Impact of methane-utilizing bacteria on rice yield, inorganic fertilizers efficiency and methane emissions

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Abstract. Methane (CH₄) is one of the greenhouse gases that contribute to climate change. Lowland rice cultivation is one of the main sources of methane emissions, accounting for around 5 to 19% of total global CH₄. One of the efforts to reduce CH₄ gas emissions that is environmentally friendly is through methane-utilizing bacteria. This study aimed to determine the effect of methane-utilizing bacteria on rice yield and methane emissions. The bacterial strains used were Mycobacterium senegalense LM1, Providencia stuartii LM18, Rhizobium rhizoryzae BMU, and Bacillus methylotrophicus N2P4. The research was conducted in the experimental field of The Indonesian Center for Rice Research, Bogor, Indonesia. The experiment was carried out by using a factorial randomized block design with two factors and three replications. The first factor was the dose of NPK inorganic fertilizer (50%, 75%, 100%). The second factor was bacterial consortium formulas (without bacteria, bacterial consortium 1, bacterial consortium 2). The application of a bacterial consortium containing all strains increased the efficiency of inorganic fertilizers by 25%, increased rice production by 33.55%, and reduced CH₄ emissions by 37.26%. It seems that the consortium of methane-utilizing bacteria has the prospect of biofertilizer and mitigation agents to mitigate the impact of global warming.

1. Introduction
Methane (CH₄) as a component of greenhouse gases causes a greenhouse effect that has macro potential to global warming. Lowland rice cultivation is one of the main sources of methane emissions, accounting for around 5-19% of total global methane. About 90% of CH₄ released from lowland rice soil to the atmosphere is emitted through rice plant [1].

Methane is produced as the result of microbial processes through the anaerobic decomposition of organic matter by methanogenic bacteria [2]. These bacteria are only active when the soil is inundated [3]. Some of the methane produced will be oxidized by methanotrophic bacteria or aerobic methane-utilizing bacteria in the soil surface and root zones. Biological oxidation of methane in paddy fields is
carried out entirely by these bacteria. So, one of the efforts to reduce methane gas emissions without reducing rice production in environmentally friendly agriculture is through the application of methanotrophic bacteria.

Some methanotrophic bacteria can reduce methane emissions and also have the potential as plant growth-promoting rhizobacteria or biofertilizer. Among various biofertilizers, methane-utilizing bacteria are good candidates to solve such problems since they could promote rice growth and yield and compete with methane-producing bacteria for substrates. The test results proved that the application of biofertilizers with active ingredients methane-utilizing bacteria and nitrogen-fixing bacteria in rice fields is significantly shown in addition to reducing 75% of chemical NPK fertilizer, also increasing rice production by 67.53% and reducing methane emissions from 18.31 mmol m$^{-2}$ h$^{-1}$ to 19.57 mmol m$^{-2}$ h$^{-1}$ [4].

Indonesian Soil Research Institute has isolated and screened 21 methane-utilizing bacteria from rice fields' sediments and rhizosphere [5]. Several bacterial strains have a high ability to reduce methane emissions and fix nitrogen (nitrogenase activity 68.1 to 92.0 nmol C$_2$H$_4$ ml$^{-1}$ h$^{-1}$), solubilize P and produce indole acetic acid or IAA (41,798 to 61,921 μg ml$^{-1}$). Hence, these species could be further formulated and used as biofertilizer and mitigation agents to reduce methane emissions. Our greenhouse experiment showed that the strains of methane-utilizing bacteria individually reduced methane emissions by 20.05%, increased inorganic fertilization efficiency by 25%, and increased rice production by 5.12%.

Biofertilizer is an active biological product consisting of microbes that can improve fertilizer efficiency, soil fertility and health [6]. Most of the biofertilizers used in Indonesia are formulated as consortia biofertilizers. Bacterial consortium formulas are composed of compatible microbial strains with different modes of action to provide a broad spectrum of usage. Strains of genetically diverse groups are also selected, with the ability to adapt to various environmental conditions, such as soil temperature, moisture, or soil pH [7]. Bacterial consortium containing the methane-utilizing bacteria strains were expected to increase rice production and reduce methane emissions. In this study, two liquid bacterial consortia were prepared, which contained a mix of methane-utilizing bacteria strains. The study evaluates the impact of two consortia of methane-utilizing bacteria on rice yield, inorganic fertilizer efficiency, and methane emissions.

