A salt tolerant *Sphingosinicella microcystinivorans* A3 isolated from soil contaminated with mercury in traditional gold mining of Jendi Village, Wonogiri District, Indonesia

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**Abstract.** Sutami, Purwanto, Rosariastuti R. 2021. A salt tolerant *Sphingosinicella microcystinivorans* A3 isolated from soil contaminated with mercury in traditional gold mining of Jendi Village, Wonogiri District, Indonesia. Biodiversitas 22: 3785-3791. Isolation and characterization of indigenous bacteria from the soil of traditional gold mining contaminated with mercury is the first step in a series of research to explore and utilize indigenous bacteria in Jendi’s area. This study was aimed to determine the characteristics and identity of bacterial isolates from soil of traditional gold mining in Jendi Village, Wonogiri contaminated by mercury. The methods used in this study included bacterial isolation, media preparation, phenotypic identification including; morphological and physiological tests and genotyping tests. The results showed that the bacterial isolate A3 grew optimally in media with the addition of 10% NaCl at a temperature of 27°C, and pH 9. There were negative reactions to the observations of gram staining, acid production from glucose, indole production, catalase and urease, and positive reactions to oxidation. A neighbor-joining phylogenetic tree based on the 16S rRNA gene sequence showed that the A3 strain was closely related to *Sphingosinicella microcystinivorans* strain Y2 (JCM 13185) with 100% Query coverage and a maximum identity of 99.56%.

**Keywords:** Gold-mining, mercury, PCR, salt tolerant, *Sphingosinicella*

**INTRODUCTION**

Indonesia is a country rich in natural resources, including mining materials (Ma’arif 2014) and is known as the largest coal and gold producer in the world (Dutu 2015). The mining industry in addition to provide many benefits, also causes environmental damage (Khanifah et al. 2020). Heavy metal is a type of pollutant widely distributed in the soil and gets special attention because of its non-degradable nature and can last for a long time in the environment (Wang et al. 2020). Different heavy metals including Cr, Cd, Pb, Hg, and As are often reported to contaminate the environmental habitats (Li et al. 2020). Mercury (Hg) is among the one of the most toxic pollutants (Selid et al. 2009; Tchounwou et al. 2012; Waheed et al. 2020). That is widely distributed in the atmosphere, lithosphere, and surface water. Mercury causes serious problems for human health, such as bioaccumulation in the brain and kidneys which ultimately leads to neurological diseases (Zulakhihah et al. 2020).

Gold mining in Jendi (Village) of Selogiri Sub-district and Wonogiri District is operating without any permit (Dewi et al. 2013). Gold mining activities are processed using an amalgamation process. The amalgamation process can cause negative impacts in the form of environmental pollution by mercury vapors. As much as 10 - 30% of the mercury used in the gold amalgamation process released to the environment (Alpers et al. 2005). The amalgamated water is dumped into ditches and flows into rivers, which are used to irrigate the surrounding paddy fields, as a result, this area has been contaminated by mercury. As per reports obtained from analyses of thirty soil samples in Jendi Village revealed that the soil contained Mercury with an average of 30.87 mgkg⁻¹. Based on Government Regulation No. 101 of 2014 Concerning Management of Hazardous and Toxic Waste, the threshold for mercury levels in the soil is 0.3 mgkg⁻¹ (Rhani 2012). Mercury levels in river water around Jendi Village vary from 0.0024 mgL⁻¹ to 0.0173 mgL⁻¹. Government Regulation No. 82 of 2001 concerning Water Quality Management and Water Pollution Control, the mercury threshold in water is 0.001 mgL⁻¹. Thus, mercury levels in the soil and river water in Jendi Village have exceeded the predetermined threshold. Therefore, bioremediation is an effective and safe alternate to deal with this problem. The efforts can be carried out by isolating indigenous bacteria from the local environment which is expected to help in overcoming pollution problems that occur in the region (Winardi et al. 2020). This study aims to determine the characteristics and identity of bacterial isolates obtained from mercury-contaminated traditional gold mining in Jendi Village, Wonogiri.

