



Contributions of available substrates and activities of trophic microbial community to methanogenesis in vegetative and reproductive rice rhizospheric soil

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Abstract: Potential of methane production and trophic microbial activities at rhizospheric soil during rice cv. Supanburi 1 cultivation were determined by laboratory anaerobic diluents vials. The methane production was higher from rhizospheric than non-rhizospheric soil, with the noticeable peaks during reproductive phase (RP) than vegetative phase (VP). Glucose, ethanol and acetate were the dominant available substrates found in rhizospheric soil during methane production at both phases. The predominance activities of trophic microbial consortium in methanogenesis, namely fermentative bacteria (FB), acetogenic bacteria (AGB), acetate utilizing bacteria (AB) and acetoclastic methanogens (AM) were also determined. At RP, these microbial groups were enhanced in the higher of methane production than VP. This correlates with our finding that methane production was greater at the rhizospheric soil with the noticeable peaks during RP ($1,150 \pm 60$ nmol g dw⁻¹ d⁻¹) compared with VP (510 ± 30 nmol g dw⁻¹ d⁻¹). The high number of AM showed the abundant (1.1×10^4 cell g dw⁻¹) with its high activity at RP, compared to the less activity with AM number at VP (9.8×10^2 cell g dw⁻¹). Levels of AM are low in the total microbial population, being less than 1% of AB. These evidences revealed that the microbial consortium of these two phases were different.

Key words: Acetoclastic methanogens, Methane production, Rice rhizospheric soil, Trophic microbial consortium

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Introduction

Rice fields have long been recognized as one of the significant sources of methane. Methane acts as an atmospheric greenhouse gas and approximately 25% of biogenic methane or 25-60 Tg is emitted from rice fields, annually (Prinn, 1994; IPCC, 2001). Methane emission from rice fields is expected to intensify in the future due to the increase of rice production in order to supply the food demand for rapidly growing population in Asia. More than 90% of global rice areas are located in developing countries especially in Asia (FAO, 2006; Ruddiman *et al.*, 2008). It is calculated the increase of methane emission from rice cultivation to 145 Tg yr⁻¹ by 2025 (Anastasi *et al.*, 1992), thus, it causes the concerning among policy makers for management practices for the methane mitigation development.

Methane production from irrigated rice fields is the result of complex interactions between rice plants and soil microorganisms under anoxic and reduced conditions that develop in soil. Plants roots and microorganisms secrete phosphatases which release inorganic phosphorus by hydrolysis of ester bonds between organic carbon and minerals for sustain plant and microorganisms growth (Sahu *et al.*, 2007). In general, rice rhizosphere is nourished by organic carbons which derive from root exudates and decaying

roots during the growing season (Dannenberg and Conrad, 1999; Lehmann Richter *et al.*, 1999; Lu *et al.*, 2000). Naturally organic matters in soil are plant residues and were hydrolyzed to fermentable sugars by variety of microbial enzymes (Kulkarni *et al.*, 2007; Huitron *et al.*, 2008). A microbial consortium consisting of fermentative, acetogenic and acetate-utilizing bacteria, involved in the degradation of organic matter in flooded rice fields, lead to the production of CO₂, H₂ and acetate, which produces methane by methanogenic *Archaea* finally. Approximately 67 and 33% of the produced methane originates from acetate and H₂/CO₂, respective (Neue *et al.*, 1996; Lehmann-Richter *et al.*, 1999; Yao and Conrad, 1999; Martin *et al.*, 2005). Anoxic soil and sediment are reported as the important niche of greenhouse gas produced by methanogenic *Archaea* (Conrad *et al.*, 2006). Methane so produced by the activity of the microbial community in the soil is predominantly emitted into the atmospheric through rice plants (Conrad, 1993; Butterbach Bahl *et al.*, 1997). Several works were conducted on methane emission in tropical rice fields. The emission from rice fields is approximately 20% of the national total emission of GHG in Thailand (Towprayoon *et al.*, 2000). Methane emissions during the vegetative phase of rice found significantly lower concentrates (50%) in the iron (ferrihydrite) fertilized plot compared to the non-supplemented control plot (Jackel *et al.*,

Table - 1: Analysis of microbial activities

Microbial group	Substrate	Activity determination
Fermentative bacteria (FB)	0.1% w/v of glucose	Rate of glucose utilization per gram soil sample
Acetogenic bacteria (AGB)	0.1% v/v of ethanol	Rate of ethanol degraded to acetate per gram soil sample
Acetate utilizing bacteria (AB)	0.1% v/v of acetate	Rate of acetate utilization per gram soil sample
Acetoclastic methanogens (AM)	0.1% v/v of acetate	Rate of methane production per gram soil sample

Table - 2: Initial substrates for methane production from fresh rice rhizospheric soil during vegetative phase (VP) and reproductive phase (RP) of rice cultivation

Substrates	Substrates concentration ($\mu\text{mol g dw}^{-1}$)	
	VP	RP
Glucose	$0.4^b \pm 0.02$	$3.3^a \pm 0.1$
Butyrate	ND	ND
Lactate	$0.3^b \pm 0.02$	$0.4^b \pm 0.06$
Propionate	ND	ND
Acetate	$3.4^a \pm 0.4$	$4.0^{ab} \pm 0.06$
Ethanol	$2.3^a \pm 0.3$	$2.3^a \pm 0.2$

