteroidetes*, and *Thermotogae*.

The application of bacteria from biogas sludge has never been reported in Indonesia for improving upland rice growth on acidic soils, include Ultisols. According to the Center for Soil and Agro-climate Research, (2000) found that the area in Indonesia covered by Ultisols was 45.8 million ha, or 24% of the total area of Indonesia. Furthermore, the area of rice growing in Indonesia was 15,712,025 ha with the yield of 81,148,617 ton ha^{-1} in 2017 and the contribution of upland rice yield reaches 4.66% (Ministry of Agriculture, 2017). The yield contribution of upland rice was classified as low and it is necessary to develop yield through biogas sludge. Thus, it is necessary to test the potential of beneficial bacterial isolates from biogas sludge to increase the availability of nitrogen and phosphate, and the growth response of upland rice due to application of the biogas sludge and selected isolates in Ultisols. The study aimed to evaluate the influence of selected superior bacterial isolates, biogas sludge, and their interaction on the mineral nutrition of the upland rice grown in Ultisols.

Materials and methods

Study area

The concentration of total N and available P in Ultisols and in the plant tissue (N and P uptake) were analyzed in the Analytical Laboratory of Socfin Indonesia Inc., Medan (Indonesia). The bacterial isolates were applied to upland rice in the village of Padang Bulan (3°37.760' N; 98°38.898' E; altitude 18 m a.s.l.), Medan Selayang Subdistrict, Medan City, Indonesia, from October 2020 to April 2021. The average temperature, air humidity, and rainfall in this study were 27.4°C, 82%, and 228.5 mm per month, respectively.

Preparation of medium and upland rice seeds

The medium to grow upland rice plants used the Ultisols from the Simalingkar area, Medan Tuntungan Subdistrict, Medan City, at a depth of 0 to 20 cm. One hundred g of soil samples were taken and analyzed for chemical characteristics such as pH using H_2O, organic C by Walkley-Black, available P by Bray-II, total N using Kjeldahl method, and cation exchange capacity (CEC) and base saturation (K, Ca, Na, Mg) by ammonium acetate pH 7 method (Tab. 1). The soil was sterilized by drying at 100°C for 2 h. For prevent heat from the sterilization process, the soil is incubated for 1 d then placed into a 10 kg polybag (18 cm × 18 cm). A basic

NPK fertilizer (16-16-16) by Meroke Tetap Jaya Inc., at a dose of 1.5 g/polybag was applied by stirring evenly with the soil. The seeds of upland rice (*Oryza sativa* L.) used in the Inpago-8 inbred variety were of Indonesia Agency for Agricultural Research and Development, then soaked in water for 24 h, followed by a Propineb fungicide (70%) application for 2 h. Upland rice was planted after 1 d of basic fertilization with two seeds per polybag at a depth of 2 cm.

TABLE 1. Chemical characteristics of the Ultisols soil samples after sterilization at 100°C.

Chemical characteristics	Value	Category*
Soil pH (H_2O)	4.80	Acid
Organic C (%)	0.44	Very low
Total N (%)	0.04	Very low
Available P (mg kg^{-1})	870.25	Very high
CEC (meq 100 g^{-1})	28.31	High
Base saturation (%)	4.85	Very low
Exchangeable cations		
K (meq 100 g^{-1})	0.60	High
Ca (meq 100 g^{-1})	0.34	Very low
Mg (meq 100 g^{-1})	0.32	Very low
Na (meq 100 g^{-1})	0.09	Very low
Al (%)	0.02	Very low

*Criteria for pH H_2O = 4.5-5.5 (acid); organic C <1% (very low); total N <0.1% (very low); available P >60 mg kg^{-1} (very high); Cation exchange capacity (CEC) = 25-40 meq 100 g^{-1} (high); base saturation <20% (very low); exchangeable K= 0.60-1.00 meq 100 g^{-1} (high); exchangeable Ca <2 meq 100 g^{-1} (very low); exchangeable Mg <0.4 meq 100 g^{-1} (very low); exchangeable Na <0.1 meq 100 g^{-1} (very low); exchangeable Al <5% (very low) (Indonesia Soil Research Institute, 2009).