2. Materials and methods

2.1. Site description and experimental design

The experiment was conducted at the experimental field of The Indonesian Center for Rice Research (ICRR) (6°36'42"S, 106°47'35"E), Bogor, West Java, Indonesia, in 2019. The characteristics of the soil in this location were presented in Table 1.

The field experiment was carried out by using a factorial randomized block design with two factors and three replications. The first factor was the dose of NPK inorganic fertilizer (F1 = 50%, F2 = 75%, and F3 = 100%). The second factor was bacterial consortium formula (C0 = without bacteria, C1 = bacterial consortium 1, and C2 = bacterial consortium 2).

The tested rice cultivar was Inpari 32. The 19-day old seedlings were manually transplanted in plots of 8 m x 5 m with the spacing of 25 cm x 12.5 cm x 50 cm (jajar legowo 2:1 method) with 3 seedlings per hole. Two t ha$^{-1}$ of cow dung was applied after tillage.

The irrigation practice followed local farmers’ traditional management practices, i.e. flooding two weeks before transplanting, with intermittent irrigation after transplanting until the tilling stage.

2.2. Preparation of bacterial inocula and the bacterial consortium

The bacterial strains used were Mycobacterium senegalense LM1, Rhizobium rhizoryzae BMU, Providencia stuartii LM18, and Bacillus methylotrophicus N2P4. They were isolated from the sediments or rhizosphere of rice fields [5]. All strains were selected based on their capability of growth-promoting abilities and methane emission reduction in the culture headspace [5]. The selection
results showed that the bacterial strains have pmxA-like gene, one of the key enzymes in the bacterial methane metabolic pathway [8], were able to fix N₂, solubilize phosphorus, and produce phytohormone indole acetic acid (IAA). These activities reflected the ability of bacteria to be used as biofertilizer and biological mitigation for reducing methane emissions.

Bacterial inocula were prepared by incubating bacterial cultures for seven days in a liquid Nitrate Mineral Salts medium with 1% (v/v) methanol [9]. The bacterial consortium was prepared by mixing the bacterial cultures of the selected strain. They were all diluted with sterile distilled water to give a concentration of approximately 10⁷ CFU (colony forming unit) ml⁻¹.

In previous studies, it was known that LM1 and BMU strains showed the highest ability to reduce methane emissions and increase rice production [5]. In this study, two bacterial consortia, i.e. C1 (consist of 2 strains M. senegalense LM1 and R. rhizoryzae BMU) and C2 (consist of 4 strains M. senegalense LM1, P. stuartii LM18, R. rhizoryzae BMU and B. methylotrophicus N2P4) were observed on growth and methane emissions.

2.3. Application of bacterial consortium on rice seeds
Rice seeds were surface sterilized with 10% NaOCl for 3 to 5 minutes, then rinsed 3 times with sterile water. The seeds were soaked in a bacterial formula for 24 h and germinated for 24 h. Each liquid inoculant then was distributed in the rice nursery.

2.4. Methane emission measurements
The CH₄ gas was collected three times during the growing season, namely at 35, 65, and 95 days after transplanting (DAT), respectively, by the closed chamber method described by Qin et al. [10]. The manual transparent closed chamber (1 m high, 0.4 m diameter) with an open bottom was made of polycarbonate (0.5 mm in thickness) with a light steel frame. Gas samples were taken in the morning starting at 06.00 am, at 60 min after the top chamber was covered. Headspace gas samples were obtained with air-tight 10 ml propylene syringes. A silicone layer was applied to septa during vial preparation to create a double barrier and prevent contamination with ambient air. Methane flux was determined by measuring the temporal increase of the CH₄ concentration of the air within the chamber. The gas samples were analyzed using a Gas Chromatograph equipped with a Flame Ionization Detector (FID) operated at 150°C. The amount of methane flux and methane emission were estimated using the equation described by Khalil et al. [11].

