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The Effect of Methanotrophic Bacteria Application on Paddy Growth and Methane Emission in Rainfed Rice of Kupang Regency, East Nusa Tenggara, Indonesia

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Abstract*—Rice productivity in province of East Nusa Tenggara (ENT) is low due to the soil condition. One of the rice-producing regency in ENT is Kupang Regency with rainfed rice type. Paddy fields have also become a major source of methane emissions (CH_4) as one of important greenhouse gases. This research aims to know the effect of methanotrophic bacteria application on paddy growth and methane emission at rainfed rice. Bacteria that used is Methylocystisrosea BGM 1, Methylobacter sp. SKM 14, Methylocystispalvus BGM 3 and Methylococcuscapsulatus BGM 9. This research used completely random design with threatment: (1) NPK 100% (P1), (2) NPK 50% (P2), (3) without fertilizer (P3), (4) NPK 100% + methanotrophic (P4), NPK 50% + methanotrophic (P5), and methanotrophic bacteria (P6). Gas sampling using closed chamber method.The application of methanotrophic bacteria increased the rice production. Treatment NPK 50% + methanotrophic (P5) from that rice field produced 7.0 t ha^{-1} dry grain weight and methanotrophic bacteria treatment without NPK (P6) with improved 6.6 t ha^{-1} dry grain weight, higher than controls of 4.9 ha^{-1} dry grain weight without any addition of synthetic fertilizer.The inoculation of methanotrophic bacteria increase rice production of 1.7 t ha^{-1}.Result of methane flux measurement showed that application of methanotrophic bacteria may decrease methane emission in treatment of 100% NPK + methanotrophic (P4) (30 DAP) and treatment of 50% NPK + methanotrophic (P5) (60 DAP), -6.27 $mg/m^2/d$ and -23.87 $mg/m^2/d$, respectively.*

Keywords*—*Kupang regency, Methane emission, Methanotrophic, Rainfed rice.

I. INTRODUCTION

Rice is a basic requirement of Indonesian society, including the province of East Nusa Tenggara (ENT). Rice productivity in ENT belongs low because the soil is less fertile and arid climate with rainfall between 201-300 mm (BMKG 2017). One of the rice-producing regency in ENT is Kupang Regency. In the year 2013 produced rice as much as 60.469 t, 13.846 ha of which is rainfed rice (BPS 2013). Farmers in the Regency of Kupang still using synthetic fertilizers to increase crop production. Practices will further lower soil fertility due to damage to physical, chemical, and biological soil condition (Havlin*et al*. 2005). In addition, the use of inorganic fertilizers also has an impact on global warming.

Wetlands such as paddy fields have also become a major source of methane emissions (CH_4) as greenhouse gases. The activity of methanogenesis by methanogen bacteria on paddy fields produce CH_4 gas (Le Mer and Roger, 2001). The global warming potential of methane gas is 25 times greater than CO_2 (IPCC, 2007). According to Conrad and Rothfus (1991), as much as 80% of methane gas in the rice fields can be oxidized by the methanotrophic bacteria. This can be a solution in mitigating the emission of methane gas in the paddy fields.

Some of the methanotrophic bacteria has been succesfully isolated from paddy fields in Sukabumi and Bogor (Hapsari, 2008). Isolates *Methylocystisrosea* BGM 1 and *Methylobacter* sp. SKM 14 are known to have *pmo*Agene whereas isolates BGM 9 have the *mmo*Xgene (Rusmana and Akhdiya, 2009). Isolates *Methylocystispalvus*BGM 3 and *Methylococcuscapsulatus*BGM 9 known to have *nif*H and *nif*D genes these play a role in the nitrogen fixation (Bintarti*et al*. 2014). Methanotrophic bacteria have been tested on organic and inorganic paddy fields. The trial reduced methane gas to 20.47% when compared with the control and improved the vegetative phase of rice growth (Pingak e*t al*. 2014; Sutanto*et al*. 2014). Trials have also been conducted on paddy fields in the lowlands. The trial

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reduced of methane gas and increased the growth of vegetative phase on rice and the generative phase (Sukmawati*et al*. 2015). This research aims to know thepaddy growthand methane emissions in the application of methanotrophic bacteria at therainfed rice.