Preparation of superior bacterial isolates suspension and biogas sludge

A total of 1 ml of the bacterial isolate suspension obtained from the characteristic stage was put into a test tube containing 9 ml of distilled water and homogenized. It put a total of 1 ml of the suspension from the dilution into 9 ml of distilled water. The dilution was made to 10^{-5}. A total of 0.1 ml of the suspension from the last dilution was spread over the James Nitrogen Free Malat Bromothymol Blue (JNFB) medium for the nitrogen-fixing bacteria isolates test. While the suspension phosphate solubilizing bacteria isolates, the test was spread over Pikovskaya (PVK) medium. The culture medium was incubated for 2 to 3 days at room temperature. The nitrogen-fixing bacteria isolate test was characterized by the presence of colonies growing on the JNFB medium. The growth of phosphate solubilizing bacteria isolates is indicated by a halo zone around the microbial colonies on the PVK medium. The result was found in seven nitrogen-fixing and seven phosphate solubilizing isolates to produce total-N and available-P. The isolates of superior bacteria were selected which had the highest phosphate and nitrogen increasing abilities, namely phosphate solubilizing bacteria (P7) and nitrogen-fixing bacteria (N3) which were confirmed by Mustamu *et al*. (2021a, 2021b).

The biogas sludge was collected by the fixed tank of digester at the palm oil mill of Nubika Jaya Inc., Pinang City, Labuhanbatu District, North Sumatra Province, Indonesia and then handle with tongs. Bacterial isolates and biogas sludge were applied to the soil surface at the base of the plants at one week after planting. Biogas sludge samples at 500 ml volume were used to analyze the chemical and biological characteristics (Tab. 2).

TABLE 2. The chemical and biological characteristics of the biogas sludge.

Characteristics	Method	Value
pH	Electrometry	7.41
Chemical oxygen demand (mg L^{-1})	Spectrophotometry	4547.8
Biological oxygen demand (mg L^{-1})	Titrimetry	1127.5
Total N (%)	Kjeldahl	0.051
Total P (%)	Spectrophotometry	0.0097
Available-P (%)	Spectrophotometry	0.013
Total K (%)	Graphite furnace - atomic absorption spectrophotometry (AAS)	0.18
Organic C (%)	Walkley-Black	0.14
Ca (%)	Graphite furnace-AAS	0.04
Mg (%)	Graphite furnace-AAS	0.04
Na (mg L^{-1})	Graphite furnace-AAS	44.41
Cu (%)	AAS	0.0001
Total nitrogen-fixing bacteria (CFU ml^{-1})	Plate count	29.4×10^5
Total phosphate solubilizing bacteria (CFU ml^{-1})	Plate count	7.0×10^4

Source: laboratory analysis

Treatment application

This study used a randomized block design with two factors and seven replicates. The first factor was the type of superior bacterial isolates (B0 = untreated; B1 = nitrogen-fixing bacterial isolate (N3); B2 = phosphate solubilizing bacteria isolate (P7); B3 = combination of isolates N3+P7) at a similar dose, namely 10 ml/polybag. The second factor was dose of biogas sludge

(S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Determination of biogas sludge based on the dose of liquid organic fertilizer at the oil palm was 126 m^3 ha^{-1} equal to 126,000 L ha^{-1} (Sutarta *et al.,* 2003), then converted to soil weight per polybag (Eq. 1). Each replicate was disassembled at 4, 8, and 12 weeks after the application (WAA) for determination of the crop growth rate (CGR).

$$\text{Biogas sludge} = \frac{\text{The dose of liquid organic fertilizer ha}^{-1}}{\text{Soil weight ha}^{-1}} \times \text{soil weight per polybag} \qquad (1)$$

$$= \frac{26{,}000 \text{ L ha}^{-1}}{2{,}000{,}000 \text{ kg ha}^{-1}} \times 10 \text{ kg} = 630 \text{ ml}$$

Parameters and data analysis

The observations of the variables were conducted by measuring the growth of upland rice (plant height, and total fresh and dry weight), nutrient contents and uptake of N and P in the shoots, and CGR. The plant height was measured by the base of the roots to the tip of leaves, and the total fresh weight was obtained by weighing the roots and shoots. The total dry weight (roots+shoots) was measured after using an oven (model VS-1202D3, Vision Scientific Co., Ltd., Korea) at 60°C for 48 h and weighed using the analytical scales. A 200 g sample of the second leaf from the shoots was collected and analyzed to determine the N content using the Kjeldahl and the P content was estimated using the destruction method trought dry ashing. The N and P uptake were measured using Equation 2. The CGR was calculated as the dry weight related to the unit area at 4-8, 8-12, and 12-16 WAA using Equation 3 (Shon *et al.,* 1997):

$$\text{Nutrient uptake} = \text{nutrient content in the shoots} \times \text{total dry weight} \qquad (2)$$

$$\text{CGR} = \frac{\Delta W}{\Delta t} = \frac{W2 - W1}{t2 - t1} \qquad (3)$$

where:

CGR = crop growth rate;

W1 = dry weight per unit area at t1;

W2 = dry weight per unit area at t2;

t1 = first sampling;

t2 = second sampling;

The parameters of the second phase of the study were analyzed by an ANOVA and if the treatment had a significant effect, followed by Duncan's multiple range test at $P<0.05$ using SPSS v.20 software (IBM, 2011).

Results

Effect of bacterial isolates and biogas sludge on upland rice growth

Plant height of upland rice

The effect of biogas sludge application was significant on the plant height of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the plant height of upland rice at 4, 8, 12, and 16 WAA (Fig. 1). A significant increase in plant height of upland rice was observed with increased doses of biogas sludge of 630 ml/polybag at 8, 12, and 16 WAA with the highest increase of 14.81% compared to the control at 16 WAA. Although the effect was not significant, the combination of isolates B3 and the interaction of B2S3 showed the highest in plant height of upland rice by 2.94 and 22.06%, respectively, compared to the control.

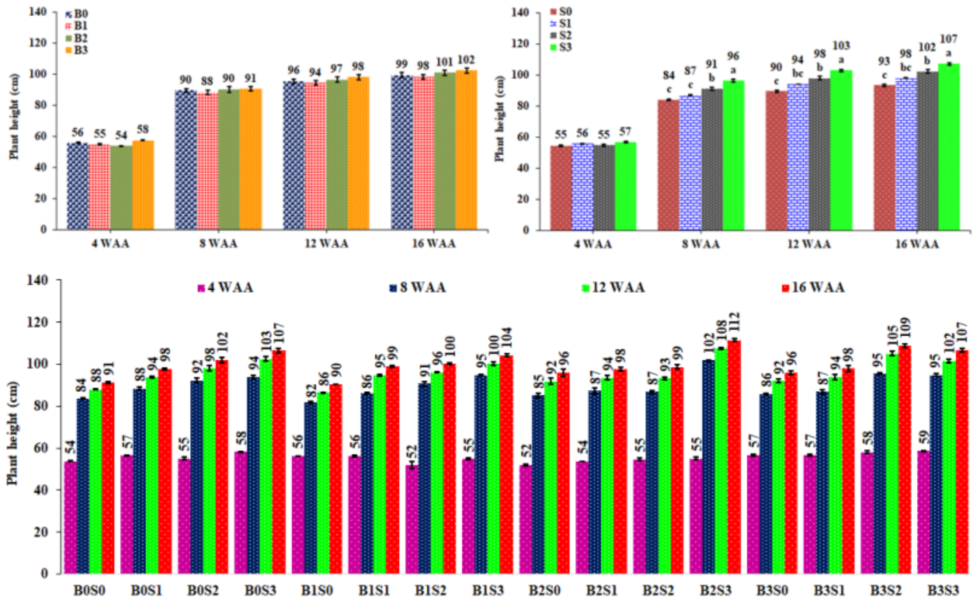

FIGURE 1. Effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on plant height of upland rice at 4, 8, 12, and 16 WAA. Values followed by the different letter in the graph significantly differed according to the Duncan test at *P*<0.05. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Biomass of upland rice

The effect of biogas sludge significantly increased the total fresh weight of upland rice at 8, 12, and 16 WAA. Superior bacterial isolates and their interactions did not have a significant effect on the total fresh weight of upland rice at 4-16 WAA (Tab. 3).

