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RESEARCH ARTICLE

Greenhouse gas emissions reduction utilizing methane-oxidizing bacteria in critical growth stages of paddy

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ABSTRACT

Paddy cultivation contributes to greenhouse gas (GHG) emissions in the agricultural sector. The consistency of the biological option to reduce GHG emissions is utilizing methane-oxidizing bacteria in paddy cultivation. This study aimed to obtain methane (CH_4) and nitrous oxide (N_2O) reduction by applying methane-oxidizing bacteria in soil incubation and the critical growth stages of paddy in paddy fields. The research was conducted at the greenhouse gas laboratory for soil incubation with four isolates (*Amorphomonas oryzae* (SI5), *Ciceribacter* sp. (OF4), *Rhodanobacter rhizosphaerae* (TH6), *Bordetella petrii* (BD4)), and combination bacterial consortium at the paddy field scale. The treatment consisted of applying bacterial consortium and organic matter at the field scale. The first factor: R0 = without bacterial application; R1 = SI5, OF4, BD4; R2 = SI5, TH6, OF4; R3 = SI5, BD4, *Priestia aryabhattai*. The second factor was organic matter addition; O0 = without manure and O1 = 2 t ha^{-1} farmyard manure. Applying four isolates had a positive effect in reducing N_2O and CH_4 concentrations in soil incubation. The SI5 reduced CH_4 concentration by 22%, and BD4 showed a 22% N_2O reduction during soil incubation. The bacterial consortium R3 was the best treatment to reduce total CH_4 and N_2O emissions at three critical growth stages of paddy and produced the highest total population of methanotrophs and denitrifying bacteria. Bacterial consortia are suggested as a potential strategy for promoting sustainable agriculture and environment.

Key words: CH_4 emission, denitrifying bacteria, methane-oxidizing bacteria, N_2O emission, paddy field.

INTRODUCTION

Paddy fields are emitters of greenhouse gas (GHG) emissions, as well as methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2), which are released into the atmosphere. The primary GHG emissions in paddy fields are CH_4 and N_2O (Pham et al., 2021). Rice (*Oryza sativa* L.) cultivation contributes 30% and 11% of CH_4 and N_2O emissions from agriculture, respectively (Gupta et al., 2021). Methane is emitted by the anaerobic decomposition of organic matter and the presence of methanogenic archaea in the soil. Nitrous oxide is also released during denitrification processes in the soil due to fertilization. Increasing CH_4 and N_2O emissions are influenced by various factors, such as water regime, organic matter amendment, soil temperature, soil moisture, fertilizer application, and rice varieties (Gupta et al., 2021; Pramono et al., 2024).

Paddy plays a crucial role in the release of GHG emissions in paddy fields. The total CH_4 emissions from the soil are released into the atmosphere through aerenchyma. Aerenchyma is found in the stems and roots of paddy and acts as chimneys for releasing CH_4 into the atmosphere (Iqbal et al., 2023). The optimum conditions for the formation of CH_4 in paddy fields are continuous flooding irrigation systems, limited oxygen (anaerobic condition), and low redox potential in the soil. The CH_4 is produced by methane-producing archaea that utilize

C sources, such as CO_2, methyl alcohol (CH_3OH), and acetate (CH_3COOH), as energy sources in the metabolic process under anoxic soil conditions (Wang et al., 2021b).

One mitigation option to reduce CH_4 emissions in paddy fields is the application of methane-oxidizing bacteria (MOB), which are known as methanotrophs. These bacteria utilize CH_4 as a C source in their metabolic processes, significantly reducing CH_4 emissions in paddy fields. The bacteria have genes to encode the enzyme methane monooxygenase (MMO), which catalyzes CH_4 into CO_2 and H_2O under aerobic conditions (Wang et al., 2021b). Davamani et al. (2020) reported that applying bacterial consortium with methanotrophic, N-fixing, and phosphate-solubilizing bacteria reduced CH_4 emissions by 60% and improved rice yields by 35%.

Rice cultivation activities are closely related to applying N fertilizers to enhance crop production. Nitrogen is an essential macro element for plant growth, where its availability determines crop yield (Ye et al., 2022). However, N fertilizer is also a significant source of N_2O emissions into the atmosphere. More than two-thirds of N_2O emissions come from nitrification and denitrification by bacteria and fungi in the soil, primarily due to N fertilizer application (Pan et al., 2022). Moreover, increasing the N fertilizer rate also increased atmospheric CH_4 emissions. Several studies have indicated that applying N, P, and K fertilizers can stimulate CH_4 production, as indicated by increased *mcrA* methanogen gene expression (Zhang et al., 2018). Reducing N input into the soil is an option to reduce N_2O emissions, but it may also reduce crop productivity. An alternative option is the application of bacteria that reduces nitrate to N_2 by producing nitrate-, nitrite-, and nitrous oxide reductase, which could reduce N_2O emissions (Jonassen et al., 2021).

Increasing the population of MOB and denitrifying bacteria in paddy fields can reduce GHG emissions by converting CH_4 and N_2O into less harmful gases. Preliminary research has carried out the isolation of Indigenous MOB at three rice agroecosystems (Adriany et al., 2021). Three rice agroecosystems have some isolates to potentially reduce CH_4 and N_2O fluxes in paddy soils, namely isolate from technically irrigated paddy fields (SI5), rice organic farming (OF4), and rainfed paddy fields (TH6). Another isolate, BD4, is an aerobic bacterium that was isolated from a slurry of biodigester. *Priestia aryabhattai*, which was previously known as *Bacillus aryabhattai* (Gupta et al., 2020), increases crop production. These four isolates were used in this research. The objective was to identify and evaluate GHG emissions reduction by utilizing MOB in soil incubation in the critical growth stages of paddy.

