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Identification of denitrifying bacteria from sediments of Rawa Jombor waters, Central Java and its trophic status

SUNARTO♥, RATNA SETYANINGSIH, ANDRI YANTI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./Fax.: +62-271-663375. ♥email: rm.sunarto @ yahoo.com

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Abstract. *Sunarto, Setyaningsih R, Yanti A. 2016. Identification of denitrifying bacteria from sediments of Rawa Jombor waters, Central Java and its trophic status. Biodiversitas 17: 578-584.* Pooled freshwater (lentic) is susceptible to contamination. One of pollution contributors on freshwater is the agricultural sector. One of them is the inclusion of waste in the form of organic and inorganic materials. The waste will increase the nutrient in waters causing sedimentation, eutrophication and pollution. The pollution is from nitrates derived from agricultural wastes using inorganic fertilizers. An alternative solution for the prevention and tackling of it is to utilize aquatic microorganisms namely denitrifying bacteria. These bacteria can convert nitrates into nitrogen gas (N_2) in the anaerobic state so that it can handle the pollution of nitrate in water. This study aimsed to identify the denitrifying bacteria isolated from aquatic sediments of Rawa Jombor and to determine trophic status of Rawa Jombor waters. Identification of denitrifying bacteria was done through the morphology and physiology testing phase and also molecular analysis of 16S rRNA gene sequence. Based on parameters of total nitrogen, phosphor and brightness referring to the criteria of the lake trophic status attached to Ministry of Environment (PerMNLH) No. 28 2009, Rawa Jombor aquatic trophic status was analyzed. The results showed that as many as 6 denitrifying bacteria were isolated from sediments in Rawa Jombor waters, Klaten. Characteristics of bacteria colonies were translucent, round in shape, Gram-negative, rod and motile shaped cells. Based on the analysis of 16S rRNA gene sequence, all of denitrifying bacteria isolates were identified as having the highest similarity to the *Shewanella* genus. TmD isolate was identified as *Shewanella putrefaciens*, while TmE, TmG and TmI isolates were identified as *Shewanella* genus and TmA isolates was new species. Trophic status of Rawa Jombor aquatic was hypereutrophication.

Keywords: denitrifying bacteria, sediment, Rawa Jombor, nitrate, 16S rRNA gene.

INTRODUCTION

Rawa Jombor is a semi-artificial lake in the Dutch period of almost 12.7 km^2 in width and is located in the village of Krakitan, Bayat Sub-district, Klaten District, Central Java, Indonesia. Rawa Jombor has extensive pool of 180 hectares surrounded by a ring road, drainage channels, hills, trees, residential and some paddy fields. Rawa Jombor is used as a source of irrigation for the east area since 1967, as the *keramba* (floating net) fishery activity since 1986 and as the floating food stall business since 1998 (Ganjarsari 2008).

Rawa Jombor is a pooled freshwater (lentic) which is a form of aquatic ecosystems where flow/stream of water does not play an important role. Problem often occurred in the waters of Rawa Jombor is water pollution due to the inclusion of pollutant sources coming from sewage of floating food stalls, from the tourists and from *keramba* fishery as well as from agricultural waste and domestic waste around the waters. The high contamination of organic and inorganic wastes increases the content of nitrogen compounds that are harmful to aquatic organisms such as nitrate, nitrite, and ammonia. Nitrate is a form of nitrogen compounds and one of essential elements for protein plants synthesis. In high concentrations, nitrates can stimulate an unlimited growth of phytoplankton if some conditions, such as phosphorus concentration, can be met. Nitrate levels of more than 0.2 mg/L may result in eutrophication of waters which can stimulate the growth of algae and aquatic plants rapidly or blooming (Effendi 2003). Eutrophication is the enrichment of water due to the presence of nitrogen and phosphorus that are badly needed by plants and can causes an increase in the waters primary productivity (Mason 1993). In addition, high nitrate in waters can also cause decrease of water quality such as lowering of dissolved oxygen, fertilizing waters.

One attempt to control high nitrate compounds in the water is by utilizing the activity of denitrifying bacteria. Denitrifying bacteria can reduce nitrate to nitrogen gas so as to reduce nitrate levels in water. Denitrifying bacteria is a group of nitrate reducing bacteria. These bacteria are heterotrophic; require an organic carbon source such as acetic acid, propionic acid, succinic acid, glycerol and glucose for growth (Teixeira and Oliveira 2002). The ideal denitrifying bacteria in the process of nitrate control are the bacteria that produce N_2 gas as their end product.

