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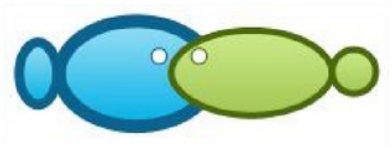

The significance of water quality parameters on the diversity of ammonia-oxidizing bacteria in the water surface of Musi river, Indonesia

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Abstract. This study was conducted in Musi river area to assess the extent of ammonia-oxidizing bacteria (AOB) diversity as affected by land-use activities. PCR-Terminal Restriction Fragment Length Polymorphism (PCR-TRFLP) analysis of 16S rRNA gene was used to evaluate the diversity of AOB. A total of 158 Terminal Restriction Fragments (TRFs) of AOB in different sizes digested with *BsuR*I and *Msp*I were obtained from all samples that were aligned to representative published sequences of 13 strains. The number of TRFs in each sample ranged from 52 to 491. There were eight strains of AOB results of digesting the two-restriction enzymes within the beta-subdivision ammonia-oxidizers namely *Nitrosomonas europaea, Nitrosomonas eutropha*, *Nitrosomonas ureae*, *Nitrosomonas europaea* strain C-31, *Nitrosospira briensis, Nitrosospira briensis* Nsp10, *Nitrosococcus mobilis Nc2*, and *Nitrosolobus multiformis,* its had the similarity coefficient in the range of 93-96%. The environmental conditions that affect the diversity of AOB are salinity, temperature, DO, and nutrients.
Key Words: AOB, diversity, Musi river, physicochemical, season, T-RFLP.

Introduction. Musi river is a large river with its drainage area covering three provinces in Sumatra Island of Indonesia, namely South Sumatra, Lampung, and Bengkulu, and with multi uses of its resources. At the downstream of Musi river, around the Palembang city, industries there are major activities with their waste products are discharged directly into Musi river. Those particular activities have negative ecological impact on the aquatic organisms including bacteria (Husnah et al 2008).

Rivers constitute the main land water resource for domestic, industrial and irrigation uses in many areas, and play an important role in hydrologic and biogeochemical cycles (Jian et al 2011). Rivers are highly vulnerable water bodies because of their role in carrying off and assimilating pollutants from both point sources (e.g., municipal wastewater and industrial discharge and non-point sources (e.g., agricultural and urban runoff, atmospheric deposition) (Carpenter et al 1998; Ouyang et al 2006). Municipal and industrial wastewater discharge constitutes a constant polluting source, whereas surface runoff is a seasonal phenomenon, largely affected by climate within the basin (Singh et al 2004). Seasonal variation in precipitation, surface runoff, interflow, groundwater flow and anthropogenic transfers have a strong effect on river discharge and, subsequently, on the concentration of pollutants in river water (Vega et al 1998). By identifying spatial and temporal patterns in river water quality, an improved understanding of the environmental conditions may help managers establish priorities for sustainable water management (Antonopoulos et al 2001).

The decreasing value of water obstructs the growth and the metabolism of aquatic organism. Nitrogen compounds, ammonia and nitrite, are the substances that decrease dissolved oxygen in water system, while the other substance such as nitrate potentially causes eutrophication (Tchobanoglous et al 2003). Those substances can be disposed

from water bodies by assimilation and dissimilation process (nitrification-denitrification) (Kirchman 2008).

Ammonia oxidation is the first, rate-limiting step in nitrification, the microbially mediated process in which ammonium is oxidized to nitrite and then to nitrate (Ward 2000). Thus, ammonia oxidation plays a critical role in the nitrogen cycle as the first and rate-limiting step in nitrification. This process is catalyzed by bacterial and archaeal microbial groups (Prosser & Nicol 2008). The community of ammonia-oxidizing bacteria might be affected by environmental factors such as temperature, salinity, pH, dissolved oxygen, ammonia concentration and organic carbon (Kim et al 2008a; Erguder et al 2009; Santos et al 2018).

Terminal restriction fragment length polymorphism (TRFLP) is a widely used by molecular technique for studying microbial community composition and diversity in waters environmental (Liu et al 1997; Zhang et al 2008). For TRFLP, PCR products or amplicons are obtained by using primers labeled with a fluorescent dye. Amplicons are digested with restriction enzymes, and the fragments generated are separated by high-resolution electrophoresis (DNA sequencer). The resulting fingerprint of the microbial community is the set of the lengths of all labeled terminal restriction fragments (TRFs). TRFLP analysis has been successfully applied for different targets, including 16S rRNA genes and genes of enzymes involved in specific metabolic processes, such as nitrogen fixation, nitrification, denitrification, or mercury resistance (Kitts 2001).

In this study, we analyze the diversity of AOB using PCR amplification and TRFLP analysis of 16S rRNA genes in the water surface of Musi river during rainy and dry seasons at sampling sites with different water quality, to evaluate the significance of the water quality parameters on the diversity of AOB, and understand their relationship.