2.5. Data processing and statistical analysis
The variables observed included vegetative growth and yield, i.e. plant height, number of tillers per hill, rice yield, total biomass, soil redox potential, soil pH, CH₄ flux, and CH₄ emissions. Data were analyzed using Analysis of Variance (ANOVA), while the differences of the mean among the treatments were analyzed by Duncan’s Multiple Range Test (DMRT) at the level of p= 0.05.

3. Results and discussion

3.1. Soil characteristics
Analysis results of the soil samples are presented in Table 1. The soil texture in experimental field is silt loam, with a content of 15% sand, 58% silt and 27% clay, and a pH of 6.1 (slightly acid).

| Table 1. The analysis result of soil in experimental field of the ICRR, Bogor |
|-----------------------------|---------|---------|-----------------|----------|---------|--------|----------|
|                             | Sand (%)| Silt (%)| Clay (%)        | K₂O (mg 100 g⁻¹) | P₂O₅ (mg 100 g⁻¹) | N (%)  | C-org (%) | pH H₂O  |
| Value                       | 15      | 58      | 27              | 20                 | 14                  | 0.13   | 1.37      | 6.1     |
| Criteria                    | Silt loam | low     | low             | low                | low                 | low    | slightly acid |
The soil is categorized as less fertile for rice growth, requiring several inputs, i.e. compost and NPK fertilizer. In this study, we added 2 t ha\(^{-1}\) of cow dung, and inorganic N, P, and K fertilizers, in the form of urea, SP-36, and KCl. Based on the analysis using the PUTS (Perangkat Uji Tanah Sawah or paddy soil test kit), the recommended dosage of 100% NPK fertilizer was 250 kg ha\(^{-1}\) urea, 100 kg ha\(^{-1}\) SP36, and 100 kg ha\(^{-1}\) KCl.

3.2. Effect of treatment on vegetative growth and production

The agronomic parameters (plant height, number of tillers per hill, and rice production) are summarized in Table 2. There was an interaction between inorganic NPK fertilizer dosage and the bacterial consortia on the parameters of the number of tillers, yield, and total biomass.

Statistical analysis showed that the combination of NPK fertilizer dosage and bacterial consortia significantly affected productive tillers, rice yield and total biomass (Table 2). Plant heights and number of tillers for all of these treatments were not significantly different at 35 days after transplanting (DAT) or at active tiller phase and 65 DAT (generative phase). After entering the reproductive phase at 95 DAT, the number of tillers began to show significant differences. At 75% NPK fertilizer with bacterial consortium 2 showed the highest number of productive tillers, i.e. 15.47 tillers.

Table 2. The effect of treatments on vegetative growth, grain yield and total biomass

| Bacterial Consortia | Inorganic Fertilizer | Average | Plant Height at 95 DAT (cm) | CV |
|---------------------|---------------------|---------|-----------------------------|----|
|                     | F1 (50%)            | F2 (75%)| F3 (100%)                   |    |
| C0                  | 101.90              | 102.27  | 102.13                      | 102.10 a |
| C1                  | 103.67              | 104.50  | 104.73                      | 104.73 a |
| C2                  | 104.10              | 106.27  | 107.20                      | 106.19 a |
| Average             | 103.22 a            | 104.68 a| 104.69 a                    |    |
| CV                  | 17.94%              |         |                             |    |
| Productive of Tillers per Hill at 95 DAT | | | | |
| C0                  | 11.10 aA            | 12.22 bB| 12.16 bB                    | 11.83 |
| C1                  | 12.90 bA            | 13.28 bB| 13.22 bB                    | 13.13 |
| C2                  | 14.01 cA            | 15.47 cB| 15.45 cB                    | 14.98 |
| Average             | 12.67               | 13.66   | 13.61                       |    |
| CV                  | 5.05%               |         |                             |    |
| Rice Yield (t ha\(^{-1}\)) | | | | |
| C0                  | 4.28 aA             | 4.85 aAB| 4.68 aB                     | 4.60 |
| C1                  | 5.13 bA             | 5.31 bA | 5.35 bA                     | 5.27 |
| C2                  | 5.48 cA             | 6.25 cC | 5.73 cB                     | 5.82 |
| Average             | 4.97                | 5.47    | 5.25                        |    |
| CV                  | 2.27%               |         |                             |    |
| Total Biomass (t ha\(^{-1}\)) | | | | |
| C0                  | 5.13 aA             | 5.82 aB | 5.61 aB                     | 5.52 |
| C1                  | 6.18 bA             | 6.38 bA | 6.48 bA                     | 6.34 |
| C2                  | 6.58 cA             | 7.50 cB | 6.88 cA                     | 6.99 |
| Average             | 5.96                | 6.57    | 6.32                        |    |
| CV                  | 2.32%               |         |                             |    |