MATERIAL AND METHODS

Experimental site

The experiment was conducted at the Agricultural Experimental Station of Indonesian Agricultural Environmental Standardization Institute, in Sidomukti village (6°46'39.7" S, 111°11'53.0" E; Figure 1), Jaken sub-district, Pati Regency, Central Java Province, Indonesia. The research started from March to September 2021.

Soil incubation

The four isolates, *Amorphomonas oryzae* (SI5), *Ciceribacter* sp. (OF4), *Rhodanobacter rhizosphaerae* (TH6), and *Bordetella petrii* (BD4), were cultured in NMS (liquid) + methanol to test their ability to oxidize methane (CH_4) and reduce nitrous oxide (N_2O) concentrations. For the experiment, 25 g sterile dry soil, 10 mL liquid medium nitrate minimal salts (NMS) without methanol, 30 mL sterile distilled water, urea 180 kg N ha^{-1}, 1 mL each isolate 10^6 CFU mL^{-1}, and CH_4 standard 11.52 mg kg^{-1} were added into an incubation bottle. The headspace settings were adjusted to a composition of 50% CH_4:50% air in the bottle. The control was without the addition of bacteria. The incubation was accomplished for 15 d with a 150 rpm shaker at room temperature. The CH_4 and N_2O concentration was measured at 2, 5, 7, 10, and 15 d during incubation by taking 5 mL gas in the bottle using a 5 mL syringe. The CH_4 and N_2O concentrations were analyzed using gas chromatography (GC) (GC-2014; Shimadzu, Kyoto, Japan). The percentage reduction of CH_4 and N_2O concentrations was calculated by comparing the control concentration with the concentration for each isolate as follows:

$$R = \frac{[C]-[T]}{[C]} \times 100\%$$

where R is the percentage reduction of CH_4 or N_2O, [C] is the concentration of control treatment, and [T] is the concentration of treatment CH_4 or N_2O.

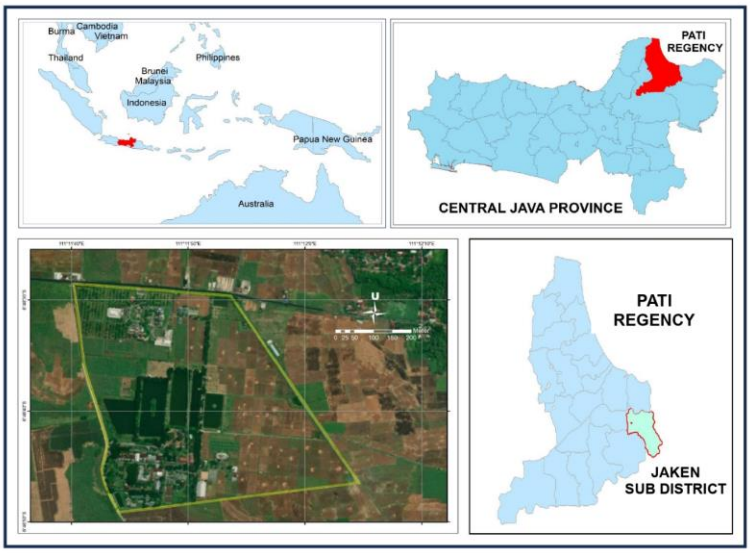

Figure 1. Experimental site at Sidomukti village, Jaken sub-district, Pati Regency, Central Java Province, Indonesia.

Rice cultivation and greenhouse gas (GHG) measurement

The treatments were conducted using a randomized block design, with two factors (bacterial consortium and organic matter) and three replicates. The treatment code for the first factor was the application of bacterial consortium: R0 = without bacterial consortium application; R1 = SI5, OF4, BD4; R2 = SI5, TH6, OF4; R3 = SI5, BD4, *Priestia aryabhattai*. The second factor was the application of organic matter: O0 = without manure and O1 = 2 t ha^{-1} farmyard manure. The nutrient content in farmyard manure was organic C 14.95%, total N 0.62%, C/N ratio of 24, total P 8.61%, total K 7.59%, and pH 8.21.

Land preparation consisted of plowing, leveling, and harrowing. A plot with the size of 6 × 6 m was used as an experimental unit. The rice (*Oryza sativa* L.) 'Inpari 32' was used with a seedling age of 15 d after sowing. The bacterial consortium was applied at three stages: Seed treatment, vegetative phase at 30 d after transplanting (DAT), and generative phase at the beginning of flowering primordia at 55 DAT. The bacterial consortium was applied during seed treatment by soaking plant seeds at 500 mL ha^{-1}, 10^5 CFU mL^{-1}, dissolved in water in a ratio of 1:100 (v/v). The plant spacing used was 20 × 20 cm with 2-3 rice seedlings per hole. The bacterial consortium was applied at vegetative and early generative phases with a rate of 6 L ha^{-1} and sprayed above the soil surface.

The inorganic fertilizers rates were 120 kg N, 45 kg P_2O_5, and 60 kg K_2O per hectare based on fertilization recommendations from the Integrated Cropping Calendar Information System of the Indonesian Ministry of Agriculture for paddy fields in Jaken sub-district, Pati Regency, Central Java, and preliminary soil analysis results. Fertilizer application was split at 7 DAT for the first fertilizer ($^1/_3$ N + $^1/_3$ K + P), 28 DAT for the second fertilizer, and 49 DAT for the third fertilizer ($^1/_3$ N + $^1/_3$ K, respectively). The irrigation system used continuous flooding with a water level of 5 cm in the soil surface to optimize GHG emissions from paddy fields. The climate data was recorded by an automatic weather station located near the experimental site. The average daily temperature and precipitation during rice plant growth are shown in Figure 2.