**MATERIALS AND METHODS**

**Sample collection**

Samples were collected during April 2020 from mercury-contaminated traditional gold mining in the
village of Jendi, Selogiri, Wonogiri, Indonesia (07°47’41.9”S, 110°52’51.1”E). Soil and wastewater samples were taken randomly in several areas where mining materials were processed. Based on the results of the analysis, the soil and wastewater contained mercury of 10.98 mgkg⁻¹ and 0.65 mgL⁻¹. Soil samples were taken from the rhizospheric soil of plant grows well in sampling location with a depth of 20 cm using a soil drill. Soil and wastewater samples were put into sterile containers and stored in a cool box. This is done to reduce the presence of bacterial contamination from the air.

Instruments and culture media used and sterilization

The tools used in this research were UV-Vis Spectrophotometer (Cory Conc 50), incubator (Heraeus), Autoclave (Astell), oven (Memmert), laminar airflow (Esco), analytical balance (Kern), microscope, pH digital meters, refrigerators, micropipettes, 100°C thermometers, Petri dishes, Bunsen, horn spoons, loop needles, spray bottles, sterile sample bottles and glassware commonly used in laboratories. Two different liquid media were used: A Nutrient Agar (NA); and a soil extract medium (SEM). NA was prepared as described previously by Fan et al. (2002) and SEM was prepared using soil and wastewater from the field site in gold mining in Jendi, Wonogiri. Antifungal, 70% alcohol, sterile distilled water, Kovac’s reagent, methyl red reagent, and yeast extract. SIM Medium, Tryptone broth, Christensen’s Urea Agar, Stuart’s Urea Broth. All glassware used were wrapped in paper, and sterilized using an autoclave at 121°C with a pressure of 1.5 bars for 30 minutes. Medium Nutrient Agar (NA) was measured as needed, then heated until dissolved. The medium was sterilized by autoclaving at 121°C at 1.5 bars pressure for 15 minutes.

Soil bacterial isolation

The isolation of soil bacteria was carried out using soil extract agar (Hii et al. 2020). Soil samples that had been taken from 5 points at each sampling location were homogenized, and then 5 g were taken and dissolved in 45 mL of physiological NaCl solution, then vortexed (10⁻¹ dilution). The 10⁻¹ soil suspension results were made in graded dilutions up to 10⁻¹¹ using physiological NaCl solution. The results of 10⁻³ to 10⁻¹¹ dilutions were then cultured on Nutrient Agar (NA) media, then incubated at room temperature for 24 hours. Bacterial growth was carried out using the pour plate technique. Colonies that grew in the range of 25-250 colonies were then observed for bacterial morphology and counted the number of bacterial colonies to determine the number of bacterial isolates obtained. Bacteria obtained from mixed cultures were separated using the streak quadrant method using a blunt loop and the media used was NA media. Separated colonies on scratch plates were transferred to slanted NA media which was used as stock culture (Aanniz et al. 2015).

The bacterial colonies that grew separately were then tested for their resistance to growth in NA media containing 10 mgkg⁻¹ Mercury. The bacteria that managed to grow were purified to obtain pure isolates. Purification of isolates using streak plate method on NA medium. Pure isolates were then identified based on characteristics that referred to Bergey’s Manual of Determinative of Microorganisms (Ludwig et al. 2010).

Bacterial identification

The identification and characterization of the selected isolates was carried out by both phenotypic test and the genotypic test.

Phenotypic characterization

The phenotypic characterization tested in this study included macroscopic observations including colony morphology, microscopic observations including cell shape and cell staining, after macroscopic observations, further tests were carried out only for isolates that grew best in media containing mercury. Further tests in the form of physiological tests include; oxygen demand test, carbohydrate fermentation ability, NaCl resistance test, and biochemical tests; motility test (Public Health England 2014), catalase test (Reiner 2010), oxidase test (Shields and Catc hart 2016), testing the ability to hydrolyze urea (Brink 2010), indole test (MacWilliams 2009), and acid production from glucose (Public Health England 2019).

