IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

Effect of C/N ratio variations on the capability of microbes from Muara Karang river sediment in the production of biogas and identification using VITEK 2

To cite this article: L M Widadri *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **308** 012024

View the article online for updates and enhancements.

This content was downloaded from IP address 114.221.41.103 on 10/10/2019 at 09:38

Effect of C/N ratio variations on the capability of microbes from Muara Karang river sediment in the production of biogas and identification using VITEK 2

L M Widadri[1], W Mangunwardoyo[1], and H Ambarsari[2]

[1]Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia
[2]Center for Environmental Technology, Building 820 Geostech, Puspiptek Serpong, Tangerang Selatan, Banten 15314, Indonesia

Email: wmarlatifa@gmail.com; wibowo.mangun@ui.ac.id, hanies.ambarsari@gmail.com

Abstract. Gas is one form of microbial metabolism products that can be identified as biogas, one example of biogas is methane gas. The production of methane gas by bacteria occurs through methanogenesis with three stages, namely hydrolysis, acetogenesis, and methanogenesis. These processes are generally performed by bacteria in an anaerobic environment. The Muara Karang River sediments contaminated with organic matters and having low oxygen are potential as the habitat for anaerobic microbes with methanogenesis ability. The ability of such sediment microbes in biogas production was tested by inoculating Muara Karang sediment in a Methanogen Enrichment Barker broth medium with variations of C/N ratio using glucose as the carbon source to analyze the biogas production. The parameters measured were the total carbon, the total nitrogen, and the biogas volume. Two isolates were obtained, namely isolate I and isolate II. These isolates were then identified by the VITEK 2 compact equipment. The result showed that C/N ratio of 25:1 could produce the highest biogas volume. Isolate I was identified by the VITEK 2 equipment as *Methylobacterium* spp. from methanotroph group bacteria and isolate II was identified as *Dermacoccus nishinomiyaensis*.

1. Introduction

Muara Karang River in North Jakarta is one of many polluted rivers caused by the build up of organic and inorganic matters from human activities. The accumulation formed an ecosystem with low level of dissolved oxygen. This condition supports the growth of anaerobic microorganisms in sediment found in layer of water body [1].

Sediment in the waters commonly contains accumulation of materials from decomposition process of organic matters. The high level of organic matters causes dissolved oxygen level to be low in sediment. Low level of dissolved oxygen in sediment is suitable for obligate anaerobic methanogenic microorganisms' growth condition so that methanogenic biomass is higher in the sedimen than in the water. Methanogenic microbe generates biogas, particularly methane (CH_4) as one of its metabolism products. Methane formation process or methanogenesis by methanogenic bacteria plays a role in the

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

global carbon cycle on anaerobic ecosystem. Accumulation of methane from methanogenesis that isreleased into the atmosphere has the potential to cause greenhouse effect and climate changes, yet methane is also potential as alternative renewable energy source [2,3,4,5].

Methanogenesis performed by methanogenic bacteria depends on the availability of essential elements for the methanogenic bacteria's metabolism, such as carbon (C) and nitrogen (N). Carbon and nitrogen are known to be macromolecules with structural and functional roles in bacteria's cell components. Carbon is mostly utilised by microbes in the form of carbon dioxide (CO_2), as well as other organic forms (glucose), function as main constituent in cellular materials. Nitrogen in the form of NH_3, NO_3, N_2, and in organic forms serve as constituents for amino acids, coenzyme, and nucleic acid [6].

This research was aimed to investigate the ability of microorganisms from sediments of Muara Karang River in producing biogas as well as to identify the types of such microorganisms using VITEK 2 identification method based on the biochemical testing results.

2. Materials and Methods

2.1 Samples and sampling

Sediment samples were collected using Ekman grab from three sites in Muara Karang river located in North Jakarta, with the coordinates were in S.66°.07.07" E 106.46'24.5" for Site 1, S.06°.07.10.2" E 106°.46'25.2" for Site 2, and S.06°.06.45.8" E 106.45'05.5" for Site 3. Sediments from three sites were composited and inserted into 1000 mL tubes, stored in a cool box with ice pack, then transported into the laboratorium on the same day.