Mean $\pm$ SD, n=3; ^{a,b,*} The different letters in the same row are significant at $p \leq 0.5$ using Duncan's multiple range test; ND – Not detectable

2005). Variations of rice cultivars, Suphanburi 1, Supanburi 60, Supanburi 90 and Chainat 1, have shown on methane emission, which is directly related to biomass yield and some sugar and organic acid constituents of root tissues (Kerdchoechuen, 2005). Kimura *et al.* (1991) suggested that rice roots in soil may significantly contribute to methane production in rice soil by supplying various organic matters to the rhizospheric soil.

However, the activity of microbial communities related to nutrients in the soil involved with methane production in tropical zone is little known. Some researchers investigated the methane production in rice soil during rice cultivation by the laboratory-incubation studies (Kimura *et al.*, 1991; Kaku *et al.*, 2000; Lu *et al.*, 2000). According to our previous report (Chawanakul *et al.*, 2002) found two noticed peaks of high methane production during the vegetative phase (VP), which is associated with the application of fertilizer, and during the reproductive phase (RP) when plant exudates are released. Since methane production is associated with microbial activity and communities in the soil and in order to foresee any methane emission mitigation options, the study of microbial consortium should be well understood for methane production which affected to methane emission. Therefore, the aim of this study was to investigate the potential of methane production, the activities of methanogens and associated microbial consortium and the available nutrients in rhizospheric soil during vegetative and reproductive phases in Thai rice fields.

Materials and Methods

Experimental rice field, cultural practice and soil descriptions: The experimental site (latitude north $5^\circ 21'$ to east $105^\circ 37'$), transplanting paddy field located in the U-thong district, Supanburi province, central region of Thailand. This region is regarded as one of the most productive areas for rice production in Thailand. Supanburi

1 is a popular rice variety for the local farmers by its non-photosensitivity, pathogenic-resistance and high yield. This experimental field ($10.7 \times 20.3 = 217.2 \text{ m}^2$) was continuously flooded, where a 5-10 cm water depth was maintained throughout the cultivation periods. The growth period is approximately 120 days. The first 1-40 days of growth is the vegetative phase, 41-80 days is the reproductive phase and 81-120 days is the ripening phase. The soil is traditionally supplemented by rice straw or plant residues before cultivation. The irrigation water is drained into the paddy field a few days before planting. After irrigation 2 weeks, transplanting was performed into the field. The water levels are kept at around 10 cm above the ground soil. Formulated fertilizer was applied twice in one crop at the initial vegetative and reproductive phases. Formulated N-P-K fertilizer of 16-20-0 was a basal fertilizer used at the first month and top dressing was carried out with $125\text{-}145 \text{ kg ha}^{-1}$ of urea at the panicle phase of rice. The characteristics of the paddy soil were pH of 5.1, sand of 20.8%, silt of 22%, clay of 57.2% and total organic carbon of 1.1% (Chawanakul *et al.*, 2002). This soil texture classified as clayey soil, close to the Ayutthaya soil type (Agricultural Statistics Division, 2002).

Soil sampling for the determination of methane production and microbial activities:

The fresh rice soil samples were obtained from the field at 5 positions, using randomized blocked design with four replicate blocks for each treatment. Sampling of rhizospheric soil (the soil surrounding the roots of plants) was done by carefully taking out the rice plants from the field by digging around growing root systems using trowel. Soil adhering around the roots was carefully removed and collected the soil as rhizospheric soil. Sampling of non-rhizospheric soil (the soil between the drill rows of rice plants) was done using core sampler. A plastic core sampler with 200 ml in volume, 10 cm height and 5 cm in inner diameter were used for soil sampling. The plow layer (0-10 cm in depth) from different sampling position in rice fields were taken and collected the soil samples at middle part of soil cores (anaerobic zone) for the methanogenesis study. The small roots or large plant residue (approximately 4-5 mm and greater) were removed from the rhizospheric and non-rhizospheric soils. The collected soils were placed in anaerobic plastic bags, which were stored in an icebox and delivered to the laboratory for analysis.

Methane production measurement: Methane production was performed during the rice cultivation period (0-120 days). Soil samples were taken every 2 weeks during 120 days of the rice cultivation area. The methane productions in rhizospheric and non-rhizospheric zones, at different periods were studied by using the serum vial technique under anaerobic condition in the laboratory.