Genotypic characterization

Genotype characterization was done by 16S rDNA gene sequencing. Genomic DNA of bacterial isolates was extracted from 5 mL bacterial cultures grown overnight following the previously described method. Bacterial pellets were suspended in 410 μL TE buffer (10 mM Tris-HCl pH 8 and 1 mM EDTA pH 8). A volume (50 μL) of 60 mg mL⁻¹ fresh lysozyme (Sigma, Milwaukee, WI) solution was added to cells suspension and incubated. For 30 minutes at 37°C with occasional mixing. Subsequently, 30 μL of 10% SDS (Sodium Dodecyl Sulfate) and 3 μL of 20 mg mL⁻¹ proteinase K were added, mixed and incubation was continued for 30 min. After that, 100 μL of NaCl 5 M and 100 μL of CTAB (N-cetyl-N, N, N, -trimethylammonium bromide) 10% were added, slowly mixed, and incubated at 65°C for 10 minutes. The mixture was extracted with 600 μL of ice-cold chloroform by gently mixed, centrifuged at 12,000 rpm for 5 min, and the aqueous phase was carefully removed and transferred to a new sterile micro centrifuge tube. The DNA in the aqueous phase was precipitated by adding 0.6 vol. ice-cold isopropanol and incubating for one hour on ice. The precipitate washed with ice-cold 70% ethanol. After drying, the precipitate was resuspended in 50 μL of TE buffer.

Universal bacterial 16S rRNA primers, 27 F (AGAGTTTGTATCMTGCTCAG) and 1492 R (TACGGYTACCTTGGTACCTACT) used to manage implication fragment of the 16S rRNA gene in genomic DNA of bacteria were isolated as a template to follow proto cabbage (Stackebrandt and Goebel 1994) with modifications in men NOTICE 2x My Taq HS Red Mix (Bioline, BIO-25048 ). PCR was performed in the GeneAmp®PCR system 9700 (Applied Biosystems, Foster City, CA). The following conditions were used for DNA
amplification. Initial denaturation at 95°C for one minute, followed by 35 cycles consisting of 15 seconds at 95°C, 30 seconds at 52°C, plus an additional final extension step of 45 seconds at 72°C. The accuracy of the PCR product (approximately 1400 bp) was confirmed by electrophoresis through a 0.8% horizontal agarose gel containing 0.5 g mL\(^{-1}\) ethidium bromide. The gel was examined under UV light and photographed. The PCR products were purified using Zymoclean \(\alpha\) Gel DNA Recovery Kit (Zymo Research).

Sequencing of 16S rRNA was performed on the ABI PRISM 3100-Avant™ Genetic Analyzer (Applied Biosystems, Foster, CA) with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Primer used for sequencing of 16S rDNA was 27 F (AGAGTTTGATCMTGGCTCAG) and 1492 R (TACGGYTACCTTGTAGAGGCTAGT). Several alignments of the order (ca. 715 bases) were determined performed with the program GENETYX WIN Ver. 3 (Software Development, Tokyo, Japan). A GenBank search with the BLAST program (www.ncbi.nlm.nih.gov/Blast.cgi) was used to identify the named bacterial species. X2.0 clusters (ftp://ftp.ncbi.nlm.nih.gov/pub/software/clusterw2) were used to construct a phylogenetic tree and then drawn using a neighbor-joining NJ Plot (http://pbil.univ-lyon1.fr/software/njplot.html).