Denitrifying bacteria lives well on the environment having relatively low oxygen content. According to Teixeira and Olivera (2002), denitrifying bacteria is anaerobic facultative or anaerobic obligate. In a system of freshwater environment, groups of denitrifying bacteria can live well on a waters base region or sediment. The oxygen content in the sediment is relatively low and at night, it can reach 0 ppm.

This study aimed to identify the denitrifying bacteria using the gene coding of 16S rRNA and to test the trophic status of Rawa Jombor waters based on environmental parameters such as total nitrogen, total phosphor and brightness. Trophic status needs to be tested in order to know the quality of the water and its allocation level.

MATERIALS AND METHODS

Sampling and analysis of water quality

The study was conducted on July till November 2013, in the waters of Rawa Jombor, Bayat, Klaten, Central Java, Indonesia. Samples of sediment and water were taken in five sampling points, namely: inlet, middle, outlet, western part and eastern part of floating food stalls (Figure 1). The analysis of water samples were conducted at the Central Laboratory of Sciences, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. As the parameters of environment, the sediment and water of Rawa Jombor waters were measured. In the sediment, parameters measured were pH, nitrate level, nitrite level, and ammonia level. In water, the parameters measured were pH, brightness level, nitrate level, nitrite level, total nitrogen (N) and total phosphor (P). Parameters of pH and brightness were measured on site. The pH was measured using pH meter; the electrode, which was previously calibrated with distilled water, was dipped into the sample. Brightness was measured with Secchi disk; it was a disc with a rope in the middle. Secchi disk was inserted into the water, if the disc became invisible, the rope was marked and if the disc became visible again when it was pulled upward, the rope was marked too. Brightness values can be determined by measuring the distance of the disc visibility from invisible into visible again. While the parameters of nitrate level, nitrite level, total N and total P were tested at the Center for Environmental Engineering and Contagious Disease Control (BBTKL PP) Yogyakarta, Indonesia.

Isolation and characterization of denitrifying bacteria

The medium used for the isolation of denitrifying bacteria was denitrification liquid and solid media consisting of $CH_3COOHNa.3H_2O$, K_2HPO_4, KH_2PO_4, NH_4Cl, $MgSO_4.7H_2O$, yeast extract, $NaNO_3$, distilled water, and agar were added for solid media (Setyaningsih et al. 2013) and also nitrient agar (NA) media. Isolation of denitrifying bacteria was carried out using a liquid denitrification medium and was added by nitrogen gas. To isolate bacteria which were fermentative negative then the molecular test was carried out for identification process using the gene coding for 16S rRNA.