TABLE 3. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total fresh weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	Total fresh weight ± standard error (g)			
	4 WAA	8 WAA	12 WAA	16 WAA
Superior bacterial isolates (B)				
B0	4.15 ± 0.21	169.31 ± 8.90	215.27 ± 8.42	229.82 ± 8.94
B1	3.12 ± 0.12	194.50 ± 9.35	235.08 ± 10.32	252.02 ± 10.22
B2	4.52 ± 0.23	162.89 ± 11.15	201.85 ± 9.89	230.70 ± 9.28
B3	3.30 ± 0.25	173.91 ± 12.55	220.40 ± 15.96	245.03 ± 16.32
Biogas sludge (S)				
S0	3.72 ± 0.24	144.07 ± 9.37 b	182.67 ± 7.14 b	197.56 ± 6.58 b
S1	3.58 ± 0.27	153.41 ± 7.93 b	190.70 ± 8.90 b	215.65 ± 7.03 b
S2	3.64 ± 0.27	199.68 ± 10.30 a	258.70 ± 9.63 a	280.15 ± 9.25 a
S3	4.15 ± 0.25	203.45 ± 1.36 a	240.52 ± 2.81 a	264.21 ± 2.42 a
Interactions (B×S)				
B0S0	4.99 ± 0.33	124.08 ± 5.60	185.64 ± 3.32	192.78 ± 2.96

B0S1	3.47 ± 0.26	160.43 ± 1.16	188.60 ± 5.76	207.05 ± 3.97
B0S2	3.42 ± 0.42	185.97 ± 6.80	232.60 ± 8.75	250.84 ± 7.40
B0S3	4.71 ± 0.42	206.76 ± 5.49	254.23 ± 10.27	268.61 ± 8.85
B1S0	2.80 ± 0.18	155.79 ± 1.12	183.96 ± 5.20	202.88 ± 2.88
B1S1	3.74 ± 0.29	174.82 ± 9.01	227.91 ± 6.38	236.60 ± 6.32
B1S2	3.28 ± 0.40	241.17 ± 5.25	283.60 ± 7.76	296.08 ± 8.05
B1S3	2.67 ± 0.22	206.20 ± 7.23	244.85 ± 6.26	272.52 ± 4.34
B2S0	3.19 ± 0.18	190.90 ± 7.77	215.36 ± 7.67	229.11 ± 6.75
B2S1	4.85 ± 0.38	106.74 ± 13.42	143.16 ± 13.02	179.61 ± 10.36
B2S2	5.20 ± 0.24	148.40 ± 11.59	219.65 ± 5.26	248.72 ± 6.94
B2S3	4.82 ± 0.45	205.53 ± 10.50	229.21 ± 16.57	265.34 ± 9.58
B3S0	3.91 ± 0.30	105.53 ± 3.94	145.72 ± 1.96	165.45 ± 1.11
B3S1	2.25 ± 0.09	171.63 ± 4.90	203.14 ± 7.07	239.34 ± 12.07
B3S2	2.66 ± 0.14	223.17 ± 7.84	298.95 ± 1.51	324.94 ± 3.03
B3S3	4.37 ± 0.07	195.31±6.77	233.79 ± 8.40	250.38 ± 8.16
CV (%)	56.09	29.68	26.31	20.78

Values followed by the different letter in the column significantly differed according to the Duncan test at $P<0.05$. ns - not significantly, CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

A significant increase in the total fresh weight of upland rice was observed with the higher dose of biogas sludge of 315 ml/polybag at 16 WAA, with the highest increase of 41.81% compared to the control. Although the effect was not significant, B1 and the interaction of B3S2 showed the highest in the total fresh weight of upland rice with 9.66 and 68.55%, respectively, compared to the control.

The effect of biogas sludge significantly increased the total dry weight of upland rice at 12 and 16 WAA. Superior bacterial isolates and their interactions had an insignificant effect on the total dry weight of upland rice at 4 -16 WAA (Tab. 4).

TABLE 4. Effect of superior bacterial isolates, biogas sludge, and their interactions on the total dry weight (shoot+roots) of individual upland rice plants at 4, 8, 12, and 16 weeks after the application (WAA).

Treatments	**Total dry weight ± standard error (g)**			
	4 WAA	**8 WAA**	**12 WAA**	**16 WAA**
Superior bacterial isolates (B)				
B0	1.38 ± 0.06	48.01 ± 1.29	73.60 ± 3.99	82.52 ± 4.18
B1	1.13 ± 0.05	54.09 ± 2.41	76.83 ± 2.66	99.72 ± 4.15
B2	1.49 ± 0.06	47.30 ± 3.30	73.20 ± 2.28	98.25 ± 3.90
B3	1.15 ± 0.07	52.32 ± 3.39	77.18 ± 4.90	98.47 ± 4.56
Biogas sludge (S)				
S0	1.26 ± 0.06	45.51 ± 2.63	62.88 ± 2.19 b	76.78 ± 1.63 c
S1	1.23 ± 0.08	44.47 ± 1.71	68.52 ± 2.00 ab	87.65 ± 2.84 bc
S2	1.26 ± 0.08	55.36 ± 3.43	85.69 ± 1.08 a	98.95 ± 1.86 b
S3	1.40 ± 0.06	56.38 ± 1.05	83.73 ± 3.44 a	115.59 ± 2.11 a
Interactions (B×S)				