Material and Method

Study area and sample collection. The study was conducted in Musi river area. Field samplings were done on rainy season in March, 2016 and dry season in August, 2016 at five sampling sites from the freshwater to seawater at high and low tides. The sampling sites were Gandus, Palembang, Upang, Sungsang, and Tg Carat. The description of these sampling sites and their respective land-use types have been described in the previous publication (Melki et al 2018). Three liters of water surface (~1 m depth) were collected by a water sampler (LaMotte, USA) from a small boat, transported to the laboratory in a cool box, and stored at -20°C.

Measurement of environmental parameters. The salinity, temperature, pH, dissolved oxygen (DO) were measured using a hand refractometer (ATAGO Co. Ltd, Tokyo, Japan), a thermometer (HI93510, Hanna Instruments Inc. USA), a pH meter (SM101, Milwaukee Instruments, Romania) and a waterproof portable dissolved oxygen and BOD meter (HI98193, Hanna Instruments Inc. USA), respectively. The concentrations of nutrients (ammonia, nitrite and nitrate) in the water surface were measured spectrophotometrically (APHA 2005).

The density of ammonium-oxidizing bacteria (AOB). The density of AOB was determined by the most probable number (MPN) method as described previously (Melki et al 2018).

DNA extraction. Each water sample collected was immediately passed through a 1.6 mm pore-sized to remove suspended particles and eukaryotes, and subsequently through a 0.22 µm millipore membrane (diameter 47 mm; MF-Millipore) to capture microbial cells. DNA was directly extracted by a modified phenol/chloroform protocol (Jinsheng et al 2011) and stored at -20°C until the use.

PCR amplification and TRFLP analysis. The 16S rRNA genes were PCR amplified using the primers: forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3'). The forward primer was fluorescently labeled at the 5' end with 56-FAM. PCR was performed in a total volume of 25 µL containing 12.5 µL master mix (Go Taq® Green Master Mix, Promega), 1 µL of each primer, 1 µL DNA

template (i.e. 1 ng of DNA per microliter) and 9.5 µL nuclease-free water. PCR amplifications were carried out in a T100 thermocycler (BioRad) with an initial denaturation at 95°C for 3 minute, followed by 30 cycles of 95°C for 30 second, 54°C for 30 second and 72°C for 1 minute followed by a final extension time at 72°C for 5 minute. The amplification of 16S rRNA genes was confirmed by running the amplification product in 1% agarose gel (Promega, USA) (Nithya & Pandian 2012).

The PCR product of each sample was split into two aliquots (10 µL), which were digested with 1 µL FastDigest enzymes (*BsuR*I and *Msp*I), 2 µL 10X FastDigest Buffer (Thermo Scientific) and 17 µL nuclease-free water in a total volume of 30 µL, incubate at 37°C in water thermostat for 5 minute. The length of the terminal-restriction fragment (T-RF) was determined on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). After that, size determination of the fluorescently labeled terminal restriction fragments (TRFs) was conducted using the software Peak Scanner v1.0, then the peak patterns were translated into a binary data matrix (presence vs absence) for further statistical analysis (Tymensen et al 2012; Winter et al 2013). TRFs with peak size between 50 to 500 bp and with a peak area ≥ 1% were only included in the analysis.

Statistical analysis. The chosen published sequences from 13 strains representing the ammonia-oxidizing bacteria (Head et al 1993; Purkhold et al 2000; Aakra et al 2001; Shinozaki & Fukui 2002) were performed by using a default database generated from NCBI (https://www.ncbi.nlm.nih.gov) then were cut in silico digestion with two restrictions (*BsuR*I and *Msp*I) enzymes by using software Bioedit v7.1.9 and were aligned with TRFs data. Similarities between samples were displayed by using software NTSYSPc v2.1. Principal component analysis (PCA) was used to correlate variations of the AOB density with the relation to environmental conditions. PCA has been tested by using software Xlstat.

Results

Environmental conditions and AOB density. The environmental conditions in the water surface of Musi river are depicted in Table 1. The salinity in the water surface ranged from 0 to 15 ppt, and the highest salinity was found at the Tg Carat site in dry season at high and low tides. The temperature in the water surface ranged from 29.07 to 31.51°C. The highest temperature was found at the Tg Carat site in dry season at high tide, and the lowest one was found at the Tg Carat site in rainy season at high tide. The pH in the water surface ranged from 4.69 to 8.33. The highest pH was found at the Gandus site in rainy season at low tide, and the lowest one was found Tg Carat site in rainy season at low tide. The dissolved oxygen (DO) in the water surface was found between 4.38 to 8.67 mg L^{-1}. The highest DO was found at the Gandus site in rainy season at high tide, and the lowest one was found at the Palembang site in rainy season at high tide.

The concentration of nutrients in both rainy and dry seasons in the water surface of Musi river varied significantly. The ammonia concentrations in the water surface ranged from 0.02 to 0.87 mg L^{-1}. The highest ammonia concentration was found at the Upang site (0.87 mg L^{-1}) in dry season at high tide, while the lowest one was found at the Gandus site (0.02 mg L^{-1}) in rainy season at high tide. The nitrite concentrations in the water surface ranged from 0.01 to 0.88 mg L^{-1}. The highest nitrite concentration was found at the Upang site (0.88 mg L^{-1}) in dry season at high tide. The nitrate concentrations in the water surface ranged from 0.02 to 2.09 mg L^{-1}. The highest and lowest levels of nitrate were found at the Palembang site (2.09 mg L^{-1}) in rainy season at low tide, and at the Gandus (0.02 mg L^{-1}) in dry season at low tide, respectively.

