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Research Article

Effect of Methanotroph Bacteria Isolated from Paddy Rice Plant (*Oryza sativa* L.) on Growth and Yield Components of Rice

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The present study was initiated to determine whether isolates from soil and roots of paddy plants can affect the paddy plant's growth and productivity. The study was conducted to answer the question, "Can paddy rice be grown when the NPK doze is reduced?" This study aims to apply the methanotroph bacteria on the growth and production of lowland rice. The research field was carried out in the rice fields of Amparita Village, TelluLimpoe District, Sidenreng Rappang Regency, South Sulawesi. This research was conducted from June to September 2020. The plants were arranged in a split plot Randomized Complete Block Design (RCBD), the main plot, namely, the NPK fertilizer dosage treatment (P) with 4 treatments, namely, without NPK fertilizer, 75 g/plot, 150 g/plot, and 360 g/plot. The subplots were methanotroph bacteria application with 4 treatments, namely: without bacteria, 10^6 CFU per ml of methanotroph, 10^7 CFU per ml of methanotroph, and 10^8 CFU per ml of methanotroph. The results showed that there was an interaction between the NPK fertilizer and methanotrophic bacteria. The best results were obtained on the combination of 360 g per plot of NPK fertilizer and methanotrophic bacteria with 10^6 CFU per ml on the observation of plant height (111.17 cm), and the combination of NPK fertilizer 0 g per plot and methanotrophic bacteria with 0 CFU per ml on the observation of fresh weight of grain was the highest (70.44 g), whereas the combination of NPK fertilizer treatment 360 g/plot and bacteria methanotroph 0 CFU per ml on the observation of dry weight of grain was 43.89 g. NPK fertilizer 360 g/plot and bacteria methanotroph 10^6 CFU per ml at an observation weight of 100 grains was the highest number (3.53 g).

1. Introduction

Rice is a commodity that is in demand by all elements of Indonesian society. Increasing productivity and rice production must continue to be carried out to increase farmers' income and welfare and ensure food security (Satria, 2017). According to the National Council for Climate Change (DNPI, 2010), the agricultural sector has contributed the third highest greenhouse effect as gas emissions (CH_4, CO_2, N_2O, and H_2O). The greenhouse gas emissions effect was from land use change which are ranked in the first and second place. This greenhouse gas emission was derived from peat lands. With the increase in rice production, CH_4 emissions will also increase if the processing is not accompanied by efforts to reduce emissions. One of the efforts to reduce CH_4 emissions is by utilizing methanotrophic microbes. These microbes will use methane as a source of carbon and energy [1].

Rice fields in Indonesia have the potential to contribute to methane (CH4) emissions, as a greenhouse gas. CH4 emissions in the atmosphere are generally influenced by the methane cycle methanogens—methanotrophs and vertical methane transfer [2]. One of the major problems facing humanity today and in the future is global warming. Global warming is related to climate change that can affect changes in biodiversity. The increase in the amount of greenhouse gases (GHG) affects the composition of gases in the atmosphere and causes global warming, due to the production of methane (CH_4) and nitrous oxide (N_2O) [2]. Rice cultivation emits methane as much as 75,419.73 Gg (gigagrams) in 2000. Although CH_4 gas only contributes about 15 percent of all greenhouse gases, this gas is 21 times more likely to cause greenhouse effects than CO_2 gas [3].

Panjaitan stated that CH_4 emissions are influenced by water management, tillage, rice varieties, and climate [4]. Excessive use of nitrogen fertilizers apart from being inefficient can also harm plants and the environment [5]. On the ecological side, the impact of increasing CH_4 emissions in the form of global warming and climate change generated by lowland rice farming to the environment must receive attention [6]. Methane gas (CH_4) is one of the greenhouse gases (GHG) whose concentration in the atmosphere is increasing every year; this increase in concentration causes an increase in global temperature. The gas is formed under anaerobic conditions in wetlands including paddy fields and is determined by the activity of two different bacteria that live in the rhizosphere of rice plants, namely, methanogenic bacteria as organisms that play a role in the formation of methane and methanotrophic bacteria that use methane as a carbon source [7].

The presence of methanotrophic bacteria in the rhizosphere of rice plants is needed to reduce methane produced by methanogenic bacteria before it is released into the atmosphere [7]. CH_4 emissions are basically determined by two different microbial processes, namely, the production of CH_4 by methanogens and consumption of CH_4 by methanotrophic bacteria. Some of the methane that has been produced will be oxidized by methanotrophic bacteria in the soil surface layer and in the root zone [8].

Many free-living bacteria actively respond to root exudates by regulating their transcription programs against those involved in chemotaxis, root colonization, and energy metabolism. Methanotrophic bacteria are aerobic microorganisms that are able to grow and develop with methane as the only source of energy. Therefore, methane oxidation can occur in microenvironments that are aerobic in the root zone and even in soil layers with high mineral toxicity [8]. Biologically, methane emissions into the atmosphere are known to be suppressed by methanotrophic bacteria that live in aerobic parts of rice fields [2].