The CH_4 and N_2O fluxes were measured ten times using the closed chamber method during the rice growing season. The critical growth stages of paddy were 23 DAT active tillering/vegetative stage, 58 DAT maximum tillering/early generative stage, and 80 DAT seed ripening stage. In addition, the number of tillers and plant height were measured in these stages. The chamber was made of transparent plastic with a thickness of 0.8 mm and an aluminum frame with a size of 50 × 50 × 100 cm to cover four clumps. The temperature changes in the chamber were recorded at each sampling interval of 0, 10, 20, 30, and 40 min. The concentrations of CH_4 and N_2O gases were analyzed using GC-2014. The daily fluxes or emissions (Q) of CH_4 and N_2O released from one area of the paddy field were calculated based on the equation (Win et al., 2021) as follows:

$$Q = \frac{\Delta c}{\Delta t} \times \frac{V}{A} \times \frac{M}{22.4} \times \frac{273}{K}$$

where Q is CH_4 or N_2O flux (mg m^{-2} min^{-1}), $\Delta c/\Delta t$ is the rate of change of gas concentration per unit time (mg kg^{-1} min^{-1}), V is the chamber volume (m^3), A is the chamber area (m^2), M is the molecular weight of CH_4 or N_2O (g), 22.4 L is the molecular volume at standard temperature and pressure, K is Kelvin temperature of the air temperature inside the chamber.

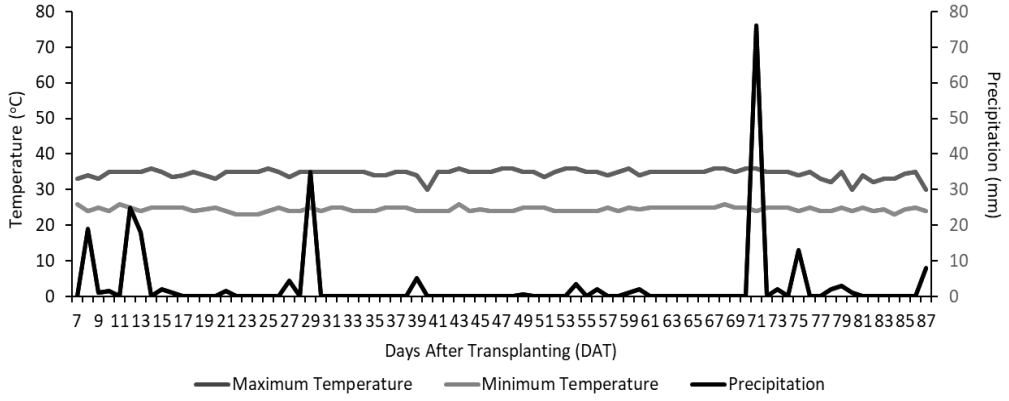

Figure 2. Daily precipitations, maximum and minimum temperatures during rice growing season.

Soil parameter

The soil sample in the experimental site was categorized in the order Inceptisols, silt loam, and subgroup Aeric Endoaquepts. Physicochemical properties for soil nutrients, pH, and soil texture before planting are shown in Table 1. Soil analysis was conducted directly after taking samples at the soil testing laboratory Indonesian Agricultural Environmental Standardization Institute. The soil samples were labeled as initial soil and post-harvesting after applying treatment. Soil fertility in the field experiment generally had a shallow C-organic, moderate total N and cation-exchange capacity, and high K and P content.

Table 1. Physicochemical properties for initial soil. CEC: Cation-exchange capacity.

Parameters	Value	Method
Organic C, %	0.48	Walkey and Black
Total N, %	0.24	H_2SO_4, Titrimetric
N-NO_3^-, mg kg^{-1}	9.34	Morgan Wolf
N-NH_4^+, mg kg^{-1}	1.97	Morgan Wolf
Available P, mg kg^{-1}	114.81	Bray
Available K, mg kg^{-1}	41.99	Morgan Wolf
CEC, $cmol_{(+)}$ kg^{-1}	17.11	Ammonium Acetate
pH	5.70	H_2O (1:5)
Soil texture		Gravimetric
Clay, %	25.20	
Sand, %	44.50	
Silt, %	30.30	

Methanotroph and denitrifying bacteria population

Methanotroph and denitrifying bacteria populations were calculated at three critical growth stages of paddy (23, 58, and 80 DAT). Soil sampling was conducted at a depth of 0-15 cm with a composite of five sample points diagonally on each plot. The calculation of soil bacterial population was conducted to determine the abundance of methanotrophs and denitrifying bacteria. The method of calculating the population of methanotrophs and denitrifying bacteria used the most probable number (MPN) (Davamani et al., 2020). The NMS + 1% methanol medium was used for methanotroph bacteria growth and nutrient broth (NB) + KNO_3 was used for denitrifying bacteria growth. The MPN method used five series of tubes with positive results characterized by the formation of gas in the Durham tube. The number of bacterial populations was determined according to the MPN table.

Data analysis

The collected data were analyzed by ANOVA to determine the effect of differences between groups, treatments, and interactions of the two factors. The Tukey test at 5% significance level was used to identify significant differences between the treatments.