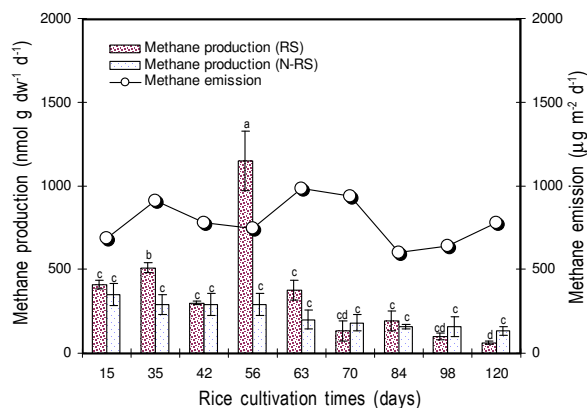


Fig. 1: Methane production in rhizospheric soil (RS) and non-rhizospheric soil (N-RS) in this study and methane emission (personal contact) during rice cultivation

The preparation of soil samples in serum vials was carried out by soil samples 25 gm, carefully mixed with 25 ml (1:1 w/v) of sterile methanogens basal medium (Zhang and Noike, 1991) into a 50 ml serum vial. The head space (40% of vial volume) was flushed with O₂-free N₂ gas before closing the sterile rubber stopper and aluminum cap. The anaerobic serum vials were incubated at 37°C for 4 weeks.

Potential of methane production was measured in the volume of biogas using a water replacement technique and multiplied with methane gas concentration. Methane concentration accumulated in the gas phase was measured by gas chromatography (Shimadzu GC-9A) equipped with thermal conductivity detector (TCD) and Porapak-N 80/100 column. Column, injection and detector temperature were set at 70, 120 and 120°C, respectively. Methane production rates were calculated by linear regression of the increase in methane with the incubation time, and expressed in nmol g dw⁻¹ d⁻¹ of soil slurry.

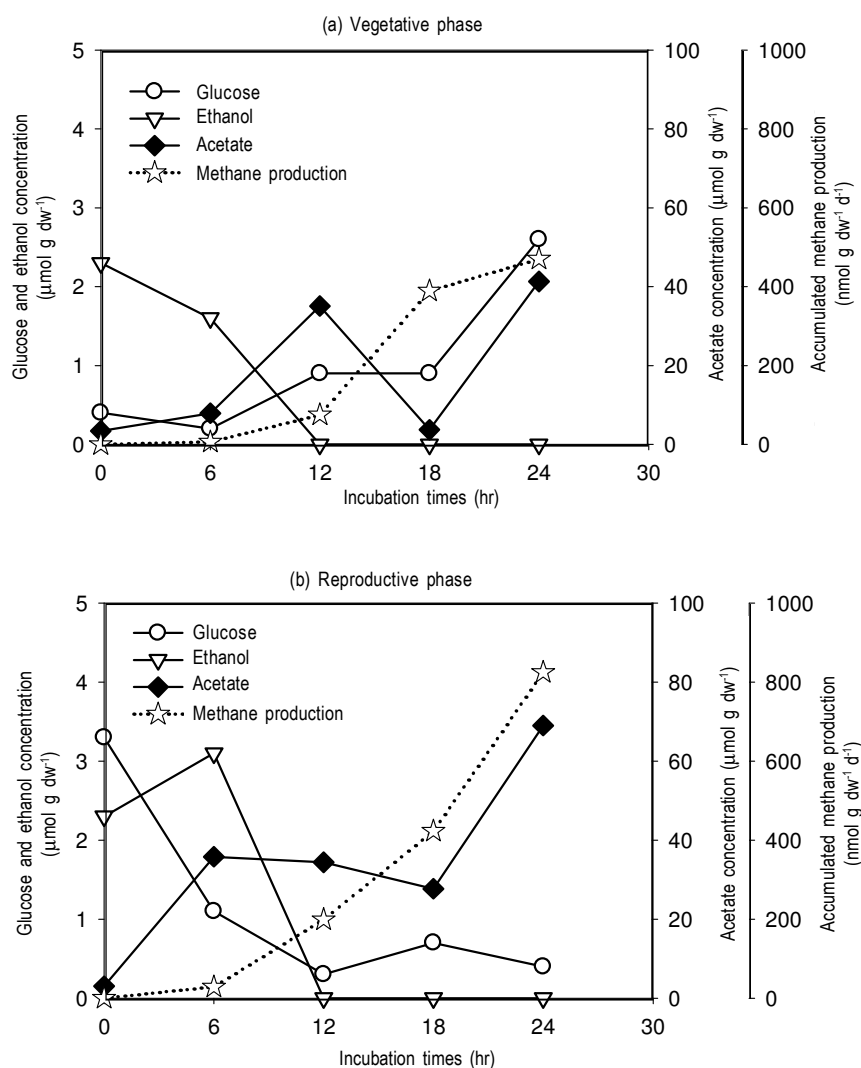


Fig. 2: Changes of available substrates for methanogens and methane production from rhizospheric soil at (a) vegetative phase and (b) reproductive phase

Determination of available substrates at the rhizospheric soil during vegetative and reproductive phases: Available substrates and methane production from rice rhizospheric soil were determined at the vegetative and reproductive phases. Soil samples 25 gm were put in a 50 ml serum vial containing 25 ml sterile basal media (Zhang and Noike, 1991) and flushed with O₂-free N₂ gas before closing the sterile rubber stopper and aluminum caps incubating at 37°C for determination of the remaining of available substrates (glucose, ethanol, lactate and volatile fatty acids) and methane production with incubation time at 0, 6, 12, 18 and 24 hr.