2. Materials and Methods Experimental Site and Design

The study consisted of two stages: the first stage was the isolation, identification, and subculture of methanotrophs in the Laboratory of Food Fungi and Biological Fertilizers, Laboratory of Bioscience and Plant Production of Hasanuddin University Faculty of Agriculture. The second stage is the field experiment that was conducted at Amparita Village, TelluLimpoe District, Sidenreng Rappang Regency, South Sulawesi.

Under rain-fed conditions, soil samples were collected from a depth of 0–30 cm. The design used was a split plot RCBD with 3 replications, consisting of the main plot, the NPK (P) fertilizer dosage treatment with 4 treatments, namely, without NPK fertilizer, 75 g/plot, 150 g/plot, and 360 g/plot. Meanwhile, the subplot was the provision of methanotroph (M) bacteria with 4 treatments, namely, without bacteria, 10^6 CFU per ml, 10^7 CFU per ml, and 10^8 CFU per ml.

2.1. Establishment and Management. Tillage was carried out using a hand tractor twice. After the first stage of processing, the soil was inundated for 2 weeks. After the second soil cultivation, manual leveling and the existing water were reduced. Experimental plots were made with a size of $4\,m \times 3\,m$ with 12 plots per replication. The experimental plots were made of 60 plots, and small bunds were made to separate the plots.

Soak the seeds with water for ±24 hours until white plumules appeared; after soaking, then drain the seeds. Rice seeds are ready to be planted around the age of 15–20 days. Planting is completed when the seeds are 20 days after sowing. Plant the seeds in the plot following rows of $4\,m \times 3\,m$ with a spacing of $25\,cm \times 25\,cm$. Before planting the rice seedlings, give 200 g of compost in the plots.

2.2. Plant Measurements. The unit area of the experimental plot was a bed with a width of 3 m and a length of 4 meters. The population per plot is 192 plants, with a spacing of $25\,cm \times 25\,cm$, and the number of seeds when planted is 5. The need for rice seeds in this experiment is the population of each plot × the number of experimental plots, namely, 192 planting holes × 48 experimental plots, namely, 9216 holes planting. The sampling method was carried out diagonally on the rice fields, namely, at each corner and in the middle of the rice planting area (subplot), each of which amounted to 10 clumps.

2.3. Statistical Analysis. The plants will be analyzed for variance using variance scale analysis. The results of analysis of variance that have a significant effect will be further tested with the smallest significant difference test (LSD) at the level of α 0.05.

3. Results and Discussion

3.1. Growth and Yield Components. The results showed that there was an interaction between the NPK fertilizer and methanotrophic bacteria. This gives the best results on the combination of NPK 360 fertilizer per plot and methanotrophic bacteria 10^6 CFU per ml on the observation of plant height (111.17 cm), the combination of NPK fertilizer treatment 0 g per plot and methanotrophic bacteria 0 CFU per ml on the observation of wet weight of grain is (70.44 g), the combination of NPK fertilizer treatment 360 g/plot and methanotrophic bacteria 0 CFU per ml on the observation of dry weight of grain is (43.89 g), and the combination of NPK fertilizer 360 g per plot and methanotrophic bacteria 10^6 CFU per ml on the observation weight of 100 grains is (3.53 g). Meanwhile, the combination of NPK fertilizer and methanotrophic bacteria had no effect on lowland rice production, in the parameters of the number of tillers, number of productive tillers, panicle length, and percentage of filled grain.

Table 1: Average plant height with the NPK fertilizer and methanotrophic bacteria.

	Methanotroph bacteria				
NPK fertilizer	M0 0 CFU per mL	M1 10-6 CFU per mL	M2 10-7 CFU per mL	M3 10-8 CFU per mL	Average
P0 (0 g)	93.22*ap*	97.22*ap*	97.78*ap*	111.17*bq*	93.11
P1 (75 g)	96.11*ap*	97.00*ap*	95.70*ap*	92.67*ap*	95.37
P2 (150 g)	97.17*ap*	97.78*ap*	97.78*ap*	111.17*bq*	93.11
P3 (360 g)	97.78*ap*	97.78*ap*	111.17*bq*	93.11*ap*	94.11
Average	96.07	102.43	90.90	92.39	95.45
NP BNT 0.05			6.81		

HSD: the numbers followed by the same letters in rows (p, q, r, s) and columns (a, b, c) are not significantly different in the BNT follow-up test for the confidence level of 0.05.

The results on BNT $\alpha = 0.05$ in Table 1 show that the treatment with a concentration of 10^6 methanotrophic bacteria CFU per ml produced the highest average plant height (111.17 cm) and was not significantly different from the concentration of methanotrophic bacteria 10^6 CFU per ml and treatment with a concentration of 0 methanotrophic bacteria CFU per ml but not significantly different from others.

