Korean Journal of Microbiology (2016) Vol. 52, No. 2, pp. 157-165
DOI http://dx.doi.org/10.7845/kjm.2016.6007

pISSN 0440-2413
eISSN 2383-9902

보 문

Metagenomics analysis of methane metabolisms in manure fertilized paddy soil

Son G. Nguyen[1,2], Cuong Tu Ho[3], Ji-Hoon Lee[4], and Tatsuya Unno[1]*

[1]*Faculty of Biotechnology, College of Applied Life Sciences, SARI, Jeju National University, Jeju 63243, Republic of Korea*
[2]*Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[3]*Institute of Environmental Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[4]*Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju 54896, Republic of Korea*

메타게놈 분석을 이용한 돈분뇨 처리에 의한 논토양에서 메탄대사에 미치는 영향 조사

응우옌 기앙 손[1,2] · 호 투 꿩[3] · 이지훈[4] · 운노타쯔야[1]*

[1]제주대학교 생명자원대학 생명공학부, [2]베트남과학기술원 생물자원학과, [3]베트남과학기술원 환경기술학과, [4]전북대학교농업생명과학대학 생물환경화학과

(Received February 22, 2016; Revised April 11, 2016; Accepted April 13, 2016)

ABSTRACT: Under flooded rice fields, methanogens produce methane which comes out through rice stalks, thus rice fields are known as one of the anthropogenic sources of atmospheric methane. Studies have shown that use of manure increases amount of methane emission from rice. To investigate mechanisms by which manure boosts methane emission, comparative soil metagenomics between inorganically (NPK) and pig manure fertilized paddy soils (PIG) were conducted. Results from taxonomy analysis showed that more abundant methanogens, methanotrophs, methylotrophs, and acetogens were found in PIG than in NPK. In addition, BLAST results indicated more abundant carbohydrate mabolisetm functional genes in PIG. Among the methane metabolism related genes, PIG sample showed higher abundance of methyl-coenzyme M reductase (*mcrB*/*mcrD*/*mcrG*) and trimethylamine-corrinoid protein Co-methyltransferase (*mttB*) genes. In contrast, genes that down regulate methane emission, such as trimethylamine monooxygenase (*tmm*) and phosphoserine/homoserine phosphotransferase (*thrH*), were observed more in NPK sample. In addition, more methanotrophic genes (*pmoB*/*amoB*/*mxaJ*), were found more abundant in PIG sample. Identifying key genes related to methane emission and methane oxidation may provide fundamental information regarding to mechanisms by which use of manure boosts methane emission from rice. The study presented here characterized molecular variation in rice paddy, introduced by the use of pig manure.

Key words: metagenomics, methane, methanogens, microbial community, rice

Methanogens are Archaea that produce methane under anaerobic conditions. Flooded rice paddy soil gives a favorable condition for methanogens to produce methane which is piped through rice stalks to the atmosphere. Methane is a potent greenhouse gas, estimated to have global warming potentials 72 times larger than that of carbon dioxide after 20 years (IPCC, 2007). Rapid growth of population in Asia had led to expansion of cultivated rice areas, which increased the amount of anthropogenic methane emission (Li *et al.*, 2009). Two centuries of human activities (i.e., industrialization) have caused the significant increase of atmospheric methane level 1.5–2.5 times higher than that of naturally emitted methane (Forster *et al.*, 2007). Factors known to influence methane emissions from

***For correspondence.** E-mail: tatsu@jejunu.ac.kr
Tel.: +82-64-754-3354; Fax: +82-64-756-3351

rice paddies include soil types (Conrad *et al.*, 2008; Bao *et al.*, 2014a), fertilizers (Kruger and Frenzel, 2003; Yuan *et al.*, 2013), varieties of cultivars (Liou *et al.*, 2003), temperature (Gaihre *et al.*, 2012), soil redox potential (Singla *et al.*, 2014), and carbon availability (Yuan *et al.*, 2013; Kim *et al.*, 2014).

Methanogenesis is a complex biosynthetic process involving various coenzymes and cofactors (Thauer, 1998). Most abundant and ubiquitous methanogens in soil environments are acetoclastic methanogens that use acetic acid as a substrate to produce methane (Ma *et al.*, 2010; Das *et al.*, 2011; Das and Adhya, 2012; Datta *et al.*, 2013). On the other hand, there exist some rice paddy specific methanogens, referred to as "Rice cluster I", members of which also contribute substantial amount of methane emission from rice (Conrad *et al.*, 2006). Rice cluster I includes hydrogenotrophic methanogens (Erkel *et al.*, 2006; Sakai *et al.*, 2007; Lü and Lu, 2012). Therefore concentrations of both hydrogens and acetic acids also affect methane emission from rice field (Das *et al.*, 2011; Datta *et al.*, 2013). Activities of both hydrogenotrophic and acetoclastic methanogens were affected by pH, carbon nitrogen ratio, rice growth stages, and moisture (Das *et al.*, 2011; Singh and Dubey, 2012; Singh *et al.*, 2012; Datta *et al.*, 2013). On the other hand, methylotrophic bacteria including methanotrophs also increase where methane is available as a substrate. Growth of these methanotrophic and methylotrophic bacteria also depend on rice variety (Ma *et al.*, 2010; Bao *et al.*, 2014b), types of fertilizers (Mohanty *et al.*, 2006; Zheng *et al.*, 2014), and number of co-existing methanogens (Edwards *et al.*, 2015). These studies suggest that methane emission system under rice paddy is very complicated, involving various microbial species and environmental factors.