Remarks:
F1 = 50% NPK, F2 = 75% NPK, F3 = 100% NPK
C0 = without bacteria, C1 = bacterial consortium 1, C2 = bacterial consortium 2
The same lowercase letters in each column and uppercase letters in each row represent no significant difference at 5% DMRT
The interaction between the inoculation of the bacterial consortium and the dose of NPK fertilizer was exist on the number of productive tillers. The combination of 75% and 100% NPK fertilizer and bacterial consortium showed higher number of productive tillers than the control treatment without bacteria. Observations on 95 DAT showed that the highest number of productive tillers resulted from the interaction of 75% NPK fertilizer and bacterial consortium 2 (C2) which had 15.47 productive tillers. The number of productive tillers in this treatment was not significantly different from the treatment of 100% NPK fertilizer + bacterial consortium 2 (C2). At 35 DAT and 65 DAT the number of tillers per hill ranged from 16.40 to 17.10 tillers and 19.93 - 20.27 tillers, respectively (data not shown). The number of tillers decreases with increasing plant age. In 95 DAT the number of tillers decreased from 11.1 to 15.47 tillers. The number of dead tillers increases the availability of organic matter as a substrate for methanogenic bacteria to produce CH$_4$ gas under anaerobic conditions.

Rice yield at the 75% NPK fertilizer with bacterial consortium 1 (C1) or bacterial consortium 2 (C2) were not significantly different from the 100% NPK with the same bacterial consortia. It's suggested that the application of bacterial consortium with methane-utilizing bacteria could increase fertilizer efficiency by 25%. These results were the same as previous studies, where each methane-utilizing bacterium could increase the efficiency of inorganic fertilizers by up 25% [5]. One of the advantages of these methane-utilizing bacteria was to fix N$_2$. Harada et al. [12] described that N$_2$ fixation by bacterial consortia containing N$_2$-fixing bacteria could promote rice growth by providing an additional nitrogen source to rice.

Analysis of variance showed that the application of bacterial consortium at three dosage levels of inorganic NPK fertilizer also significantly affected the total biomass or straw's dry weight (Table 2). The higher the NPK dose, the more the straw dry weight elevated. According to Jha et al. [13] the more amino acids formed, the plant's dry weight will increase. Amino acids are formed from N$_2$, which is converted to NO$_3$. The more N$_2$ input to the plant, the greater the amino acids produced, and the higher the plant's dry weight. The amount of N$_2$ input is thought to be caused by the activity of methane-utilizing bacteria capable of fixing nitrogen.

### 3.3. Effect of treatment on CH$_4$ flux and CH$_4$ emissions

Methane emission was measured three times during the growing season, at 35 DAT (active tillering phase), 65 DAT (generative phase), and 95 DAP (ripening phase). The growth of rice plants influences methane dynamics in paddy fields. The fluctuation of CH$_4$ flux and CH$_4$ emissions in paddy fields observed is shown in Table 3. Analysis of variance showed that the application of bacterial consortium at three dosage levels of inorganic fertilizer significantly affected the CH$_4$ flux and CH$_4$ emissions.