B0S0	1.58 ± 0.08	41.73 ± 2.78	58.08 ± 1.54	67.23 ± 0.96
B0S1	1.12 ± 0.08	45.87 ± 0.83	62.74 ± 1.83	71.08 ± 1.91
B0S2	1.20 ± 0.12	52.25 ± 2.07	81.39 ± 5.48	88.28 ± 5.02
B0S3	1.60 ± 0.12	52.18 ± 0.29	92.20 ± 3.05	103.49 ± 2.43
B1S0	0.97 ± 0.04	46.64 ± 1.39	69.53 ± 4.90	80.30 ± 4.51
B1S1	1.40 ± 0.07	48.13 ± 2.78	78.91 ± 0.53	96.23 ± 1.50
B1S2	1.12 ± 0.10	67.79 ± 1.44	91.05 ± 2.25	101.80 ± 2.40
B1S3	1.02 ± 0.08	53.81 ± 3.76	67.84 ± 1.77	120.54 ± 2.15
B2S0	1.17 ± 0.05	59.32 ± 2.33	70.92 ± 4.20	81.43 ± 3.82
B2S1	1.54 ± 0.10	34.47 ± 2.16	61.69 ± 1.97	89.84 ± 1.41
B2S2	1.73 ± 0.05	37.37 ± 3.74	83.10 ± 1.19	105.46 ± 1.37
B2S3	1.53 ± 0.10	58.05 ± 1.76	77.07 ± 4.27	116.28 ± 1.30
B3S0	1.30 ± 0.07	34.35 ± 7.04	52.98 ± 0.73	78.16 ± 0.48
B3S1	0.85 ± 0.03	49.40 ± 0.08	70.72 ± 1.29	93.44 ± 2.19
B3S2	0.99 ± 0.05	64.05 ± 4.68	87.22 ± 2.90	100.26 ± 1.93
B3S3	1.44 ± 0.02	61.48 ± 2.47	97.80 ± 0.77	122.04 ± 0.20
CV (%)	43.80	31.22	26.54	18.38

Values followed by the different letter in the column significantly differed according to the Duncan test at $P<0.05$. ns= not significantly, CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

A significant increase in total dry weight of upland rice was observed with the increase in the dosage of biogas sludge of 630 ml/polybag at 16 WAA, with the highest increase of 50.55% compared to the control. Although the effect was not significant, B1 and the interaction of B3S3 showed the highest in the total dry weight of upland rice with 20.84 and 81.53%, respectively, compared to the control.

Crop growth rate of upland rice

The effect of superior bacterial isolates, biogas sludge, and their interactions significantly increased the crop growth rate of upland rice at 12 to 16 WAA, but it did not have a significant effect at 4-8 and 8-12 WAA (Tab. 5).

TABLE 5. Effect of superior bacterial isolates, biogas sludge, and their interactions on the crop growth rate of the upland rice.

Superior bacterial isolates (B)	**Biogas sludge (S)**				**Average**
	S0	**S1**	**S2**	**S3**	
			4-8 WAA		
B0	1.434	1.598	1.823	1.806	1.665
B1	1.631	1.669	2.381	1.885	1.892
B2	2.077	1.176	1.273	2.019	1.636
B3	1.180	1.734	2.252	2.144	1.828
Average	1.580	1.544	1.932	1.964	CV = 32.28%

			8-12 WAA		
B0	0.584	0.602	1.041	1.430	0.914
B1	0.818	1.099	0.831	0.501	0.812
B2	0.414	0.972	1.633	0.679	0.925
B3	0.665	0.761	0.828	1.297	0.888
Average	0.620	0.859	1.083	0.977	CV = 56.17%
			12-16 WAA		
B0	0.327 fgh	0.298 gh	0.246 h	0.403b-h	0.318 b
B1	0.385 c-h	0.619 a-h	0.384 d-h	1.882a	0.817 a
B2	0.375 e-h	1.005 a-h	0.798 a-h	1.400a-h	0.895 a
B3	0.899 a-h	0.811 a-h	0.466 a-h	0.866a-h	0.761 a
Average	0.496 b	0.683 b	0.474 b	1.138 a	CV = 51.07%