Manure is a commonly used fertilizer in paddy fields in South East Asia. The use of manure could directly introduce fecal methanogens and other carbohydrates degrading bacteria, or manure itself serves as a source of carbon for methane emission. Previously, effects of cow and swine manure on methane emission from rice paddy were compared (Kim *et al.*, 2014). According to the study, introduction of fecal methanogens were the direct cause of methane emission increase in case of cow manure, whereas swine fecal methanogens were not detected in paddy field, although high methane emission was observed. Other studies also reported high methane emission from rice fields due to pig manure addition (Pandey *et al.*, 2014; Nguyen *et al.*, 2015). Pig feces contain prevalent microbes that metabolize carbohydrates (Lamendella *et al.*, 2011), therefore, use of pig manure could introduce substrate producers for indigenous methanogens. While methane increase caused by cow manure is likely due to the addition of fecal methanogens, mechanisms by which pig manure enhances methane emission in paddy fields is not clear. In this study, we conducted comparative soil metagenomics between pig manure fertilized paddy soil and inorganically fertilized paddy soil. The study presented here should provide fundamental information regarding to molecular mechanisms involved with respect to the methane emission boots due to pig manure fertilizer.

Materials and Methods

Sample collection and DNA sequencing

A field experiment was established in rice paddy fields in Thuong Cat, Bac Tu Liem, Hanoi, Vietnam (21.0884°N and 105.7273°E). The experiment was carried out during the spring crop in April, 2014. Approximately 100 kg/ha of inorganic fertilizer, a mixture of Nitrogen, Phosphate, and Potassium at 10:9:6, was introduced to rice field one week after transplantation. One plot was amended with approximately 10 ton/ha pig manure (PIG), while the other plot served as a control (NPK). Soil samples were collected at a depth of 12 cm in triplicates, and total DNA was extracted from 250 mg paddy soil using the MOBIO Power Soil DNA isolation kit (MO BIO Laboratories Inc.). Obtained DNA samples were pooled and concentrated prior to sequencing. Library construction and sequencing were performed with Illumina HiSeq 2000 (100 bp × 2) by Macrogen Inc. according to the manufacture's instruction.

Sequence processing and analysis

Fastq files obtained from HiSeq paired-end sequencing were deposited at short read archives (SRA) with a registration number SRP059668 (PRJNA287178). Quality of each sequencing library was assessed using FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) to determine quality cutoff. Trimming was performed with Pearf software (option : -q 28 -f 0.25 -t 0.05 -l 30) (Bengtsson-Palme *et al.*, 2014) to trim reads with lower quality scores than 28 over than 75% of the read, and if more

than 5% of the bases were below a quality score of 28, reads were trimmed at the first low quality base, or discarded if the resulting read was less than 30 bp. Taxonomic classification was done using Metaxa2 (Bengtsson-Palme, 2015). Methanogens, acetogens, methanotrophs, and methylotrophs were screened based on taxonomy (Supplementary data Table S1) and abundance of them was visualized with a heatmap (R heatmap2 package) after converting read abundance to a log scale.

De-novo assembly was done using Velvet v1.2.10 (velveth kmer 35, velvetg -exp_cov auto -ins_length 350) (Birney, 2011). Contigs less than 300 bp were removed from further analyses. MG-RAST (Meyer *et al.*, 2008) was used to predict open reading frames (ORF) and annotation was done by BLASTp (Camacho *et al.*, 2009) against NCBI non-redundant (nr) database with e-value $\leq 10^{-5}$. Abundance of each contig was calculated by mapping reads against ORFs. In-house perl script was used to replicate Blast output to meet the contig abundance, then loaded to MEGAN v5.10.3 (Huson *et al.*, 2011) for COG, SEED, and KEGG annotation. Genes in methane metabolism KEGG Orthologous genes (map00680) were retrieved using GenomeNet LinkDB search (Kanehisa *et al.*, 2002) and used as a methane metabolism related gene database (CH_4-DB). Read abundance of the methane related genes were calculated using RSEM (Li and Dewey, 2011) to obtain number of mapped reads, which is further normalized with a total number of mapped reads and multiplied with one million.

Results and Discussion

Sequencing, assembly, and ORF prediction results

As a result of shotgun sequencing, we obtained 74,733,934 and 62,071,877 raw paired reads for NPK and PIG samples, respectively (Supplementary data Table S2). Almost all reads showed 60% GC contents with average Phred score 36 (Supplementary data Fig. S1). After trimming, there were 53,370,919 and 43,895,780 paired reads remained for NPK and PIG samples, respectively. By *de-novo* assembly, 243,915 (269,003 ORFs, average length 443 bp) and 75,441 (81,191 ORFs, average length 98 bp) contigs ($\geq$ 300 bp) were obtained for NPK and PIG samples, respectively. Assembly results are summarized in Supplementary data Table S3.