The highest CH$_4$ flux was obtained from the active tillering phase (35 DAT), and after that, the CH$_4$ flux decreased. In the active tillering phase, the paddy fields were flooded to increase the number of tillers. Flooded causes a decrease of soil redox potential (Eh), the pH value closer to neutral, and anaerobic decomposition of organic matter occurs, which causes CH$_4$ gas formation [14].

The early CH$_4$ flux observed during the early vegetative stage could be due to the decomposition of remaining organic matter in the soil from the previous season. At 65 DAT observed during the reproductive phase, it was probably due to the actions of methanogenic soil bacteria on organic compounds released by rice plants as root exudates. At 95 DAT, the peak may be related to the deterioration of root senescence and the release of soil entrapped CH$_4$.

In Table 3 it can be observed that the higher the NPK fertilizer dose, the higher the CH$_4$ flux and CH$_4$ emissions. Wang et al. [13] explained that CH$_4$ fluxes are strongly affected by the rate, mode and methods of application of fertilizers. Nitrogen application in the form of urea enhances CH$_4$ emissions due to a drop in redox potential and increasing soil pH, which favors methanogenesis processes. The impact of fertilizers on CH$_4$ emission has mainly reported due to stimulatory and inhibitory effect on methanogens and methanotrophs.

The highest CH$_4$ emission was observed in plots receiving inorganic fertilizer only. The application of bacterial consortia at all NPK fertilization dosage reduced methane emissions (Table 3). At 100%
NPK fertilizer doses, inoculation with the bacterial consortium 1 and bacterial consortium 2 decreased CH$_4$ emissions from 123.09 kg ha$^{-1}$ season$^{-1}$ to 89.35 kg ha$^{-1}$ season$^{-1}$ and 79.07 kg ha$^{-1}$ season$^{-1}$, respectively. A bacterial consortia application containing methane-utilizing bacteria at all fertilizer doses reduces CH$_4$ flux and CH$_4$ emissions. It is suspected that this was due to methane-utilizing bacteria's activity, increasing the CH$_4$ oxidation in soil. It was found that paddy CH$_4$ emissions significantly decreased under biofertilizer application most likely caused by (i) increased methane-utilizing bacteria abundances significantly and (ii) decreased the ratio of methanogenic to methane-utilizing bacteria abundances greatly [15].

Table 3. The effect of treatments on seasonal CH$_4$ flux and CH$_4$ emissions

| Bacterial Consortia | Inorganic Fertilizer | Average |
|---------------------|----------------------|---------|
|                     | F1 (50%)             | F2 (75%) | F3 (100%) |         |
| CH$_4$ flux (mg m$^{-2}$ day$^{-1}$) at 35 DAT |
| C0                  | 261.00 bA            | 295.00 bA | 371.50 bB | 309.17   |
| C1                  | 173.00 aA            | 245.00 aB | 234.00 aB | 217.33   |
| C2                  | 210.00 aA            | 235.00 aA | 217.50 aA | 220.83   |
| Average             | 214.67               | 258.33   | 274.33     |         |
| CV                  | 11.03%               |         |           |         |
| CH$_4$ flux (mg m$^{-2}$ day$^{-1}$) at 65 DAT |
| C0                  | 168.00 bA            | 221.00 bA | 267.50 bB | 218.83   |
| C1                  | 170.00 aA            | 205.00 bB | 215.00 aB | 196.67   |
| C2                  | 118.00 aA            | 194.50 aB | 220.50 aC | 177.67   |
| Average             | 152.00               | 206.83   | 234.33     |         |
| CV                  | 7.63%                |         |           |         |
| CH$_4$ flux (mg m$^{-2}$ day$^{-1}$) at 95 DAT |
| C0                  | 38.00 bA             | 73.00 bB | 105.00 cC | 72.00    |
| C1                  | 20.00 aA             | 54.50 aB | 97.00 bC  | 57.17    |
| C2                  | 11.50 aA             | 37.00 aB | 43.50 aB  | 30.67    |
| Average             | 23.17                | 54.83    | 81.83      |         |
| CV                  | 2.27%                |         |           |         |
| CH$_4$ emission (kg ha$^{-1}$ season$^{-1}$) |
| C0                  | 77.97 bA             | 97.31 bB | 123.09 cC | 99.46    |
| C1                  | 59.84 aA             | 83.29 aB | 89.35 bB  | 77.49    |
| C2                  | 57.37 aA             | 77.23 aB | 79.07 aB  | 71.22    |
| Average             | 65.06                | 85.94    | 97.17      |         |
| CV                  | 7.63%                |         |           |         |