RESULTS AND DISCUSSION

Characteristics and potential of bacteria

The result indicated that the four bacterial isolates (SI5, OF4, TH6, and BD4) had the potential to reduce CH_4 and N_2O emissions in paddy fields. The identification and characteristics of these bacterial isolates are shown in Table 2. The SI5 and OF4 have the ability for N_2 fixation and indole acetic acid (IAA) production. The BD4 and TH6 are classified as denitrifying bacteria.

Table 2. Identification and characteristics of bacteria were observed on NMS medium + methanol 1%, a selective medium for the growth of methanotrophic bacteria. IAA: Indole-3-acetic acid.

No.	Isolate	Similarity (%)	Species name	Accession	Characteristics
1	SI5	99.93	*Amorphomonas oryzae*	AB233493.1	Gram-negative, aerobic, yellow-cream, bacillus, motile, N_2 fixer, IAA-producing
2	OF4	99.93	*Ciceribacter* sp.	MH579729.1	Gram-negative, aerobic, dark brown, bacillus, motile, N_2 fixer, IAA-producing
3	BD4	99.68	*Bordetella petrii*	EU082174.1	Gram-negative, aerobic, white-cream, bacillus, non-motile
4	TH6	99.93	*Rhodanobacter rhizosphaerae*	NR_156938.1	Gram-negative, aerobic, brown-yellow, bacillus, non-motile

Amorphomonas oryzae (SI5) and *Ciceribacter* sp. (OF4) are groups of Gram-negative bacteria, *Rhizobiaceae* family, and non-symbiotic N_2 fixation (Yousuf et al., 2014; Siddiqi et al., 2018). *Amorphomonas oryzae* survives under neutral soil up to high salt concentrations (saline soil) and adapts to dry and stressful environmental conditions (Yousuf et al., 2014). Genus *Ciceribacter* grows in a wide pH range, as an N_2 fixer and positive catalase (Zhang et al., 2023). *Rhodanobacter rhizosphaerae* (TH6) is an aerobic bacterium. The genus *Rhodanobacter*, the class *Gammaproteobacteria*, is a denitrifying bacterium that is tolerant to lower pH conditions, rich in nitrate, and contaminated with heavy metals in soil (Prakash et al., 2021). *Bordetella petrii* (BD4) is the only species in the genus *Bordetella* found in environments that have encoding nitrate-, nitrite-, and nitrous oxide reductases genes, which play a crucial role in the denitrification process to convert nitrate into N_2O and N_2. It also has a monooxygenase enzyme for the degradation of aromatic compounds (Gross et al., 2008).

Reduction of CH_4 and N_2O concentrations in soil incubation

The ability to reduce CH_4 and N_2O concentrations was shown in percentages of CH_4 and N_2O reduction compared to the control treatment. Percentage reduction in CH_4 and N_2O concentrations from the four isolates with the addition of urea 180 kg N ha^{-1} for 15 d incubation compared to control are shown in Figure 3. Four isolates and adding urea positive responded to reducing CH_4 and N_2O concentrations. The SI5 had the highest average decrease in CH_4 concentration by 22% for 15 d incubation. Moreover, TH6 had the lowest CH_4 concentration reduction for 15 d incubation by 8%. Three isolates namely, *A. oryzae* (SI5), *Ciceribacter* sp. (OF4), and *B. petrii* (BD4), had a positive response in reducing N_2O concentration in sterile soil incubation with the addition of urea, as shown in Figure 3. The N_2O concentration reduction occurred after 5 d incubation. The highest reduction of N_2O concentration was BD4, with an average percentage of reduction of N_2O concentration by 22% for 15 d incubation. *Rhodanobacter rhizosphaerae* (TH6) did not give a response in reducing N_2O concentration during incubation.

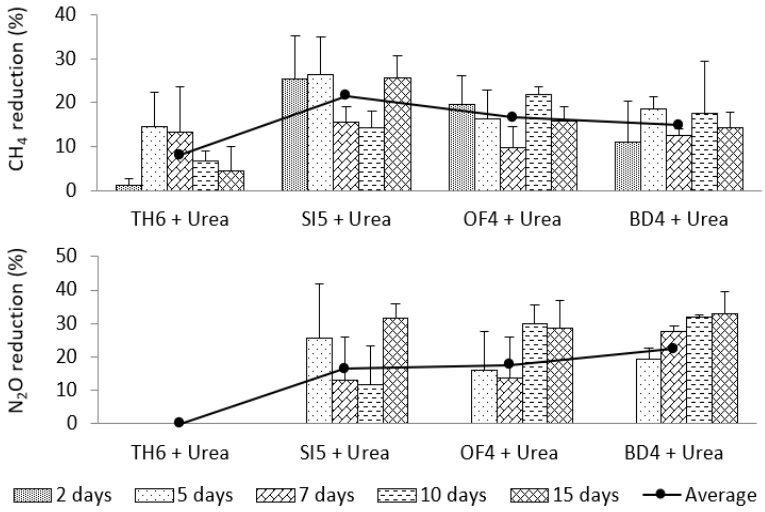

Figure 3. Percentage reduction in CH_4 and N_2O concentrations during soil incubation with the addition of urea and four isolates.

Only three bacterial isolates, SI5, OF4, and BD4, simultaneously reduced CH_4 and N_2O concentrations, while only TH6 reduced CH_4 concentration singularly. However, these four bacterial isolates are not included in the 13 genera of methanotrophic bacteria known to have methane monooxygenase (MMO) enzymes that can oxidize methane. The addition of urea to the growth medium of methanotrophic bacteria affected the rate of methane oxidation. Wei et al. (2016) reported that applying more urea fertilizer will accelerate methane oxidation rate and stimulate methanotrophic activity.