II. METHODS

2.1 Culturing Bacterial Isolates

Methanotrophic bacteria isolates i.e. BGM 1, 3, 9, and SKM 14 were cultured in NMS (Nitrate Mineral Salt) plus 1% methanol (v/v), incubated at room temperature (± 28^{o}C) for 7-10 days and shakedup to reach 10^8 CFU cell/mL.

2.2 Seedling and Plantation

Seeds of paddy variety Ciherang were germinated for 48 h. After that, the seed was sowed in the field for 20 days to make seedling. Before transplanting, the seedling was dipped in a mixture of methanotrophic bacteriafor 15-20 minutes, then plantated with a distance of 20 x 20 cm which 3 seedling in every hole. Five plants selected from every plot of treatment for measurement of growth parameters.

2.3 Experimental Design

The experimental design used was completly random design with one factor i.e. fertilization. The treatment consists of: (1) NPK100% (P1), (2) NPK 50% (P2), (3) without fertilizer (P3), (4) NPK 100% + methanotrophic (P4), NPK 50% + methanotrophic (P5), and methanotrophic bacteria (P6). Each treatment has 4 replications.

2.4 Measurement of Growth Parameters

Paddy growth was observed at 30, 60, and 90 day after plant(DAP). During the vegetative growth plant height and number of tillers was measurement. The shoot dry weight, number of panicles per plants, grains per panicle, empty grain, weight 1000 grain, and the dry grain weight was measured of the harvest.

2.5 Gas Sampling and Measurement Methane Fluxes

Gas sampling was using closed chamber method. Gas sampling is done at 30, 60, and 90 day after plant DAPwith time taking between 06.00-11.00 am. Gas sampling was done every 10 minutes from 0 to 30 minutes. Methane fluxes were calculated as follows by IAEA (1993) :

$$E = \frac{dc}{dt} \times \frac{Vch}{Ach} \times \frac{mW}{mV} \times \frac{273{,}2}{(273{,}2 + T)}$$

E = CH_4 emission rate(mg/m^2/d)
dc = Difference concentration (ppm)
dt = Time interval (min)
Vch = Volume of the chamber (m^3)
Ach = Basal area of the chamber (m^2)
mW = Molecular weight
mV = Molecular volume
T = Temperature (^{o}C)

2.6 Data Analysis

Data was analysed using microsoftexel software and software SAS 9 portable at the confidence level of 95%. The data showed a significant difference, was tested with Duncan multiple range test (DMRT).

III. RESULTS

3.1 Paddy Growth and Production

Observation of plant height and number of tillers were at 30, 60, and 90 DAP (Table 1 and Table 2). The observations showed that the treatment combination of NPK with methanotrophic bacteria was not significantly different from the treatment without combinations, but all treatment was significantly different with control without fertilization (P3). Treatment of NPK 100% + methanotrophic (P4) and treatment of methanotrophic bacteria (P6) without fertilizer higher showed plant height than other treatments at 30 DAP. Treatment NPK 100% + methanotrophic (P4) showed the highest plants height on 90 DAP than other treatment, while treatment of methanotrophic bacteria (P6) showed the lowest plant height.

Observation of the number of tillers showed that the treatment combination of NPK with methanotrophicbacteria was not significantly different with the treatment without the combination at 30 and 60 DAP, but all treatment was significantly different with the treatment without fertilization (P3). Treatment NPK 50% + methanotrophic (P5) was not significantly different with the control treatment without fertilization (P3) on 90 DAP. Treatment of methanotrophic bacteria (P6) was significantly different with the control treatment without fertilization at 60 and 90 DAP.