Microbial community analysis

After trimming, 14,317 (0.0268%) and 14,775 (0.0337%) paired sequences were assigned to archaeal/bacterial 16S rRNA

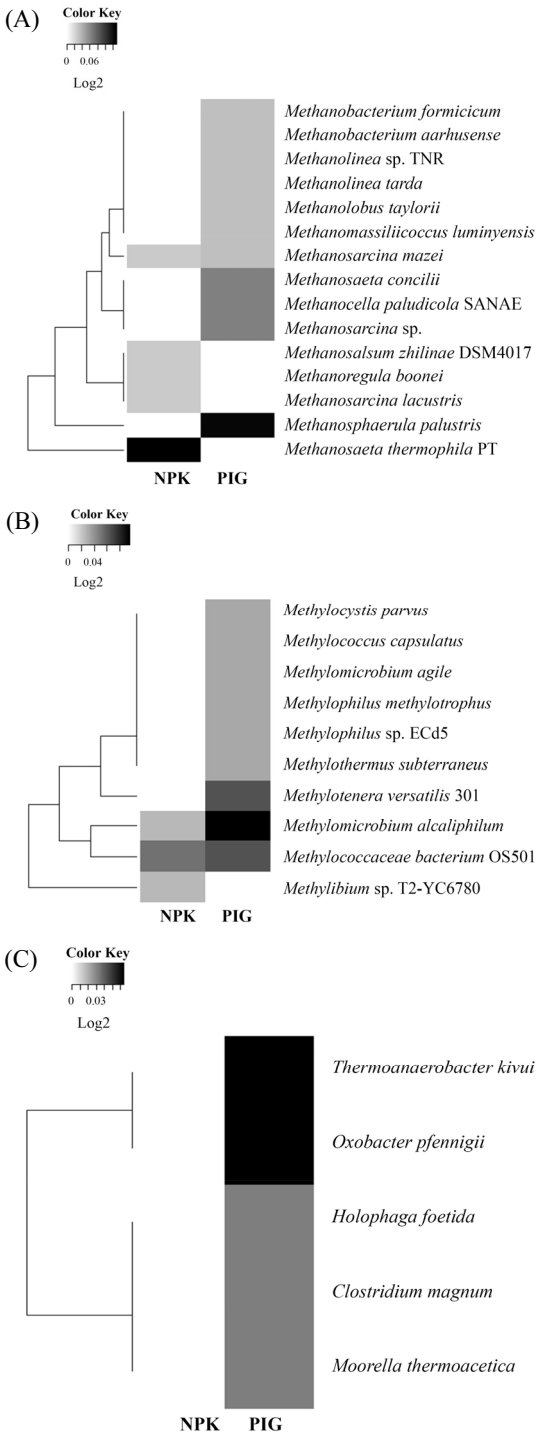

Fig. 1. Microbial community comparison between NPK and PIG among Methanogens (A), Methanotrophic/Methylotrophic bacteria (B), and Acetogens (C).

genes for NPK and PIG samples, respectively. Although no significant difference was observed in the total soil microbial communities between NPK and PIG samples (Supplementary data Fig. S2), species diversity and relative abundance of methanogenic archaea were higher in PIG sample than in NPK sample (4.11% and 2.53%, respectively) (Fig. 1A). On the other hand, relative abundance of methanotrophs and methylotrophs was also higher in PIG sample (Fig. 1B). Acetogenic bacteria, including *Holophaga foetida*, *Moorella thermoacetica*, *Oxobacter pfennigii*, *Thermoanaerobacter kivui* (Muller, 2003; Drake *et al.*, 2008; Fenchel, 2011; Müller and Frerichs, 2013), are possible substrate producers to acetoclastic methanogens. In this study, acetogenic bacteria were identified only in PIG sample (Fig. 1C). Higher abundance of methanogens and acetogenic bacteria could result in high methane emission (Peng *et al.*, 2008; Bao *et al.*, 2014a), whereas presence of methanotrophic/methylotrophic bacteria may indirectly indicate the methane availability in the environment.

Functional gene distribution analysis

Blastp successfully annotated 204,938 (76.20%) and 65,236 (80.35%) genes in NPK and PIG samples, respectively. Results in Fig. 2 show that distributions of functional genes in NPK and PIG were almost similar (Fig. 2). Results from the COG annotation showed that the largest difference between PIG and NPK samples were observed for "unknown functions" by 5.27%, followed by "energy production and conversion" (by 2.98%) and "general function and prediction only" (by 2.11%) (Fig. 2A, Supplementary data Table S4). Results from SEED analysis showed that the largest difference between the two samples was "carbohydrates" (by 1.78%), followed by "stress response" (by 1.40%) and "membrane transport" (by 1.22%) (Fig. 2B). Carbohydrate metabolism was also observed as the largest difference between the samples based on KEGG mapping (Fig. 2C). Therefore, our results suggest that the largest difference in functional genes between the two samples likely involve carbohydrates metabolisms. Lamendella *et al.* (2011) reported that carbohydrate metabolism functional genes were highly abundant in pig feces (>13%), therefore there may be a chance that the use of pig manure may have introduced microbes owning genes related to carbohydrate metabolisms to paddy soils. Recently, genetically modified rice was developed in order to increase aboveground biomass, which consequently reduced root exudates (Su *et al.*, 2015). According to the study, the alteration suppressed the carbohydrate availability in rhizosphere, which consequently reduced significant amount of methane emission from rice. Therefore, the abundance of genes involved in carbohydrate metabolism may also play a key role in controlling methane emission from rice.