The application of N-urea fertilizer enhances ammonium concentration and microorganism activities, such as those of methanotrophic bacteria. The bacteria utilize ammonium as a nutrient for their growth. When a large amount of N-ammonium is applied to the soil, it is used as the food source for methanotrophic bacteria. According to van Dijk et al. (2021), ammonia acts more as a nutrient than as an inhibitor when methane concentrations are high enough to support the growth rate of methanotrophic bacteria. The abundance of methane and oxygen in the soil also influenced the high and low rates of methane oxidation. High levels of methane and oxygen availability in the soil increased methane oxidation rates, effectively reducing methane emissions into the atmosphere (Nwokolo and Enebe, 2024).

Effect of bacterial consortium application on reducing GHG emissions in critical growth stages of paddy

The results showed that CH_4 fluxes in critical growth stages of paddy with the application of bacterial consortium had a significant effect at 23, 51, and 58 DAT (Table 3). The R3 bacterial consortium showed the lowest CH_4 flux by 337.56 mg CH_4 m^{-2} d^{-1} with a reduction of 17.6% compared to the control (without application) at 23 DAT. At 51 and 58 DAT, R2 and R3 bacterial consortium applications had low CH_4 fluxes. In the early generative stage (58 DAT), both consortia emitted 264.22 and 262.52 mg CH_4 m^{-2} d^{-1}, reducing 26.7% and 27.1% compared to the control. The total CH_4 emission calculation from the application of bacterial consortium effected reducing CH_4 emissions in rice fields. Application of bacterial consortium R3 emitted the lowest CH_4 emission by 217 kg ha^{-1} $season^{-1}$ compared to the control (Table 5).

Table 3. Daily fluxes of CH_4 concentrations during the rice growing season. ^{ns}Nonsignificant, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Means followed by different letters within each group of values indicate significant differences ($p < 0.05$) within the same column by Tukey's test. SI5: *Amorphomonas oryzae*, OF4: *Ciceribacter* sp., TH6: *Rhodanobacter rhizosphaerae*, BD4: *Bordetella petrii*.

Treatments	CH_4 fluxes (mg CH_4 m^{-2} d^{-1})									
	9 DAT	16 DAT	23 DAT	30 DAT	37 DAT	51 DAT	58 DAT	65 DAT	72 DAT	80 DAT
Block										
1	169.38	260.97^{ab}	354.37^{b}	325.39^{b}	328.33^{b}	340.89	292.98^{ab}	281.88^{b}	212.89^{b}	249.14
2	236.65	192.48^{b}	340.79^{ab}	346.18^{ab}	350.45^{b}	331.89	264.67^{b}	301.79^{b}	315.39^{ab}	265.21
3	279.40	341.12^{a}	460.79^{a}	410.76^{a}	479.37^{a}	403.98	357.48^{a}	448.77^{a}	359.96^{a}	330.77
Bacterial consortium (R)										
R0 (without application)	243.82	276.05	409.80^{a}	367.95	379.12	449.95^{a}	360.33^{a}	407.36	290.30	357.10
R1 (SI5, OF4, BD4)	201.44	268.73	406.30^{ab}	386.51	406.53	376.92^{ab}	333.09^{ab}	375.33	339.91	237.84
R2 (SI5, TH6, OF4)	284.77	256.83	387.59^{ab}	360.39	388.06	309.88^{b}	264.22^{b}	286.85	329.73	262.81
R3 (SI5, BD4, *Priestia aryabhattai*)	183.87	257.57	337.56^{b}	328.24	370.49	298.93^{b}	262.52^{b}	307.04	224.39	269.07
Organic matter (O)										
O0 (without application)	235.81	250.16	381.64	371.05	370.47	398.67	325.94	333.70	304.76	290.29
O1 (2 t ha^{-1})	221.14	279.43	387.99	350.50	401.62	319.17	284.15	345.58	287.38	273.12
Pr > F										
Block	ns	**	***	*	**	ns	*	**	*	ns
Bacterial consortium (R)	ns	ns	*	ns	ns	*	*	ns	ns	ns
Organic matter (O)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R × O	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The dynamics of daily N_2O fluxes are influenced by fertilization, soil conditions, and nutrient content in the soil. The N_2O fluxes at critical growth stages of paddy with the application of bacterial consortium showed significant effects at 58 and 80 DAT (Table 4). Bacterial consortium (R1, R2, or R3) showed the lowest N_2O flux at 58 DAT by 0.49, 0.72, and 0.57 mg N_2O m^{-2} d^{-1}, decreasing 69.9%, 55.8%, and 65.0% compared to the control, respectively. At 80 DAT, the daily flux of N_2O emissions was 0.58, 0.56, and 0.48 mg N_2O m^{-2} d^{-1}, a decrease of 54%, 55.6%, and 61.9%, respectively. The application of 2 t ha^{-1} farmyard manure showed a significant effect at 23 DAT by 31.3% reduction from the control treatment. However, organic matter application increased N_2O emissions at 51 DAT (Table 4). The calculation of total N_2O emissions during the rice growing season with the application of this bacterial consortium had a significant effect in reducing N_2O emissions in paddy fields. These bacterial consortia (R1, R2, and R3) produced N_2O emissions lower than the control; 0.52, 0.57, and 0.53 kg ha^{-1} $season^{-1}$ (Table 5).

Table 4. Daily fluxes of N_2O concentrations during the rice growing season. ^{ns}Nonsignificant, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Means followed by different letters within each group of values indicate significant differences ($p < 0.05$) within the same column by Tukey's test. SI5: *Amorphomonas oryzae*, OF4: *Ciceribacter* sp., TH6: *Rhodanobacter rhizosphaerae*, BD4: *Bordetella petrii*.

