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Screening and molecular identification of lipolytic bacteria from spent bleaching earth

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Abstract. *Soleha S, Retnaningrum E. 2020. Screening and molecular identification of lipolytic bacteria from spent bleaching earth. Biodiversitas 21: 4155-4161.* Lipolytic bacteria can produce nanobioremediation materials that encourage researchers to explore the diversity of these bacteria. Lipolytic bacteria were isolated from spent bleaching earth (SBE). Bacteria isolates were screened for their lipolytic index and crude lipase activity. Strain with the highest lipolytic index and crude lipase activity were identified using the 16S rRNA gene as genetic markers. The maximum likelihood method in MEGA6 software has been used to analyze the relationship of the isolate. The results showed that strain SBE01 had the highest lipolytic index and crude lipase activity of 8.8 ± 0.7 mm and 5.7 U/mL, respectively. Based on 16S rRNA gene analysis, the isolate was identified as *Moraxella* sp. Phylogenetic tree was shown that SBE01 isolate was closely related to *Moraxella osloensis*. *Moraxella* sp. has the potential to be used as a bioremediation agent from oil-contaminated environment.

Keywords: 16S rRNA, lipolytic index, *Moraxella* sp, nanobioremediation, phylogenetic tree

INTRODUCTION

Based on the Indonesian Palm Oil Association (2018), crude palm oil (CPO) production in Indonesia increased rapidly to reach 38.17 million tons in 2017. The oil refining process involves degumming and bleaching and releases an abundance of solid waste known as spent bleaching earth (SBE). This waste includes about 20-40% of residual oil, metallic impurities, and other organic compounds, therefore classified as hazardous waste. The conventional methods of waste disposal were waste disposal at farm-land or burial in local landfill sites (Kheang et al. 2006; Loh et al. 2006). However, the disposal causes the release of hazardous chemical compounds and green-house gases (GHGs) emissions which can endanger living things and the environment. Alternative ways of SBE utilization have been practices as material blocks for construction (Beshara et al. 2014; Nalobile et al. 2019), biolubricants (Syahir et al. 2017), fertilizer (Loh et al. 2017), pollutants adsorbent (Tang et al. 2017), the substrate for biodiesel production (Sedghamiz et al. 2019; Xu et al. 2020) and catalyst (Liu et al. 2020). Besides, the high oil content in SBE is a good nutrient for lipolytic bacterial growth.

Lipolytic bacteria are well-known for their ability to produce bionanomaterials called extracellular lipases. These enzymes act as catalysts and hydrolyze triacylglycerols to diacylglycerols, monoacylglycerols, fatty acids, and glycerol (Odeyemi et al. 2013; Rizwan et al. 2014). Some of the bionanomaterials are non-specific, catalyzing reactions in triacylglycerols at all positions, whereas others are regiospecific, catalyzing reactions on lipid molecules at particular positions. Therefore, these bacteria can be used in the bioremediation process of special pollution, including oil, hydrocarbons, plastics, and pesticides pollution.

The utilization of bacterial lipolytic in the bioremediation process is more energy intensive operation, eco-friendly, and effective compared to conventional methods (Corsi et al. 2018; Tripathi et al. 2018). Several studies on biological agents for reducing oil pollution have been reported, including *Staphylococcus pasteuri*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus pumilus* (Kanmani et al. 2015; Lauprasert et al. 2017; Saranya et al. 2019). Other studies on the ability of the biological agents to degrade hydrocarbon pollutants have also been reported, including *Pseudomonas aeruginosa*, *Pseudomonas* sp, *Bacillus* sp (Retnaningrum and Wilopo 2017; Xia et al. 2019; Sari and Retnaningrum 2019; Benget and Retnaningrum 2020). Bacterial isolates such as *Pseudomonas* sp, *Rhodococcus* sp, and *Comamonas* sp have been investigated for their potential in reducing plastics pollution (Urbanek et al. 2018; Danso et al. 2019), while *Pseudomonas fluorescens* can reduce pollutant pesticides (Wang et al. 2018). Lipolytic bacteria isolated from various sources including soil, rhizosphere soil, seawater, wastewater containing fat from slaughterhouses have been investigated, however, a study on lipolytic bacteria isolated from SBE was still limited. Therefore, the exploration of lipolytic bacteria from SBE needs to be done to increase diversity and to enrich nano-bioremediation agents.

