Effect of Irradiated Rice Varieties on CH$_4$, Population of Bacteria and Fungi in tidal Swamp Soil in Kalimantan

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Abstract. Recently, paddy fields have encountered extreme environmental conditions like flood and storm which caused decreased grain production. To overcome this problem, National Atomic Energy Agency (BATAN) of Republic of Indonesia has developed hybrid rice varieties like Atomita, Sulutan Unsrat, Woyla, etc. However, there are few reports on population of bacteria, fungi, and the methane (CH$_4$) emissions as affected by these rice varieties in tidal swamp area of Kalimantan. A field experiment has been carried in tidal swamp of South Kalimantan aiming at elucidating the effect of irradiated rice varieties on CH$_4$, population of bacteria and fungi. Sixteen plots, each sizing 3 m x 4 m, were demarcated by plastic tape. At the same time with land preparation, rice varieties of Atomita (irradiated), Sulutan Unsrat (irradiated), Woyla (irradiated) and Karang Dukuh (local variety) were nursed in nearby upland until one month old. Three of the plots were cultivated to Atomita rice variety, three of the plots were cultivated to Sulutan Unsrat (irradiated), Woyla (irradiated) and Karang Dukuh (local variety) were nursed in nearby upland until one month old. Three of the plots were cultivated to Atomita rice variety, three of the plots were cultivated to Sulutan Unsrat rice variety, while the other three were cultivated to Woyla rice variety. The rest of the plots were cultivated to Karang Dukuh and considered as control treatment. Air samples were collected by closed chambers in biweekly bases until harvest of rice. Air samples were then transferred to laboratory and used for the determination of CH$_4$ concentration. Soil samples were taken at 15, 45, 75 and 105 days after transplanting and used for determinations of bacterial and fungal numbers using plate count method. The results showed that irradiated rice varieties suppressed CH$_4$ emissions from tidal swamp paddy field. More suppression was given by Sulutan Unsrat, while Atomita and Woyla gave similar affects. There were no differences in population of bacteria in the four rice variety except at 15 days after transplanting when Atomita gave highest number. Similarly, there was no difference in population of fungi in the four rice variety except at 45 days after rice transplanting when Sulutan Unsrat gave highest number. These suggest that irradiated rice varieties suppressed CH$_4$ emissions without negative impact on the bacterial and fungal population in tidal swamp soil of Kalimantan.

1. Introduction
The human population of Indonesia in the last five years has increased at 1.4% annually, which had put Indonesia as the forth populated country in the world with population more than 267 million heads [1]. In line with the high population, coupled with changing eating habit, the rice demand has raised at 2.8 % annually [2]. During 2012 to 2014, the rice harvest increased from 69.1 million ton to 70.9 million ton or 2.6% annually [1]. These data indicated gaps between rice supplies and demand in Indonesia.
To improve the taste, flavor, and function of rice, Indonesian government promotes the use of hybrid rice variety [3]. High tolerance to pest and diseases, drought, and water logging are other required characteristics of hybrid rice. Rice with low sugar content which is good for consumers with diabetic problem is among the useful rice variety improvement [4]. To improve the rice grain quality, National Atomic Energy Agency of Republic of Indonesia (known as BATAN) has employing gamma radiation at 20 Gy. By using the gamma radiation, BATAN has produced 22 hybrid rice varieties which are considered as highly productive irradiated rice varieties [5].

Rice with high tolerance to pest and diseases, drought, and water logging are specially needed upon the present climate change. The climate change enhances the storm which can attack rice plant and causes grain un-filled. The rice plant should also release minimum amount of methane (CH$_4$), a gas which is precursor for global warming and subsequent climate change. Additionally, the use of radiation in producing grain should not have negative impact on organisms like soil bacteria and/or fungi.

Atomita, Sulutan Unsrat, and Woyla rice variety are among hybrid rice produced and promoted to cultivate in irrigated area by BATAN. These rice varieties are strong against wind and relatively resistant to blast diseases. However, these rice varieties have not been cultivated in swamp of Kalimantan where the local rice variety like Karang Dukuh is more common. Moreover, there is no study on effect of these rice varieties on CH$_4$ emissions and bacterial and fungal population. Therefore, the objective of the present research is to compare the CH$_4$ emission and bacterial and fungal population of Atomita, Woyla, and Sulutan Unsrat with Karang Dukuh.