2.2 Media

Methanogen Enrichment Barker medium with glucose as the carbon source contained (per liter) 1.0 g NH_4Cl, 0.4 g $K_2HPO_4.3H_2O$, 0.1 g $MgCl_2.6H_2O$, and variations of $C_6H_{12}O_6$ for C/N ratio with 9.35 g for 15:1, 15.58 g for 25:1, and 24.94 g for 40:1. Addition of 20 g Agar was used to make the agar medium [7].

2.3 Assessment of microbial growth and gas production with C/N ratio variation

Several 1000 mL anaerobic incubators connected with the water displacement system were used to accomodate Methanogen Enrichment Barker (MEB) medium broth with 750 mL working volume and 15 mL composite sediments. Five incubators were set to incubate for 0 day, 7 days, 14 days, 21 days, and 28 days. Sample collecting was performed at the end of each incubation period. The liquid sample from each incubators was transported to Analytical Laboratory in UI-Chem Departement of Chemistry Universitas Indonesia then measured to determine the concentrations of carbon and nitrate with procedure of Total Organic Carbon and Total Kjehdahl Nitrogens. Samples in gas form were measured inside the water displacement system to determine the amount of biogas production [8,9].

2.4 Identification of potential biogas-producing bacteria

The samples from each incubator at 28 days of incubation were inoculated into Methanogen Enrichment Barker (MEB) medium agar for isolation, the purification process using the MEB medium agar was performed after 14 days of incubation. The identification of microorganism was performed using VITEK 2 with Gram positive and Gram negative identification cards in Tangerang Regency General Hospital, Tangerang. The VITEK 2 system uses fluorogenic and turbidimetric methods for identification of microorganism [10].

2.5 Measurement of carbon and nitrogen concentration in the medium
The measurement of total carbon was performed using total carbon organic method according to SNI standard. Total organic carbon was measured as the main parameter of the microbial metabolism to determine the amount of carbon in each batch fermentor namely, Methanogen Enrichment Barker (MEB) broth with glucose ($C_6H_{12}O_6$) as the carbon source. The total nitrogen in the medium was performed using the total Kjehdahl Nitrogen method according to SNI standard.

2.6 Statistical analysis and graphical work
Statistical analysis and graphical works were conducted using Microsoft Excel 2011.

3. Results and Discussion

3.1 Carbon concentration changes
The results of carbon concentration measurement in each incubation time can be seen in Figure 1. The amount of carbon on 0 day incubation, the control medium was lower than at incubation 7, 14, 21 and 28 days. This can be caused by the loss of volatile type carbon compounds in the digestion process of the analysis total chemical of organic carbon [11, 12].

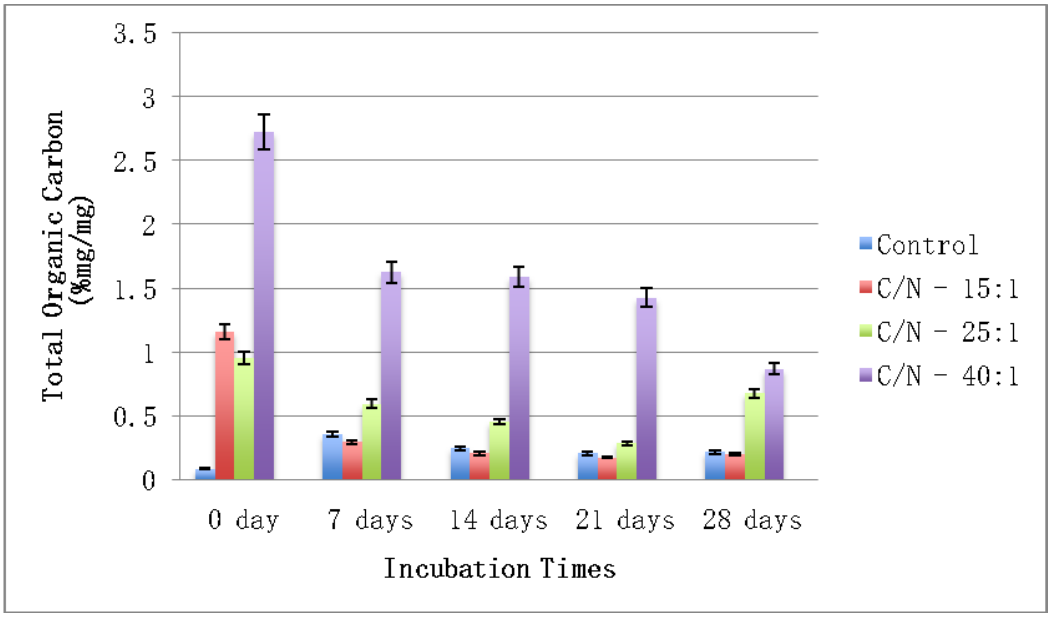

Figure 1. Total Carbon concentration (%mg/mg) in each incubation times.