The purposes of the present study are to isolate lipolytic bacteria from SBE samples and to identify the isolates based on the 16S rRNA gene sequence. This gene is

commonly used to identify bacteria based on several reasons, i.e. 16S rRNA genes present in all bacteria, sustainable genes (conserved), and large enough for informatics purposes (Senthilraj et al. 2016). Based on these reasons, taxonomy and evolution (phylogenetic) of lipolytic bacteria can be determined using the 16S rRNA gene sequence.

MATERIALS AND METHODS

Isolation and screening of lipolytic bacteria

As much as 10% (w/v) of SBE was put into 250 mL enrichment Nutrient Broth (5 g peptone, 3 g yeast extract, and 10 mL CPO/L). The enrichment culture was subcultured in the same medium three times at 30 °C, 150 rpm. The third subculture was serially diluted from 10^{-1} to 10^{-6} using sterile distilled water. Diluted cultures from 10^{-4} to 10^{-6} were inoculated using pour plate method in modified CPO agar (5 g peptone, 3 g yeast extract, 20 g agar, 10 mL CPO and 10 mL tween-80/L), then incubated at 30 °C for 48 hours. After incubation, bacterial colonies were purified using a quadrant streak (Chary and Devi 2018).

Isolated bacteria was screened based on their lipolytic activity and crude lipase activity that were veryfied using tween-80 agar and phenol red agar, respectively. Bacterial isolates were inoculated on tween-80 agar and phenol red agar and incubated at 30°C for 7 days. Tween-80 agar consist of 1% peptone (w/v); 0.5% NaCl (w/v); 0.01% $CaCl_2.2H_2O$ (w/v); 2% agar (w/v); 1% tween-80 (v/v) and phenol red agar consist of 0.1% CPO (v/v); 0.01% phenol red (w/v); 0.1% $CaCl_2$ (w/v); 2% agar (w/v).

A visible precipitate or a clear zone around the isolate on tween-80 agar indicated lipolytic activity. Besides, red color changes to yellow in the phenol red test indicates lipolytic activity (Lee et al. 2015). The isolate on phenol red agar with the highest lipolytic index was further studied. Lipolytic index was calculated using equation 1.

$$\text{Lipolytic index} = \frac{Yellow\ zone\ diameter\ (mm)}{Bacteria\ colony\ diameter\ (mm)} \quad \text{(eq. 1)}$$

Crude lipase activity analysis was carried out as follows: bacterial lipolytic was inoculated in the modified minimal medium consisted of (% w/v) $NH_4H_2PO_4$ 0.1; $MgSO_4.7H_2O$ 0.04; NaCl 0.25; $CaCl_2.2H_2O$ 0.04; peptone 0.2; CPO 2% (v/v); 1-2 drops of tween-80. As much as 20% of bacterial inoculums in the final logarithmic phase were put into 100 mL of a modified minimal medium and incubated at 30 °C, 150 rpm. After 24 hours of incubation, bacterial culture was centrifuged for 20 minutes at 4 °C and a speed of 10,000 rpm. The supernatant was taken as a crude lipase (Veerapagu et al. 2013).

Lipase activity was calculated using the spectrophotometric approach with nitrophenyl palmitate as a substrate. The reaction combination was composed of 0.1 mL lipase, 0.8 mL of 0.05 M Tris buffer (pH 8) and 0.1 mL of 0.01 M para-nitrophenyl palmitate. Before using, para-nitrophenyl palmitate was dissolved in isopropanol. The combination has been incubated at 37°C for 30 minutes and then added with 0.25 mL of 0.1 M Na_2CO_3. The mixture was centrifuged at 11,000 g for 15 minutes, and the optical density (OD) of the supernatant was determined at wavelength of 410 nm. Crude lipase activity was measured using the standard curve of the nitrophenol (equation 2). One unit of lipase activity was described as being capable of releasing several enzymes that release 1 µmol of nitrophenol from the nitrophenyl palmitate (Tripathi et al. 2014).

$$y = 0.0044x - 0.0742 \quad \text{(eq. 2)}$$

Where;
y = absorbance
x = lipase activity

Identification of lipolytic bacteria

The identification of lipolytic bacteria was carried out based on morphological characters of the selected lipolytic bacteria as well as molecular identification. Morphological characters observed were the shape of the colony, elevation, margin, surface texture, cell shape, and Gram properties. The lipolytic bacterium was then identified using the 16S rRNA gene sequence as a genetic marker. The process of DNA isolation was carried out following the protocol kit from Zymo Research.