RESULTS AND DISCUSSION

Phenotypic characterization

**Morphological observation**

The results of morphological observations of 3 bacteria isolated coded T (Cassava rhizosphere) and A (wastewater) are shown in Table 1.

| Isolate Code | Colony form | Elevation | Surface | Edge  | Color           |
|--------------|-------------|-----------|---------|-------|-----------------|
| T5           | Round       | Flat      | Glossy  | Flat  | Creamy White    |
| T7           | Round       | As droplets | Glossy  | Flat  | Creamy White    |
| A3           | Round       | Convex    | Glossy  | Flat  | Yellow          |

*Table I. Results of observation of bacterial colony morphology*

*Sphingosinicella humi*. has white, smooth, circular, convex, and slightly transparent colonies on R2A media (Qiao et al. 2007). *Sphingosinicella xenopeptidilytica* has pale yellow colonies, round and convex. Cells are aerobic and measure 0.6-0.861, 5-2.5 mm (Geueke et al. 2007). *Sphingosinicella gene* has yellow colonies, truly aerobic and chemo-organ trophic (Maruyama et al. 2006).

**Microscopic observations**

Microscopic observations were made by Gram staining. The results of microscopic observations of bacterial isolates obtained are shown in Table 2.

Bacteria are grouped into two groups, namely gram-positive bacteria and gram-negative bacteria. Gram staining is based on differences in the structure of the bacterial cell wall, resulting in different reactions in the permeability of the dye and the addition of a washing solution. Gram-positive bacteria’s cell wall consists of a thick layer of peptidoglycan while the cell wall of Gram-negative bacteria has thick lipid content.

The results of microscopic observations (Table 2) showed that, all bacterial isolates belong to the Gram-negative group of bacteria. In addition, bacterial isolates formed single colonies with the shape of rod cells (*Bacillus*) in isolate A3 while isolates T5 and T7 were spherical (*coccus*). To give optimal results in Gram staining, it is better to use fresh cultures aged 24-48 hours, because in old cultures many cells are damaged in their cell walls, this results in the release of dye when washed with a bleach solution, thus disguising the results. According to Geueke et al. (2007), *Sphingosinicella xenopeptidilytica* is Gram-negative bacteria, as determined by KOH and aminopeptidase assays. According to Maruyama et al. (2006) *Sphingosinicella* gene has rod cell shape, Gram-negative, does not form spores, motile using polar flagella and completely aerobic and chemo-organ trophic.

The discovery of Gram-negative isolates in Jendi water sources related to environmental conditions. Gram-negative bacteria require relatively simple nutrition compared to Gram-positive bacteria. This means that the ability of this group of bacteria to grow in an environment is greater than that of Gram-positive bacteria.

**Biochemical test**

The biochemical tests were carried out for the confirmation and identification of the bacteria isolated. The results of the observation of the biochemical test of bacterial isolates can be seen in Table 3.
The formation of genus of the bacterial isolates. This means that bacterial isolates can almost all spiral bacterial cells and ammonium in the culture medium distinguish three parameters, namely Sulfur. Sphingomonas paucimobilis are immotile. Microcytochrome oxidase, namely yellow; this was because the isolate does not produce acid in glucose fermentation (Public Health England 2019). Sphingomonas paucimobilis JCM 7516T had a positive nitrate reduction test.

**Sulfide-indole-motility (SIM) test**

The Sulfide-Indole-Motility (SIM) test used media to distinguish three parameters, namely Sulfur reduction to distinguish enteric bacteria, Indole test for part of the IMViC test, to distinguish the family, and motility test to distinguish common types of bacteria (MacWilliams 2009). In sulfur reduction, bacterial isolates did not change. According to MacWilliams (2009) bacteria that can reduce sulfur to hydrogen sulfide, then hydrogen sulfide reacts with iron (Iron) to form ferric sulfide, which precipitates black. The indole test on bacterial isolates showed negative results, bacteria that produced a negative reaction on the addition of Kovac's reagent containing HCl, n-ethyl alcohol, and p-dimethylaminobenzaldehyde (DMABA) into SIM medium, then p-dimethylaminobenzaldehyde react with indole, producing red Quinoidal compounds (MacWilliams 2009).

Motility is the ability of an organism to move on its own. It was found positive on bacterial isolates isolated in the present study. Motility plays an important role in determining species survival (Miyata et al. 2020; Aygan and Arikan 2007). Almost all spiral bacterial cells and some of the Bacillus bacterial cells are motile, while cocci-shaped bacteria are immotile (Aygan and Arikan 2007).