The different reductions of total carbon in the medium could be due to the number of microbes in the sediment were not homogeneous, even though the weight of the sediment inoculated was the same. The number of microbes affects the use of nutrients in the medium. The highest biggest decrease in carbon concentration occurred in the medium of Methanogen Enrichment Barker (MEB) broth with a C/N ratio of 40: 1. This could be caused by the large number of microbes in the sediment so that the carbon content in the medium was used maximally by the microbial sediment of Muara Karang in the metabolic process.

For example carbohydrates in the methanogenesis fermentation process went through three main stages, namely, hydrolysis, acetogenesis, and methanogenesis as also described in several previous studies [13, 14, 15,16].

3.2 Nitrogen concentration changes

Nitrogen is one of the building blocks of the organism. The presence of nitrogen elements in the form of ammonium chloride (NH_4Cl) in Barker Enrichment Methanogen medium was needed for the growth and metabolism of sedimentary microorganisms in Muara Karang. The total nitrogen in the medium was performed using the total Kjehdahl Nitrogen method according to SNI standard. Nitrogen concentration changes in Methanogen Enrichment Barker (MEB) broth can be seen in Figure 2.

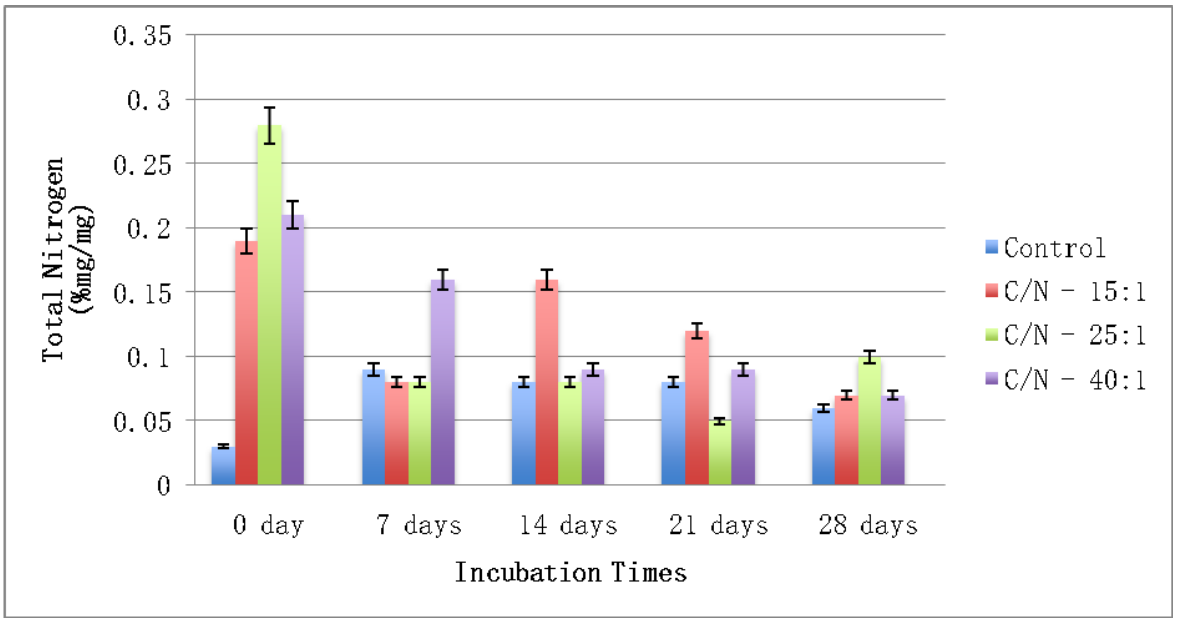

Figure 2. Total Nitrogen concentration (% mg/mg) in each incubation times.