DNA isolation consists of several steps including bacterial cell preparation, cell lysis, DNA binding, DNA washing, and DNA elution. Bacterial cells (50-100 mg) are put in the micro tube and added 750 µL BashingBead TM Buffer for bacterial cell lysis. Bacterial cells were lysed using a vortex at high speed for 1 minute, and then they were exposed to cold shock at 4ºC for 3 minutes (repeated 5 times). The mixture was centrifuged at a speed of 10,000 g for 1 minute. The supernatant that contains DNA was taken out for further analysis.

The DNA binding step carried out by adding the supernatant with the Zymo-SpinTM III-F Filter. Te supernatant was then centrifuged at 8000 g for 1 minute. The filtrate was taken out and added with 1000 µL Genomic Lysis Buffer. As much as 800 µL of the mixture transferred to the Zymo-SpinTM IIC Column and Collection tube. This mixture centrifuged at 10,000 g for 1 minute.

A 200 µL DNA Pre-Wash Buffer was added to the Zymo-SpinTM IIC Column in a new collection tube and centrifuged for 1 minute at 10,000 g. A total of 500 µL gDNA Wash Buffer was added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 g for 1 minute. The DNA elution was carried out by placing the Zymo-SpinTM IIC Column into a new 1.5 mL microtube and adding 100 µL of DNA Elution Buffer. Eluted samples were then centrifuged at 10,000 g for 30 seconds.

The purity of isolated DNA was verified qualitatively using electrophoresis and quantitatively using spectrophotometric nanodrop at the wavelength ratio of 260/280 nm. Three µL DNA, 1 µL loading dye, and 2 µL ddH_2O were homogenized on parafilm and put in 1% agarose gel. Agarose gel electrophoresis was carried out for 30 minutes at 50 volts. The result was observed under UV

and the pure DNA was used for 16S rRNA gene sequence analysis.

The 16S rRNA gene was amplified using universal primers 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT). Those primers produced 1478 bp sequence 16S rRNA gene (Narayanan 2014; Nurhikmayani et al. 2019). Amplification of the 16S rRNA gene follows the manufacture's protocol from GoTaq® Green Master Mix. The mixture for PCR reaction consists of 12.5 µL GoTaq® Green Master Mix, 1 µL forward primer, 1 µL reverse primer, 1 µL DNA template and 9.5 µL Nuclease-Free Water. The pre-denaturation stage in the amplification process takes place at 95°C for 3 minutes, followed by 30 cycles of denaturation (95°C) for 30 seconds, annealing (57°C) for 1 minute, elongation (72°C) for 1 minute, final elongation (72 °C) for 10 minutes and cooling.

The amplification of DNA was carried out using 1% agarose gel electrophoresis and visualized under UV using 1 kb DNA as a marker. PCR products were sequenced to confirm the amplified DNA sequence. The results of sequencing aligned using the GeneStudio software. The DNA sequence in FASTA format compared for homology using BLASTN (https://www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic analysis

Homology 16S rRNA gene sequences obtained from NCBI were aligned using the MEGA6 software. The phylogenetic tree was then reconstructed with the maximum likelihood method in the MEGA6 program with 1000 bootstrap values (Tamura et al. 2013). Relations between species were investigated based on GC content in the 16S rRNA gene sequence. The analysis uses Models-Computational Composition in MEGA6.