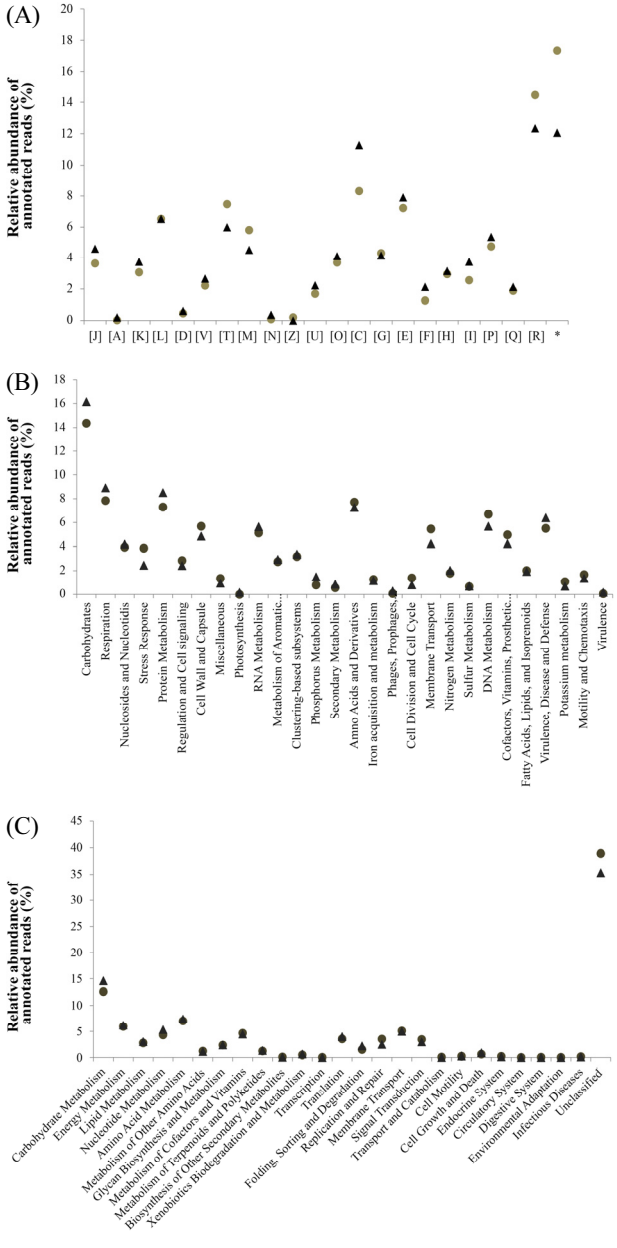

Fig. 2. Total gene (ORF) comparison using MEGAN based on BLASTP results: COG (A); SEED (B); and KEGG (C). Circles and triangles denote for NPK and PIG samples, respectively.

Methane metabolism genes

There are 158 KEGG orthologous genes (KO) in the methane metabolism KEGG map, among which we retrieved 91,345 sequences and constructed CH_4-DB. By mapping reads to CH_4-DB, we obtained abundance of genes involved in methane metabolism in NPK and PIG samples. RSEM mapped reads to 1,236 sequences in CH_4-DB. Heatmap clustering divided 461 and 568 genes based on the read-abundance (Supplementary data Fig. S3). Genes in the cluster I and II were further analyzed for KEGG annotation and total 40 KO were identified (Fig. 3A and Supplementary data Table S5).

KO of these differentially abundant genes were further mapped on the methane metabolism KEGG map (Fig. 3B). Roles of the mapped genes found in PIG and NPK are summarized in Tables 1 and 2, respectively. Our results show that PIG specific KO were found in various pathways towards production of methane.

Table 1. Role of mapped genes found more abundant in PIG

Gene	Enzyme code	Role
mcrB/mcrD/mcrG	[EC:2.8.4.1]	A key enzyme of biological methane formation from Methyl-CoM
mtaA	[EC:2.1.1.246]	Conversion of methanol into methane
mttB	[EC:2.1.1.250]	Involved in Methyl-CoM synthesis
dmd-tmd	NA*	Involved in Methyl-CoM synthesis
coxM/coxS	[EC:1.2.99.2]	Involved in acetogenesis
cdhA	[EC:1.2.7.4]	Involved in acetogenesis
pmoB-amoB	[EC: 1.14.18.3 1.14.99.39]	Conversion of methane to formaldehyde Oxidization of ammonia to nitrite
mxaJ	NA*	Oxidization of methanol to formaldehyde
fwdE/fwdF	[EC:1.2.99.5]	Involved in methanogenesis from CO_2
ftr	[EC:2.3.1.101]	Involved in methanogenesis from CO_2
mdo	[EC:1.2.98.1]	Formaldehyde formation
gfa	[EC:4.4.1.22]	Formaldehyde formation
fae-hps	[EC:4.2.1.147 4.1.2.43]	Formaldehyde assimilation
hps-phi	[EC:4.1.2.43]	Involved in Ribulose-P pathway
gpmB	[EC:5.4.2.12]	Involved in Ribulose-P pathway
Fructose-bisphosphate aldolase	[EC:4.1.2.13 2.2.1.10]	Involved in Ribulose-P pathway
fbp-SEBP	[EC:3.1.3.11 3.1.3.37]	Involved in Ribulose-P pathway
cofH	[EC:2.5.1.77]	Coenzyme F420 biosynthesis
AGXT	[EC:2.6.1.44 2.6.1.45 2.6.1.51]	Involved in Serine pathway
aksA	[EC:2.3.3.14 2.3.3.-]	Coenzyme B biosynthesis