The density of AOB in the water surface of Musi river was detectable in all seasons (Table 1) in the range of $4.9x10^2$ to $9.4x10^2$ cells mL^{-1} during the rainy season. In this season, the high densities of AOB were found at the Gandus site at high tide, the Upang site at high and low tide, and the Tg Carat site at low tide. The lowest density of these bacteria was found at the Sungsang site at high tide. In the dry season, the density of AOB ranged from $4.9x10^2$ to $5.3x10^3$ cells mL^{-1}. The high densities of AOB were found at the Palembang site at low tide, and the Sungsang site at low tide. The lowest one was found at the Upang site at low tide.

Table 1

Environmental conditions and density of AOB at the water surface of sampling sites in the Musi river

Sites	Salinity (ppt)		Temperature (°C)		pH		DO (mg L^{-1})		Concentration (mg L^{-1})						Density of AOB (cells mL^{-1})	
									Ammonia		Nitrite		Nitrate			
	ht	lt	ht	lt	ht	lt	ht	lt	ht	lt	ht	lt	ht	lt	ht	lt
Rainy season																
Gandus	0	0	29.57	29.48	6.92	8.33	8.67	6.33	0.02	0.42	0.06	0.52	0.54	1.32	$9.4x10^2$	$8.4x10^2$
Palembang	0	0	29.26	29.74	5.79	7.01	4.38	5.89	0.07	0.20	0.03	1.00	1.99	2.09	$5.3x10^2$	$6.1x10^2$
Upang	0	0	29.53	29.92	5.65	5.68	6.06	7.42	0.44	0.22	0.06	0.12	1.42	1.87	$9.4x10^2$	$9.4x10^2$
Sungsang	0	0	29.48	29.70	7.70	5.56	7.80	7.69	0.21	0.19	0.01	0.09	0.92	0.65	$4.9x10^2$	$5.8x10^2$
Tg Carat	12	10	29.07	29.81	6.71	4.69	7.97	7.91	0.19	0.27	0.07	0.08	0.74	1.65	$8.4x10^2$	$9.4x10^2$
Dry season																
Gandus	0	0	31.35	30.96	6.83	8.26	6.53	6.57	0.19	0.04	0.43	0.02	1.66	0.02	$1.1x10^3$	$1.1x10^3$
Palembang	0	0	30.88	31.21	5.68	6.35	4.95	6.20	0.54	0.33	0.76	0.01	0.98	2.03	$1.1x10^3$	$5.3x10^3$
Upang	0	0	30.55	30.79	7.12	6.12	4.97	5.24	0.87	0.22	0.88	0.02	0.92	1.66	$5.3x10^2$	$4.9x10^2$
Sungsang	8	5	31.20	30.83	7.26	6.24	6.51	6.71	0.33	0.43	0.04	0.04	0.70	0.76	$1.1x10^3$	$5.3x10^3$
Tg Carat	15	15	31.51	31.27	7.84	7.01	7.86	7.33	0.55	0.30	0.70	0.05	2.04	0.63	$9.4x10^2$	$9.4x10^2$

ht = high tide; lt = low tide.

Terminal restriction fragments (TRFs) of AOB. Absence or presence of labeled terminal restriction fragments (TRFs) in community profiles were used to compare community composition. A total of 158 TRFs of AOB using the two restrictions enzymes (*BsuR*I and *Msp*I) of different sizes were obtained from all samples that aligned to the published sequences from representative 13 strains of AOB. The number of fragments in each sample ranged from 52 to 491. In rainy season there were a total of 58 fragments ranged from 52 to 491, and in dry season, a total of 100 fragments was found in the range from 52 to 491 (data not shown).

The restriction results using the two-restriction enzymes identified eight strains of AOB (Table 2), namely *Nitrosomonas europaea, Nitrosomonas eutropha*, *Nitrosomonas ureae*, *Nitrosomonas europaea* strain C-31, *Nitrosospira briensis, Nitrosospira briensis* Nsp10, *Nitrosococcus mobilis* Nc2, and *Nitrosolobus multiformis* which were within the beta-subdivision ammonia-oxidizers.

Table 2

TRFs absence of AOB were classified based on the NCBI library match by two different rectriction enzymes

Ribotype fingerprint		*Strain of AOB**	*Sites*
BsuR1	*Msp1*		
		Rainy season	
301	65	*Nitrosomonas eutropha*	Gandus at low tide
60	65	*Nitrosomonas eutropha*	Gandus at high tide, Palembang at high tide
207	488	*Nitrosospira briensis* Nsp10	Sungsang at low tide
		Dry season	
130	300	*Nitrosomonas ureae*	Gandus at high tide
219, 253	82, 486	*Nitrosococcus mobilis* Nc2	Gandus at high tide
400	486	*Nitrosomonas europaea*	Gandus at high tide, Palembang at high tide
220	191	*Nitrosolobus multiformis*	Gandus at high tide, Palembang at high tide
217	488	*Nitrosospira briensis*	Palembang at high tide
292	488	*Nitrosospira briensis* Nsp10	Palembang at low tide
219	82, 486	*Nitrosococcus mobilis* Nc2	Palembang at high tide
52	52	*Nitrosomonas europaea* C-31	Upang at low tide
253	82	*Nitrosococcus mobilis* Nc2	Sungsang at low tide
402	488	*Nitrosospira briensis*	Tg Carat at high tide
253	82	*Nitrosococcus mobilis* Nc2	Tg Carat at high tide
401	82	*Nitrosococcus mobilis* Nc2	Tg Carat at low tide