RESULTS AND DISCUSSION

Isolation and screening of lipolytic bacteria from spent bleaching earth (SBE)

This study successfully isolated 11 bacterial strains that could utilize CPO as a carbon source for growth, namely SBE01, SBE02, SBE03, SBE04, SBE05, SBE06, SBE07, SBE08, SBE09, SBE10, and SBE11. These strains were screened for their ability to produce lipase. Lipolytic activity of isolates in tween-80 was characterized by visible precipitate or clear zones around the colony (Kumar et al. 2012; Lee et al. 2015). These visible precipitation is formed due to the reaction between the fatty acids derived from tween-80 hydrolysis and calcium in the medium. In addition, isolates which have lipolytic activity form a yellow zone around the colony in the phenol red medium caused by a decrease in pH (Lee et al. 2015). The decrease in the pH of the media is due to the production of fatty acids resulting from CPO hydrolysis by bacterial lipase. As shown in Table 1, three lipolytic bacteria (SBE01, SBE03, and SBE04) were obtained from qualitative observation in tween-80 agar while four lipolytic bacteria (SBE01, SBE02, SBE03, and SBE04) were obtained from observation in phenol red agar. Some lipolytic bacteria were therefore further analysed to quantitatively assess their function.

The quantitative screening was carried out by measuring the lipolytic index (LI) on phenol red agar medium and crude lipase activity. Lipolytic index values describe the magnitude of the bacterial lipolytic activity. Based on the lipolytic index value, the SBE01 isolate had the highest lipolytic index followed by SBE02, SBE03, and SBE04 isolates. In addition, results of the crude lipase activity assay showed that SBE01 isolate had the highest activity of 5.7 U/mL (Table 2). Therefore, the SBE01 isolate was identified using 16S rRNA gene sequence analysis.

Identification of lipolytic bacteria from SBE

The SBE01 isolate as the selected lipolytic bacteria was characterized based on its morphological characters to support molecular identification. The morphological colony of SBE01 was circular, convex, entire, smooth, and white. The SBE01 isolates classified as Gram-negative cocci (Figure 1). Based on morphological characters following Bergey's Manual Systematic of Bacteriology, SBE01 isolate was included in the genus Moraxella (Brenner et al. 2005). Embers et al. (2011) reported that the Moraxella genus is a family group of Moraxellaceae which are Gram-negative aerobic cocci.

The first step in molecular identification of bacteria based on 16S rRNA gene sequences is DNA extraction. The DNA extraction is performed to isolate the DNA genome from the cells and other components of the cells. The Zymo Research Kit was used to extract DNA genomes from SBE01 isolate. The result of DNA extraction showed that the crude DNA concentration of SBE01 was 176.96 ng/µL (Table 3).

Table 1. Screening of lipolytic activity of bacterial strains from SBE

Isolate	Tween-80 agar	Phenol red agar
SBE01	+	+
SBE02	-	+
SBE03	+	+
SBE04	+	+
SBE05	-	-
SBE06	-	-
SBE07	-	-
SBE08	-	-
SBE09	-	-
SBE10	-	-
SBE11	-	-

Note: lipolytic activity (+); no lipolytic activity (-).

Table 2. Lipolytic index and crude lipase activity of bacterial isolates from SBE

Isolate	Lipolytic index (mm)	Crude lipase activity (U/mL)
SBE01	8.8 ± 0.7	5.7
SBE02	2.6 ± 0.3	4.6
SBE03	2.8 ± 0.4	2.8
SBE04	2.6 ± 0.3	4.0

Table 3. DNA purity and concentration of SBE01 isolate using NanoDrop spectrophotometer

Parameters	Result
Concentration (ng/µL)	176.96 ± 1.500
DNA purity value (A260/280)	1.90 ± 0.002
Volume (µL)	30.00 ± 0.060

The purity of extracted DNA should be determined before it was used as a PCR template. Concentration and purity of DNA decide next step progress. According to Boesenberg-Smith et al. (2012), the concentration and purity of DNA are very crucial because the chemical residues from the extraction and the amount of DNA strongly influence the downstream investigation. DNA purity was measured using electrophoresis and spectrophotometry methods. In the electrophoresis method, the quality of DNA from SBE01 isolate was examined using 1% agarose (Figure 2). It showed that the molecular weight of the DNA band was greater than 10,000 bp. The DNA band result was very thick, sharp, and clear. The thickness of the DNA band can estimate the concentration of DNA. The thicker band indicates higher DNA concentration. According to Sambrook and Russell (2001), DNA quality was demonstrated by the sharpness and clarity of the bands in agarose gel.