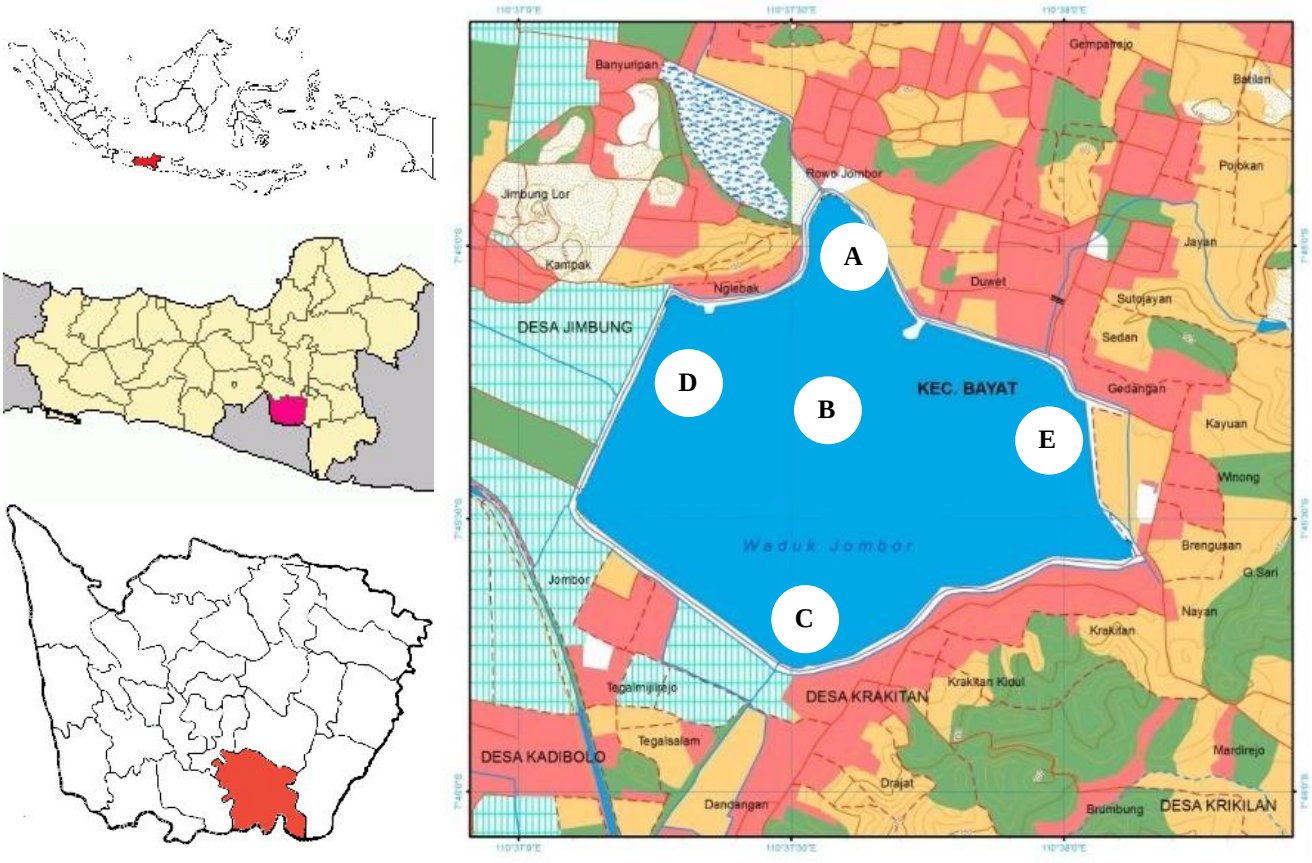

Figure 1. The study site in Rawa Jombor, Bayat, Klaten, Central Java, Indonesia. A. inlet, B. middle, C. outlet, D. western part and D. eastern part of floating food stalls

Sediment samples were put into sterile glass bottles of 100 mL for as much as 20 grams and mixed with 80 mL of liquid denitrification medium. Anaerobic condition was created by removing the oxygen in the bottle, by inserting nitrogen gas at a pressure of ± 250 kPa, via syringe aseptically into the bottle for 15 minutes. Then the mixture was homogenized in an orbital shaker (130 rpm, 28-30^{o}C) for 5 days (Widiyanto et al. 2008). With inoculating needle, 1 loopful supernatant was streaked onto Petri dishes containing solid denitrification media with quadrant method and was incubated at a temperature of 28^{o}C for ± 2 to 3 days in an incubator (Waluyo 2008). The colonies which were grown separately were streaked and incubated again on NA medium with the same incubation conditions for purification. The obtained pure isolates were stored on oblique NA media as cultured stocks.

Nitrate reduction test

The next selection phase was to test the nitrate reduction which aimed to select bacteria that could reduce nitrate to nitrite. Nitrate reduction test was performed in the Laboratory of Microbiology Health Laboratory (BLK) Yogyakarta. Bacterial isolates were inoculated into the medium of nitrate broth (DifcoTM) and were incubated for 24 h at 37 ° C in an incubator. The bacterial cultures were dripped with 2-3 drops of solution A, and then were dripped again with 2-3 drops of solution B. If nitrite were found in the tested cultures, it would be marked by the formation of red which meant that nitrate was reduced into nitrite (positive test). If there were no visible change in color within 3-5 minutes, a bit of zinc powder would be added into the culture. If red color were formed, it would mean that the test was negative, whereas if there were no color change, it would mean that the test was positive (Hanum 2005).

Oxidative/fermentative (O/F) test

The last selection stage was the oxidative-fermentative test (OF) which aimed to select bacteria isolates that were fermentative. Oxidative/fermentative test was performed to select oxidative bacteria since most denitrifying bacteria were generally oxidative and also to distinguish the fermentative bacteria which reduce $NO_3^-$to NH_4^+. The medium used was O/F media (Hugh and Leifson 1953). A total of 5 mL of medium was poured into a 12 mL test tube with a screw lid, then bacteria isolates were inoculated by a puncture. On culture, liquid paraffin was poured into to create anaerobic conditions. Incubation was performed at room temperature for 1-2 days. Group of fermentative bacteria produced acids so that green medium changed to yellow.