Values followed by the different letter in the column significantly differed according to the Duncan test at $P<0.05$. ns - not significantly, CV - coefficient of variation. Dosage of biogas sludge (S0 = untreated; S1 = 157.5; S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = un-treated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

The biogas sludge dose of 630 ml/polybag (S3) significantly increased the highest crop growth rate for upland rice at 12 to 16 WAA by 129.44% compared to the control. The isolates B1-B3 significantly increased the crop growth rate of upland rice with the highest increase for B2 of 181.45% compared to the controls at 12 to 16 WAA. The interaction of the B1S3 significantly increased the crop growth rate of upland rice, showing values 5.76-times greater than those of the control.

Effect of bacterial isolates and biogas sludge on upland rice nutrition

Nutrient content of N and P in the upland rice

The effect of biogas sludge, superior bacterial isolates, and their interactions did not have a significant effect on the nutrient content of N and P in the upland rice (Fig. 2). The biogas sludge doses of 315 and 630 ml/polybag (S2 and S3) explained that the contents of P and N in the plant tissue of upland rice were 33.33 and 4.53% higher, respectively, compared to the control. The isolate B2 showed the highest content of N in the plant tissue of upland rice with values 1.63% higher than those of the control; however, all isolates (B1-B3) showed a similar level of P in the plant tissue of upland rice compared to the control.

Nutrient uptake of N and P in the upland rice

The effect of biogas sludge significantly increased in the nutrient uptake of N and P. The superior bacterial isolates significantly increased in the nutrient uptake of nitrogen. The interaction of biogas sludge with superior bacterial isolates did not show a significant effect on the nutrient uptake of N and P in the upland rice (Fig. 2).

A significant increase in the nutrient uptake of N and P in upland rice was observed with a higher dose of biogas sludge of 630 ml/polybag, with the highest increases of 54.11 and 65.20%, respectively, compared to the control. The superior bacterial isolates (B1-B3) also significantly increased the nutrient uptake of N in the upland rice with the highest increase with the B2 of 20.77% compared to the control. Although the effect was not significant, B3 showed the highest in nutrient uptake of P in the upland rice of 19.93% compared to the control.

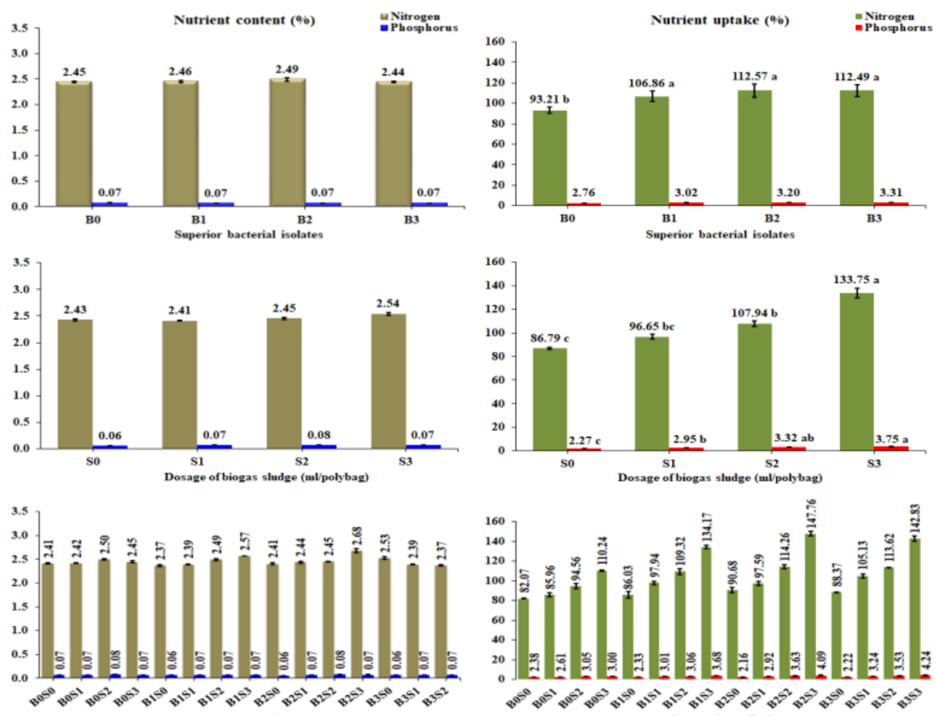

FIGURE 2. The effect of superior bacterial isolates, dosage of biogas sludge, and their interactions on the nutrient content and uptake of N and P in the upland rice. Values followed by different letters significantly differed according to the Duncan test at $P<0.05$. ns - not significant. Dosage of biogas sludge (S0 = untreated; S1 = 157.5;

S2 = 315; S3 = 630 ml/polybag). Superior bacterial isolates (B0 = untreated; B1 = isolate N3, B2 = isolate P7, B3 = N3+P7 isolates).