	N_2O fluxes (mg N_2O m^{-2} d^{-1})									
Treatments	9 DAT	16 DAT	23 DAT	30 DAT	37 DAT	51 DAT	58 DAT	65 DAT	72 DAT	80 DAT
Block										
1	1.34	1.59^{a}	1.57^{a}	0.32	0.46	0.98	1.17^{a}	0.99	0.48	0.78
2	1.44	0.34^{b}	0.44^{b}	0.36	0.58	0.93	1.00^{a}	0.44	0.33	0.62
3	1.73	1.13^{ab}	0.89^{b}	0.32	0.82	0.60	0.39^{b}	0.44	0.56	0.76
Bacterial consortium (R)										
R0 (without application)	1.77	0.52	1.14	0.44	0.92	1.15	1.63^{a}	0.93	0.61	1.26^{a}
R1 (SI5, OF4, BD4)	1.37	0.89	0.94	0.34	0.43	0.77	0.49^{b}	0.69	0.35	0.58^{b}
R2 (SI5, TH6, OF4)	1.66	1.06	0.97	0.30	0.56	0.86	0.72^{b}	0.32	0.41	0.56^{b}
R3 (SI5, BD4, *Priestia aryabhattai*)	1.21	1.06	0.82	0.26	0.57	0.54	0.57^{b}	0.54	0.47	0.48^{b}
Organic matter (O)										
O0 (without application)	1.95^{a}	1.02	1.15^{a}	0.38	0.76	0.47^{b}	0.85	0.79	0.46	0.80
O1 (2 t ha^{-1})	1.06^{b}	1.02	0.79^{b}	0.28	0.48	1.19^{a}	0.84	0.45	0.45	0.65
Pr > F										
Block	ns	*	***	ns	ns	ns	**	ns	ns	ns
Bacterial consortium (R)	ns	ns	ns	ns	ns	ns	***	ns	ns	**
Organic matter (O)	*	ns	*	ns	ns	**	ns	ns	ns	ns
R × O	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 5. Yield and total CH_4, and N_2O emissions of rice cultivation. ^{ns}Nonsignificant, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Means followed by different letters within each group of values indicate significant differences ($p < 0.05$) within the same column by Tukey's test. SI5: *Amorphomonas oryzae*, OF4: *Ciceribacter* sp., TH6: *Rhodanobacter rhizosphaerae*, BD4: *Bordetella petrii*.

Treatments	Grain yield	CH_4 emission	N_2O emission
	t ha^{-1}	kg ha^{-1} $season^{-1}$	
Block			
1	3.38	216^{b}	0.72^{a}
2	3.24	226^{b}	0.42^{b}
3	2.90	297^{a}	0.62^{a}
Bacterial consortium (R)			
R0 (without application)	3.27	270^{a}	0.78^{a}
R1 (SI5, OF4, BD4)	3.16	257^{ab}	0.52^{b}
R2 (SI5, TH6, OF4)	3.09	240^{ab}	0.57^{b}
R3 (SI5, BD4, *Priestia aryabhattai*)	3.15	217^{b}	0.53^{b}
Organic matter (O)			
O0 (without application)	3.13	251	0.62
O1 (2 t ha^{-1})	3.21	242	0.58
Pr > F			
Block	ns	***	***
Bacterial consortium (R)	ns	**	**
Organic matter (O)	ns	ns	ns
R × O	ns	ns	ns
CV, %	12.42	19.49	28.86

On the other hand, the application of 2 t ha^{-1} farmyard manure did not positively respond to reducing CH_4 emissions in paddy fields. This result indicated that the growth phase, both the active and maximum tillering stage (early flowering primordia of rice paddy), had a dominant effect on the total CH_4 emissions during the rice growing season. In corroboration, Habib et al. (2023) reported that CH_4 emission rates rose with the development and growth of rice plants up to flowering. It happens due to several factors, such as the good development of aerenchyma tissue and the release of more root exudates. Hence, during this phase, there is an increase in the number of tillers that play a role in the translocation of CH_4 release from the soil to the atmosphere.

Potentially increased N_2O emissions are influenced by increasing N fertilizer application in agricultural cultivation. The N fertilizer will enhance nitrification and denitrification activities and increase N_2O emissions in the soil (Kim et al., 2021). Some of the N_2O emissions in the soil can be reduced to N_2 by microbes that have N_2O reductase enzymes (encoded by the *nosZ* gene) in denitrifying bacteria (Jonassen et al., 2021). The result of the denitrification process, N_2, will remain partially trapped in the soil and will not be released into the atmosphere until the soil dries out, as in flooded paddy ecosystems. The N_2O emissions, a by-product of the denitrification mechanisms, are released through paddy plants and soil.

Based on experimental block design, the daily fluxes at 23 and 58 DAT with differences in the elevation of paddy fields affected CH_4 and N_2O emissions. Water is an essential factor in the availability of oxygen in the soil, and it will affect the methane oxidation rate and denitrification processes. The difference in the water table in the paddy fields caused the variance in water level, oxic, and anoxic conditions among the experimental blocks. The difference in land surface elevation was 20-25 cm between blocks. Block three has the lowest land surface elevation and is longer than the flooding time of other blocks. Water availability in the soil affects the methane oxidation rate by influencing the diffusiveness of methane and oxygen, and the activity of methanotrophic bacteria (Guerrero-Cruz et al., 2021). The methane oxidation process uses oxygen in aerobic conditions to activate the methane monooxygenase enzyme. Moreover, oxygen availability in the soil is crucial for the complete nitrification and denitrification process. The production and reduction of N_2O depend on the soil's spatial extent of anoxic conditions (Rohe et al., 2021).

