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Analysis of Population and Growth Rate *Metanotrof* Bacteria as Reducers Methane Gases Emission in Rice Field

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Abstract: ***The life cycle of rice plant has three phases of growth; they are the vegetative, reproductive and maturation phase. They are greatly affect the life of dynamics metanotrof bacterial as reducer methane emissions in the rice field, both of population and on the rate of growth. The aim of this study was to analyze the population and growth rate of methanotrof isolates which has been isolated in previous studies. Isolates were taken at all the life cycle of rice plant. Population of analysis was conducted by standard plate count method and growth rate was analyzed by logarithmic calculation. The results showed that each isolate varied in population and growth rate. The highest population was obtained in the isolates GowaMethanotrof Reproductive (GMR 8) about 7.06 x 10 11cfu / ml on 3 days of incubation and the lowest populationwas obtained in the GowaMethanotrof Maturation (GMP 5) about 0.27 x 10 11 cfu / ml on 7 days of incubation. Some isolate were demonstrated in long growth rate about 5 days of incubation and another are 3 days.***

Keywords: population, metanotrof, methane

1. Introduction

The life cycle of Rice has three phases of growth, they are. the vegetative, reproductive and maturation phase. The presence of *methanotrof* bacteria in rhizosphere area are needed to reduce the methane produced by *methanogenic* bacteria before released into the atmosphere, through the vessel *aerenchim* of rice, the diffusion and ebullition process.

Methane gases (CH4) is one of the greenhouse gases (GHG), thatcan cause increase of global temperature.Emissions of methane gases was formed in anaerobic conditions in wetlands, including rice field and it was determined by the activity of two different bacteria, they are*methanogenic* bacteria as organisms of methane producers and *metanotrof*bacteria as organism of methane sourcers.

The life cycle of Rice has three phases of growth, they are.the vegetative, reproductive and maturation phase. The presence of *methanotrof* bacteria in rhizosphere area are needed to reduce the methane produced by*methanogenic* bacteria before released into the atmosphere, through the vessel*aerenchim* of rice, the diffusion and ebullition process. However, each phase of the rate growth in rice plant affects to population dynamic and the rate growth of *mehanotrofic* bacteria.Base on these reasons,itis necessary to study about analysis population and growth rate *methanotrof* bacteria to support reduction of efforts methane emissions through mitigation technology in paddy fields.

2. Material and Method

2.1. Using of Isolate

Isolates was screened results of previous studies i.e. 11 isolates that there are same characteristic of physiological for*metanotrof* bacteria. The isolates were obtained from the rhizosphere area in rice field at Gowa, South Sulawesi, on three growth phases. There namely Gowa *Methanotrof* Vegetative (GMV) 1,3,4 and9 for isolates were obtained from vegetative phase, Gowamethanotr of reproductive (GMR) 1,4,5 and 8for isolates were obtained from reproductive phase and Gowa *Metanotrof* maturation (GMR) 2,4,5for isolates were obtained from maturation phase. The collection of Isolates have been analyzed.

2.2. Purification of Isolates

Isolates of bacteria were purified by streaking the colonies on Nitrate mineral salt solid medium[1]. Medium containing 1,0 g Mg $SO_4.7H_2O$; 1,0 g KNO_3 ; 0,717 g Na_2 HPO4.12 H_2O; 0,272 g KH2PO_4; 0,2 $CaCL_2$.$6H_2O$; 4,0 g NH_4CL; 20 g/L Bacto , 0,5 ml *trace Element Solution* with composition: 0,5 gr Na_2EDTA; 0,2 g $FeSO_4$ .7H2O; 0,03 g H_3BO_3 ; 0,02 g $CoCL_2$ .6H2O; 0,01 $ZnSO_4$.$7H_2O$; 3,0 mg Mn CL_2 .$4H_2O$; 3,0 mg Na_2 MoO_4 ; 2,0 mg $NiCl_2$.$6H_2O$; 1,0 mg $CaCl_2$.$2H_2O$, then incubated for 7 days to use in the process of inoculation.

2.3. Inoculation and Dilution

Each isolate was inoculated in liquid culture that incubated on 100 rpm shaker in room temperature for 24 hours. The isolates were dilution for 10^{-1}-10^{-8} on water sterile, then it

was grown on Nutrient Broth solid media with three replications, then incubated for 7 days in room temperature and do calculations for number of colonies on first, thirty, fifth, and seventh day by a colony counter.