* The chosen published sequences from 13 strain to representing the AOB were performed by using a default database generated from NCBI (AF037106 *Nitrosomonas europaea,* NR_040879 *Nitrosomonas europaea* C-31, AY123795 *Nitrosomonas eutropha,* NR_114771 *Nitrosomonas eutropha* Nm 57, AF272418 *Nitrosomonas marina,* M96400 *Nitrosomonas* sp. C-56, AJ245757 *Nitrosomonas ureae,* M96396 *Nitrosospira briensis,* AJ298741 *Nitrosospira briensis* Nsp10, M96397 *Nitrosovibrio tenuis,* M96401 *Nitrosolobus multiformis,* AF037105 *Nitrosococcus mobilis* Nc2, DQ068704 Uncultured AOB clone CL1-3/C).

Similarity-based estimation of the AOB fragments. Analysis of AOB fragments by using *BsuR*1 restriction enzyme in the rainy season resulted in the highest similarity coefficient of 94.5% with *Nitrosomonas eutropha*. This similarity was found in the sample from St1 (Gandus site) at high tide. Other high similarities were also found to *Nitrosomonas europaea*, *N. ureae, N. eutropha* Nm 57, *Nitrosospira briensis* and *Nitrosolobus multiformis* in the sample from Sungsang site at low tide (Figure 1A). In the dry season, the highest similarity coefficient 95.5% was found to *Nitrosomonas europaea, N. ureae, N. eutropha* Nm 57, *Nitrosospira briensis* and *Nitrosolobus multiformis* in the sample of Gandus site at low tide (Figure 1B).

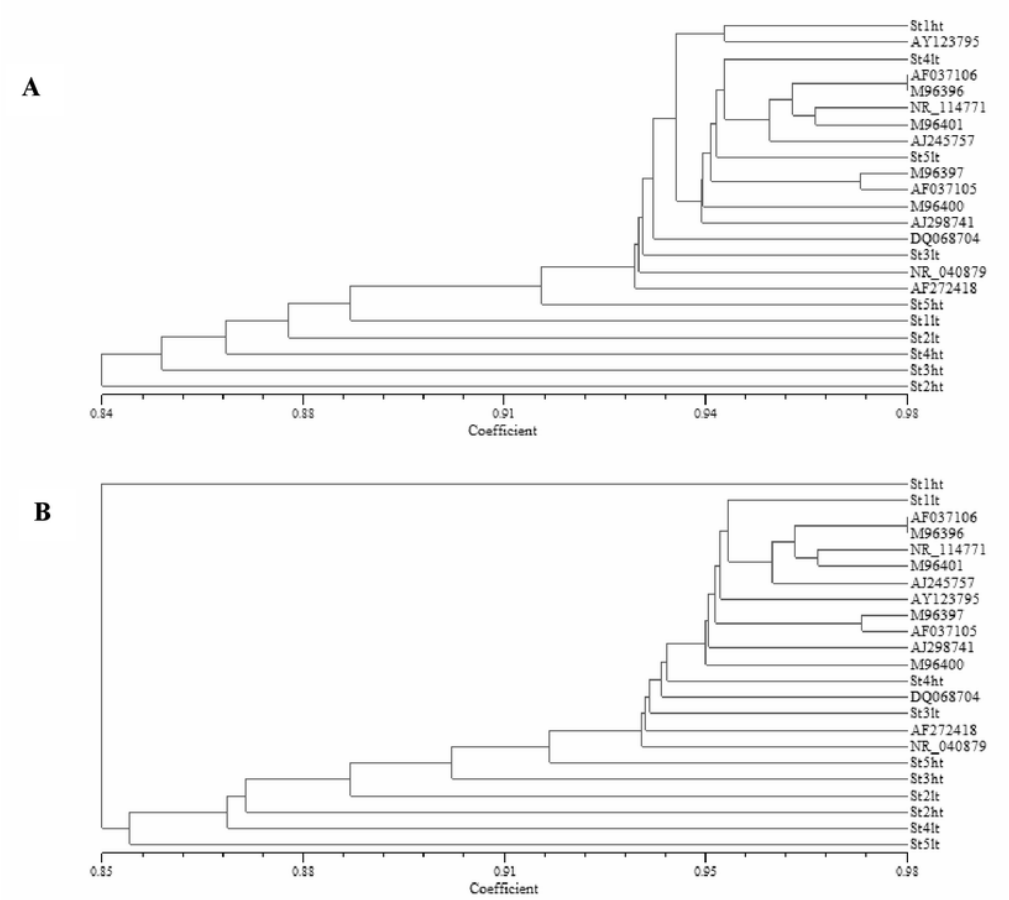

Figure 1. Dendrogram showing the similarity of AOB communities in the water surface of Musi river, revealed by T-RFLP analysis using *BsuR*1 restriction enzyme (A - rainy season; B - dry season; St1 - Gandus; St2 - Palembang; St3 - Upang; St4 - Sungsang; St5 - Tg Carat; ht - high tide; lt - low tide).