Remarks:
F1 = 50% NPK; F2 = 75% NPK; F3 = 100% NPK;
C0 = without bacteria; C1 = bacterial consortium 1; C2 = bacterial consortium 2
The same lowercase letters in each column and uppercase letters in each row represent no significant difference at 5% DMRT

The soil reduction-oxidation (redox) potential is a measure of the degree of aeration in soil and is important for methane production since the methanogens are dependent on anaerobic conditions. A high redox potential indicates a high oxygen level. Low redox values may be an indication that conditions are anaerobic. The potential redox value of soil Eh of -150 mV is considered the critical point of Eh to initiate methane production [16]. The values of soil redox potential during the study were quite oxidative ranging from -195.5 mV to -252.9 mV, possibly because the rice fields were not irrigated continuously. So that the methane gas emissions released during one growing season are lower, around 57.37 to 123.09 kg ha$^{-1}$ season$^{-1}$; these methane emissions are much smaller than methane emissions from other experiments, rice cultivation in irrigated paddy fields reaching 218 kg ha$^{-1}$ season$^{-1}$ [17].
This quite oxidative rice condition increases oxidation of methane by methane-utilizing bacteria, resulting in low methane emission. The presence of oxygen increases in the oxidizing layer thickness, and reducing carbon into CH$_4$ gets suspended due to the inactivity of methanogens [18]. Further, methane-utilizing bacteria increases the CH$_4$ oxidation in soil [13]. The availability of oxygen in flooded rice field is influenced by several factors like soil texture, availability of light, photosynthetic of aquatic plants and rice cultivars. Wassman and Aulakh [19] reported that the CH$_4$ oxidation in paddy fields is localized near surface aerobic layer and in the rhizosphere. In rhizosphere, the concentration gradients of O$_2$ and CH$_4$ overlap. Aerenchymal transportation of O$_2$ from atmosphere to rhizosphere enhances the process of CH$_4$ oxidation in rice soil. As the soil porosity increases more O$_2$ diffusion occurs, while increased water content reduces O$_2$ diffusion into the soil [20].

Soil pH during observation increased from 6.1 to 6.65 - 7.10. Soil pH plays a vital role in methane production with maximum production rates at neutral pH conditions [12]. Methanogens are usually more active in neutral (pH 6.5 to 7.5) or slightly alkaline and very sensitive to soil pH fluctuations.

*M. senegalense*, *P. stuartii*, *R. rhizoryzae*, and *B. methylotrophicus* could live in the rhizosphere and plant tissue [21-24]. These bacteria can be used as biofertilizers. The combined application of *M. senegalense* LR73 and *Bacillus formis* JR80 and arbuscular mycorrhizal fungus has significant effects on plant height, biomass production, sugar content, P and K uptake of sweet sorghum [21]. *Providencia* spp. significantly increased plant growth of Crucifers under greenhouse condition. Hence, they may be used as biofertilizers to promote the plant growth of Crucifers in field conditions [22]. While *R. rhizoryzae* is a Gram-negative, free living, saprotrophic soil bacterium, has an enzyme nitrogenase responsible for the nitrogen-fixing ability. The bacteria did not form rice nodules as host plants [23]. *Bacillus methylotrophicus* was methanol-utilizing, a plant-growth-promoting bacterium isolated from rice rhizosphere soil [24].

The bacterial consortium application at 75% and 100% NPK fertilizer on rice showed that vegetative growth and rice production were not significantly different. This application impact is that the bacterial consortia could increase the efficiency of NPK fertilizer by 25%. From the results of comparing the data on the treatment of 100% NPK fertilizer without inoculation of bacterial consortia (Table 4), it can be observed that the two bacterial consortia could increase rice yield and reduce methane emissions.