The potential of methane production in rhizospheric soil samples was measured in serum vials with the incubation times of 6, 12, 18 and 24 hr, respectively. Methane production rate were calculated by the increase in methane at the incubation time of 6, 12, 18 and 24 hr and expressed in nmol g dw⁻¹ d⁻¹ of soil slurry.

Population size of acetoclastic methanogens enumeration:

The number of acetoclastic methanogens (AM) of rhizospheric soil at the vegetative and reproductive phases was counted by three tube, most probable number (MPN) technique at a dilution of 10 using tube, incubated under O₂-free N₂ gas and the sterile basal medium (Zhang and Noike, 1991) added with 0.5% of sodium acetate as precursor for acetoclastic methanogens. Soil samples mixed with medium 1:1 (v/v) by 3.5 ml at each sample dilution were mixed with 3.5 ml of double strength MPN medium in 10 ml of serum vials. Then, serum vials were flushed with O₂-free N₂ gas for anaerobic condition. The vials were closed with rubber stopper and aluminum cap, incubated at 37°C for 4 weeks. After incubation, the pattern of positive tube (produced methane gas) and negative tube (no produced methane gas) is noted and a standardized MPN table is consulted to determine the most probable number of acetoclastic methanogens (causing the positive results) per unit weight of the rhizospheric soil sample.

Microbial activities determination: The activities of methanogens and associated microbial consortium at rice rhizospheric soil were investigated in the vegetative and reproductive phases. Fresh rhizospheric soil samples were collected as described in 2.2. The microbial groups in methanogenesis were separated into three groups; i.e. acidogens or fermentative bacteria, acetogens, and acetoclastic methanogens. These microbial groups are different mainly with respect to their nutritional requirements. The substrate used to test each microbial group activity was shown in Table 1.

Acetoclastic methanogenic (AM) activity: Each 10 ml serum vial containing 3.5 gm of soil sample and 3.5 ml of sterile basal medium (Zhang and Noike, 1991) spiced with 0.1% sodium acetate in order to determine the AM activity compared with the control vial without sodium acetate spicing. The headspace of serum vial was flushed with O₂-free N₂ gas before closing the sterile rubber stopper and aluminum cap, incubated at 37°C and checked during 0-24 hr, of incubation time. The methanogenic activity was calculated by linear regression of the increase in methane production from the

control with the incubation time, and expressed in $\mu\text{mol g dw}^{-1} \text{ hr}^{-1}$ of soil slurry.

Associated microbial consortium activities: The activities of associated microbial consortium namely fermentative bacteria (FB), acetogenic bacteria (AGB) and acetate utilizing bacteria (AB) in rhizospheric soil were determined. Each 10 ml serum vial containing 3.5 g of soil sample and 3.5 ml of sterile basal medium (Zhang and Noike, 1991) spiced with 0.1% glucose, ethanol and acetate in order to determine the activities of fermentative, acetogenic and acetate utilizing bacteria, respectively compared with the control vial without substrate spicing. The headspace of serum vial was flushed with O₂-free N₂ gas before closing the sterile rubber stopper and aluminum cap, incubated at 37°C and checked during 0-24 hr, of incubation time. The activities of FB, AGB and AB were calculated by linear regression of the increase of substrate utilization from the control with the incubation time as described in Table 1 and expressed in $\mu\text{mol g dw}^{-1} \text{ hr}^{-1}$ of soil slurry.

This study would like to estimate the microbial population by determining the activity of substrate utilization of each trophic microbial group (Kalyuzhnyi *et al.*, 1996). We calculated the ratios or percentage of trophic microbial groups such as FB, AGB and AB in the consortium of methane production from microbial activity based on carbon (COD) of substrate utilization. Acetoclastic methanogens (AM) is one of microbial groups in AB which consist of either AM or other acetate utilizing bacteria such as sulfate reducing bacteria (SRB).

Statistical analysis: The experiment was conducted using randomized block design with four replicate blocks for each treatment. The significance of the difference between treatments was assessed by analysis of variance and subsequently by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Methane production in rhizospheric and non-rhizospheric soils during rice cultivation: The methane productions were measured in rhizospheric soil (RS) and non-rhizospheric soil (N-RS) at various rice cultivation times as shown in Fig. 1. It was clear that methane production rate from rhizosphere is higher than non-rhizosphere in two notice phases of rice vegetation (day 35) and reproduction (day 56). It is due to rice rhizosphere is nourished by organic carbons which derive from root exudates and decaying roots during the growth phase, which provides methanogenic substrate (Dannenberg and Conrad, 1999, Lehmann-Richter *et al.*, 1999; Lu *et al.*, 2000). In addition, inorganic fertilizer was applied twice in this experimental rice field at the initial vegetative and reproductive phases. In this experiment, it was found obvious peak of methane production from rhizospheric soil at reproductive phase of day 56 ($1,150 \pm 60 \text{ nmol g dw}^{-1} \text{ d}^{-1}$) and another small peak at vegetative phase of day 35 ($510 \pm 30 \text{ nmol g dw}^{-1} \text{ d}^{-1}$). Methane emissions shows in Fig. 1 was obtained data by personal contact and measured by the static box technique from the real rice field. Fig. 1 shows the two equivalent peaks of high methane production and emission at vegetative and reproductive phases. Similar result