**Oxidase test**

An oxidation test was carried out for the Gram-negative bacterial isolate. Based on the results, bacterial isolates showed positive results, which were indicated by the appearance of a purple color on the surface of the filter paper (Shields and Cathcart 2016). This means that the isolate belongs to a group of bacteria that have cytochrome oxidase enzymes. Cytochrome oxidase is a complex enzyme that plays a role in oxidative phosphorylation. According to Mayugama (2006), the bacteria that produce cytochrome oxidase, namely sphingosinicella. Oxidase-positive bacteria allow them to oxidize reagents containing certain amine.

**Urea hydrolyzing test**

The urea test on bacterial isolates gave a negative result in the form of the color of the media that did not change, namely yellow; this was because the bacterial isolates did not produce the urease enzyme. Microorganisms that produce the enzyme urease will break down urea into ammonium and CO₂. When urea is hydrolyzed, the culture medium containing urea and a pH indicator (phenol red) accumulate ammonium in the culture medium and cause the pH to become alkaline. The color change from yellow to pink is an indication of urea hydrolysis (Brink 2010).

**Table 2. Results of microscopic observations of bacterial isolates**

| Isolate code | Cell shape   | Gram's properties |
|--------------|--------------|-------------------|
| T5           | Coccus (round) | Gram-negative     |
| T7           | Coccus (round) | Gram-negative     |
| A3           | Basil (stem)  | Gram-negative     |

**Table 3. Biochemical Test Results of A3 Bacterial Isolates**

| Observation Parameter | Result |
|-----------------------|--------|
| Catalase Test         | +      |
| Oxidase Test          | +      |
| Urea hydrolyzing Test | -      |
| SIM test:             |        |
| Sulfur reduction      | -      |
| Indole test           | -      |
| Motility              | +      |
| Acid production from glucose | -      |

Note: (+): indicates positive, (-): indicates negative.

The catalase test was carried out to obtain a more specific information of genus of the bacterial isolates. Based on the results of the study, bacterial isolates showed positive results, which were indicated by the formation of many oxygen bubbles shortly after the addition of 3% H₂O₂ solution (Reiner 2010). This means that bacterial isolates can produce catalase enzyme and have tolerance for oxygen availability. Catalase enzyme is an enzyme that can catalyze the direct conversion of hydrogen peroxide (H₂O₂) which is toxic to cells into water and oxygen (Lionel 2016). According to Mayugama (2006) Sphingosinicella microcystinivorans JCM 13185T; S. soli KSL-125T, Sphingomonas paucimobilis JCM 7516T had a positive catalase test.

**Oxidase test**

An oxidation test was carried out for the Gram-negative bacterial isolate. Based on the results, bacterial isolates showed positive results, which were indicated by the appearance of a purple color on the surface of the filter paper (Shields and Cathcart 2016). This means that the isolate belongs to a group of bacteria that have cytochrome oxidase enzymes. Cytochrome oxidase is a complex enzyme that plays a role in oxidative phosphorylation. According to Mayugama (2006), the bacteria that produce cytochrome oxidase, namely sphingosinicella. Oxidase-positive bacteria allow them to oxidize reagents containing certain amine.

The fermentation media of the isolate showed a green color after incubation for 2 days. The green color indicated that the isolate does not produce acid in glucose fermentation (Public Health England 2019). Isolate A3 has positive characteristics in the oxidation test, Gram-negative bacteria are rod-shaped and do not produce gas and acid in the simple sugar fermentation process. Based on the research of Qiao et al. (2019) on S. humi acid produced from D-galactose, but not from D-ribose, lactose, D-xylene, D-sorbitol, D-rihamnose, L arabinose, sucrose, myoinositol,
halophilic bacteria are typical. Each bacterium has an optimal sequence Sphingosinicella microcystinivorans. They were aerobic bacteria. Microorganisms require oxygen (growing at high sugar content) can survive at high salt or sugar levels, namely osmophilic microorganisms that can grow optimally in an environment with high salt concentrations by maintaining osmotic balance (Oren 2008). Sphingosinicella humi can grow with 0-0.4% NaCl and grows best without the addition of NaCl and can grow up to 1mM NaAsO2 (Qiao et al. 2007). Based on the capacity of A3 to grow in a medium containing NaCl higher than 2%, A3 does not belong to Sphingosinicella xenopeptidilytica (Geueke et al. 2007). Unfortunately, information of Sphingosinicella xenopeptidilytica about NaCl tolerance is unavailable from the literature (Maruyama et al. 2006).