2.4. Populations of Bacterial

Calculation of the number of bacteria colonies was conducted by Standard Plate Count method[2]. Total population of bacteria was obtained from multiplication of the number of colonies and the dilution rate and converted into cfu / ml units. Bacteria which grow was calculated with the following formula:

$TP = \sum koloni \times DT \times 10 \frac{cfu}{ml}$ [3].

Description:
TP= Total of Population
DT= Dilution Rate

2.5 The growth of curve

The growth of curve was made by converting total population into a logarithmic value, then graphed the number of population log cfu / ml as the y-axis and time of incubation as the x-axis. Slope is the growth rate of isolates.

3. Result and Discussion

3.1. Total population For GMVIsolates

Result of calculation(Table 1) showed that GMV 9 has the highest population(1.84 x 10^{11}cfu / ml)and GMV 4 has the lowest number of populations (1,42x 10^{11}cfu / ml) on the first day of incubation. GMV 9 has the highest number of populations (2.72 x 10^{11}cfu / ml) and GMV 1 has the lowest number of populations (1.28 x 10^{11}cfu / ml) on the third day. GMV 1 increased to 1.70 x 10^{11}cfu/ml and GMV 4 increased to 2.54 x 10^{11}cfu / ml, GMV 3 decreased to 1.76 x 10^{11}cfu / ml and GMV 9 also decreased to 1.84 x 1011 cfu / ml on the fifth day . All isolates of bacteria has decreased population on the seventh day in incubation period

Table 1: The number of populatin for GMV

Isolate Kode	Population for GMV on concentration of 10^{11} (cfu / ml)/ day			
	1	3	5	6
GMV 1	1.68	1.28	1.70	1.51
GMV 3	1.44	2.37	1.76	1.70
GMV 4	1.42	1.61	2.54	2.02
GMV 9	1.84	2.72	1.84	1.80

Dynamic behavior of microorganism cells has four phases of growthgenerally, they are (1) the lag phase (adaptation) is the adjustment phase to the environment with a slow process, (2) The rapid growth phase (exponential) is the increaseof volume and cell size that can be expressed by exponential function, (3) The static phase(stationary) is decline phase of growth where the number of cells were regenerated almost similar to the number of death, (4) the death phase (decline) is the population decline which date of same cells larger that life.

Based on observations for growth of curve (Figure 1) showed that GMV 4 and 1 had needed same for time to exponential and stationary phase about five day of incubation, this is very beneficial for the paddy crop, because the bacteria produce metabolites on the phase for long time. Beside that the bacteria also took a lot of energy for this phase, so that the absorption of methane as a carbon source for the bacteria *metanotrof* is very high. The exponential and stationary phase are the critical point on the cultivation process, where microorganism produced a lot of metabolite. After the adaptation phase is completed, the microorganism entered to exponential phase where the rate growth is maximum to constant, so that the maximum amount of biomass contained in this phase. GMV 3 and GMV 9 have exponential and stationary phases for three days, but nevertheless has the highest number of population on that day. The number of Population for all GMV isolate still on the early concentration 1 x 10^{8} cfu / ml.

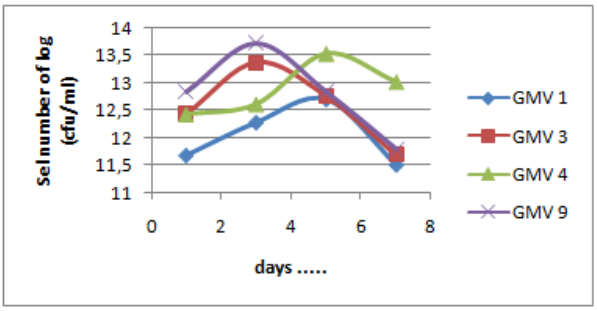

Figure 1: Growth of Curvefor GMV Isolate

Rice on the vegetative phase was characterized by increase number of tillers, the condition is very stimulating for *metanotrof* bacteria to growth because the increase number of tillers were released exudates by plant roots even greater. [4]in the process of growth, the root of plant produce exudates as a result the entered fotosinteties about 5-23% (amino acid, vitamin, sugar, tannin. The exudates affect to growth and activity of microorganisms in the rhizosphere, including the *methanogenic* bacteria produced methane, it will also stimulate *methanotrof* bacteria as reducer methane, so it will be a balance between the population of *methanogenic* and *metanotrof* bacteria. If in these conditions can be improved *metanotrof* population by introduction the long phase (GMV1 and GMV 4) and the highest population (GMV 9), so the absorption of methane will increase and it will reduce the emission of methane into the atmosphere. [5]the emission of methane by aerenchyma in paddy field can up to 90%.