Table.1: Plant height at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Plant Height (cm)*)		
	30 DAP	60 DAP	90 DAP
P1	49.35ab	89.40a	88.40a
P2	49.30ab	85.90a	90.00a
P3	45.70b	80.70b	81.85bc
P4	50.90a	87.50a	90.60a
P5	48.15ab	89.25a	85.65ab
P6	50.70a	81.15b	79.10c

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**) Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

Table.2: Number of tillers at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Number of Tillers*)		
	30 DAP	60 DAP	90 DAP
P1	31.25a	29.75a	27.60a
P2	27.05ab	26.00ab	26.50ab
P3	22.00b	23.20b	21.00b
P4	32.30a	24.80ab	25.35ab
P5	28.05a	24.15ab	21.65b
P6	28.35a	14.60c	14.95c

**) Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

Harvest parameters observation showedin Table 3. Observation of shoot dry weight showed the treatment combination of NPK with the methanotrophic bacteria was not significantly different with the treatment without the combination, while the treatment NPK 50% + methanotrophic(P5) and treatment of methanotrophicbacteria (P6) was not significantly different with control without NPK (P3). Average shoot dry grain weight of P5 and P6 treatment was higher than treatment of P3.Treatment of 100% NPK (P1)produced the highest number of panicles per plants, while treatment of methanotrophic bacteria (P6) produced the lowest panicles per plants. Treatment of NPK 50% (P2) and treatment of NPK 100% + methanotrophic (P4) was not significantly different with treatment of 100% NPK (P1), whereas treatment of NPK 50% + methanotrophic (P5) was not significantly different with the treatment of 50% NPK (P2), control without NPK (P3), and treatment of NPK 100% + methanotrophic (P4).

All the treatments were not significantly different in the number of grains per panicle parameter. But treatment combination of NPK with methanotrophic bacteria produced the number of grains per panicle higher than treatment without the combination. Treatment of NPK 50% + methanotrophic (P5) produced the highest number of panicles, followed by treatment of methanotrophic bacteria (P6) and treatment of NPK 100% + methanotrophic (P4). Although it produced the highest number of grains per panicle, treatment NPK 50% + methanotrophic(P5) has highest empty grain, while treatment of methanotrophic bacteria (P6) produced the lowest empty grain. Weight 1000 grain measurements were not significantly different in all treatments.

Treatment of NPK 50% + methanotrophic (P5) produced highest dry grain weight, followed by treatment of 100% NPK (P1) and treatment of NPK 100% + methanotrophic(P4). Treatment of methanotrophic bacteria (P6) produced dry grain weighthigher than the control without NPK (P3).

Table.3: Measurement of harvest parameters (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Shoot Dry Weight (g)	No. of Panicles per Plants	Grains per Panicle	Empty Grain	Weight 1000 Grain (g)
P1	114.03a	27.16a	97.94a	20.00ab	18.75a
P2	118.83a	23.55ab	98.11a	21.49ab	20.25a
P3	64.43b	19.16b	99.27a	13.08b	20.00a
P4	115.33a	24.33ab	99.47a	18.16ab	20.25a
P5	95.25ab	20.74b	109.80a	24.46a	20.00a
P6	97.57ab	13.93c	108.80a	12.63b	20.50a

Table.4: Dry grain weight parameters (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. Methanotrophic + NPK 100%; P5. Methanotrophic + NPK 50%; P6. Methanotrophic)

Treatment	Dry Grain Weight (t ha^{-1})
P1	6.8ab
P2	5.6bc
P3	4.9c
P4	6.7ab
P5	7.0a
P6	6.6ab

**)Numbers within a column followed by the same letter are not significantly different at 5% level by DMRT (α = 0.05*

3.2 Methane Flux

The highest methane flux was shown in 30 DAP. Treatment of NPK without inoculation of methanotrophic bacteria showed highest emissions. Treatment of 50% NPK (P2) emmited 60.69 CH_4 mg/m^2/d, followed by treatment NPK 100% (P1) of 54.72 mg/m^2/d. Treatment of NPK with bacterial inoculation of P5 (NPK50% +methanotrophic) emitted 61.60 CH_4mg/m^2/d and treatment of methanotrophic bacterial alone without fertilizer (P6) produced 18.97 CH_4 mg/m^2/d.