Characterization of morphology and nature of bacteria Gram

The characterization of colonies and cells were the observation of cell morphology and colony morphology of denitrifying bacteria. Observation of cell morphology included Gram staining and observation of the bacteria cell shape. Observations on colony morphology included colony color observations, observation on the form and on the edge of colony. Gram staining was performed by passing an object glass over a Bunsen flame. Then 1 drop of sterile physiological saline was dripped on the object glass and continued by a drop of pure isolates. Isolates and the physiological saline were spread out evenly and then dried up and a fixation was carried out on it. 1-2 drops of Gram **A** was dripped on its surface and left for 30-60 seconds. Preparation was washed with flowing water, and then was dried up. 1-2 drops of Gram B solution was dripped onto the surface of preparations, left for 30-60 seconds, washed with flowing water and dried up. Furthermore, Gram C (96% alcohol) was dropped on the preparation for as much as 1-2 drops and left for 30 seconds, then it was dripped with Gram D of 1-2 drops and left for 30-60 seconds. Finally, preparation was washed with flowing water and was observed under a microscope to find out the microscopic character of the bacteria in the form of Gram reaction and cell shape of bacteria (Waluyo 2008). If the results of the bacteria cell staining were red, it would mean that the cells are Gram-negative, while if the color were purple, it would indicate that the nature was Gram-positive.

Identification of denitrifying bacteria using sequences gene coding for16S rRNA

Bacteria DNA extraction

Prior to the extraction of DNA, bacteria isolates were cultured in Luria Bertani media (LB) for 24 hours on an orbital shaker (130 rpm, 37 ° C). DNA extraction of 6 denitrifying bacteria isolates from aquatic sediments of Rawa Jombor, Klaten used geneJET genomic DNA purification kit (Fermentas). One mL of liquid culture was poured into 1.5 mL eppendorf tube and was centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and the pellet was added with 180 µL of digestion solution and 20 µL of proteinase K, and then was mixed using a vortex. The mixture was incubated in an incubator shaker 56^{o}C for 30 minutes with a speed of 150 rpm. After incubation 20 µL of RNase solution was added to the previous solution and was mixed using a vortex before it was incubated at 37 ° C for 10 minutes. After the second phase incubation 200 µL of lysis solution was added to it and was mixed using a vortex for 5 seconds until homogeneous. After it was homogeneous, the solution was added with 400 µL of 50% ethanol and was mixed using a vortex. Supernatant was poured in geneJet tube which was equipped with a flow-through tube, and then it was centrifuged for 1 minute at a speed of 6000 rpm. Furthermore, the liquid inside the flow-through tube was removed and the flow-through tube was reassembled. A total of 500 µL of washing buffer I was added and the solution was centrifuged for 1 minute at a speed of 8000 rpm. The residue (liquid) inside the flow-through tube was removed and reassembled. A total of 500 µL of washing buffer II was added and was re-centrifuged for 3 minutes at a speed of 12,000 rpm. If the residue (liquid) were still present, the flow-through tube could be emptied and was centrifuged again for 1 minute at a speed

of 12,000 rpm. Furthermore, the flow-through tube was removed and replaced with a new 1.5 mL eppendorf. The next phase 200 µL of elution buffer was added, and then was incubated for 2 minutes at room temperature, and then was centrifuged again for 1 minute at a speed of 8000 rpm. At the last phase, the genJet tube was removed and the supernatant which was left inside eppendorf tubes (microtube) was genomic DNA. Results of the extraction or isolation of genomic DNA was visualized on a 0.8% agarose gel and was stored at-21 ° C.

Amplification of 16S rRNA Gene

Amplification was performed with universal primers for group of bacteria namely primer 63F (5'CAG GCC CAC TAA GTC ATG CAA) and 1387R (WTG 5'GGG GTA CAA CGG GGC) (Marchesi et al. 1998). The reaction mixture consists of: 18.8 µL ddH2O, 10x 2.5 µL dream taq buffer, 0.5 µL dNTP, 0.5 µL of each primer, 0.2 µL DNA polymerase (Fermentas) dream taq and 1 µL DNA samples (DNA Template), so that the total volume was 25 µL. PCR conditions took place in the following stages: pre-PCR for 5 minutes at a temperature of 95° C, denaturation for 30 seconds at 94° C, annealing for 30 second at 55^{o} C, elongation for 30 seconds at 72^{o} C, post PCR for 7 minutes at 72^{o} C and storage at 4° C. The PCR process lasted for 30 cycles. PCR products were visualized on 0.8% agarose gel (Salupi 2011). DNA amplification product was then sequenced to obtain sequences of DNA isolates of denitrifying bacteria isolated from aquatic sediments of Rawa Jombor, Klaten. The purification and sequencing of rRNA 16S genes were conducted by PT Genetika Science Indonesia-1st Base, Singapore. The base sequence of the sequencing results were then compared to sequences in the data bank of the National Center for Biotechnology Information (NCBI) using the program of Basic Local Alignment Search Tool for Nucleotides (BlastN) (http:// www.ncbi.nlm.nih.gov/BLAST/).