Discussion

Effect of selected superior bacterial isolates

The selected superior bacterial isolates (N3 and P7) significantly increased the nutrient uptake of nitrogen and crop growth rate of upland rice on Ultisols at 12 to 16 WAA, but it did not have a significant effect on plant height, total fresh weight, total dry weight, nutrient content (N and P) in leaf tissue, nutrient uptake of phosphorus, and crop growth rate of upland rice at 4 to 8 and 8 to 12 WAA. The superior bacterial isolates (N3, P7, and N3+P7) could increase the nutrient uptake of nitrogen in upland rice by 14.64%, 20.77%, and 20.68%, respectively, compared to the control (Fig. 2). Similar results are also shown in Table 5, where can be observed that the crop growth rate of upland rice at 12 to 16 WAA has increased 2.57, 2.81, and 2.39 times, respectively due to the selected superior bacterial isolates (N3, P7, N3+P7), compared to the control. The results indicate that the ability of a single P7 bacterial isolate was greater in increasing the nitrogen and crop growth rate of upland rice compared to a single N3 isolate and the combination of N3+P7 isolates. This was due to the presence of several organic acids and hormones produced by P7 that can increase the nutrient uptake of nitrogen and crop growth rate of upland rice. This result is supported by Mustamu *et al*. (2021a) who found that the phosphate solubilizing bacterial isolate (P7) from the biogas sludge contains organic acids such as lactic, oxalic, acetic, and citric acids, and had the highest ability to solubilize phosphate from calcium triphosphate and rock phosphate with values 4.62 and 2.66 times higher, respectively, compared to the control. Meena *et al*. (2016) reported that the availability of nitrogen and phosphorus in soils slightly increased with the application of bio fertilization with *Bacillus cereus*; this was due to the production of organic acids and other chemicals such as citric, tartaric, and oxalic acids that can stimulate plant growth and nutrient availability. Youssef and Eissa (2017) reported that the increase in vegetative growth and total biomass was due to increased photosynthesis, translocation, and accumulation of mineral nutrients. Khan *et al*. (2020) reported that *Bacillus cereus* strain SA1 can produce the hormones gibberellin, indole-acetic acid (IAA), and organic acids. Ferrara *et al*. (2012) reported that the hormone gibberellin and IAA, can increase plant growth under stressful conditions. Kang *et al*. (2014) said that the Plant Growth-Promoting Bacteria (PGPB) has several mechanisms to increase plant growth with nitrogen-fixation and phosphate solubilization, increasing nutrient availability. Suksong *et al*. (2016) reported that bacteria of palm oil solid waste from an anaerobic digester include *Ruminococcus* sp., *Thiomargarita* sp., *Clostridium* sp. *Anaerobacter* sp., *Bacillus* sp., *Sporobacterium* sp., *Saccharofermentans* sp., *Oscillibacter* sp., *Sporobacter* sp., and *Enterobacter* sp. Liaquat *et al*. (2017) also reported abundance of *Bacillus, Clostridium*, and *Enterobacter* spp. in an anaerobic digester of wastewater when producing biogas.

Effect of biogas sludge

The dose of biogas sludge significantly increased plant height, total fresh weight (8, 12, and 16 WAA), total dry weight (12 and 16 WAA), nutrient uptake (N and P), and the crop growth rate of upland rice at 8 to 12 WAA. However, it did not have a significant effect on nutrient content (N and P) in leaf tissue, and crop growth rate of upland rice (4-8 and 8-12 WAA). An increase in plant height, total dry weight, nutrient uptake in terms of nitrogen and phosphorus, and also crop growth rate of upland rice on Ultisols with a higher dose of biogas sludge of 630 ml/polybag at the end of this study (16 WAA). In contrast, the total fresh weight had an increasing along with the increase at the dose of biogas sludge to 315 ml/polybag then decreased at the dose of 630 ml/polybag. This result is supported the biogas sludge had chemical characteristics such as pH (7.41), total N (0.051%), available P (0.013%), organic C (0.14%), total K (0.18%), and biological characteristics such as total nitrogen-fixing bacteria (29.4×10^5