The results of BNT test with $\alpha = 0.05$ in Table 2 show that the interaction of no fertilizers with the concentration of methanotrophic bacteria was 0 CFU per ml which produced the highest average value of fresh grain weight (70.44 g), and it was not significantly different from the treatment of 360 g NPK and interaction with an average of 66.22 g with a concentration of 10^6 methanotrophic bacteria CFU per ml and 360 G NPK treatment with an average of 63.82 g with a concentration of 10^8 methanotrophic bacteria CFU per ml but not significantly different from other treatments.

The results of the observation of dry weight grain showed that there was a significant effect on the NPK fertilizer treatment, and there was no effect on the methanotroph bacterial treatment and the interaction on the dry weight grain of rice plants. Test results of BNT $\alpha = 0.05$ in Table 3 showed that the interaction of treatment 360 gram fertilizers with the concentration of methanotrophic bacteria was 0 CFU per ml which yielded the highest average of the dry weight value of grain, that is 43.89 g, and it was not significantly different from the interaction of no fertilizer treatment with the concentration of 0 methanotroph bacteria CFU per ml. Meanwhile, 360 G fertilizers with a concentration of 10^6 methanotrophic bacteria CFU per ml was also not significantly different from other treatments.

The observation results of grain dry weight showed no significant effect on NPK (P) fertilizer treatment and had a significant effect on the treatment of methanotroph bacteria; it had no significant effect on the 100 grain weight of rice as shown in Table 4.

3.2. Discussion Effect of Interaction. The combination of NPK fertilizer with the concentration of methanotrophic bacteria had a significant effect on the observations of plant height, weight of 100 grains of rice, wet weight of plotted grain, and dry weight of unhulled grain, but there was no significant effect on other parameters. Parameters such as the highest plant height in treatment of NPK 360 g per plot with a concentration of 10^6 methanotrophic bacteria CFU per ml produced an average value of 111.17 cm, and the highest weight of 100 grains in the NPK treatment of 360 g/plot with a concentration of 10^6 methanotrophic bacteria CFU per ml produced the highest average weight of 100 grains with an average of 3.53 g; in the treatment, the highest wet weight of grain was in the treatment of 0 g NPK fertilizer and 0 methanotroph bacteria CFU per ml and the highest dry weight of grain in the NPK fertilizer treatment 360 g/plot and bacteria methanotroph 0 CFU per ml.

Based on the results of the analysis of the variety of fertilizers on methanotrophic bacteria, it can be seen that the growth and production of lowland rice have an effect on plant height, weight of 100 grains, wet weight of grain, and dry weight. Methanotrophic bacteria are able to reduce methane gas in the soil, the effect of high NPK fertilizer can be suppressed by methanotrophic bacteria, and it can produce monooxygenase enzymes, which plays an important role in the oxidation process used in the methane gas process in paddy fields. This was in accordance with the opinion of Hapsary [9], who states that methanotrophic bacteria are one type of soil microbe capable of oxidizing methane gas compounds. Methanotrophic bacteria have the enzyme monooxygenase which was used in the methane oxidation process. Bacteria that are active in aerobic conditions have an important role in the methane gas cycle and are a solution to reduce methane gas expansion in the air.

The effect of NPK fertilizer on plant height, the weight of 100 grains of wet weight of grain, and the dry weight of grain have a significant effect because the NPK fertilizer was able to have an effect or impact on lowland rice plants because NPK fertilizers are contained in it, such as nitrogen, phosphorus, and potassium, which are suitable for lowland rice plants, both of vegetative or generative stages. It was in accordance with the opinion of Simanjuntak [10], which states that the fertilizer (NPK) was one of the inorganic fertilizers that can be used very efficiently in increasing the availability of macronutrients (N, P, and K), replacing single fertilizers such as urea, SP-36, and KCl which was difficult to obtain in the market and were very expensive. The highest plant height was treated fertilizer NPK 360 g per plot with a concentration of 10^6 methanotrophic bacteria CFU per ml. The highest weight of 100 grains in NPK treatment was 360 g/plot with a concentration of 10^6 methanotrophic bacteria CFU per ml. The highest wet weight of grain was in the treatment of NPK 0 g fertilizer and without methanotroph bacteria. The highest dry weight of grain was in the treatment of NPK fertilizer 360 g per plot and without

Table 2: Average wet weight of grain with NPK fertilizer treatment and methanotrophic bacteria.