The concentration of control medium nitrogen and C/N ratio variation 15: 1, 25: 1, and 40: 1 were not the same. This could be caused by the loss of nitrogen elements in the sample when the digestion or distillation stage was carried out in the total Kjehdahl nitrogen method. Nitrogen concentrations with variations in C/N ratio of 15:1, 25:1, and 40:1 were reduced during each incubation days of 0, 7, 14, 21, and 28. Reduced nitrogen concentrations were caused by the use of nitrogen from the medium into the microorganism cells. This was supported by reports on the use of medium with NH_4^+ -N form nitrogen content in biogas production by the microorganisms. Nitrogen concentration can inhibit methanogenesis process by depleting substrates for methanogens bacteria [17, 18, 19, 20, 21,22].

3.3 Biogas production

The biogas volumes in the reactors were measured using the water displacement method. Figure 3 shows the volume of biogas produced per incubation day in the reactor system. Gas was formed in all media with variations in C/N ratio. The average biogas volume in the control medium, the variation of the C/N ratio of 15: 1, 25: 1, and 40: 1 respectively are: in 0 day incubation were 0 cm^3, 0 cm^3, 0 cm^3, and 0 cm^3; 7-day incubation were 0 cm^3, 15.3 cm^3, 21 cm^3, and 18.3 cm^3; 14-day incubation were 0 cm^3, 5.6 cm^3, 12.3 cm^3,

and 20 cm^3; 21-day incubation were 0 cm^3, 9 cm^3, 20.3 cm^3, and 12.3 cm^3; and 28-days incubation were 0 cm^3, 11.6 cm^3, 23 cm^3, and 7.6 cm^3.

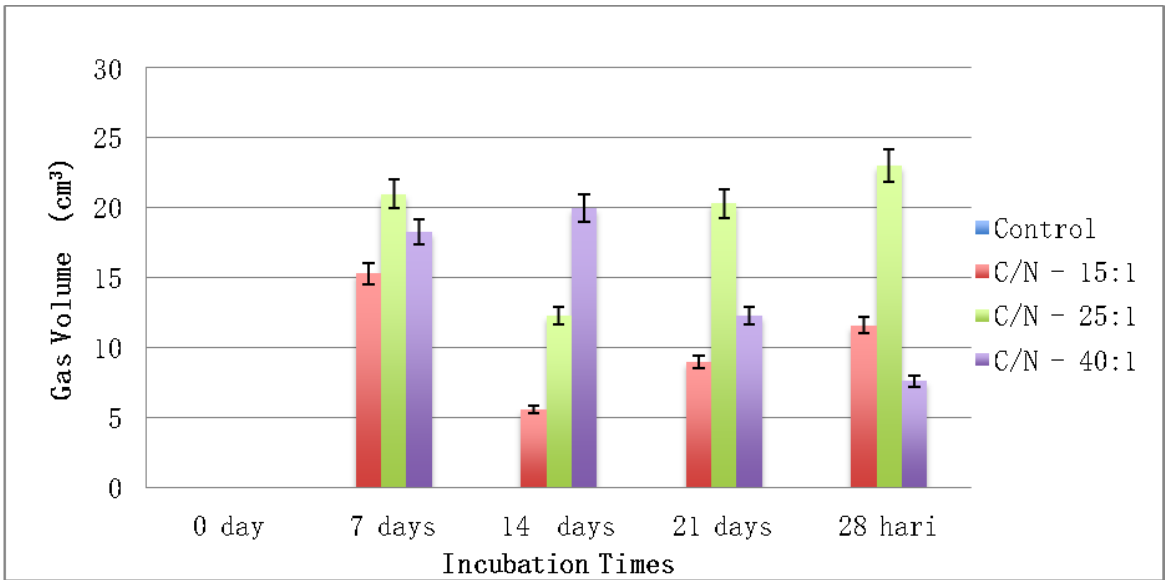

Figure 3. Average Biogas production (cm^3) in eachincubation Times.