The test of trophic status

In this study, water samples were taken from waters of Rawa Jombor at 5 locations of sampling namely; inlet, middle, outlet, western and eastern of floating food stalls. The parameters tested to determine the trophic status in these waters were the total nitrogen, total phosphorus and brightness. The results obtained were in accordance to the Regulation of the Minister of Environment No. 28 of 2009 on Water Pollution Load Capacity Lake and/or reservoir.

RESULTS AND DISCUSSION

The condition of water quality of sampling sites

The observation of water quality in aquatic sediments in Rawa Jombor of five different sampling locations was presented in Table 1. In general, the sediment in the waters of Rawa Jombor has a neutral pH of 6.91 to 7.05, but the pH at the inlet was higher with 8.27. The highest content of ammonia was at the west of floating foodstall and the lowest was at the outlet. The content of nitrate and nitrite at the east of floating foodstall was the highest and the lowest was at the inlet. The highest result of DO measurement was at the outlet, and the lowest was at the west of the floating foodstall.

Table 1. Condition of water quality in Rawa Jombor, Klaten

Sample location	pH	Ammo-nia (mg/L)	Nitrate (mg/L)	Nitrite mg/L)	DO (ppm)
Inlet	8.27	0.28	0.08	0.06	7.0
Middle	6.91	0.20	0.23	0.17	6.5
Outlet	6.95	0.02	0.10	0.07	8.0
West of floating foodstall	7.05	0.43	0.31	0.23	5.5
East of floating foodstall	6.99	0.33	0.54	0.40	6

The observation on the trophic parameters, namely ammonia, nitrite and nitrate resulted that the highest number was at the vicinity of the floating foodstalls. It was closely related to the waste produced by the activity of the floating foodstalls and agriculture. In an anaerobic circumstance and the availability of high nutrient especially nitrate, denitrifying bacteria could grow and perform optimal denitrification process. The presence of ammonia, nitrates and nitrites in the water was not the only the result of the nitrogen cycle, but mostly was from pollutants originating from outside of waters that goes into the waters such as the remnants of the pesticides use flowing in with rain water, fertilizer use and domestic waste from surrounding waters.

A total of 45 bacteria isolates were obtained from 5 sediment samples from different locations, namely 5 isolates were found in the inlet (In), 8 isolates were found in the middle (Te), 6 isolates were found in outlet (Ot), 11 isolates were found at west of floating foodstall (Br), and 15 isolates were found at east of floating foodstall (Tm). Isolation was also as an initial selection stage to obtain denitrifying bacteria isolates. Based on results of nitrate reduction test, it was shown that as much as 35 bacteria isolates were positive results as nitrate-reducing bacteria and one isolate was weak nitrate reducing.

Oxidative-fermentative test results showed that out of 34 isolates tested, 28 isolates were fermentative and 6 of them were negative fermentative. These six isolates which were negative fermentative were denitrifying bacteria and all obtained from sediment samples at east of floating foodstalls (Tm). At this point of sampling, the number of nitrate and nitrite were the highest, so the denitrifying bacteria could be found at this location. Denitrifying bacteria live and grow in an environment that has a high content of nitrogen compounds, especially nitrates and nitrites in an anaerobic circumstance (Effendi 2003). Denitrification was carried out by microorganisms using nitrate and nitrite compounds as an electron acceptor and gaseous nitrogen compounds. Group of positive fermentative bacteria will reduce nitrate to ammonium (Rusmana and Nedwell 2004). Group of positive fermentative bacteria could not use nitrate as an electron acceptor compounds, on the contrary, they used it as a source of electrons (Widiyanto et al. 2008).

Characteristic of colonies and isolates cell of denitrifying bacteria

A total of six isolates of denitrifying bacteria found in aquatic sediments of Rawa Jombor had similar characteristics, namely translucent colored colonies, round with flat edges, rod-shaped bacterial cell and Gram-negative bacteria (Figure 2 and Table 2). Characteristics of denitrifying bacteria obtained in this study was relatively similar to that reported by Widiyanto et al. (2008) which states that the denitrifying bacteria found in the shrimp ponds have rod-shaped cell, motile and Gram negative.