Significant methane absorption (sink) was showed in the treatment of NPK100% + methanotrophic (P4) and

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emmited -6.27 mg/m^2/d at 30 DAP , treatment of 50% NPK (P2) of -10.72 mg/m^2/d at 60 DAP, and treatment of NPK 50%+ methanotrophic (P5) of -23.87 mg/m^2/d at 60 DAP. All the treatments showed a low methane flux on 90 DAP. This because of low rainfall so there was no formation of anaerobic environment as a habitat of methanogenic bacteria that produce methane gas.

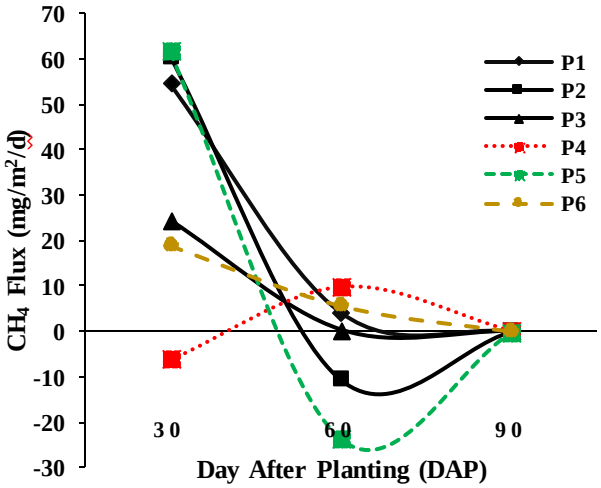

Fig.1: CH_4 Flux at 30, 60, 90 DAP. (P1. NPK 100%; P2. NPK 50%; P3. Without Fertilization; P4. NPK 100% + Methanotrophic; P5. NPK 50% + Methanotrophic; P6. Methanotrophic)

IV. DISCUSSION

Generally, the combination of methanotrophic bacteria and NPK have no effect in stimulating the growth of paddy in the vegetative phase, based on plant height parameters (Table 1) and the number of tillers (Table 2). According to Suparthaet *al*. (2012) treatment of solid organic fertilizers and organic liquid fertilizer has no effect against paddy height. Plant height and numbers of tillers has decreased at each observation. This is because of the low fertility of the soil. According to Lamberset *al*. (2008) plant height and the formation of tillers is an indicator of growth as a result of the interaction of the processes of photosynthesis, respiration, and nutrient transport.

Observations on harvest parameters generally do not indicate a difference between the treatment and control treatment. The results obtained in contrast to previous research by Sukmawatiet *al*. (2015) and Hadiantaet *al*. (2014). Both of these studies showed the application of methanotrophicbacteria effective in improving crop parameter. This is because of the content of soil chemical imbalance on every patch of the experiment. According to Zeigler and Puckridge (1995), the soil chemical imbalance to be another major constraint to the productivity of rainfed lowland rice. Most rainfed lowlands, particularly in Southeast Asia, have soils with potentially major fertility constraints. They list the main soil problems to be salinity, alkalinity, Fe toxicity, P deficiency, Zn deficiency, and organic and acid sulfate conditions.

There are differences in the parameters of dry grain weight. Treatment of NPK 50% + methanotrophic (P5) can produce 7.0 t ha^{-1}, whereas the methanotrophic bacteria treatment without NPK (P6) produces 6.6 t ha^{-1}. This indicates that the application of methanotrophic bacteria effective in increasing production in rainfed rice. Methanotrophic bacteria which applicated is a consortium of several isolates (Hapsari, 2008) i.e. *Methylocystisrosea* BGM 1,*Methylobacter* sp. SKM 14, *Methylocystispalvus* BGM 3 and *Methylococcuscapsulatus* BGM 9. Isolates *Methylocystispalvus* and *Methylobacter* sp. known to have *nif*H and *nif*Dgenes, the role gene in nitrogen fixation (Bintartiet *al*. 2014). This makes those methanotrophic bacteria can increase the availability of nitrogen for paddy growth. Nitrogen acts as a constituent of chlorophyll which is involved in the process of photosynthesis thus can increase the amount of productive grain, increase the percentage of protein and was instrumental in the preparation of the essential components of plant organs (Chaturvedi, 2005; Nettoet *al*. 2005; Watanabe and Kitagawa, 2000).