Furthermore, similarity-based estimation of AOB fragments by using *Msp*I restriction enzyme in the rainy season resulted in the highest similarity coefficient of 92.5% with 13 strains of AOB (*Nitrosomonas europaea, N. europaea* C-31, *N. eutropha, N. eutropha* Nm 57, *N. marina, N.* sp. C-56, *N. ureae, Nitrosospira briensis, N. briensis* Nsp10, *Nitrosovibrio tenuis, Nitrosolobus multiformis, Nitrosococcus mobilis* Nc2, Uncultured AOB clone CL1-3/C) found in the sample from St2 (Palembang site) at low tide (Figure 2A). Similar results were obtained in the dry season, the highest similarity coefficient of 93% was found to 13 strains of AOB (*Nitrosomonas europaea, N. europaea* C-31, *N. eutropha, N. eutropha* Nm 57, *N. marina, N.* sp. C-56, *N. ureae, Nitrosospira briensis, N. briensis* Nsp10, *Nitrosovibrio tenuis, Nitrosolobus multiformis, Nitrosococcus mobilis* Nc2, Uncultured AOB clone CL1-3/C) in the sample of St3 (Upang site) at low tide (Figure 2B).

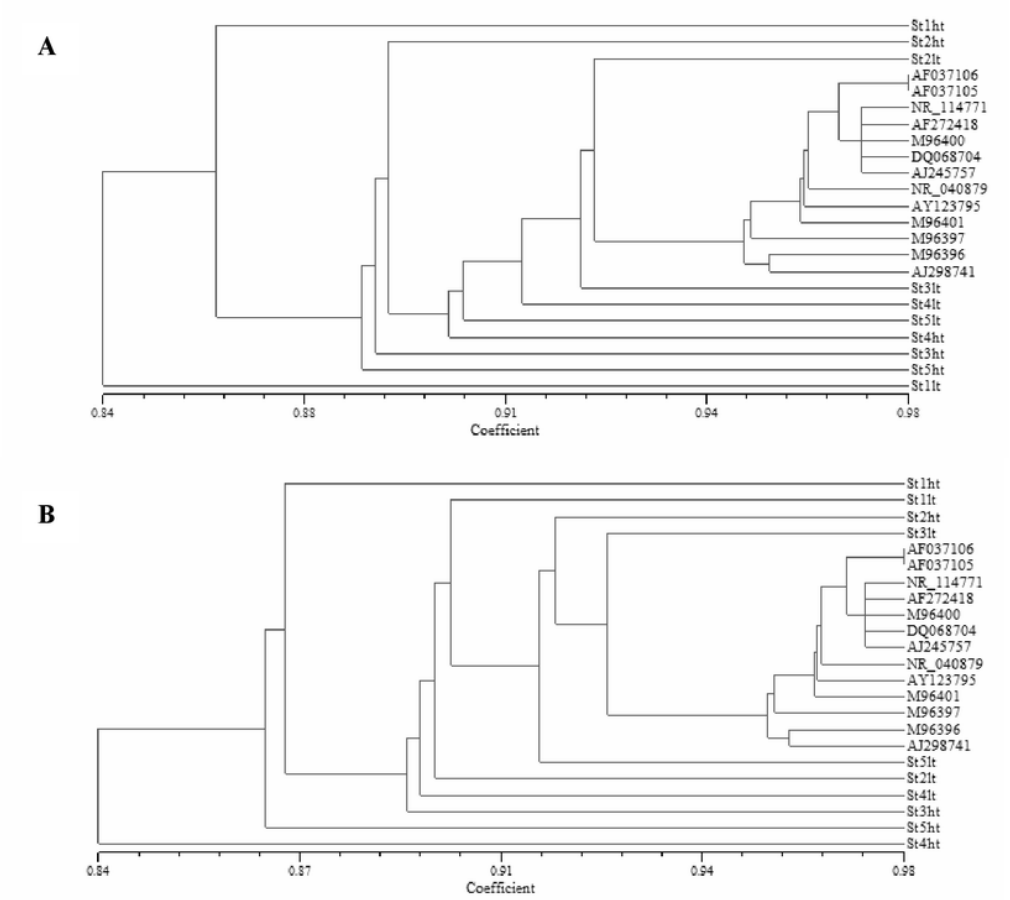

Figure 2. Dendrogram showing the similarity of AOB communities in the water surface of Musi river, revealed by T-RFLP analysis using *Msp*1 restriction enzyme (A - rainy season; B - dry season; St1 - Gandus; St2 - Palembang; St3 - Upang; St4 - Sungsang; St5 - Tg Carat; ht - high tide; lt - low tide).