Denitrifying bacteria identification base on 16S rRNA gene sequence

A total of six isolates of bacteria which were capable of reducing nitrate and were thought to be group of denitrifying bacteria that isolate the TmA, TmD, TmE, TmG, TmI and TmK were identified base on the sequences of 16S rRNA gene. Fragment of PCR product had a size of about 1300 bp which was the expected size by using a combination of 63F primer for forward direction and 1387R primer for reverse direction (Figure 3).

PCR products which could be analyzed further by sequencing were DNA fragment of the five bacterial isolates. One PCR product was damage, so it was not sequencing analyzed, namely DNA fragment of TmK. The data results which were analyzed by BlastN showed that all five isolates have the highest similarity to the genus *Shewanella*. One isolate which was identified as *Shewanella putrefaciens* was TmD isolates with the percentage of similarity of 99%, two other isolates, namely the isolates TmE and isolates TmG has similarities with *Sh. putrefaciens* with the percentage of similarity respectively 98% and 97%, while the isolates TmI

Table 2. Characteristics of denitrifying bacteria isolated from aquatic sediment of Rawa Jombor, Klaten, Central Java

Bacteria isolates	Colony color	Colony shape	Colony shape	Cell shape	Gram
Tm A	Yellowish Trans.	Round	Flat	Rod	Negative
Tm D	Brownish Trans.	Round	Flat	Rod	Negative
Tm E	Translucent	Round	Flat	Rod	Negative
Tm G	Yellowish Trans.	Round	Flat	Rod	Negative
Tm I	Translucent	Round	Flat	Rod	Negative
Tm K	Translucent	Round	Flat	Rod	Negative

Note: Trans. = Translucent

Table 3. Identification of denitrifying bacteria on sediment water of Rawa Jombor using BlastN

Isolate code	Closest relatives	Access number	% Simila-rity
Tm A	*S. putrefaciens* strain KOl2	KC607511.1	96
Tm D	*S. putrefaciens* strain KOl2	KC607511.1	99
Tm E	*S. putrefaciens* strain K717	KC607526.1	98
Tm G	*S. putrefaciens* strain KOl2	KC607511.1	97
Tm I	*S. xiamenensis* strain H3	HQ418493.1	98

Note: *S.* = *Shewanella*

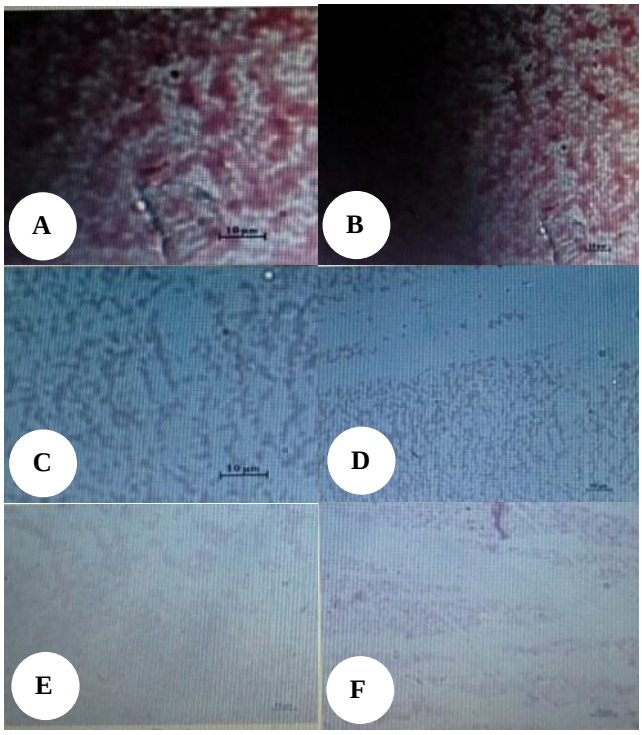

Figure 2. The shape of the cell of 6 denitrifying bacteria. A. Tm A, B. Tm D, C. Tm E, D. Tm G, E. Tm I, F. Tm K.