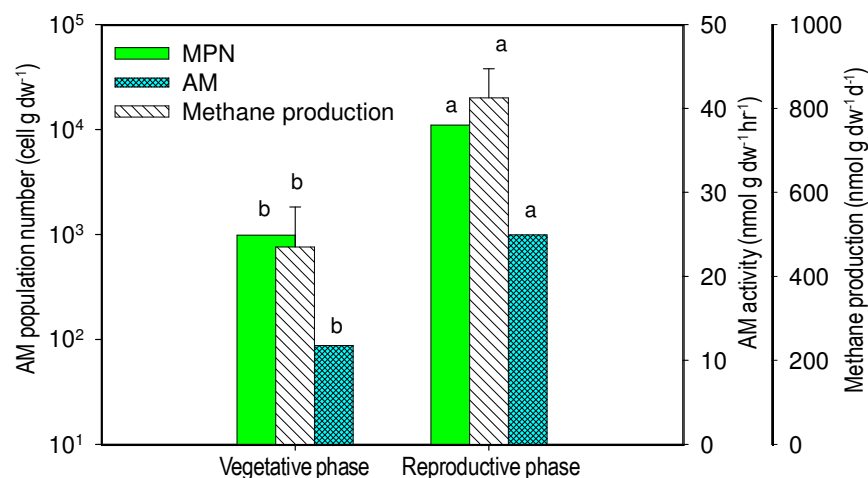


Fig. 3: Population size and activity of acetoclastic methanogens (AM) and methane production from rhizospheric soil at vegetative and reproductive phases

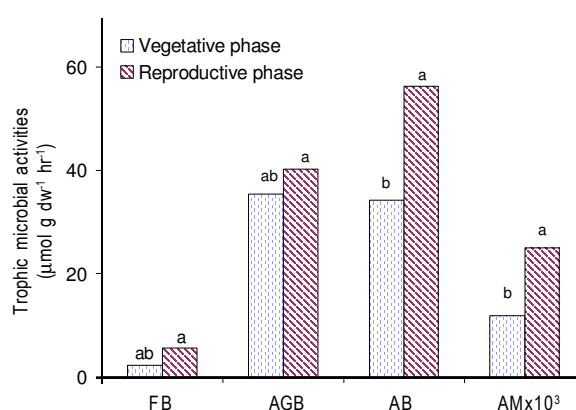


Fig. 4: Microbial activities of fermentative bacteria (FB), acetogenic bacteria (AGB), acetate utilizing bacteria (AB) and acetoclastic methanogens (AM) in rhizospheric soil at vegetative and reproductive phases

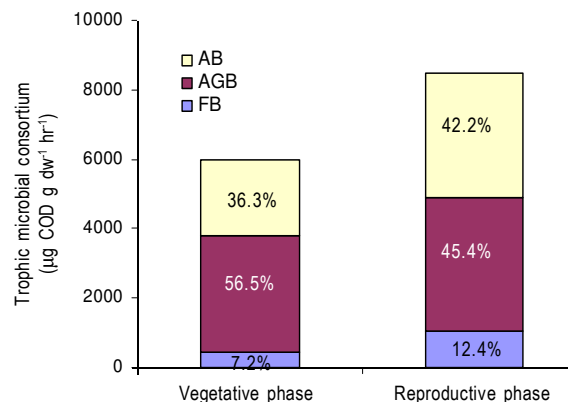


Fig. 5: Microbial consortium in methanogenesis of fermentative bacteria (FB), acetogenic bacteria (AGB) and acetate-utilizing bacteria (AB) in vegetative and reproductive rice rhizospheric soils

of methane emission was also found by Das and Baruah (2008). It was noticed that high in methane production induce in emission of methane through rice plants.

The high production of methane of rhizospheric soil at vegetative phase (day 35) and reproductive phase (day 56) may account for intensive metabolic activity, the variation of methanogens and associated microbial consortium and amount of organic materials as substrates for methane formation. Therefore microbial consortium in rhizospheric soil and their activities related to available substrates were investigated and compared between vegetative phase at day 35 (VP) and reproductive phase at day 56 (RP).

Change of substrates in methane production of vegetative and reproductive rhizospheric soils: The rice plant also provides methanogenic substrates through root exudates into rhizosphere as a major source for methanogenic and associated microbial consortium in production and emission of methane from rice soils. Kaku *et al.* (2000) indicated that several substrates such as saccharides, amino acids and organic acid were provided from

rice rhizosphere during the growing period of rice. We characterized the available substrates related to methane production as well as population size and activity of methanogens inhabiting the rhizospheric soil at vegetative phase (VP) and reproductive phase (RP) where the noticed peak of methane production was found.

Glucose, ethanol, lactate, butyrate, propionate and acetate contents of rhizospheric soil show marked variations between development phases (Table 2). The RP rhizospheric soil had higher glucose and acetate contents than VP rhizospheric soil; similar levels of ethanol and lactate were found; and contents of butyrate and propionate were undetectable in these both phases. Correspondent to Kerdchoechuen study (2005), it was also found total sugars and acetate levels in root exudates of Supanburi 1 and Supanburi 90 cultivar at RP higher than VP, while propionate and butyrate contents were not found in these both phases.