Correlation of the environmental conditions and AOB density. The principal component analysis (PCA) on the correlation matrix of the environmental conditions and AOB density in the water surface of Musi river in rainy season indicated that the cumulative eigenvalue was 68.28% and squared minimum was 0.5 from F1 and F2 axis (Figure 3A). The variability at the F1 axis (35.87%) and F2 axis (32.41%) showed that there were two groups. The first group consisted of the Tg Carat site related to salinity at high and low tides (10 ppt and 12 ppt), DO at low tide (7.91 mg L^{-1}), and this environmental condition contributed to the density of AOB between $8.4x10^2$ cells mL^{-1} at high tide and $9.4x10^2$ cells mL^{-1} at low tide. The second group was the Upang site related to temperature at low tide (29.92°C), nitrate at high and low tides (1.42 mg L^{-1} and 1.87 mg L^{-1}), and this environmental condition contributed to the AOB density of $9.4x10^2$ cells mL^{-1} at high and low tides.

At dry season, the cumulative eigenvalues was 74.20% and squared minimum was 0.5 from F1 and F2 axis (Figure 3B). The variability at the F1 axis (51.94%) and F2 axis (22.26%) showed that there were two groups. The first group consisted of the Tg Carat site related to salinity at high and low tides (15 ppt), DO at high and low tides (7.86 mg L^{-1} and 7.33 mg L^{-1}), temperature at high tide (31.51°C), pH at high tide (7.84), nitrite at low tide (0.05 mg L^{-1}), nitrate at high tide (2.04 mg L^{-1}), and this

environmental condition contributed to the AOB density of $9.4x10^2$ cells mL^{-1} at high and low tides.

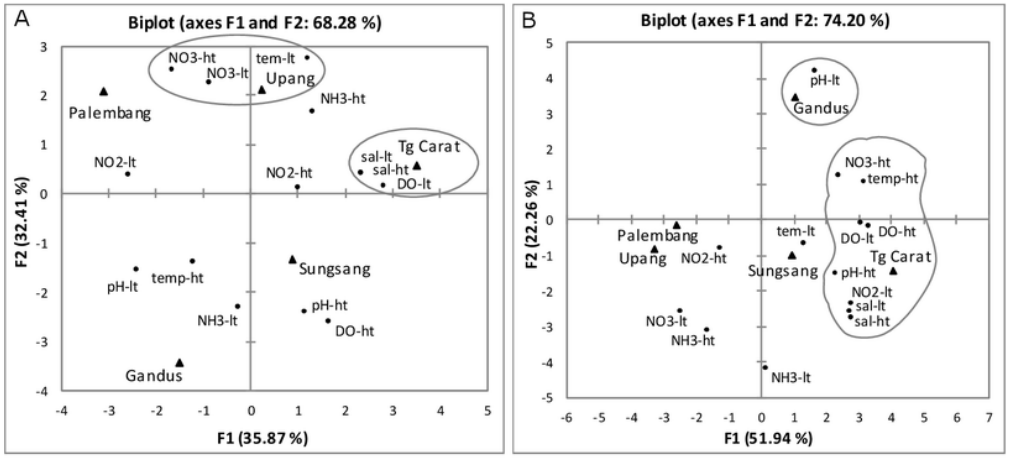

Figure 3. PCA of the relationship between environmental conditions and AOB density in the water surface of Musi river (A - rainy season; B - dry season; sal - salinity; temp - temperature; DO - dissolved oxygen; NH3 - ammonia; NO2 - nitrite; NO3 - nitrate; ht - high tide; lt - low tide).

Discussion. The present study was the first attempt to explore the unculturable bacterial community of ammonia-oxidizing bacteria (AOB) in the water surface of of Musi river. By using the restriction enzyme of *Msp*I, the number of AOB fragments generated by PCR-TRFLP was higher comparing to that from *BsuR*I enzyme. Within dry season, the number of AOB fragments was higher than that of in rainy season. The results indicated that the estimation of the bacterial community in a particular environment would not be affected by the restriction enzyme used. The use of different restriction enzymes invariably resulted in the same diversity index (Dunbar et al 2000). The biases occurred during DNA extraction and PCR amplification, so they did not explore the actual bacterial abundance in a particular environment (Frey et al 2006).

The two-restriction enzymes absence/presence of AOB have indicated eight species of AOB, there were *Nitrosomonas europaea, Nitrosomonas eutropha*, *Nitrosomonas ureae*, *Nitrosomonas europaea* strain C-31, *Nitrosospira briensis, Nitrosospira briensis* Nsp10, *Nitrosococcus mobilis* Nc2, and *Nitrosolobus multiformis* which were within the beta-subdivision ammonia-oxidizers. Genera *Nitrosomonas, Nitrosospira,* and *Nitrosococcus* were dominant in the water surface of Musi river. Li et al (2015) reported that *Nitrosomonas* spp. are dominant in the AOB community in the Colne Estuary, United Kingdom. In a study of the three aquatic systems studied (two lakes: Plußsee, Schöhsee, and the Baltic Sea) *Nitrosomonas* spp. are also found to be dominant over *Nitrosospira* spp. (Kim et al 2008b). Further, *Nitrosomonas* and *Nitrosospira* are found dominant in intertidal sediments of the Yangtze Estuary (Zheng et al 2014). *Nitrosomonas* and *Nitrosospira* are also dominant in estuary ecosystem (Santos et al 2018).