*NA, not available

Table 2. Role of mapped genes found more abundant in NPK

Gene	Enzyme code	Role
tmm	[EC:1.14.13.148]	Oxidization of trimethylamine (pre-cursor of methane)
aksE	[EC:4.2.1.114]	Coenzyme B biosynthesis
cdhC/cdhD/cdhE	[EC:2.3.1.- 2.1.1.245]	Involved in acetogenesis
comE	[EC:4.1.1.79]	Coenzyme M biosynthesis
fdhA1	[EC:1.2.1.43]	Involved in Wood-Ljungdahl pathway
mcl	[EC:4.1.3.24 4.1.3.25]	Involved in Serine pathway
mttC	NA*	Catalysis of Methyl-CoM synthesis
thrH	[EC:3.1.3.3 2.7.1.39]	Involved in Ribulose-P pathway

*NA, not available

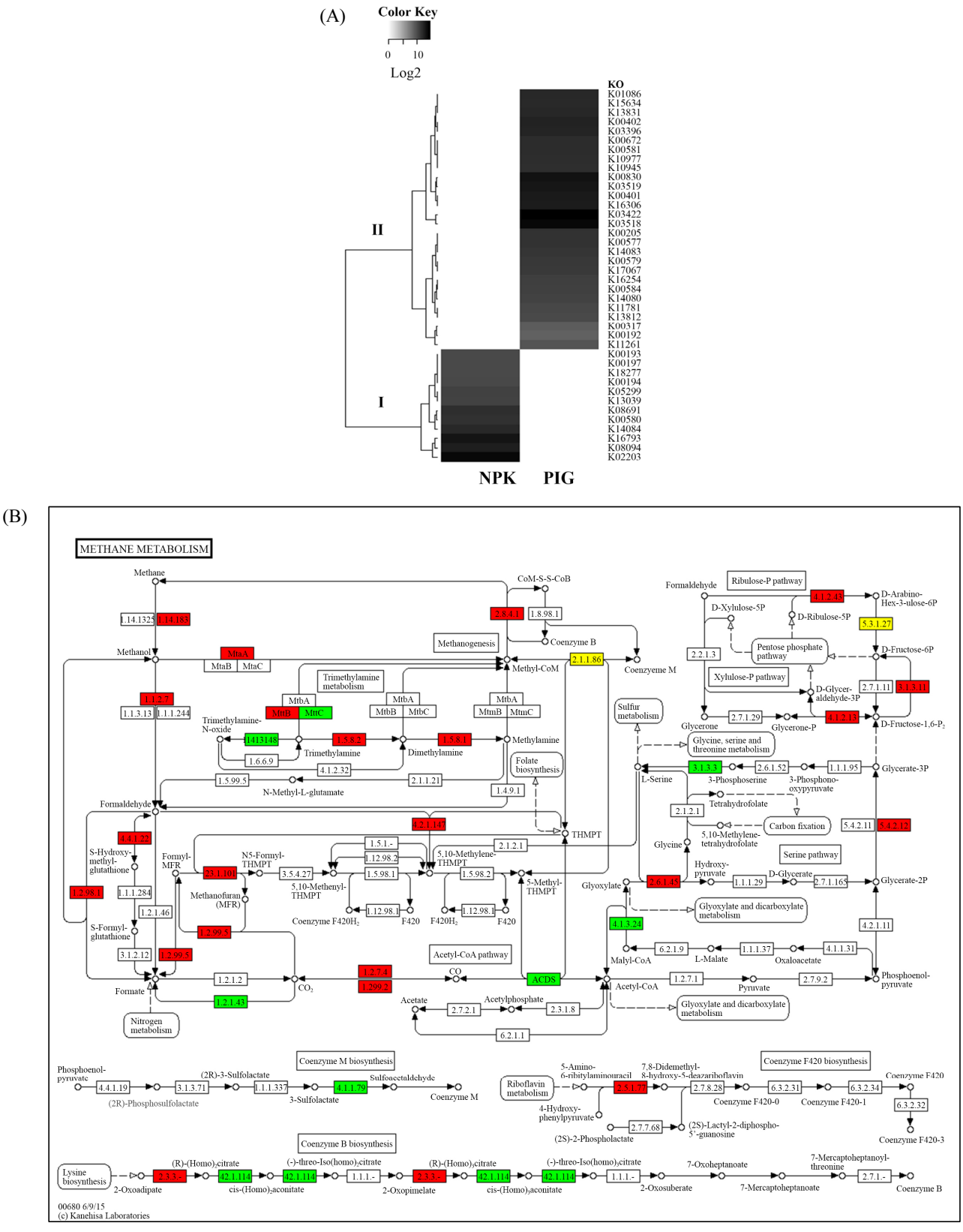

Fig. 3. Abundance comparison between NPK and PIG for methane metabolism related genes based on KEGG annotation (A) and methane-metabolism KEGG mapping (green NPK; red PIG; and yellow both) (B).