DNA purity is also measured using a NanoDrop spectrophotometer at the absorbance ratio A260/280. This ratio is used to calculate DNA and protein concentrations in wavelengths of 260 nm and 280 nm, respectively. At those of the wavelengths, both of DNA and protein will absorb maximally. The result showed that the absorbance ratio of A260 / A280 from DNA isolate was 1.90 (Table 3). The purity of nucleic acids are categorized as good if the value were in the range of 1.8 to 2.0 (Gallacher and Desjardin 2006). Hence, the DNA purity value of SBE isolate was 1.90, it suggested high purity and good efficiency of DNA.

In this study, primers 27F and 1492R were used to amplify the 16S rRNA gene from SBE01 isolate. Primers 27F and 1492R were the best universal primers capable of amplifying almost all 16S rRNA gene sequences (Fredriksson et al. 2013). Visualization of PCR products showed the presence of a single band of more than 1500 bp (Figure 3).

Using Basic Local Alignment Search Tool Nucleotide (BLASTN) (www.ncbi.nlm.nih.gov), the homology for the 16S rRNA gene sequence from SBE01 isolates was compared with the GenBank database. The result revealed that the sequence 16S rRNA gene of SBE01 isolate had similarities with the genus *Moraxella* at various percentages of similarity (Table 4). The BLASTN analysis showed that SBE01 isolates had a similarity range of 92.29-98.60% with GenBank data. The SBE isolate had a similarity of 98.53-98.60% with *Moraxella osloensis*. Based on the range of similarity values, SBE01 isolate could not be identified as *Moraxella osloensis*. According to Drancourt et al. (2000), bacteria was identified in the same species level if it had a similarity value was ≥ 99%. Whereas, the bacteria was identified in the same genus if the similarity value was ≥ 97%. Therefore, SBE01 isolate could be identified as *Moraxella* sp.

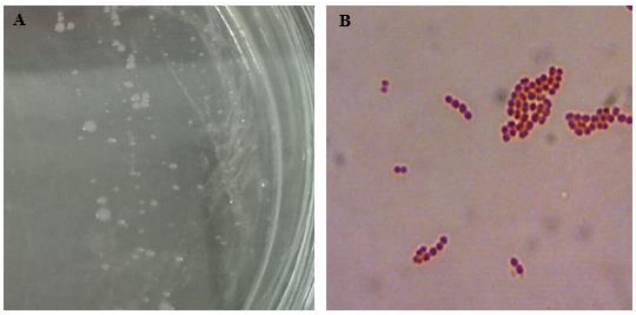

Figure 1. Morphology of colony (A) and bacterial cell with 1000 × magnification using a light microscope (B)

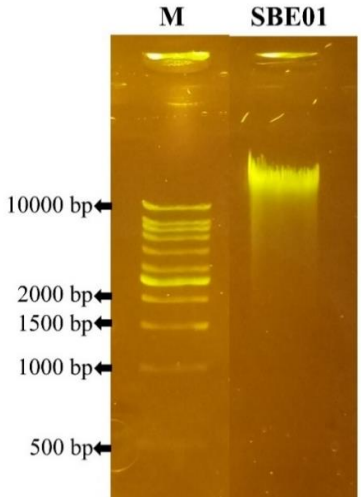

Figure 2. Electropherogram of genomic DNA of the SBE01 isolate on 1% agarose. Note: M: Marker 1 kb; SBE01: SBE01 isolate

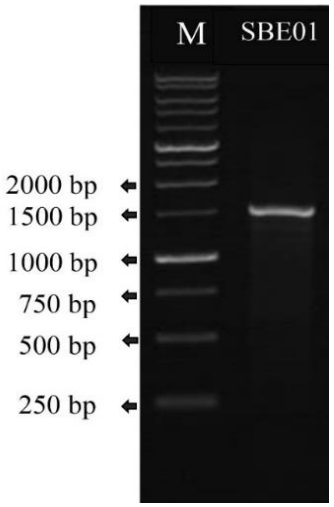

Figure 3. Electropherogram of 16S rRNA gene sequence of the SBE01 isolate using 27F and 1492R primer. Note: M: Marker 1 kb; SBE01: SBE01 isolate