The differences in the soil leveling affected the length of flooding at each block. The conditions increased CH_4 emissions into the atmosphere. It aligns with Wihardjaka et al. (2023), who reported that the tillage and flooding treatment influenced the CH_4 emission from the paddy, particularly the maximum tillage treatment, which resulted in higher CH_4 emissions. However, it did not significantly affect the number of tillers and plant height. The results showed that the number of tillers and plant height were not significantly different in each of the critical stages of paddy (Table 6). The number of tillers and plant height will affect the root biomass of rice plants. Yao et al. (2024) reported that root biomass indirectly affects CH_4 emissions by promoting Fe plaque formation, which accelerates CH_4 emissions from paddy soils. Root diameter, malic acid, citric acid, and succinic acid contents also significantly influenced increasing CH_4 emission fluxes (Qi et al., 2024). Root exudates also influenced microbial activity for GHG production and consumption (Chari et al., 2024).

Table 6. Number of tillers and plant height at three critical growth stages of paddy. SI5: *Amorphomonas oryzae*, OF4: *Ciceribacter* sp., TH6: *Rhodanobacter rhizosphaerae*, BD4: *Bordetella petrii*.

Treatments	Number of tillers			Plant height (cm)		
	23 DAT	58 DAT	80 DAT	23 DAT	58 DAT	80 DAT
Bacterial consortium (R)						
R0 (without application)	11.17 ± 2.45	12.40 ± 1.68	10.85 ± 1.39	39.67 ± 2.44	82.53 ± 3.92	100.27 ± 1.33
R1 (SI5, OF4, BD4)	11.67 ± 2.61	11.25 ± 1.56	10.60 ± 1.58	41.00 ± 2.33	81.84 ± 2.96	97.48 ± 2.29
R2 (SI5, TH6, OF4)	10.10 ± 2.05	11.56 ± 1.76	10.46 ± 1.34	38.39 ± 1.33	78.29 ± 3.07	96.17 ± 2.64
R3 (SI5, BD4, *Priestia aryabhattai*)	11.71 ± 1.92	11.75 ± 2.14	11.10 ± 1.96	40.17 ± 3.95	80.35 ± 7.36	97.04 ± 3.61
Organic matter (O)						
O0 (without application)	10.90 ± 2.23	12.07 ± 1.58	10.61 ± 1.42	38.97 ± 2.25	79.06 ± 3.77	97.40 ± 3.17
O1 (2 t ha^{-1})	11.33 ± 2.34	11.41 ± 1.88	10.40 ± 1.64	40.64 ± 2.91	82.45 ± 5.02	98.08 ± 2.67
CV, %	6.72	5.79	2.96	20.21	14.75	14.32

Effect of organic matter and bacterial consortium on soil fertility improvement

The results showed that all the treatments did not significantly affect pH, C-organic, total N, N-NO_3^-, N-NH_4^+, available P, and K in general post-harvesting soil samples. Several factors that might be affected by this study were the application methods, dosages, timing application, and the short-term effect on the interaction between the bacteria consortium and the organic matter in the soil (Ríos-Ruiz et al., 2020). Considering this study was conducted in the first period. Nevertheless, compared to the initial soil and control treatment, the treatments could increase pH, C-organic, and total N. It was predicted due to the application of synthetic fertilizer and continuous flooding of irrigation systems. Flooding irrigation promotes anoxic conditions that trigger various bacteria populations. It also was depicted in Figure 4 that the bacteria not only inhabited the bacteria consortium and organic matter application but also on the without application treatment.

Flooded conditions enhance the number of N_2-fixing bacteria that convert atmospheric N_2 into available N for plants (Guo et al., 2023). In this specific condition, anaerobic bacteria convert NO_3^- to N_2O and N_2 gases by denitrification. Nitrate levels are also reduced in this process. However, it can increase other N forms, such as NH_4^+ (Wang et al., 2021a). It also was revealed in Table 7 that N-NH_4^+ increased compared to the initial soil. Decreasing N-NO_3^- in this study was depicted on R0 without applying bacterial consortium. The conditions also increase the availability of P by promoting the dissolution of colloidal Fe-bound P. This process involves the conversion of Fe^{3+} to Fe^{2+}, potentially releasing P into more available forms (Maranguit et al., 2017). This modification would decrease the compound of insoluble Fe-phosphate complexes, such as $FePO_4$, and stimulate the available P for plants.

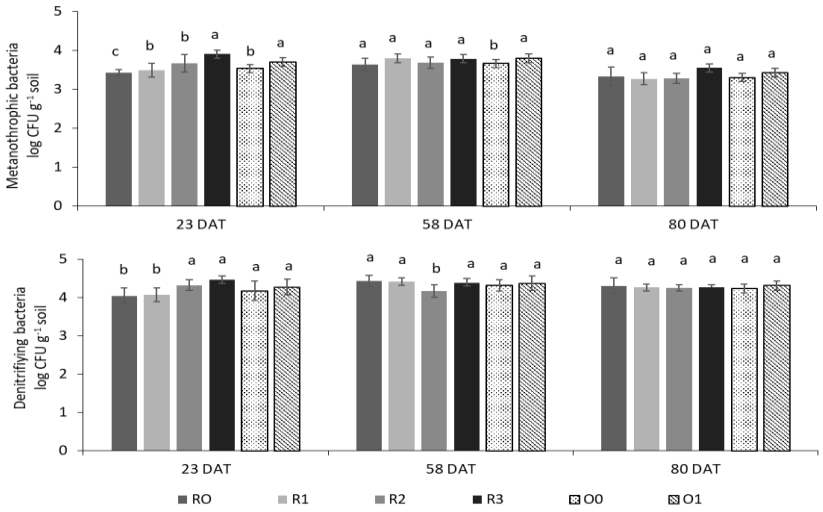

Figure 4. Total population of methanotrophic and denitrifying bacteria.