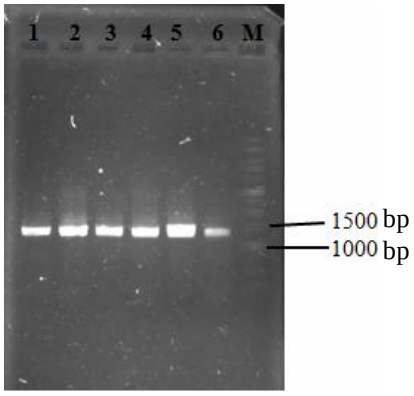

Figure 3. The DNA fragment as a result of amplification of 16S rRNA gene on six denitrifying bacteria isolates. M = 1 kb DNA Marker; 1 = isolate the TmA; 2 = isolates TmD; 3 = isolates TmE; 4 = isolates TmG; 5 = isolates TmI; 6 = isolates TmK

resemblance to the *Shewanella xiamenensis* with 98% similarity percentage. One other isolates namely isolates TmA had a percentage of similarity of 96%. This isolates was possibly a new species (Table 3). According to Dancourt et al. (2000), if the similarity with the database were ≥ 99%, then the isolates could be identified at the species level. If it were ≥ 97%, it could be identified at the genus level, whereas if the similarity were <97%, it might be a new species due to the lack of data on the database or the size of the sequencing results was too short to be compared to database.

Shewanella genus was one of metal-reducing bacterium genus. These bacteria were found many in the marine environment, freshwater, lakes, ground or terrestrial, rivers, Arctic and Antarctic oceans, the rusty or corroded oil pipeline and contaminated uranium aquifer environment. These bacteria were widely used for bioremediation or for cleaning the environment from pollutants such as compounds that underwent chlorination, radionuclides and other environmental pollutants (Venkateswaran et al. 1999). *Shewanella* belong to the Gram-negative bacteria and some species were pathogens that cause disease in humans. Other characteristics of these bacteria were rod-shaped, motile (moving) with polar flagella and had a metabolism as a facultative anaerobic organisms (Huang et al. 2010). To survive, these bacteria were capable of using a variety of electron acceptors such as oxygen, iron, manganese, uranium, nitrate, nitrite, fumaric and others. *Shewanella putrefaciens* could be found in the marine environment, motile, and was facultative anaerobic bacteria that had the ability to reduce iron and manganese as a terminal electron acceptor in the electron transport chain. This bacterium was also an organism associated with the stench of decaying fish, such as marine organisms that produce trimethylamine (Johansen et al. 1996). S. *putrefaciens* could grow on solid media as well as on liquid media. On solid media, bacteria colonies were round, pink and grew fast. These bacteria also grew fast on liquid medium and the liquid medium was made entirely into pink (Khashe and Janda 1998). *Shewanella xiamenensis* was motile with single nonpolar flagella and was facultative anaerobic. Colonies were circular-shaped, brown, grew at temperatures between 4 ° C-37 ° C but if it were below 37 ° C, they would not grow. pH growth ranged from 6.0 to 9.0 with a pH optimum of 7.0, positive to hydrolyze gelatin, DNA and Tween 80 and could reduce nitrate, nitrite, fumaric (Huang et al. 2010).

Trophic Status of Rawa Jombor Waters

Rawa Jombor had high level of total-N and total-P high and low brightness making it to have hypereutrophication status according to the Regulation of the Minister of Environment No. 28 of 2009 (Table 4). Status hypereutrophication (very fertile) is a status of waters containing nutrients in very high levels.

Table 4. Total nitrogen, total phosphor and brightness on water of Rawa Jombor, Klaten

Sample	Level of Total-N (µg/l)	Level of Total-P (µg/l)	Brightness (m)
Inlet	5643	667.1	0.12
Middle	19903	260.4	0.12
Outlet	4807	416.1	0.18
West of floating foodstall	6273	542.2	0.14
East of floating foodstall	3827	601.6	0.17
Average	8090.6	497.48	0.146
Criteria of Regulation of the Minister of Environment (Hypereutrophication)	>1900	$\geq$ 100	< 2.5

According to Suryono et al. (2008), the amount of nutrients contained in the waters of lakes or reservoirs can be used for the assessment of the trophic status. This status indicates the water was heavily contaminated by elevated levels of nitrogen and phosphate. Machbub et al. (2003) suggested that the occurrence of eutrophication in the waters of the lake and reservoirs can be detected by a variety of indicators, namely: (i) decrease in the concentration of dissolved oxygen in the zone of hypolimnion, (ii) increase of nutrients i.e. nitrogen and phosphorus in bodies of waters, (iii) the decrease of water transparency, and (iv) increase in suspended solids, especially those containing organic material. The indicators are a common sign, but the monitoring of water quality parameter remains to be done, especially parameters associated with the process of eutrophication.