For example, methyl-coenzyme M reductase (MCR) (*mcrB/mcrD/mcrG*) is a key enzyme of biological methane formation from Methyl-CoM (Scheller *et al.*, 2013), coenzyme M methyl transferase (*mtaA*) is involved in transferring methanol into methane (Harms, 1996), and trimethylamine-corrinoid protein Co-methyltransferase (*mttB*) and dimethylamine/trimethylamine dehydrogenase (*dmd-tmd*) catalyze the synthesize of Methyl-CoM, a precursor of methane (Yang *et al.*, 1995; Tallant and Krzycki, 1997; Jones *et al.*, 2002; Pritchett and Metcalf, 2005). On the other hand, other KO were involved in acetogenesis (i.e., carbon-monoxide dehydrogenase and acetyl-CoA decarbonylase/synthase complex [Maupin-Furlow and Ferry, 1996]), suggesting that more acetic acid, a substrate for methanogens, would be available in PIG sample compared to NPK sample. Moreover, *pmoB-amoB* and *mxaJ* in PIG sample indicate the presence of methanotrophs (Amaratunga *et al.*, 1997; Tavormina *et al.*, 2011), suggesting that more methane was available in PIG sample. In contrast, most of genes found more abundant in NPK sample are indirectly involved in methane emission, such as biosynthesis of coenzymes and serine pathways. Moreover, NPK specific KO includes trimethylamine monooxygenase (*tmm*) which oxidizes trimethylamine, a pre-cursor of methane (Chen *et al.*, 2011).

In summary, use of pig manure may have increased the abundance of microbes involved in methane emissions such as methanogens and acetogens, which may have resulted in higher abundance of genes that could enhance methane emission. The present studies provide useful information for further studies to understand microbial methane production mechanisms under rice paddy.

적 요

침수된 논토양에서는 메탄생성균이 벼 줄기를 타고 올라오는 메탄을 생성하는 것으로 알려져 있고, 그래서 논토양은 대기 메탄의 인위적인 발생원 중 하나로 알려져 있다. 또한 (분뇨)거름을 사용하면 벼로부터 메탄 배출이 증가하는 것으로 연구 결과 알려져 있다. 어떠한 기작으로 (분뇨)거름이 메탄 배출을 증가시키는지 알아보기 위하여, 무기비료를 사용한 논토양(NPK)과 돈분뇨를 처리한 논토양(PIG)에서의 미생물의 메타게놈에 대해 비교분석을 수행하였다.

미생물군집 분류 분석 결과, 메탄생성균과 메탄영양균, 메틸영양균, 초산생성균(acetogen)이 NPK에서 보다 PIG에서 더 풍부하였다. 더욱이 BLAST 비교 분석 결과 탄수화물 대사 기능 유전자가 PIG에 더 풍부하였다. 메탄 대사와 관련된 유전자 중에서 메틸-조효소-M-환원효소(*mcrB/mcrD/mcrG*)와 트리메틸아민-코리노이드 단백질 Co-메틸전달효소(*mttB*)가 PIG 시료에 더 풍부하였다. 그와는 상대적으로, 트리메틸아민 모노산소첨가효소(*tmm*)와 포스포세린/호모세린 인산전달효소(*thrH*) 같은 메탄 배출을 하향 조절하는 유전자는 NPK 시료에서 더 관찰되었다. 메탄영양과 관련된 유전자(*pmoB/amoB/mxaJ*)들 또한 PIG에서 더 풍부하게 발견되었다.

메탄 배출과 메탄 산화와 관련된 핵심 유전자들을 환경에서 확인함으로써, (분뇨)거름 사용에 의해 벼로부터 메탄 배출이 증가하는 기작에 대해 기초적인 정보를 얻을 수 있을 것이다. 본 연구에 제시된 내용을 통해 돈분료거름을 처리한 논토양 내 미생물의 분자적 변이를 알 수 있었다.

Acknowledgements

This research was supported by the 2015 scientific promotion program funded by Jeju National University.

References

Amaratunga, K., Goodwin, P.M., O'Connor, C.D., and Anthony, C. 1997. The methanol oxidation genes *mxaFJGIR (S) ACKLD* in *Methylobacterium extorquens*. *FEMS Microbiol. Lett.* **146**, 31–38.

Bao, Z., Watanabe, A., Sasaki, K., Okubo, T., Tokida, T., Liu, D., Ikeda, S., Imaizumi-Anraku, H., Asakawa, S., Sato, T., *et al.* 2014b. A rice gene for microbial symbiosis, *Oryza sativa CCaMK*, reduces CH_4 flux in a paddy field with low nitrogen input. *Appl. Environ. Microbiol.* **80**, 1995–2003.