Table 7. pH and soil nutrients after harvesting. SI5: *Amorphomonas oryzae*, OF4: *Ciceribacter* sp., TH6: *Rhodanobacter rhizosphaerae*, BD4: *Bordetella petrii*.

Treatments	pH	Organic C	Total N	Available P	Available K	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$
		%		mg kg^{-1}			
Bacterial consortium (R)							
R0 (without application)	5.71 ± 0.60	0.67 ± 0.10	0.28 ± 0.07	117.74 ± 33.04	37.81 ± 2.52	2.50 ± 0.69	7.63 ± 0.97
R1 (SI5, OF4, BD4)	5.84 ± 0.88	0.69 ± 0.16	0.30 ± 0.06	109.75 ± 16.31	37.66 ± 2.91	2.71 ± 0.84	8.86 ± 1.03
R2 (SI5, TH6, OF4)	6.35 ± 0.65	0.69 ± 0.12	0.30 ± 0.06	119.72 ± 23.72	38.83 ± 2.92	2.13 ± 0.46	9.96 ± 1.50
R3 (SI5, BD4, *Priestia aryabhattai*)	5.54 ± 0.81	0.73 ± 0.13	0.29 ± 0.04	116.84 ± 23.99	37.73 ± 4.71	2.37 ± 0.31	9.75 ± 1.25
Organic matter (O)							
O0 (without application)	5.70 ± 0.77	0.72 ± 0.12	0.31 ± 0.06	106.87 ± 20.03	39.13 ± 3.36	2.45 ± 0.49	8.87 ± 1.57
O1 (2 t ha^{-1})	6.03 ± 0.75	0.67 ± 0.12	0.29 ± 0.05	125.15 ± 24.12	36.89 ± 2.23	2.41 ± 0.73	9.23 ± 1.39
CV, %	12.97	17.46	18.73	20.35	7.93	25.02	16.19

Waterlogged conditions also reduce soil acidity (Ding et al., 2019). In this study, the soil pH trend increased compared to the initial soil. The highest soil pH value appeared on bacterial consortium R2 and applied organic matter 2 t ha^{-1} (O1). Besides an increase in soil pH, there was also an increase in available P content at the treatments. It is indicated that applying organic matter and bacterial consortia was well adapted to promote soil nutrients in paddy fields.

The dynamic of methanotroph and denitrifying bacteria population

The oxygen content influences methanotroph and denitrifying bacteria activities in the soil as a growth-limiting factor. The application of bacterial consortiums significantly affected the total population of methanotrophic bacteria at 23 DAT (Figure 4). The R3 application (*A. oryzae, B. petrii, P. aryabhattai*) had the highest total population of methanotrophic bacteria during the critical growth stages of paddy. It also reduced CH_4 emissions in paddy fields. It indicated the ability of the R3 bacterial consortium to reduce CH_4 emission, even though the bacterial species are not classified as groups of methanotrophs bacteria. The application of farmyard manure as organic fertilizer also increased the abundance of methanotrophic bacteria at 23 and 58 DAT (Figure 4). Kong et al. (2019) reported that organic or synthetic N, P, and K fertilizers influenced the activity and abundance of methanogenic and methanotrophic communities in paddy fields. A higher total population of methanotrophic bacteria indicated their role in methane oxidation in the soil.

The increasing denitrification activity indicated an increase in the reduction of nitrate to N_2 in the soil. However, the side effect of the resulting N_2O gas will be released into the atmosphere and cause an increase in N_2O emissions. The denitrifying bacteria could produce an N_2O reductase enzyme (Nos) to reduce N_2O emissions into the atmosphere (Jonassen et al., 2021). The results showed that bacterial consortium application (R1, R2, and R3) significantly influenced the total population of denitrifying bacteria at 23 and 58 DAT (Figure 4). On the other hand, the application of organic matter did not significantly affect the total population of denitrifying bacteria in the three critical growth stages of paddy growth.

CONCLUSIONS

The greenhouse gas (GHG) emissions reduction in rice paddy is a critical concern, especially utilizing methane-oxidizing bacteria. The application of four isolates has a positive effect in reducing CH_4 and N_2O concentration in soil incubation. *Amorphomonas oryzae* (SI5) and *Bordetella petrii* (BD4) were the best isolates at decreasing CH_4 and N_2O concentrations up to 22%, respectively, for 15 d of soil incubation. Likewise, in the observation of the critical growth stages of paddy, the bacterial consortium R3, specifically *A. oryzae, B. petrii, Priestia aryabhattai*, produced the lowest CH_4 fluxes during vegetative and early generative stages by the reduction of 17.6% and 27.1%, and the highest total population of methanotrophs. Moreover, the application of bacterial consortia R1 (SI5, *Ciceribacter* sp.), R2 (SI5, *Rhodanobacter rhizosphaerae*, *Ciceribacter* sp.), and R3 emitted the lowest N_2O fluxes at 58 and 80 DAT, which also affected the population of denitrifying bacteria in paddy fields.

However, applying these bacterial consortiums did not significantly improve soil fertility. Thus, bacterial consortia are considered an option to reduce GHG emissions from paddy fields.

Author contribution

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