3.2. Total population For GMR Isolate

Results of calculation (Table 2) showed that GMR 4has the highest population (1.66 x 10^{11}cfu / ml) and GMR 8 has the lowest population (1.16 x 10^{11}cfu / ml) on the first day of the incubation period . On the thirdday all isolates increased population specially for GMR 8 increased very significant to 7.06 x 10^{11}cfu / ml. The fifth day of incubation GMR 6 constant, GMR 1 increased to 2.90 x 10^{11}cfu / m, while GMR 4 and 8 declined. All of isolate decreased population on the seventh day except GMR 8 constant on 1.10 x 10^{11}cfu / ml.

Table 2: The number of population for GMR

Isolate Kode	Population for GMR on consentration of 10^{11} (cfu/ml) / day			
	1	3	5	7
GMR 1	1.45	2.32	2.90	1.32
GMR 4	1.66	2.52	1.45	1.33
GMR 6	1.48	2.73	2.73	0.97
GMR 8	1.16	7.06	1.10	1.10

Based on observations for growth of curve (Figure 2) showed that GMR 6 and GMR 1 had needed same for time to exponential and stationary phase about five days of incubation after entered to the death phase. Unlike for GMR 4 and GMV 8 have exponential and stationary phase only three days of incubation after entered to death phase.

Rice on the reproductive phase was characterized by reduce number of tillers and panicle emergence, in this phase the population of growth for *methanogen* bacteria as methane-producer were decreased, it was caused by low exudates from the root plant. Besides that all the reproductive phase, flooding in paddy fields began intermittently so the population *methanogenic*as anaerobic bacteria was begun to decrease, contrary for *metanotrof* bacteria would increase because of aerobic, [6] *methanotrof* bacteria were obligate aerobic. The availability of oxygen was very important because without oxygen the *methanotrof* bacteria unabled to replace methane into carbon dioxide, water, cells biomass and get energy to growth [7].

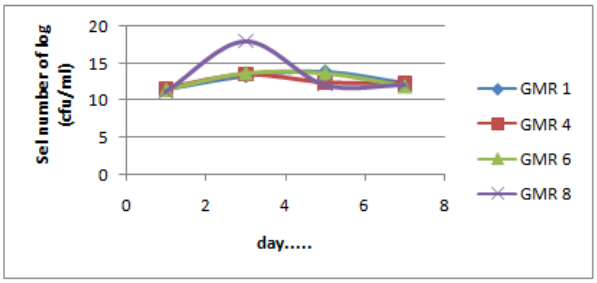

Figure 2: Growth of Curve forGMR Isolate

Isolates were found in the reproductive phase on paddy field indicated that the isolates were able to adapt on conditions the lack of nutrition source from exudates, but still obtain nutrients from other sources organic and anorganic fertilizers. The presence of GMR 6 and GMR1(exponential and stationery phase in 5 days of incubation) and GMR 8(the highest population on the 3rd day) very favorable for paddy field because in this phase the bacteria releasedsome metabolites for the plants. [8]inthe exponential phase until stationary phase bacterial isolates can release a variety of secondary metabolites for stimulating plant growth, mobilization nutrients by decomposition, and controlling the root pathogens.[9]*methanotrof*bacteria were capable to fixing nitrogen . The ability of bacteria to fix nitrogen very beneficial for plant growth. Nitrogen is one of the macro nutrients for plant to formation protein.[10]the natural biological system, the ability of prokaryotic organisms to fix nitrogen is needed for plant growth and maintain the food chain.