2. Materials & Methods

2.1. Field Preparation & Rice Cultivation

Experiment site was situated in tidal swamp of Banyiur village, Anjir Pasar sub-district, Barito Kuala regency, Indonesia (S 03°08.030’, E 114°30.913’). The soil is classified according to Indonesian Soil Research Center as Alluvial soil and has been cultivated to paddy for the last 12 years.

Sixteen plots, each sizing 3 m x 4 m, were demarcated by plastic tape following weeding to remove grass and weed from the land. At the same time with land preparation, rice varieties of Atomita (irradiated), Sulutan Unsrat (irradiated), Woyla (irradiated) and Karang Dukuh (local variety) were nursed in nearby upland until one month old. Three of the plots were cultivated to Atomita rice variety, three of the plots were cultivated to Sulutan Unsrat rice variety, while the other three were cultivated to Woyla rice variety. The rest of the plots were cultivated to Karang Dukuh and considered as control treatment. The treatments were arranged to follow Randomized Block Design with four replicates.

NPK 16:16:16 was applied at the day of transplanting at the rate of 312.5 kg ha$^{-1}$ (equivalent to 50 kg of N, P, and K, respectively). Top dressings of Urea were carried out one month after transplanting and maximum vegetative growths (8 weeks after transplanting for hybrid rice or 12 week after transplanting for Karang Dukuh) at the rate of 56 kg ha$^{-1}$ was applied, respectively. Wedding was done manually at maximum plant growth after gas sampling.

2.2. Soil & Gas Samplings

Air samples were collected by closed chamber method [6] in biweekly bases until harvest of rice. The chamber was made from PVC pipe with diameter of 8 inches and capable of covering area of 0.05 m$^2$ with one plant hill. The chamber has a removable cover equipped with a tube on its center. The high of chambers are 50 cm or 100 cm, depending on the high of the plant to cover. Gas samples were taken by sucking the air following the withdrawal of two mL air assumed stay in the tube. The first gas sample was taken two minutes after the closure of the chamber. The second and third gas samples were taken 12 and 22 minutes after the chamber closure, respectively. Twelve mL air samples were taken from each plot at each sampling time. Air samples were taken at 15, 45, 75 and 105 days after transplanting.
Following the air sampling, soil samplings were then taken from 0-15 cm soil depth using a knife. About one kg fresh soil was taken from each plots and placed in a polyethylene plastic. Trap air was released manually from the plastic bag to keep the soil at field conditions. The soil samples were brought to the laboratory and stored in refrigerator until the time of use. The soil samples were used for determinations of bacterial and fungal numbers using plate count method as described by [6].

2.3. Measurement

Air samples were then transferred to laboratory and used for the determination of CH$_4$ concentration. One mL of gas sample was transferred to glass syringe and injected to gas chromatography equipped with flame ionizing detector. The working conditions of the gas chromatography were as described by [7]. CH$_4$ concentrations were calibrated with gas standard series with concentrations ranging from 1.7 to 50 ppm.

Nutrient agar and potato dextrose agar (PDA) were prepared for cultivations of bacteria and fungi, respectively. Nutrient agar was made by dissolving 3 g of beef extract, 5 g of peptone, and 2.5 g of glucose in an one L Erlenmeyer flask containing 0.5 L distilled water. Concurrently, 20 g of agar was dissolved in another Erlenmeyer flask containing 0.5 L distilled water. The dissolved agar was then poured into the other flask to make up to one L volume. The PDA media was made by tearing and chopping 200 g of potato. The chopped potato was then boiled with one L deionized water until soft. The water containing potato extract was transferred to one L beaker glass. 0.1 g each of KH$_2$PO$_4$ and K$_2$HPO$_4$ were then added. The volume of the mixture was finally adjusted to one L. Fifteen mL of Nutrient agar or PDA was poured into Petri dishes and was allowed to solidify.