In conclusion, a total of six isolates of denitrifying bacteria were found in aquatic sediments of Rawa Jombor waters with characteristic colonies are round, translucent color, are Gram negative and rod-shaped cells. Five isolates were identified as having the highest similarity to the genus *Shewanella*. Isolates TmD is identified as *Shewanella putrefaciens* with the percentage of similarity of 99%, isolates TmE and TmG has similarities with *Shewanella putrefaciens* with percentages respectively 98% and 97%, isolates TmI has similarities with *Shewanella xiamenensis* with a percentage of 98% and isolates TmA having percentage of similarity of 96 % by *Shewanella putrefaciens* has possibility as a new species. The trophic status of Rawa Jombor aquatic based on parameters of total nitrogen, total phosphate and brightness is hypereutrophication (very fertile), that these waters contain nutrients with very high levels.

REFERENCES

Dancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 2000. 16S Ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacteria isolates. J Clin Microbiol 38 (10): 3623-3630.

Effendi H. 2003. Assessing Water Quality for Water Environment Resources Management. PT. Kanisius, Yogyakarta. [Indonesia]

Ganjarsari S. 2008. Characteristics of Local Community Empowerment in Development Sustainability of Rawa Jombor Region, District of Klaten: Differences Empowerment Efforts Conducted by the Internal and External Parties. [Research Report] Universitas Diponegoro, Semarang. [Indonesia]

Hanum DR. 2005. Selection and identification of halophilic bacterial isolates producing amylase and protease enzymes of pickled mustard (*Brassica juncea* (L.) (Zern & Coss)). [Hon. Thesis]. Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta. [Indonesia]

Huang J, Sun B, Zhang X. 2010. *Shewanella xiamenensis* sp. nov, Isolated from Coastal Sea Sediment. Intl J Syst Evol Microbiol 60: 1585-1589.

Hugh R, Leifson E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. J Bacteriol 66: 24-26.

Johansen C, Gill T, Gram L. 1996. Changes in Morphology of *Listeria monocytogenes* and *Shewanella putrefaciens* resulting from the action of protamine. Appl Environ Microbiol 62 (3): 1058-1064.

Khashe S, Janda JM. 1998. Biochemical and pathogenic properties of *Shewanella alga* and *Shewanella putrefaciens*. J Clin Microbiol 36 (3): 783-787.

Machbub B, Fulazzaky MA, Brahmana S, Yusuf IA. 2003. Euthrophication of lakes and reservoir and its restoration in Indonesia. Jurnal Litbang Pengairan Bandung 17 (50): 72-78.

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64: 795-799.

Mason CF. 1993. Biology of Freshwater Pollution. 2nd ed. Longman, New York.

Regulation of the Minister of Environment (PerMNLH) No. 28 of 2009 on Water Pollution Load Capacity Lake and/or reservoir. Minister of Environment, GoI, Jakarta. [Indonesia]

Rusmana I, Nedwell DB. 2004. Use of chlorate as a selective inhibitor to distinguish membrane-bound nitrate reductase (Nar) and periplasmic nitrate reductase (Nap) of dissimilative nitrate reducing bacteria in sediment. J FEMS Microbiol Ecol 48: 379-386.

Salupi W. 2011. Screening xilanolitik activity of bacterial isolates from *Attacus atlas* L, the identification of the 16S rRNA gene sequences [Hon. Thesis]. Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta. [Indonesia]

Setyaningsih R, Rusmana I, Setyanto P, Suwanto A. 2013. Nitrous oxide reduction activity of denitrifying *Ochrobactrum anthropi* isolated from rice Field. Microbiol Indon 7 (2): 45-50.

Suryono T, Nomosatryo S, Mulyana E. 2008. The fertility level of lakes in West Sumatra and Bali. Jurnal Limnotek 15 (2): 99-111. . [Indonesia]

Teixeira P, Oliveira R. 2002. Metabolism of *Alcaligenes denitrificans* in bio film vs planktonic cells. J Appl Microbiol 92 (2): 256-260.

Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffarini DA, MacGregor BJ, Ringelberg DB, White DC, Nishijima M, Sano H, Burghardt J, Stackebrandt E, Nealson KH. 1999. Polyphasic Taxonomy of the Genus *Shewanella* and Description of *Shewanella oneidensis* sp. nov. Interl J Syst Bacteriol 49: 705-724.

Waluyo L. 2008. Basic Microbiology Techniques. Universitas Muhammadiyah Malang Press, Malang. [Indonesia]

Widiyanto T, Rusmana I, Hermawan T. 2008. The ability of denitrification bacteria of shrimp pond to reduce nitrate and nitrite compounds. Limnotek 15 (1): 22-30. [Indonesia]