**Oxygen needs**

A3 bacterial isolates after being incubated for 24 hours showed signs of growth on the surface of the tube so that they were aerobic bacteria. Microorganisms require oxygen for growth and their enzyme systems and use O2 as the final hydrogen acceptor incomplete oxidation changes in higher molecules, such as glucose. Members of the genus Sphingosinella are Gram-negative, rod-shaped, and highly aerobic (Qiao et al. 2007). S. xenopeptidilytica has pale yellow colonies, circular and convex. Cells are aerobic and measure 0.6-0.86 x 1.5-2.5 mm (Geueke et al. 2007). S. gene. have yellow colonies. Truly aerobic and chemo-organotrophic (Maruyama et al. 2006).

**Optimum temperature**

A temperature test has been carried out to determine the optimal temperature of bacteria, isolate A3 has the optimum temperature at 27°C (Table 4). The optimal temperature usually reflects the normal environment of the microorganism. Temperature plays an important role in enzyme activity. An increase in temperature of 10°C can increase the enzyme activity by two times. At very high temperatures, irreversible protein denaturation occurs, while at very low temperatures, enzyme activity stops. At the optimal growth temperature, there will be an optimal growth rate and the maximum number of cells will be produced (Ji et al. 2021). Each bacterium has an optimal temperature at which they can grow very fast and has a temperature range in which they can grow. The genus Sphingosinella can grow at a temperature of 16-42°C and the optimum temperature is 28°C (Qiao et al. 2007).

### Table 4. Results of physical properties of A3 bacterial isolates

| Observation Parameter | Result |
|-----------------------|--------|
| Resistance to NaCl    | 10%    |
| Oxygen Needs          | Aerobic|
| Optimum Temperature (°C) | 27    |
| Optimum pH            | 9      |
| Carbon Source         | Glucose|

**Table 5. BLAST results from A3 bacterial isolates**

| Description                                      | Max score | Total score | Query E value | Per. ident | Accession |
|--------------------------------------------------|-----------|-------------|---------------|------------|-----------|
| Sphingomonadaceae bacterium CBFR-1 16 S ribosomal RNA gene, partial sequence | 2503      | 2503        | 0.0           | 99.93%     | EF066484.1 |
| Alpha proteobacterium 7CY gene for 16S ribosomal RNA partial sequence | 2503      | 2503        | 0.0           | 99.93%     | AB076083.1 |
| Sphingosinicella sp. OC5S gene for 16S rRNA partial sequence | 2497      | 2497        | 0.0           | 99.85%     | AB429069.1 |
| Sphingosinicella microcystinivorans gene for 16S rRNA partial sequence strain MDB2 | 2486      | 2486        | 0.0           | 99.71%     | AB219940.1 |
| Sphingosinicella xenopeptidilytica strain R25-56 16S ribosomal RNA gene, partial sequence | 2477      | 2477        | 0.0           | 99.70%     | MN330511.1 |
| Sphingosinicella microcystinivorans B9 DNA complete genome | 2481      | 2481        | 0.0           | 99.63%     | AP018711.1 |
| Sphingosinicella microcystinivorans strain 3-2WA 16S ribosomal RNA, partial sequence | 2481      | 2481        | 0.0           | 99.63%     | NR_043288.1 |
| Sphingosinicella sp. strain JEZ-8L 16S ribosomal RNA gene, partial sequence | 2471      | 2471        | 0.0           | 99.63%     | KY046734.1 |
| Sphingosinicella xenopeptidilytica strain LA-50 16S ribosomal RNA gene, partial sequence | 2477      | 2477        | 0.0           | 99.56%     | MK039097.1 |
| Sphingosinicella microcystinivorans strain Y2 16S ribosomal RNA, partial sequence | 2475      | 2475        | 0.0           | 99.56%     | NR.040927.1 |
**Optimum pH**