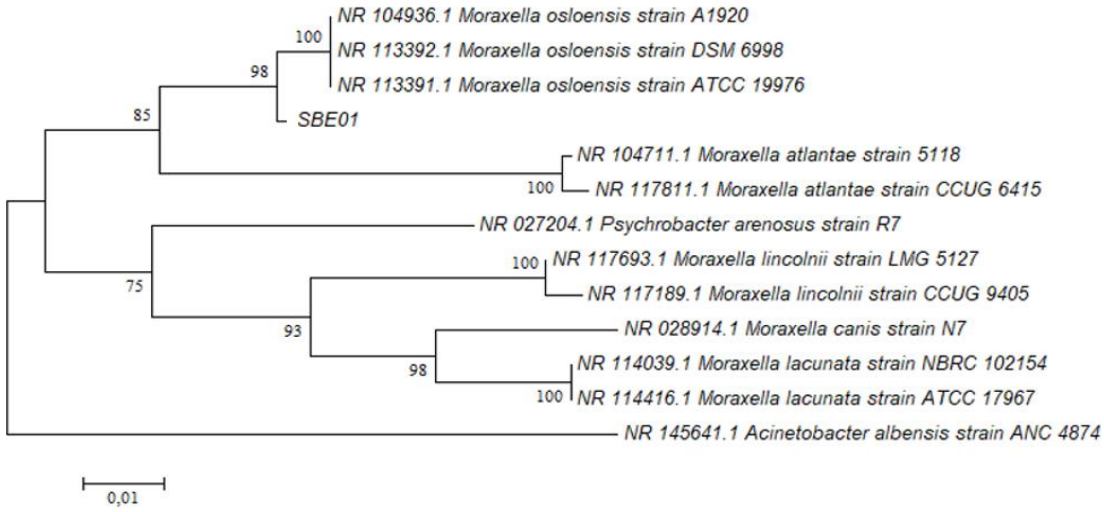

Figure 4. Phylogenetic tree of 16S rRNA gene of SBE01 isolate with *Moraxella* retrieved from GenBank using the Maximum likelihood algorithm in MEGA6 software. Note: The node value shows the bootstrap percentage of 1000 bootstrap replications. The bar value indicates the evolutionary distance of 0.01%.

Phylogenetic analysis

Based on the findings of the BLASTN study as shown in Table 4, the species that have the homologous 16S rRNA gene sequence were then used to reconstruct phylogenetic tree. This phylogenetic tree reconstruction was carried out using the Maximum likelihood method in MEGA6 software. In this phylogenetic tree reconstruction as shown in Figure 4, bacteria species of *Acinetobacter albensis* have been selected as an out group, since this species originates from the same family as the target species (Moraxellaceae).

Phylogenetic tree analysis confirmed that there was a match with the BLASTN analysis of the 16S rRNA gene of the SBS01 isolate. The previous result of BLASTN analysis showed that SBE01 isolate had a similarity value of ≤ 99% with *Moraxella osloensis*. This value was not strong enough to ensure that SBE01 isolate was *Moraxella osloensis*. In comparison with phylogenetic tree result, the SBE01 isolate formed separate clusters but its closer to *Moraxella osloensis* than other species. This result of the phylogenetic tree suggested that the isolate SBE01 and Moraxella osloensis were related and derived from the same common ancestor.

Bacteria were reported have a variation genomic GC content between 13-75% (Venton 2012). These variations of genomic GC content reflected the relationship between species. The consequence of the relationship between SBE01 isolate and *Moraxella osloensis* bacteria species performed in the MEGA6 program using Model-Computational Composition was shown in Table 5. This result showed that the percentage of GC content ranged from 51.2-52.8%.

The genomic GC content calculated at SBE01 isolate was 51.5%. The composition of genomic GC content in SBE01 isolate has similarities with three bacteria isolates from GenBank data, i.e. *Moraxella osloensis* strain A1920, *Moraxella osloensis* strain DSM 6998 and *Moraxella osloensis* strain ATCC 19976. The findings of the GC content review confirm the results from phylogenetic tree that SBE01 isolate derived from the Moraxella genus, which is closely related to the *Moraxella* sp. This result was in accordance with a result analysis by Haywood-Farmer and Otto (2003) showing that *Moraxella* was a bacterial genus with 51.55% GC content.