The lowest smallest biogas as average was produced was in medium with C/N ratio of 15: 1. The amount of carbon in the medium affected influences the amount of gas produced, because the element of carbon is one of the essential elements for bacterial growth. The carbon element supports the growth of bacteria with two important roles namely as with t.wo important roles namely, as building blocks for bacterial growth and for producing energy. For example methanogens bacteria require glucose or other carbon sources to produce methane gas in the methanogenesis process [23].

The C/N ratio of 25: 1 resulted in the highest average biogas volume during incubation of 7, 21, and 28 days. The results according to the previous reports using growth medium in the form of cow dung, poultry droppings, rice husks, sugarcane dregs, and Apu wood were optimal for the growth of biogas-producing bacteria with a C/N ratio ranging from 25: 1-30: 1. The biogas volume ratio of C / N 25:1 is smaller than the 40:1 ratio at 14 days incubation, it is likely that the number of microbes in the sediment were not homogeneous in the medium. It was known previously that the difference in the number of bacteria in the sediment could affect the amount of gas produced by metabolism, more metabolic processes result in increased biogas production [24, 25, 26].

3.4 Identification of potential biogas-producing bacteria

Identification for potential biogas-producing bacteria was performed using VITEK 2. Two species were identified as *Methylobacterium* spp. and *Dermacoccus nishinomiyaensis*. *Methylobacterium* spp. is also known as a common bacteria isolated from soil, leaf surface, and some of them can perform endosymbiosis with plant. They are member of *Methylobacteriaceae* group with the ability to oxidize methane (CH_4) gasses from environment as their energy source, also known as methanotrophic bacteria.

Their ability to oxidize methane plays a role in the reduction of environmental methane which can potentially cause a greenhouse effect [27].

Dermacoccus nishinomiyaensis was identified as one of the microbes that produce biogas in the reactor, which pressumed as carbon dioxide (CO_2) and hydrogen (H_2) from the bacterial metabolism. *Dermacoccus nishinomiyaensis* formerly known as *Micrococcus nishinomiyaensis* belongs to the group *Dermacoccaceae* and commonly isolated from aquatic sediment, soft corals, and mammalian skins [28]. The absence of methanogen species identified from the reactors was suspected since there were no methane gas produced in the reactor.

4. Conclusion

In this study the highest biogas production was found at C/N ratio variation of 25:1, compared to C/N ratio variation of 15:1 and 40:1, and two isolates respectively *Methylobacterium* spp. from methanotroph group bacteria and *Dermacoccus nishinomiyaensis*were identified, none of these bacteria were belong to methanogen group. Nevertheless, the type of gasses produced in the reactor need to be further analyzed and studied to determine the type of gas produced by the microbes in Muara Karang sediment.

5. References

[1] Badan Pengelola Lingkungan Hidup Daerah (BPLHD) Provinsi DKI Jakarta *2015 Laporan status lingkungan daerah provinsi daerah khusus ibukota Jakarta tahun 2015* (DKI Jakarta Pemerintah Provinsi DKI Jakarta) p 398

[2] Chaban B, Ng Sy, and Jarrel K F 2006 Archaeal habitats-from the extreme to the ordinary *Canada J.Microbiol* **52** 73-116

[3] Ciais P *et al.* 2013 Carbon and other biogeochemical cycles *Physi. Sci. Basis*. Cambridge University Press pp 465-570

[4] Gruca-Rokosz R and Tomaszek J A 2015 Methane and carbon dioxide in the sediment of a eutrophic reservoir: production pathways and diffusion fluxes at the sediment-water interface *Water Air Soil Pollution* 1-16

[5] Yoon J 2015 The benefits and downsides of methane 1 phttp://large.stanford.edu/ courses/2016/ph240/yoon2/. Accessed 25 February 2018, 21.58 WIB.