The similarity coefficient of AOB fragments by using *BsuR*1 and *Msp*1 restriction enzymes resulted ranged from 92.5% to 95.5% with the published sequences from 13 strains chosen to represent the ammonia-oxidizing bacteria (Head et al 1993; Purkhold et al 2000; Aakra et al 2001; Shinozaki & Fukui 2002).

The density of AOB was determined by the most probable number (MPN) method, the dry season had much higher density than rainy season. The density of AOB during rainy and dry season ranged from $4.9x10^2$ to $5.3x10^3$ cells mL^{-1}. These densities are comparable with the AOB density in seawater samples from the Ariake Sea ranged from $3.488x10^2$ to $4.781x10^3$ cells mL^{-1} (Isnansetyo et al 2014). The density of AOB in the

Musi river using the culture-dependent method give the lower density at several orders of magnitude than that detected by molecular techniques.

Environmental conditions in sampling locations of Musi river did not fluctuate significantly. The salinity in this study showed a typical characteristic range of estuarine salinity (0 to 15 ppt). Salinity is considered important in controlling the abundance and community structure of ammonia oxidizers bacteria (Li et al 2015) and nitrification rates (Rysgaard et al 1999). Ammonia oxidizers bacteria can adjust and tolerance to salinity gradients ranged from 0 to 30 ppt (Bernhard et al 2007; Santos et al 2018). The water surface of temperature in the Musi river waters during in the rainy and dry season was between 29.07 to 31.51^{o}C and pH ranged from 4.69 to 8.33. This temperature and pH is likely to be appropriate conditions for nitrification that supported by this ammonia oxidation. Kim et al (2008a) reported that the maximum ammonia and nitrite oxidation rate increases significantly with the increasing temperature ranged from 10 to 30^{o}C and pH ranged from 7.5 to 8.1.

In this study, dissolved oxygen ranged from 4.38 to 8.67 mg L^{-1} and ammonia concentrations ranged from 0.02 to 0.87 mg L^{-1} significantly affected the density of AOB in the water surface of Musi river. The previous publication also reported that high concentrations of ammonia partially inhibited the activity of ammonia-oxidizing bacteria (Melki et al 2018). These results might be vary amng the groups of AOB because the each AOB group possess differences ability in adaptation to the limitation of ammonia and oxygen concentrations (Geets et al 2006).

Conclusions. The environmental conditions that affects the diversity of AOB in the water surface of Musi river area either in rainy or in dry seasons are salinity, temperature, DO, and nutrients. The community profiles analysis indicates that the higher number of fragments is found in the dry season with 100 of the average number of fragments, while lower number of fragments is found in the rainy season with 58 of the average number of fragments. Restriction analysis by using *BsuR*I and *Msp*I enzymes reveals that there are eight the most predominant species of the beta-subdivision ammonia-oxidizers in the Musi river.

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References

Aakra A., Utaker J. B., Pommerening-Röser A., Koops H. P., Nes I. F., 2001 Detailed phylogeny of ammonia-oxidizing bacteria determined by rDNA sequences and DNA homology values. International Journal of Systematic and Evolutionary Microbiology 51:2021-2030.

Antonopoulos V. Z., Papamichail D. M., Mitsiou K. A., 2001 Statistical and trend analysis of water quality and quantity data for the Strymon River in Greece. Hydrology and Earth System Sciences 5:679-692.

APHA (American Public Health Association), 2005 Standard methods for the examination of water and wastewater. 20th edition, Washington, DC, USA.

Bernhard A. E., Tucker J., Giblin A. E., Stahl D. A., 2007 Functionally distinct communities of ammonia-oxidizing bacteria along an estuarine salinity gradient. Environmental Microbiology 9(6):1439–1447.

Carpenter S. R., Caraco N. F., Correll D. L., Howarth R. W., Sharpley A. N., Smith V. H., 1998 Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8:559-568.

Dunbar J., Ticknor L. O., Kuske C. R., 2000 Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology 66(7):2943–2950.

Erguder T. H., Boon N., Wittebolle L., Marzorati M., Verstraete W., 2009 Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiology Reviews 33:855-869.