Substrates involved in methanogenesis such as glucose, ethanol, lactate and short chain volatile fatty acids (butyrate, propionate and acetate) were measured the change with time as well as methane

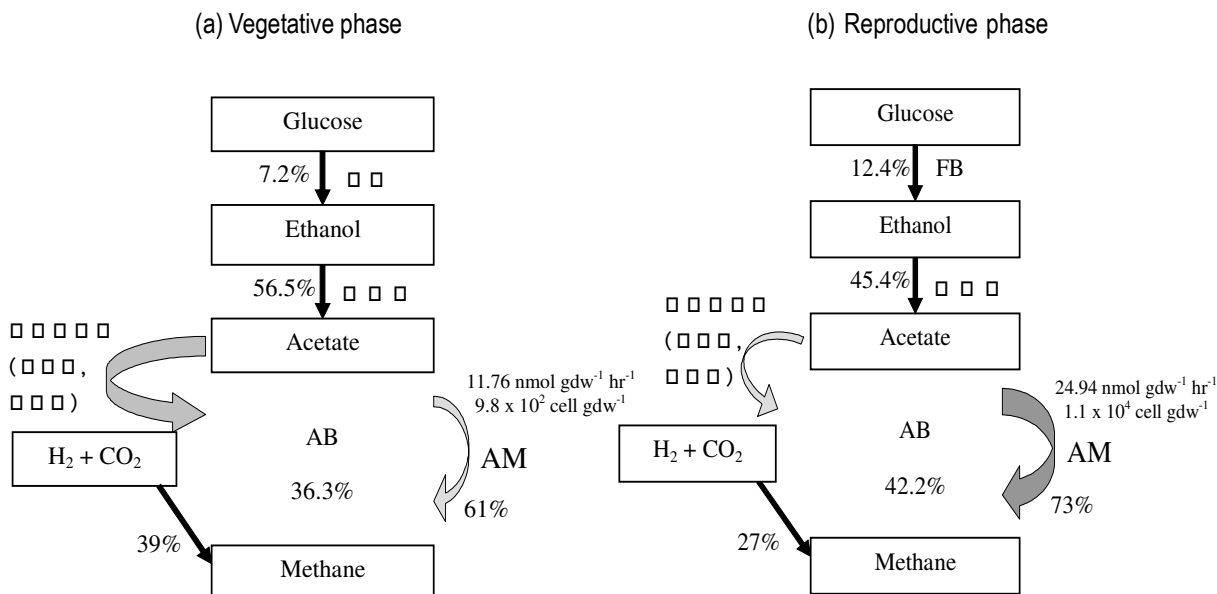


Fig. 6: Proposed methanogenesis scheme of (a) vegetative rhizospheric soil and (b) reproductive rhizospheric soil

production in rhizospheric soil at VP and RP. Fig. 2 illustrates a change of these available substrates as the result of microbial consortium in rhizospheric soil. At VP and RP, the initial concentration of lactate, acetate and ethanol were both at close proximity to each other except glucose but butyrate and propionate were undetectable as shown in Table 2. Lactate was disappeared when incubating and measuring at 6 hr. Fig. 2 shows the change of substrate profile during methane production in 24 hr incubating of VP and RP rhizospheric soils. Ethanol concentration which was one of the important intermediates for methanogenesis showed the same pattern in both VP and RP (Fig. 2) which indicated that ethanol was rapidly consumed by the microbial consortium after 12 hr. Hence, ethanol was not the significant intermediate to differentiate the discrepancy of methane production. Acetate concentration in both phases also showed a similar pattern. However, acetate was found accumulated in both phase after 18 hr while methane productions were still increased. The accumulated acetate was found due to the anaerobic digestion from available glucose still remain and methane production from acetate consumption was on going which the concentration of acetate in solution was lower than the inhibitory levels for methanogenesis. The inhibitory of acetate on methane production occurred in the range of 0.5-1.0 mM (Babel et al., 2004).

On the other hand, glucose concentration showed a discrepancy among VP and RP. The slightly accumulated glucose was found in the case of VP while the rate of methane production at the same time was slightly slow. In RP, the amount of glucose was nearly stable at $3.3 \pm 0.1 \mu\text{mol g dw}^{-1}$ and supported higher production of methane when compared to VP. The evidence was shown in Fig. 2(b) suggested microbial consortium in RP rhizospheric soil has a good performance process of methanogenesis. These phenomena were different in VP rhizospheric soil (Fig. 2a) where

the accumulation of glucose and lower amounts of methane production were observed. The results revealed that microbial consortium in VP rhizospheric soil did not perform in the same manner as RP rhizospheric soil and lead the difference level on methane production. The production of methane from VP and RP rhizospheric soils were 470 ± 95 and $825 \pm 70 \text{ nmol g dw}^{-1} \text{ d}^{-1}$, respectively, which this methane production is brought by methanogenic *Archaea*, which either convert acetate to CH_4 and CO_2 (acetoclastic methanogenesis) or convert H_2 plus CO_2 to CH_4 (hydrogenotrophic methanogenesis).