A series of dilution tube was prepared by diluting NaCl to reach concentration of 0.085%. Ninety mL of the dilution solution was placed into 250 mL BOD bottle, along with ten g of fresh soil. BOD bottle containing soil was shaken gently by hand for 2 x 10 minutes. Nine mL of the dilution solution was transferred to five reaction tubes for each soil sample. One mL of soil extract from BOD bottle was transferred to reaction tubes and was considered as $10^{-2}$ dilution factor. Following the shaking of the reaction tube on a vortex mixer, 1 mL of soil solution was then transferred into the following tube and is considered as $10^{-3}$ dilution factor. The dilution was stop when the dilution factor reached as $10^{-6}$ dilution factor.

One mL of soil solutions from $10^{-4}$ - $10^{-6}$ dilution factors were spread above the NA and PDA media in Petri dish. The dishes were kept in dark room. Bacterial and fungal colonies formed were observed and counted at every two days. The observations was stopped when the count become constant.

2.4. Calculation

Methane emission was calculated based on formula [8]:

$$F = k \cdot h \cdot \frac{\delta C}{\delta t} \left(\frac{273}{T}\right)$$

where: $k =$ constant for conversion from volume to weight (0.536); $h =$ chamber height above water level (meter), $\delta C/\delta t =$ change in concentration (ppmv) per unit time (hour), and $T =$ air temperature inside the chamber ($^\circ$K). Population of bacteria and fungi were calculated based on root weight of counts from $10^{-4}$ - $10^{-6}$ dilution factors.

Effect of treatment on CH$_4$ emissions, bacterial population, and fungal population was assessed by Analysis of Variance. The analysis was preceded by Least Significant Difference (LSD) to assess the different between treatments. Statistical analysis was carried out with Minitab statistical package [9]. All statistical considerations were based on 95% significant level.
3. Results & Discussion

The CH$_4$ emission from all rice varieties were varied and exhibited seasonal changes (Figure 1).

![Figure 1: Methane emission from paddy field as affected by rice variety.](image)

Figure 1: Methane emission from paddy field as affected by rice variety.

The CH$_4$ emissions fluctuated up to 75 days after transplanting, and remained relatively constant afterward. The CH$_4$ emission from Karang Dukuh was the highest, while CH$_4$ emissions from Sulutan Unsrat were below zero. The CH$_4$ emissions from Woyla and Atomita remained near zero. These suggest that the hybrid rice release less CH$_4$ to the atmosphere, hence less harmful to the environment.

Methane is formed in root zone of rice, transported through steam, and finally released through stomata [11, 12]. The differences in CH$_4$ emissions from different rice varieties were probably due to the difference in stem structure and stomata’s activity. Karang Dukuh with longer steam and probably more open stomata release more CH$_4$. Formations of CH$_4$ in root zones of the tested rice variety were unlikely the reason for the different CH$_4$ emissions since the bacterial and fungal population remained similar (will be explained later).

![Figure 2: Population of bacteria as affected by rice variety. Means followed by different letters indicate statistical different according to LSD test at 95% different level.](image)

Figure 2: Population of bacteria as affected by rice variety. Means followed by different letters indicate statistical different according to LSD test at 95% different level.
Figure 2 showed the population of bacteria, while figure 3 showed the population of fungi as affected by rice variety.

Figure 3: Population of fungi as affected by rice variety. Means followed by different letters indicate statistical different according to LSD test at 95% different level.

Figure 2 showed that there were no differences in population of bacteria in the four rice variety except at 15 days after transplanting when Atomica gave highest number. Similarly, figure 3 showed that there were no differences in population of fungi in the four rice variety except at 45 days after rice transplanting when Sulutan Unsrat gave highest number.

Methane was formed in anaerobic zone of wetland plant by archae, nematode, and/or fungi [12]. With the help of F420 co-enzyme, substrates are converted to CH$_4$ reduced soil conditions [13, 14]. Substrates include carbon dioxide, acetic acid, formic acid, and formaldehyde [10]. The rates of CH$_4$ formation in current study was considered to be the same since the population of bacteria and fungi remained the same (Figures 2 and 3).

4. Conclusions
It could be concluded that irradiated rice varieties suppressed CH$_4$ emissions without negative impact on the bacterial and fungal population in tidal swamp soil of Kalimantan.

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