Overall, bacterial consortium 2 showed better results than bacterial consortium 1, most likely due to the additive effect of all strains on increased lowland rice production and decreased methane emissions. The application of bacterial consortium 2 could increase inorganic fertilizers efficiency by 25%, increase rice production by 33.55%, and reduce methane emissions by 37.26%. It seems that this consortium of methane-utilizing bacteria has the prospect of biofertilizer and biological mitigation agents in paddy fields to mitigate the impact of global warming.

**Table 4.** The effect of treatments on increasing rice production and reducing of CH$_4$ emissions

| Treatments                     | Rice Yield (t ha$^{-1}$) | Increasing of yield (%) | CH$_4$ emissions (kg ha$^{-1}$ season$^{-1}$) | Reduction of CH$_4$ emissions (%) |
|-------------------------------|--------------------------|--------------------------|---------------------------------------------|----------------------------------|
| NPK 50% + bacterial consortium 1 | 5.13                     | 9.61                     | 59.84                                      | 51.39                             |
| NPK 50% + bacterial consortium 2 | 5.48                     | 17.09                    | 57.37                                      | 53.39                             |
| NPK 75% + bacterial consortium 1 | 5.31                     | 13.46                    | 83.29                                      | 32.33                             |
| NPK 75% + bacterial consortium 2 | 6.25                     | 33.55                    | 77.23                                      | 37.26                             |
| NPK 100% without bacteria     | 4.68                     | -                        | 123.09                                     | -                                |

4. Conclusions
The bacterial strains of *Mycobacterium senegalense* LM1, *Providencia stuartii* LM18, *Rhizobium rhizoryzae* BMU, and *Bacillus methylothrophicus* N2P4 isolated from rice field sediments have pmoA-like gene (one of the key enzymes in the bacterial methane metabolic pathway), can fix N$_2$, solubilize P, and produce IAA. The application of bacterial consortium containing all strains increased inorganic fertilizers efficiency by 25%, increased rice production by 33.55%, and reduced methane emissions by 37.26%. The consortium of these methane-utilizing bacteria has the prospect as biofertilizer and mitigation agents to mitigate the impact of global warming.

**Acknowledgments**

The research has been supported by Budget Implementation List or DIPA in the fiscal year 2019, Ministry of Agriculture of Indonesia. All the authors contributed equally as the main contributors to this paper.

**References**

[1] Wassmann R, Schultz H, Papen H, Rennenber H, Seiler W, Dai A G, Shen R X, Shangguan X J and Wang M X 1993 Quantification of methane emissions from Chinese rice fields (Zhejiang Province) *Biogeochemistry* 20: 83-101

[2] Zehnder A J B and Stumm W 1988 Geochemistry and biogeochemistry of anaerobic habitats. *In: Zehnder AJB (Ed.)* Biology of anaerobic microorganisms John Wiley & Sons New York p 1–38

[3] Mancinelli R L 1995 The regulation of methane in soil *Annu. Rev. Microbiol.* 49: 581–605

[4] Pingak G M F, Sutanto H, Akhdija A and Rusmana I 2014 Effectivity of methanotrophic bacteria and *Ochrobactrum anthropi* as biofertilizer and emission reducer of CH$_4$ and N$_2$O in inorganic paddy fields *J. Medical Bioengin.* 3: 217-221

[5] Pratiwi E 2018 Penelitian Pemanfaatan Bakteri Pereduksi Emisi Gas Metana Peningkat Efisiensi Serapan Hara Tanaman Padi (in Bahasa) *Laporan Hasil Kegiatan Penelitian DIPA 2019* Satker Balai Penelitian Tanah

[6] Anonymous 2019 Peraturan Menteri Pertanian Republik Indonesia Nomor 01 Tahun 2019 tentang *Pendaftaran Pupuk Organik, Pupuk Hayati, dan Pembenah Tanah* (in Bahasa) Jakarta