This discrepancy could be explained in Fig. 3 which illustrated the number of acetoclastic methanogens (MPN cell g dw⁻¹) and its activity (nmol g dw⁻¹ hr⁻¹). It is clear that the number of and the activity of acetoclastic methanogens (AM) at VP were slightly lower than RP, respectively. The number of AM in RP and VP rhizospheric soils were 9.8×10^2 and 1.1×10^4 MPN cell g dw⁻¹, respectively. The population size of methanogens in various ricefields (USA, China, India, Italy etc.) were reported at $<10^1 - 2.3 \times 10^6 \text{ cell g dw}^{-1}$ (Joulain et al., 1997). Acetoclastic methanogenic activity of RP and VP rhizospheric soils were $12 \text{ nmol g dw}^{-1} \text{ hr}^{-1}$ and $25 \text{ nmol g dw}^{-1} \text{ hr}^{-1}$ or corresponded to acetoclastic methane production of $288 \text{ nmol g dw}^{-1} \text{ d}^{-1}$ and $600 \text{ nmol g dw}^{-1} \text{ d}^{-1}$, respectively. It was appeared that the methane produced from acetate in VP and RP rhizospheric soils were account to 61% and 73% of total methane production, respectively. Therefore, the development phase in methanogenic process was probably not caused by changes in the size of methanogens but in its activity. It is noted that the amount of studied available substrates were different especially glucose and acetate as a major carbon sources for methanogenesis. In addition, report (Bolton et al., 1992) confirmed that substrates derived from the roots are the major source of carbon, energy and metabolism of microbial growth of methane production in the root rhizosphere. The imbalance

between acetate utilization and production is the reason for the accumulation of acetate, which has regularly been observed in most rice fields within 1-2 weeks of flooding (Inubushi *et al.*, 1997; Watanabe *et al.*, 1997). Therefore, we expected that the difference should be attributed to the proportion of microbial consortium occurring in the different phase of VP and RP in rice field (Martin *et al.*, 2005; Conrad *et al.*, 2006).

Microbial activities related to methane production in vegetative and reproductive rhizospheric soils: Methane is produced by a microbial consortium consisting of fermentative bacteria that degrade organic matter to organic acids and alcohol, and then acetogenic bacteria degrade these substrates ultimately to acetate, H_2 and CO_2 . The actual methane production is from acetoclastic and hydrogenotrophic methanogenesis by converting acetate and H_2 plus CO_2 , respectively (Inubushi *et al.*, 1997; Kaku *et al.*, 2000; Conrad *et al.*, 2006). Due to the available substrates (glucose, ethanol and acetate) found in vegetative and reproductive rhizospheric soils, therefore the activities of microbial consortium related to available substrates were studied to investigate the effectiveness of microorganisms in each trophic group for methanogenesis. Fig. 4 shows the activities of fermentative bacteria (FB), acetogenic bacteria (AGB), acetate utilizing bacteria (AB) and acetoclastic methanogens (AM). In this experiment, the activities of FB, AGB and AB were measured from the utilization of a spiced specific substrate as glucose, ethanol and acetate, respectively. The activity of AM was measured in term of methane production from the spiced acetate.

As shown in Fig. 4 the microbial activities of FB, AGB, AB and AM in RP rhizospheric soil were more active than that in VP rhizospheric soil. Types of available substrates and their concentrations found in VP and RP rhizospheric soils influence on the activity of methanogens and associated microbial consortium in methanogenesis. Available sugar and organic acid contents in rhizosphere could provide and induce the potential amount of methane production through the activities of trophic microbial groups doing concert in methanogenic processes to enhance methane production in the developmental phase of rice growing (Dannenberg and Conrad, 1999; Kerdchoechuen, 2005). Methanogens with potential acetoclastic activity made up a larger fraction of total methane production in RP (73%) than in VP (61%) rhizospheric soils. Indeed, acetate concentrations were slightly higher in RP than in VP. This observation is in agreement with domination of methane production by acetate-dependent methanogenesis in rhizosphere either VP or RP. Interestingly, the activity pattern was reflected in the composition of available substrates found *in situ*.

Microbial consortium in vegetative and reproductive rhizospheric soil ecosystem: The ratios of trophic microbial groups in microbial consortium of vegetative and rhizospheric soils were determined. The differences of microbial consortiums can be expressed by the percentage of the bacterial communities at difference of rice developmental phase. Fig. 5 shows the different share of trophic microbial groups in VP and RP microbial consortium at

rhizosphere. The proportion of FB, AGB and AB in rhizospheric soil occurred in a percentage of 7.2%, 56.5% and 36.3% at the vegetative phase and 12.4%, 45.4% and 42.2% at reproductive phase, respectively. Acetoclastic methanogens (AM) is one of the groups in acetate utilizing bacteria (AB) and occupied in 0.17% and 0.38% of AB community in VP and RP rhizospheric soils, respectively, which were the small population in microbial consortium. Most of AB community in rhizospheric soil either at VP or RP was non-acetoclastic methanogens population but slightly higher population found in RP than that in VP. The ratio of these groups is the essential factor for monitoring the community of an anaerobic ecosystem influencing in methane production.