Tabel 4. Species similarity of SBE01 isolate with the GenBank data

Isolate	Species	Similarity (%)	Accession number
SBE01	*Moraxella osloensis* strain A1920	98.60	NR_104936.1
	Moraxella osloensis strain DSM 6998	98.53	NR_113392.1
	Moraxella osloensis strain ATCC 19976	98.53	NR_113391.1
	Moraxella atlantae strain 5118	92.84	NR_104711.1
	Moraxella atlantae strain CCUG 6415	92.29	NR_117811.1
	Moraxella lacunata strain ATCC 17967	92.73	NR_114416.1
	Moraxella lacunata strain NBRC 102154	92.73	NR_114059.1
	Moraxella lincolnii strain CCUG 9405	92.29	NR_119189.1
	Moraxella lincolnii strain LMG 5127	92.71	NR_117693.1
	Moraxella canis strain N7	92.67	NR_028914.1
	Psychrobacter arenosus strain R7	93.59	NR_027204.1

Tabel 5. Composition of 16S rRNA nucleotide genes and gaps in SBE01 isolate

Species	Nucleic acid composition (%)						Total	Gaps (%)
	T(U)	C	A	G	GC	AT		
SBE01	21.9	21.3	26.6	30.2	51.5	48.5	1411	1.2
Moraxella osloensis strain A1920	21.8	21.5	26.7	30.0	51.5	48.5	1409	1.3
Moraxella osloensis strain DSM 6998	21.7	21.5	26.7	30.0	51.5	48.4	1409	1.3
Moraxella osloensis strain ATCC 19976	21.7	21.5	26.7	30.0	51.5	48.4	1409	1.3
Moraxella atlantae strain 5118	20.1	21.6	28.0	30.2	51.8	48.1	1405	1.6
Moraxella lacunata strain NBRC 102154	21.6	21.7	26.8	29.9	51.6	48.4	1407	1.5
Moraxella lacunata strain ATCC 17967	21.6	21.7	26.8	29.9	51.6	48.4	1407	1.5
Moraxella lincolnii strain LMG 5127	21.4	21.6	27.3	29.7	51.3	48.7	1405	1.6
Psychrobacter arenosus strain R7	20.8	22.3	26.4	30.5	52.8	47.2	1414	1.0
Moraxella lincolnii strain CCUG 9405	21.5	21.6	27.3	29.6	51.2	48.8	1405	1.6
Moraxella canis strain N7	21.1	22.1	26.5	30.2	52.3	47.7	1409	1.3
Moraxella atlantae strain CCUG 6415	20.2	21.5	27.9	30.4	51.9	48.1	1407	1.5
Acinetobacter gandensis strain UG 60467	21.7	21.7	26.3	30.3	52.0	48.0	1417	0.8

Based on the GC content analysis, the SBE01 isolate was closely related to *Moraxella* sp although the two bacteria were different species. The two species comprised two clusters in the phylogenetic tree as displayed at Figure 3. This difference was caused by the presence of gaps in the nucleotide sequence. Gaps indicated mutations in the sequences during evolution due to insertions and deletions. Gaps are very important information in determining an organism's evolutionary relationship. The presence of a gap can influence the length of the branching in the topology of the phylogenetic tree. According to Ashkenazy et al. (2014), insertions and deletions played an important role in determining various taxa and increasing the accuracy of phylogenetic reconstruction. Gaps in the 16S rRNA gene sequence of some species as shown in Table 5 were analyzed in the multiple sequence alignment process. The analysis reported that there was a 1.2% gap that made the difference between SBE01 isolate and other species.

It can be concluded based on the screening of 11 bacterial isolates from spent bleaching earth obtained SBE01 isolate with the highest lipolytic index and crude lipase activity of 8.8 ± 0.7 mm and 5.7 U/mL, respectively. This isolate was identified as *Moraxella* sp. based on the 16S rRNA gene sequence. On a microcosm scale, this isolate could degrade lipids. Further investigation of lipases from this bacteria needs to be carried out to obtain more detailed information and determine the potential of lipase in removing pollutants, including oil, hydrocarbons, plastics, and pesticides.

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