CFU ml^{-1}) and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2). The organic C content and the total population of nitrogen-fixing and phosphate solubilizing bacteria from the biogas sludge could increase the nutrient uptake in terms of nitrogen and phosphorus in upland rice with an increasing dose of biogas sludge of 630 ml/polybag (Fig. 2). Therefore, the nutrients absorbed are used for plant metabolic processes and stimulate the plant height, biomass, and crop growth rate of the upland rice. A similar result was reported by Mustamu and Triyanto (2020) who determinedthe macro and micronutrients from the biogas sludge and the population of nitrogen-fixing and phosphate solubilizing bacteria of 480×10^4 and 42×10^4 CFU ml^{-1}, respectively. Ndubuisi-Nnaji *et al*. (2020) reported that the total phosphate solubilizing bacteria (1.6 to 2.5 CFU ml^{-1}) was significantly higher compared to nitrogen-fixing bacteria (0.5-1.4 CFU ml^{-1}) showing a significant increase in nutrient concentration in the order of N>K>P>Ca>Mg>S in all anaerobic digester bioreactors. Möller and Müller (2012) reported that an increase in concentrations of NH_4^+-N ranged from 45 to 80% in the anaerobic waste.

Interaction of selected superior bacterial isolates and biogas sludge

The interaction of biogas sludge and superior bacterial isolates only significantly increased the crop growth rate of upland rice on Ultisols at 12-16 WAA, but it did not have a significant effect on the other parameters in this study. The interaction of B1 with biogas sludge at the dose of 630 ml/polybag (B1S3) showed the highest crop growth rate of upland rice compared to other interactions and was 5.76-times greater compared to the control. This was caused by the application of biogas sludge that could have increased soil organic matter and the total population of beneficial bacteria. Likewise, the biogas sludge contained organic C (0.14%), total nitrogen-fixing bacteria (29.4×10^5 CFU ml^{-1}), and total phosphate solubilizing bacteria (7.0×10^4 CFU ml^{-1}) (Tab. 2) that could improve soil quality and support the crop growth rate. This result is supported by Urra *et al*. (2019) who found that the application of sewage sludge in the long term significantly increases the organic matter contents in the soil, causing a decrease in soil pH due to the nitrification of ammonium in sewage sludge and the production of organic acids along with the decomposition of the organic matter. Bhardwaj *et al*. (2014); Carvajal-Muñoz and Carmona-Garcia (2012) showed that the application of a biofertilizer had advantages in the plant such as availability of nutrients that are balanced for plant health. It also stimulates nutrient mobilization that can increase soil biological activity and the availability of microbial food to encourage the growth of beneficial microorganisms, increasing the soil organic matter content and, therefore, the cation exchange capacity. Siswanti and Lestari (2019) indicated that the interaction of biogas sludge+biofertilizer (36 ml+10 L ha^{-1}) significantly increased the plant height, number of leaves, and capcaicin content in chili pepper compared to a single treatment of biogas sludge and biofertilizer.

Conclusions

The isolates N3, P7, N3+P7 from the biogas sludge significantly increased the nutrient uptake of nitrogen (20.77%) and crop growth rate (2.81 times higher than the control) of upland rice on Ultisols with the highest increase found with the P7 isolate. The dose of biogas sludge significantly increased plant height (14.81%), total dry weight (50.55%), nutrient uptake of nitrogen (54.11%) and phosphorus (65.20%), and also crop growth rate (129.44%) of upland rice on Ultisols with the highest increase at a dose of 630 ml/polybag. Likewise, the dose of biogas sludge significantly increased the total fresh weight of upland rice by 41.81% with the highest increase at the dose of 315 ml/polybag. The interaction of isolates N3, P7, N3+P7 with the dose of biogas sludge only significantly increased the crop growth rate of upland rice on Ultisols 5.76 times with the highest increase found with B1S3.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors formulated the overarching research goals and aims, provided the study materials, developed or designed the methodology. NEM analyzed and interpreted the study data. NEM and MS wrote the initial draft, managed and coordinated the research activity in the field, and collected the data. ZN and I verified the overall reproducibility of results and the other research outputs. All authors conducted the critical review/commentary/revision of the manuscript.

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