Bao, Q., Xiao, K.Q., Chen, Z., Yao, H.Y., and Zhu, Y.G. 2014a. Methane production and methanogenic archaeal communities in two types of paddy soil amended with different amounts of rice straw. *FEMS Microbiol. Ecol*. **88**, 372–385.

Bengtsson-Palme, J., Boulund, F., Fick, J., Kristiansson, E., and Larsson, D.G. 2014. Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. *Front. Microbiol.* **5**, 648.

Bengtsson-Palme, J., Hartmann, M., Eriksson, K.M., Pal, C., Thorell, K., Larsson, D.G., and Nilsson, R.H. 2015. METAXA2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. *Mol. Ecol.*

Resour. **15**, 1403−1414.

Birney, D.R.Z.a.E. 2011. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* **18**, 821−829.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421.

Chen, Y., Patel, N.A., Crombie, A., Scrivens, J.H., and Murrell, J.C. 2011. Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase. *Proc. Natl. Acad. Sci. USA* **108**, 17791−17796.

Conrad, R., Erkel, C., and Liesack, W. 2006. Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. *Curr. Opin. Biotechnol.* **17**, 262−267.

Conrad, R., Klose, M., Noll, M., Kemnitz, D., and Bodelier, P.L.F. 2008. Soil types links microbial colonization of rice roots to methane emission. *Glob Change Biol.* **14**, 657−669.

Das, S. and Adhya, T.K. 2012. Dynamics of methanogenesis and methanotrophy in tropical paddy soils as influenced by elevated CO_2 and temperature interaction. *Soil Biol. Biochem.* **47**, 36−45.

Das, S., Ghosh, A., and Adhya, T.K. 2011. Nitrous oxide and methane emission from a flooded rice field as influenced by separate and combined application of herbicides bensulfuron methyl and pretilachlor. *Chemosphere* **84**, 54−62.

Datta, A., Santra, S.C., and Adhya, T.K. 2013. Effect of inorganic fertilizers (N, P, K) on methane emission from tropical rice field of India. *Atmospheric Environment* **66**, 123−130.

Drake, H.L., Gossner, A.S., and Daniel, S.L. 2008. Old acetogens, new light. *Ann. N. Y. Acad. Sci.* **1125**, 100−128.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.a., and Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. USA* **112**, E911−E920.

Erkel, C., Kube, M., Reinhardt, R., and Liesack, W. 2006. Genome of rice cluster I archaea—the key methane producers in the rice rhizosphere. *Science* **313**, 370−372.

Fenchel, T. 2011. Bacterial Ecology. DOI: 10.1002/9780470015902.a0000339.pub3.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., and Myhre, G. 2007. Changes in atmospheric constituents and in radiative forcing. Chapter 2. In *Climate Change 2007. The Physical Science Basis*.

Gaihre, Y., Wassmann, R., Pangga, G., and Aquino, E. 2012. Effect of elevated temperature and rice straw application on methane emission from lowland rice paddy soils. *Philippine J. Crop Sci. (Philippines)* **37**, 10.

Harms, U. and Thauer, R.K. 1996. Methylcobalamin: coenzyme M methyltransferase isoenzymes MtaA and MtbA from *Methanosarcina barkeri*. Cloning, sequencing and differential transcription of the encoding genes, and functional overexpression of the *mtaA* gene in *Escherichia coli*. *Eur. J. Biochem.* **235**, 653−659.

Huson, D.H., Mitra, S., Ruscheweyh, H.J., Weber, N., and Schuster, S.C. 2011. Integrative analysis of environmental sequences using MEGAN4. *Genome Res.* **21**, 1552−1560.

IPCC. 2007. IPCC fourth assessment report: climate change 2007 synthesis report.

Jones, M., Talfournier, F., Bobrov, A., Grossmann, J.G., Vekshin, N., Sutcliffe, M.J., and Scrutton, N.S. 2002. Electron transfer and conformational change in complexes of trimethylamine dehydrogenase and electron transferring flavoprotein. *J. Biol. Chem.* **277**, 8457−8465.

Kanehisa, M., Goto, S., Kawashima, S., and Nakaya, A. 2002. The KEGG databases at GenomeNet. *Nucleic Acids Res.* **30**, 42−46.

Kim, S.Y., Pramanik, P., Bodelier, P.L., and Kim, P.J. 2014. Cattle manure enhances methanogens diversity and methane emissions compared to swine manure under rice paddy. *PLoS One* **9**, e113593.

Kruger, M. and Frenzel, P. 2003. Effect of N-fertilization on CH_4 oxidation and production, and consequences for CH_4 emissions from microcosms and rice fields. *Glob Change Biol.* **9**, 773−784.

Lamendella, R., Domingo, J.W., Ghosh, S., Martinson, J., and Oerther, D.B. 2011. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. *BMC Microbiol.* **11**, 103.

Li, B. and Dewey, C.N. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**, 323.

Li, X., Dodson, J., Zhou, J., and Zhou, X. 2009. Increases of population and expansion of rice agriculture in Asia, and anthropogenic methane emissions since 5000 BP. *Quat. Int.* **202**, 41−50.

Liou, R.M., Huang, S.N., and Lin, C.W. 2003. Methane emission from fields with differences in nitrogen fertilizers and rice varieties in Taiwan paddy soils. *Chemosphere* **50**, 237−246.