3.3. Total population For GMP Isolate

Result of calculation (table 3) showed that GMP 4 has the highest population (2.97 x 1011 cfu / ml) and GMP 5 has the lowest population (0.22 x 1011 cfu / ml) on the first day of incubation period. Population for GMP 2 and GMP 4 have constanton the 3rd day of the incubation period, while GMP 5 increased to 2.50 x 1011 cfu / ml. Population all isolate decreased on the 5th and 7th day of incubation period.

Table 3: The number of population for GMPIsolat

Isolate Kode	Population for GMP on Consentration of 10^{11} (cfu/ml) day.....			
	1	3	5	7
GMP 2	2.76	2.76	0.86	0.45
GMP 4	2.97	2.97	1.78	1.12
GMP 5	0.22	2.50	2.50	0.27

Base on observation for growth of curve (figure 3) showed that GMV 2 and GMV 4 hadneeded same for time to exponential and stationary phase about three days of incubation , then began to enter the death phase after 4th day. GMV 5 had needed more time for exponential and stationary phase about five days of incubation period.

Rice on the maturation phase was characterized by the formation of grain which began full fledged. In this condition has not flooded for rice field so reach maximum aerobic conditions in the soil surface layer. Thereby isolate the bacteria found in the maturation phase can use the methane to be released into the atmosphere, through the process diffusion andebullition in surface sediments and soil. So that emission of methane into the atmosphere will not happen[11] because of the oxidation of methane may occur in the microenvironment that are aerobic in the root zone and the toxic of soil on the surface layer, with the presence of *methanotrof* bacteria has able to catalyze the transformation of various pollutants.

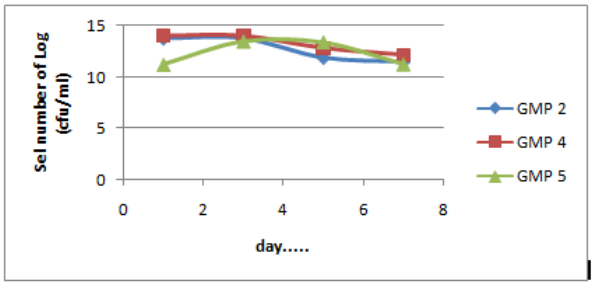

Figure 3: Growth of Curve for GMP Isolate

The presence of GMP isolates can indicate that the bacteria are able to adapt on the aerobic conditions so that it can help rice plants to get nitrogen, because [12] *methanotrof* bacteria utilize methane as a carbon source and as a electron suplayer to produce energy, this process occurs in aerobic conditions on the surface sediment. Then the *methanotrof* bacteria can also fixating nitrogen that can convert to ammonium so that can be utilized by plants[1].

Some types of bacteria such as *Azotobacte*r and *Azospirillum* also has the ability to fix nitrogen in the air that are beneficial to fertility plant using multi component

nitrogenase system[13]. Other bacteria such as *Rhizobium* and *Clostridium* also been known to play a role in the process of nitrogen fixation. *Azospirilliumlipoferum* can fix nitrogen that found in the roots of plants grass in tropical climates.[14]*the anthropic Ochrobactrum* (denitrifying bacteria) have nif genes to associated with nitrogen fixation. The combination of bacteria *metanotrof* with bacteria *Azotobacter* and *Azospirillum*also declared able to tiefix free nitrogen. The ability of bacteria to fix nitrogen very beneficial for plant growth.

Result of calculation obtained that the growth of populations for all isolates on the three phases of growth showed that the number of population still on the standards, it was found the growth activity for all isolates. The bacteria of viability is influenced by organic matter in form of soluble particles and materials used are a source of carbon and energy for the activity of microorganisms. Beside that the good of viabilities bacteria and stable is also determined by the composition of the substances used, the ability of isolate to utilize materials on the media as a source of carbon and the survival of strategy bacteria using the mechanism of efficiency the bacteria.

4. Conclusion

The growth of *methanotrof* bacteria that obtained on three phases growth rice of plants have varied on the number of population and the growth of curve. The ability of bacterial to grow began to look on the first day for all isolates, obtained the highest number of population in the reproductive phase that GMR 8 about 7.06 x 10 11 cfu / ml on 3 days of incubation, the lowest population in maturation phase that GMP 5 about 0.27 x 10 11 cfu / ml on 7 days of incubation. Exponential and stationary growth phase longest isolates demonstrated by GMV 1, GMV 4, GMR 1, GMR 6 and GMP 5, which is 5 days of incubation.

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