However, the share of AB activity was greater in reproductive phase and indicated the dominant group of active acetate consumption bacteria in RP rhizospheric soil. We also found that activity of AM was significantly different in VP and RP (Fig. 4). The number and activity of acetoclastic methanogens in RP rhizospheric soil were 1.1×10^4 cell g dw⁻¹ and 24.9 nmol g dw⁻¹ hr⁻¹ while only 9.8×10^2 cell g dw⁻¹ and 11.76 nmol g dw⁻¹ hr⁻¹ were found in VP rhizospheric soil. Moreover, methane was produced higher during reproductive phase, which corresponded with a higher number and activity of AM when compared with vegetative phase. Therefore, it is suggested that in RP rhizospheric soil, more available acetate is converted to more methane by acetoclastic methanogen (AM). This might be taken into account that less AM population number and AM activity appeared in VP rhizospheric soil. It is confirmed that not only less amount of AM and its weak activity that lead to lower production of methane but there are another groups of AB that compete utilizing of acetate. Unfortunately, with limitation of equipment and experimental design, we did not measure hydrogen producing bacteria (HPB) and sulfate reducing bacteria (SRB) using acetate as carbon source which can be expect as a competitor to AM resulted in methane production.

In general, SRB and AM can be distinguished by the condition of oxidation-reduction potential (Eh). Wang *et al.* (1993) reported that SRB is active when redox potential is upper -200 mV and methane producing bacteria is active at -200 mV or lower. In our experiment, we measured redox potential in rhizospheric soil before taken a sample. It was found that redox potential during VP was -219 mV which was slightly higher than RP (-270 mV). The Eh during VP suggested that SRB can be more favorable occurred. Rath *et al.* (2002) has studied the number of SRB in the rice soil applied with urea and ammonium thiosulfate. It was reported higher than those without application. In our experimental field, local farmers used urea plus ammonium sulfate as the basal fertilizer; this may result in the occurrence of SRB and other competitive non-methanogens during VP (Lantin *et al.*, 2000; Rath *et al.*, 2002; Towprayoon *et al.*, 2005).

Methane production during the developmental phase of Supanburi 1 rice cultivation was remarkable higher in rhizospheric soil than that in non-rhizospheric soil at VP and RP. The similar profile was also found in methane emission. Glucose, ethanol and

acetate were dominant substrates available in VP and RP rhizospheric soils. Different concentrations of glucose and acetate were found higher in RP than that in VP. Therefore, the activities of methanogens and associated consortium namely glucose fermentative bacteria (FB), ethanol acetogenic bacteria (AGB), acetate utilizing bacteria (AB) and acetoclastic methanogens (AM) were detected and show the different share of trophic microbial community in VP and RP. Considering the available substrates as related to microbial consortium activities as well as the amount of AM and its activity in both phase for methane production, we proposed the community of methanogens in VP and RP rhizosphere as illustrated in Fig. 6. Higher methane production was found in RP rhizospheric soil than in VP rhizospheric soil which resulted from the higher concentration of glucose and acetate, higher activity of trophic microbial groups in consortium and higher number of acetoclastic methanogens. In this study, it was observed that 73% and 61% of the methane produced in RP and VP rhizospheric soil were originated from acetate, respectively. Even though the tiny activity of acetoclastic methanogens was found in VP and RP rhizosphere when compared to associated microbial community, however methane can produce in the range of 500-800 nmol g dw⁻¹ d⁻¹. We found the competition of acetate utilizing bacteria (AB) in VP rhizospheric soil as seen by less amounts of AM and its weak activity as well as lower production of methane. The evidence was supported by the condition of redox potential in the field and the typical cultural practice of fertilizer application during VP of rice cultivation.

Trophic microbial groups of FB, AGB and AB play the important role in the methanogenic consortium in rhizospheric soil. Competition of acetate may occur and lead to less activity of AM and methane production. The suggested competitor may be HPB or SRB. Therefore, the major route of methane production as recognized by number of AM and its activity in the developmental phase at vegetation and reproduction of rhizosphere was shown in Fig. 6a and Fig. 6b. Methane production significantly depends on the balance among AM and associated microbial consortium namely FB, AGB and AB populations. Fertilizer management, interventions in the water management and types of rice varieties were an affect certain microbial guilds with key functions in monitoring the fertility of tropical rice soils and greenhouse gas reduction (Reichardt et al., 2001). Fertilization of ammonium sulfate instead of urea is also known as a mitigation strategy (Lantin et al., 2000; Rath et al., 2002) and is based on an increase of sulfate will increase SRB which is the competitor of AM. Therefore, understanding the enhancement of other acetate utilizing bacteria as non-acetoclastic methanogens (SRB) by ammonium sulfate instead of urea fertilizer that related to typical cultural practice will be another way to cut off methane production which on the other hand, it can be proposed as one criteria on the methane mitigation options.

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