Frey J. C., Angert E. R., Pell A. N., 2006 Assessment of biases associated with profiling simple, model communities using terminal-restriction fragment length polymorphism-based analyses. Journal of Microbiological Methods 67:9-19.
Geets J., Boon N., Verstraete W., 2006 Strategies of aerobic ammonia-oxidizing bacteria for coping with nutrient and oxygen fluctuations. FEMS Microbiology Ecology 58:1-13.
Head I. M., Hiorns W. D., Embley T. M., McCarthy A. J., Saunders J. R., 1993 The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. Journal of General Microbiology 139:1147-1153.
Husnah, Prianto E., Makri, Dahlan H. Z., 2008 Fish community structure in relation to water quality of the downstream of Musi river, South Sumatera, Indonesia. Indonesian Fisheries Research Journal 14:51-65.
Isnansetyo A., Getsu S., Seguchi M., Koriyama M., 2014 Independent effects of temperature, salinity, ammonium concentration and pH on nitrification rate of the Ariake seawater above mud sediment. HAYATI Journal of Biosciences 21:21-30.
Jian Z., Guo F., Kun L., Yanwu L., Xiaomin L., Juan L., Jun Q., Hongjin T., Ying Z., 2011 Assessment of spatial-temporal patterns of surface water quality in the Min River (China) and implications for management. In: International Conference on Computer Distributed Control and Intelligent Environmental Monitoring, IEEE, pp. 1983-1989.
Jinsheng S., Fei G., Xuyun G., Junli W., Xiang L., Jingjing L., 2011 Seasonal changes and diversity of bacteria in Bohai Bay by RFLP analysis of PCR-amplified 16S rDNA gene fragments. World Journal of Microbiology and Biotechnology 27:275-284.
Kim J. H., Guo X., Park H. S., 2008a Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation. Process Biochemistry 43:154-160.
Kim O. S., Junier P., Imhoff J. F., Witzel K. P., 2008b Comparative analysis of ammonia monooxygenase (*amo*A) genes in the water column and sediment-water interface of two lakes and the Baltic Sea. FEMS Microbiology Ecology 66:367-378.
Kirchman D. L., 2008 Introduction and overview. In: Microbial ecology of the oceans. Kirchman D. L. (ed), John Wiley and Sons, Inc., New Jersey, pp. 1-26.
Kitts C. L., 2001 Terminal restriction fragment patterns: a tool for comparing microbial communities and assessing community dynamics. Current Issues in Intestinal Microbiology 2:17-25.
Li J., Nedwell D. B., Beddow J., Dumbrell A. J., McKew B. A., Thorpe E. L., Whitby C., 2015 *amo*A gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria and not archaea dominate N cycling in the Colne Estuary, United Kingdom. Applied and Environmental Microbiology 81(1):159-165.
Liu W. T., Marsh T. L., Cheng H., Forney L. J., 1997 Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology 63:4516-4522.
Melki, Isnansetyo A., Widada J., Murwantoko, 2018 Distribution of ammonium-oxidizing bacteria in sediment with relation to water quality at the Musi River, Indonesia. HAYATI Journal of Biosciences 25(4):198-205.
Nithya C., Pandian S. K., 2012 Evaluation of bacterial diversity in Palk Bay sediments using terminal-restriction fragment length polymorphisms (T-RFLP). Applied Biochemistry and Biotechnology 167:1763-1777.
Ouyang Y., Nkedi-Kizza P., Wu Q. T., Shinde D., Huang C. H., 2006 Assessment of seasonal variations in surface water quality. Water Research 40:3800-3810.
Prosser J. I., Nicol G. W., 2008 Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environmental Microbiology 10:2931-2941.
Purkhold U., Pommerening-Röser A., Juretschko S., Schmid M. C., Koops H. P., Wagner M., 2000 Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amo*A sequence analysis: implications for molecular diversity surveys. Applied and Environmental Microbiology 66:5368-5382.

Rysgaard S., Thastum P., Dalsgaard T., Christensen P. B., Sloth N. P., 1999 Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 22:21–30.
Santos J. P., Mendes D., Monteiro M., Ribeiro H., Baptista M. S., Borges M. T., Magalhães C., 2018 Salinity impact on ammonia oxidizers activity and *amo*A expression in estuarine sediments. Estuarine, Coastal and Shelf Science 211:177-187.
Shinozaki H., Fukui M., 2002 Comparison of 16S rRNA, ammonia monooxygenase subunit A and hydroxylamine oxidoreductase gene, in chemolithotrophic ammonia-oxidizing bacteria. Journal of General and Applied Microbiology 48:173-176.
Singh K. P., Malik A., Mohan D., Sinha S., 2004 Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India) - a case study. Water Research 38:3980-3992.
Tchobanoglous G., Burton F. L., Stensel H. D., 2003 Wastewater engineering: treatment and reuse. 4th edition, Metcalf & Eddy, Inc., China.
Tymensen L., Barkley C., McAllister T. A., 2012 Relative diversity and community structure analysis of rumen protozoa according to T-RFLP and microscopic methods. Journal of Microbiological Methods 88:1-6.
Vega M., Pardo R., Barrado E., Debán L., 1998 Assessment of seasonal and polluting effects on the quality of river water by exploratory data analysis. Water Research 32:3581-3592.
Ward B. B., 2000 Nitrification and the marine nitrogen cycle. In: Microbial ecology of the oceans. Kirchman D. L. (ed), Wiley-Liss, New York, pp. 427-454.
Winter C., Matthews B., Suttle C. A., 2013 Effects of environmental variation and spatial distance on bacteria, archaea and viruses in sub-polar and arctic waters. The ISME Journal 7:1507-1518 .
Zhang R., Thiyagarajan V., Qian P. Y., 2008 Evaluation of terminal-restriction fragment length polymorphism analysis in contrasting marine environments. FEMS Microbiology Ecology 65:169-178.
Zheng Y., Hou L., Newell S., Liu M., Zhou J., Zhao H., You L., Cheng X., 2014 Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze Estuary. Applied and Environmental Microbiology 80(1):408-419.

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