The Intergorvenmental Panel on Climate Change (IPCC) guidelines for compiling national inventories of greenhouse gas emissions (IPCC, 1997) distinguish between rice fields that are (1) permanently flooded and (2) those with unstable flooding regime. Rainfed rice belongs to the latter category (Wassmannet *al*. 2000). According to Phillips *et al*. (2009), one of the key factors that affect the production and consumption of methane is fertilization. Input of NPK emmited methane gas emissions range between 54.72 - 61.60 CH_4 mg/m^2/d at 30 DAP, higher than control without NPK ranging from 18.97-24.44 CH_4 mg/m^2/d. Setyantoet *al*. (2000) report the range of methane emissions in rainfed rice between 19-123 mg/m^2/d. The highest methane emissions occur at the beginning of the growth period and the decline in reproductive phase and the maturation phase. The intensity of the rain on the vegetative phase of 371 mm and declined on the reproductive phase and maturation phase, 10 and 11 mm, respectively. Rainfall is higher in the early growth period in rainfed rice trigger high methane emissions (Wassmannet *al*. 2000). Methane formed by the anaerobic conditions was temporary stay stuck on flooding condition. When drying, most methane is trapped will be oxidized, however, most will escape into the atmosphere as soon as flooding recedes and macro pores aerated (Neueet *al*. 1995).Strong rainfall triggered high emissions in the rainfed plots while relatively dry periods resulted in lower emission rates (Setyantoet *al*.2000). This causes the emission of methane

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gas was low in the maturation phase from 0.0072--0.15 mg/m^2/d.

The use of methane (sink) showed in the treatment of NPK 100% + methanotrophic (P4) at 30 DAP of -6.27 mg/m^2/d and treatment of NPK 50% + methanotrophic (P5) at 60 DAP of -23.87 mg/m^2/d. Methanotrophic bacteria including obligate aerobic bacteria that can use methane as a source of carbon and energy for growth (Roslev and King, 1994). According to Dubey (2005), methanotrophic bacteria is the only biological system which acts as a reservoir of methane. Methanotrophic bacteria capable of transforming CO_2 into methane oxidation process by using the enzyme methane monooxygenase (MMO). Methane oxidation can occur in the microenvironment aerobic condition on rooting zone and toxic part in the surface layer of the soil (Semrau*et al*. 2010).

Synthetic fertilizer can increase methane emission. Based on the observation, methane flux was increased in treatment with addition of synthetic at 30 DAP. Treatment of methanotrophic bacteria without NPK (P6) produced the lowest methane flux in 30 DAP (18.97 mg/m^2/d), followed by control without fertilization (P3) (24.44 mg/m^2/d). Inorganic fertilizer enhanced soil porosity by increasing regular and irregular pores and caused a priming effect of native soil organic matter (Tiquia*et al*. 2002) ultimately affecting CH_4 and N_2O emissions (Ge *et al*. 2010).

V. CONCLUSION

The application of methanotrophic bacteria (*Methylocystisrosea* BGM 1, *Methylobacter* sp. SKM 14, *Methylocystispalvus* BGM 3, *Methylococcuscapsulatus* BGM 9) increased the rice production in rainfed rice. Treatment NPK 50% + methanotrophic (P5) from that rice field produced 7.0 t ha^{-1} dry grain weight and methanotrophic bacteria treatment without NPK (P6) with improved 6.6 t ha^{-1} dry grain weight, higher than controls of 4.9 ha^{-1} dry grain weight without any addition of synthetic fertilizer.The application of methanotrophic bacteria may decrease methane gas emissions at rainfed rice. Treatment 100% NPK + methanotrophic (P4) emmited -6.27 mg/m^2/d at 30 DAP and NPK treatment 50% + methanotrophic (P5) emmited -23.87 mg/m^2/d at 60 DAP.

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