[7] Sekar J, Raj R, Prabavathy V R. 2016 Microbial consortial products for sustainable agriculture: Commercialization and regulatory issues in India p 107–131 *In: Singh H B, Sarma B K and Keswani C (Eds.)* *Agriculturally Important Microorganisms* Science+Business Media: Singapore

[8] Jhala Y K, Vyas R V, Sheat H N, Patel H K and Patel K T 2014. Isolation and characterization of methane utilizing bacteria from wetland paddy ecosystem *World J. Microbiol. Biotechnol.* 30: 1845–1860

[9] Whittenbury R, Phillips K C, Wilkinson J F and Enrichment W J F 1970 Enrichment, isolation and some properties of methane-utilizing bacteria *J. Gen. Microbiol.* 61: 205–218

[10] Qin X, Li Y, Wang H, Li J, Wan Y and Gao Q 2015 Effect of rice cultivars on yield-scaled methane emissions in a double rice field in South China *Integr. Environ. Sci.* 12: 47–66

[11] Khalil M A K, Rasmussen R A, Wang M X and Ren L 1991 Methane emissions from rice fields in China *Environ. Sci. Technol.* 25: 979-981

[12] Harada N, Otsuda S, Nishiyama M and Matsumoto S 2005 Influences of indigenous phototrophs on methane emissions from a straw-amended paddy soil *Biol. Fertil. Soils* 41: 46–51

[13] Jha P, Shashi R A, Agnihotri P K, Kulkarni V M and Bhat V 2011 Efficient *Agrobacterium*-mediated transformation of *Pennisetum glaucum* (L.) R. Br. using shoot apices as explant source *Plant Cell Tiss. Organ Cult.* 107: 501–512

[14] Wang Z, Lindau C W, Delaune R D and Patrick Jr W H 1993 Methane emission and entrapment in flooded rice soils as affected by soil properties *Biol. Fertil. Soils* 16: 163-168
[15] Kightley D, Nedwell D B and Cooper M 1995 Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms Appl. Environ. Microbiol. 61: 592-601

[16] Masscheleyyn P H, DeLaune R D, and Patrick W H Jr 1993 Methane and nitrous oxide emissions from laboratory measurements of rice soil suspension: effect of soil oxidation-reduction status. Chemosphere 26: 251–260

[17] Wihardjaka A, Soeprapto and Mamaril C P 1999 Response of rainfed lowland rice and soybean to sulphur in light textured soils in Central Java Indonesian J. Crop Sci. 14: 29-34

[18] Bender M and Conrad R. 1995 Effect of CH\textsubscript{4} concentrations and soil conditions on the induction of CH\textsubscript{4} oxidation activity Soil Biol. Biochem. 27: 1517-1527

[19] Wassmann R and Aulakh M S 2000 The role of rice plants in regulating mechanisms of methane emissions: A review Biol. Fertil. Soils 32: 20-29

[20] Dubey S K 2005 Microbial ecology of methane emission in a rice agroecosystem Appl. Ecol. Environ. Res. 3: 1–27

[21] Rupaedah B, Anas I, Santosa D A, Sumaryono W and Budi S W 2014 Role of rhizobacteria and arbuscular mycorrhizae on enhancing nutrient absorption efficiency of sweet sorghum (Sorghum bicolor L. Moench) J. Tanah Lingk. 16: 45-52

[22] Gowtham H G, Singh S B and Niranjana S R 2015 Evaluation of plant growth promoting ability of Providencia spp. collected from North Eastern Region of India in Crucifers Int. J. Agric. Sci. Res. 5: 321-327

[23] Zhang X X, Tang X, Sheirdil R A, Sun L and Ma X T 2014 Rhizobium rhizoryzae sp. nov., isolated from rice roots Int. J. Syst. Evol. Microbiol. 64: 1373–1377

[24] Madhaiyan M, Poonguzhali S, Kwon S W and Sa T M 2010 Bacillus methylotrophicus sp. nov., a methanol-utilizing, plant-growth-promoting bacterium isolated from rice rhizosphere soil Int. J. Syst. Evol. Microbiol. 60: 2490–2495