The pH tests have been carried out to determine the level of soil acidity, isolate A3 has an optimal pH of 9. The pH is an important for bacterial growth because each bacterial species has an optimum temperature and pH for its growth (Gondal et al. 2021). The pH of the culture medium affects the growth rate, for bacterial growth, there is also an optimal pH and pH range. *Sphingosinicella humi* can grow at pH ranges from 6.5 to 9.0. (Qiao et al. 2007).

**Carbon source**

Glucose, sucrose, lactose, and maltose are some carbon source used by different bacteria. A3 isolate grew well on glucose carbon sources. Fermentation media must contain compounds that can be oxidized and fermented by microorganisms. To determine the presence of carbohydrate fermentation, simple sugars in the form of glucose, sucrose, lactose, mannotiol, and maltose are used. 10% carbohydrate compounds in the form of glucose, sucrose, lactose, maltose, and mannotiol, were sterilized separately from the medium. The use of carbohydrates as a carbon source was characterized by the formation of acid which was indicated by a change in the color of the medium to yellow.

**Genotypic characterization**

DNA isolation was only carried out on A3 isolates as they had good growth in mercury-treated media. The primer used was a 16S rRNA primer with a primary base sequence of 27 F (AGAGTTTGATCMTGGCTCAG) and 1492 R (TACGTYACCTGTAGACTT) (Stackebrandt and Goebel 1994). The 16S rRNA primer is a universal primer that can identify various kinds of bacteria by molecular method. The initial stage of this identification process is the DNA isolation process using the alkaline lysis method, and then followed by the amplification process by PCR to the electrophoresis process. The result of the 1kb ladder electrophoresis marker showed that the DNA band of A3 isolates was 1358 bp. The results of the amplification that have shown a band measuring 1358bp are then continued for the final process of purification and sequencing. After getting the results of the sequencing of bacteria, the bioinformatics process is continued. Table 5 was obtained from the BLAST program at www.ncbi.nlm.nih.gov.

Based on Table 5, it is known that isolate A3 has a very close relationship with *Sphingosinicella microcystinivorans* strain Y2. The nucleotide similarity rate of about 80% belongs to the high similarity level (Addinilia 2012). To confirm the position of the isolates in the phylogeny, a number of representative strains of *Sphingosinicella* were selected for the construction of the phylogenetic tree, using *Alpha proteobacterium* as the out-group, see Figure 1.

Based on Figure 1, it is known that the position of Isolate A3 is closely related to *S. microcystinivorans* strain Y2. The genus *Sphingosinicella* belongs to the family Sphingomonadaceae in the Alphaproteobacteria class. *S. microcystinivorans* Y2 was first discovered by Maruyama et al. as microcystin-degrading bacteria (Maruyama et al. 2006; Qiao et al. 2007). Microcystins (MCs) are toxins produced by cyanobacteria, photosynthetic microorganisms that preferentially inhabit aquatic environments (Bittencourt-Oliveira et al. 2014). So far, there are only four species of *Sphingosinicella* including *S. microcystinivorans* (Maruyama et al. 2006), *S. xenopeptidilytica* (Gueuke et al. 2007), *S. soli* (Yoon et al. 2008) and *S. vermicomposti* (Yasir et al. 2010) represented by strains isolated from eutrophic lakes, wastewater aeration tanks, alkaline soils, and vermicompost. The characteristics of members of the genus *Sphingosinicella* include Gram-stain-negative, rod-shaped and highly aerobic (Maruyama et al. 2006). This is in accordance with the characteristics of the isolate A3.

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