[6] Todar K 2012 Nutrition and growth of bacteria 1 phttp://textbookofbacteriology.net/ nutgro.html. Accessed 23 March 2018, 09.55 WIB

[7] Atlas R M 2010*Handbook of microbiological media* 3rded (CRC Press: USA) p 1960

[8] Patil J H, M A Lourdu, A Raj and Gavimath C C 2011 Study on effect of pretreatment methods on biomethanation of water hyacinth *Intl. J. Adv. Biotech. Research.* **2** 143-147

[9] Osuagwu C G, O U Levi and A Pariatamby 2014 Bio-hydrogen production from food waste through anaerobic fermentation *Sains Malaysiana* **43** 1927-36

[10] Pincus D H 2005 Chapter 1 microbial identification using the Biomérieux VITEK® 2 system Biomérieux 32 p https://store.pda.org/tableofcontents/ermm_v2_ch01.pdf. Accesed 19 June 2018, 10.42 WIB

[11] Badan Standardisasi Nasional 2005 *Standar Nasional Indonesia: air dan air limbah-bagian 28: cara uji karbon organik total (TOC) (*Jakarta: BadanStandardisasiNasional) p 10

[12] Schumacher B A 2002 *Methods for the determination of total organic carbon (TOC) in soils and sediments* United States Environmental Protection Agency p 25

[13] Winter J U 1980 Glucose fermentation to methane and CO_2 by defined mixed culture *Zentralblattlntl. Bakteriol. Hyg., I AbteilungOri* **1** 293-305

[14] Cammen L M 1982 Effect of particle size on organic content and microbial abundance within four marine sediments *Marine Ecol. – Prog. Series* **9** 273-80

[15] Dworkin M, Falkow S, Rosenberg E, Schleifer K L, Stackedbrandt E 2006 The prokaryotes volume 4, a handbook on the biology of bacteria: bacteria: *Firmicutes, Cyanobacteria*. 3rd ed (USA: Springer) p 1182
[16] Maier R M, Pepper I, Gerba C, Gentry T 2008 *Environmental microbiology* 2nded (Academic Press:USA) p 624
[17] Lynch J M and Barbano D M 1999 Kjehdahl nitrogen analysis as reference method for protein determination in dairy products *J. AOAC Intl.* **82** 1389-98
[18] Chen Y, Cheng J J, Creamer K S 2008 Inhibition of anaerobic digestion process: a review *Bioresour. Tech.* **99** 4044-64
[19] Fotidis I A, Karakashev D, Kotsopoulos T A, Martzopoulos G G, Angelidaki I 2013 Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition *Fed. Eu. Microbiol. Soc. Microbiol. Ecology* **83** 38-48
[20] Wagner A O, Hohlbrugger P, Lins P and Illmer P 2012 Effects on different nitrogen sources on biogas production – a lab scale investigation *Microbiol. R.***167** 630-6
[21] BadanStandardisasiNasional 2013 *Standar nasional Indonesia: cara uji kadar nitrogen total sedimen dengan distilasi Kjeldahl secara titrasi* (Jakarta: Badan Standardisasi Nasional) p 19
[22] Bae H-S, Morrison E, Chanton J.P, Ogram A 2018 Methanogens are major contributors to nitrogen fixation in soils of the floridaeverglades *Appl. Environ. Microbiol* **84** p 17
[23] Wawrik B K, Kerkhof L, Kukor J and Zylstra G 2005 Effect of different carbon sources on community composition of bacterial enrichments from soil. *Appld. Envi. Microbiol.* **71** 6776-83
[24] Maishanu S M and Hussani H B N 1991 *Studies on factors affecting biogas generation from pistiastariote* University of Ilorin: 7-1
[25] Dioha I J, Ikeme C H, Nafi'u T, Soba N I, Yusuf M B S 2013 Effect of carbon to nitrogen ratio in biogas production. *Intl. R. J. Nat. Sci*. **1** 1-10
[26] Tanimu M I, Ghazi T I M, Harun R M, Idris A 2014 Effect of carbon to nitrogen ratio of food waste on biogas methane production in a batch mesophilic anaerobic digester *Intl. J. Innov. Mgmt. Tech.* **5** 116-9
[27] Kovaleva J, Degener J E, and Van der Mel H 2014 Taxonomic dissection of the genus *Micrococcus*: *Kocuria*gen.nov., *Nesterenkonia* gen. nov., *Kytococcus*gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend *J. Clinical. Microbiol.* **52** 1317-21
[28] Stackebrandt E, Koch C, Gvozdiak O, Schumann P 1995 *Intl. J. Sys. Bacteriol.* **45** 682-92

Acknowledgements
This study was supported by Agency for Assessment and Application of Technology (BPPT) and INSINAS 2017-2018 Ministry of Research, Technology and Higher Education.