Lü, Z. and Lu, Y. 2012. *Methanocella conradii* sp. nov., a thermophilic, obligate hydrogenotrophic methanogen, isolated from chinese rice field soil. *PLoS One* **7**, e35279.

Ma, K.E., Qiu, Q., and Lu, Y. 2010. Microbial mechanism for rice variety control on methane emission from rice field soil. *Global Change Biol.* **16**, 3085−3095.

Maupin-Furlow, J.A. and Ferry, J.G. 1996. Analysis of the CO dehydrogenase/acetyl-coenzyme A synthase operon of *Methanosarcina thermophila*. *J. Bacteriol.* **178**, 6849−6856.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., *et al.* 2008. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* **9**, 386.

Mohanty, S.R., Bodelier, P.L.E., Floris, V., and Conrad, R. 2006. Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. *Appl. Environ. Microbiol.* **72**, 1346−1354.

Muller, V. 2003. Energy conservation in acetogenic bacteria. *Appl. Environ. Microbiol.* **69**, 6345−6353.

Müller, V. and Frerichs, J. 2013. Acetogenic Bacteria. *In* Encyclopedia of life sciences. www.els.net.

Nguyen, S., Guevarra, R., Kim, J., Ho, C., Trinh, M., and Unno, T.

2015. Impacts of initial fertilizers and irrigation systems on paddy methanogens and methane emission. *Water Air Soil Pollut.* **226**, 1−11.

Pandey, A., Mai, V.T., Vu, D.Q., Bui, T.P.L., Mai, T.L.A., Jensen, L.S., and de Neergaard, A. 2014. Organic matter and water management strategies to reduce methane and nitrous oxide emissions from rice paddies in Vietnam. *Agriculture Ecosystems & Environment* **196**, 137−146.

Peng, J., Lu, Z., Rui, J., and Lu, Y. 2008. Dynamics of the methanogenic archaeal community during plant residue decomposition in an anoxic rice field soil. *Appl. Environ. Microbiol.* **74**, 2894−2901.

Pritchett, M.A. and Metcalf, W.W. 2005. Genetic, physiological and biochemical characterization of multiple methanol methyltransferase isozymes in *Methanosarcina acetivorans* C2A. *Mol. Microbiol.* **56**, 1183−1194.

Sakai, S., Imachi, H., Sekiguchi, Y., Ohashi, A., Harada, H., and Kamagata, Y. 2007. Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate affiliated with the clone cluster rice cluster I. *Appl. Environ. Microbiol.* **73**, 4326−4331.

Scheller, S., Goenrich, M., Thauer, R.K., and Jaun, B. 2013. Methyl-coenzyme M reductase from methanogenic archaea: isotope effects on the formation and anaerobic oxidation of methane. *J. Am. Chem. Soc.* **135**, 14975−14984.

Singh, A. and Dubey, S.K. 2012. Temporal variation in methanogenic community structure and methane production potential of tropical rice ecosystem. *Soil Biol. Biochem.* **48**, 162−166.

Singh, A., Singh, R.S., Upadhyay, S.N., Joshi, C.G., Tripathi, A.K., and Dubey, S.K. 2012. Community structure of methanogenic archaea and methane production associated with compost-treated tropical rice-field soil. *FEMS Microbiol. Ecol.* **82**, 118−134.

Singla, A., Sakata, R., Hanazawa, S., and Inubushi, K. 2014. Methane production/oxidation potential and methanogenic archaeal diversity in two paddy soils of Japan. *Int. J. Ecol. Environ. Sci.* **40**, 49−55.

Su, J., Hu, C., Yan, X., Jin, Y., Chen, Z., Guan, Q., Wang, Y., Zhong, D., Jansson, C., Wang, F., *et al.* 2015. Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice. *Nature* **523**, 602−606.

Tallant, T.C. and Krzycki, J.A. 1997. Methylthiol:coenzyme M methyltransferase from *Methanosarcina barkeri*, an enzyme of methanogenesis from dimethylsulfide and methylmercaptopropionate. *J. Bacteriol.* **179**, 6902−6911.

Tavormina, P.L., Orphan, V.J., Kalyuzhnaya, M.G., Jetten, M.S., and Klotz, M.G. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ. Microbiol. Rep.* **3**, 91−100.

Thauer, R.K. 1998. Biochemistry of methanogenesis, a tribute to Marjory Stephenson. 1998 Marjory Stephenson Prize Lecture. *MIcrobiology* **144**, 2377−2406.

Yang, C.C., Packman, L.C., and Scrutton, N.S. 1995. The primary structure of *Hyphomicrobium X* dimethylamine dehydrogenase: Relationship to trimethylamine dehydrogenase and implications for substrate recognition. *Eur. J. Biochem.* **232**, 264−271.

Yuan, Q., Pump, J., and Conrad, R. 2013. Straw application in paddy soil enhances methane production also from other carbon sources. *Biogeosciences Discussions* **10**, 14169−14193.

Zheng, Y., Huang, R., Wang, B.Z., Bodelier, P.L.E., and Jia, Z.J. 2014. Competitive interactions between methane- and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil. *Biogeosciences* **11**, 3353−3368.