	Methanotroph bacteria				
NPK fertilizer	M0 0 CFU per mL	M1 10-6 CFU per mL	M2 10-7 CFU per mL	M3 10-8 CFU per mL	Average
P0 (0 g)	70.44*bq*	45.11 *ap*	41.44 *ap*	51.67 *ap*	52.17
P1 (75 g)	54.11*abp*	45.44 *ap*	52.11 *abp*	56.11 *ap*	56.11
P2 (150 g)	46.55 *ap*	46.67 *ap*	52.00 *abp*	54.22 *ap*	54.22
P3 (360 g)	63.44 *abp*	66.22 *bp*	59.78 *bp*	63.82 *ap*	63.82
Average	58.64	50.86	51.33	56.46	56.56
NP BNT 0.05			17.89		

HSD: the numbers followed by the same letters in rows (p, q) and columns (a, b) are not significantly different in the BNT follow-up test for the confidence level of 0.05.

Table 3: Average dry weight of grain with NPK fertilizer treatment and methanotrophic bacteria.

	Methanotroph bacteria				
NPK fertilizer	M0 0 CFU per mL	M1 10-6 CFU per mL	M2 10-7 CFU per mL	M3 10-8 CFU per mL	Average
P0 (0 g)	40.67 *abq*	24.56 *ap*	23.56 *ap*	27.00 *ap*	28.95
P1 (75 g)	31.56 *abp*	25.22 *ap*	29.44 *aq*	31.11 *ap*	31.11
P2 (150 g)	27.67 *ap*	26.67 *ap*	30.78 *ap*	30.55 *ap*	30.55
P3 (360 g)	43.89 *bp*	37.44 *ap*	36.33 *ap*	36.78 *ap*	36.78
Average	35.94	28.47	30.03	31.36	31.85
NP BNT 0.05			13.08		

HSD: the numbers followed by the same letters in rows (p, q) and columns (a, b) are not significantly different in the BNT follow-up test for the confidence level of 0.05.

Table 4: The average weight of 100 grains with NPK fertilizer treatment and methanotrophic bacteria.

NPK fertilizer (a)	M0 0 CFU per mL	M1 10-6 CFU per mL	M3 10-8 CFU per mL	Average
P0 (0 g)	3.26 *aq*	3.51 *ap*	3.43 *ap*	3.40
P1 (75 g)	3.01 *br*	3.40 *ap*	3.49 *ap*	3.29
P2 (150 g)	3.06 *aq*	2.94 *bqr*	3.42 *ap*	3.18
P3 (360 g)	3.19 *aqr*	3.53 *ap*	3.30 *aq*	3.36
Average	3.13	3.35	3.41	3.31

HSD: the numbers followed by the same letters in rows (p, q, r) and columns (a, b) are not significantly different in the BNT follow-up test with a confidence level of 0.05.

bacteria methanotroph. It can be seen that giving methanotrophic bacteria with several concentrations can affect the result. It was according to the opinion of Theowidavitya et al. [8], which states that mutualistic interactions between plants and microbes can increase the availability or absorption of nutrients for plant growth.

Based on the results of soil analysis after the research, there was C (Carbon) 3.43 ppm, N (Nitrogen) 0.21 ppm, and K (Calcium) 0.28 ppm. It was due to the addition of methanotrophic bacteria into the rhizosphere (the area around the roots) which was one of the bacteria that breaks down the methane gas cycle in lowland rice fields.

The results of statistical analysis showed that NPK treatment had a significant effect on wet weight and dry weight. The effect of NPK fertilizers showed that with a dose of NPK fertilizer 360 g per plot and methanotrophic bacteria of 10^6 CFU per ml, resulting the highest number of average plant height, which was 111.17 cm. It was because the amount of methanotrophic bacteria (10^6 CFU per ml) can reduce methane gas produced by excessive NPK fertilizers. This was in accordance with the opinion of Schaefer et al. [11], who reported that the cause of the increase in methane gas in the atmosphere has changed the source from thermogenic to biogenic. They identified that most likely the source of the methane gas came from agriculture and livestock rather than from wetlands. Biogenic methane was produced from biological processes (for example, produced by wetlands or agriculture and livestock) [12].

Plant height (cm)

Grain wet weight (g)

Dry weight of grain (g)

Weights 100 grains

4. Conclusion

Based on the research results, it can be concluded that NPK fertilizer treatment significantly affected the wet weight of grain and dry weight of grain per plot.

The highest fresh weight of grain was 70.44 g on the treatment of without NPK fertilizer and methanotropic bacteria, meanwhile the highest dry weight of grain on the treatment of 300 g per plot NPK without methanotrophic bacteria was 43.89 g.

Methanotrophic bacteria treatment had a significant effect on the plant height and weight of 100 ears. The highest plant height in the treatment concentration of methanotrophic bacteria 10^6 CFU per ml produced an average value of 111.17 cm. The weight of 100 grains was the highest in the absence of concentration of methanotrophic bacteria, which produced the highest average grain weight value of 3.53 g.

Data Availability

Data had been included in this research article. The full data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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