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Isolation and Identification of some Heterotrophic Sulfur Oxidizing Bacteria Isolated in Mangrove along to Haiphong - Quangninh Coastal

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ABSTRACT: The study was conducted to isolate and identify heterotrophic sulfur oxidizing bacteria isolated in the mangrove along to Haiphong - Quangninh coastal. Results showed that out of 15 isolated bacterial strains, 9 of them can reduce the pH of the environment compared to the initial pH 8.0. They also can produce sulfate ion in broth medium with thiosulfate substrate. Based on the phenotypic and molecular characteristics, they are placed into *Bacillus cereus*, *B. anthracis*, *Bacillus spp.* SO2, *Bacillus spp.* SO4, *Bacillus spp.* SO5, *Bacillus spp.* SO8, *Pseudomonas plecoglossicida* SO7, *Micrococcus spp.* SO9 and *Klesiella spp.* SO6.

Keywords: Mangrove, sulfur oxidizing bacteria, phenotypic, molecular, Haiphong - Quangninh coastal.

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INTRODUCTION

In mangroves, inorganic sulfur usually exists in high concentrations and is an important factor in the biogeochemical cycle of sedimentary environment (Alongi, 1992; Behera *et al*., 2014). The presence of sulfur has a great influence on the content and status of other elements such as oxygen, nitrogen, carbon, phosphorus and iron (Bruser *et al*., 2000; Babana *et al*., 2011; Jian *et al*., 2017). Inorganic sulfur is created by decomposing organic sulfur compounds and exists in reducing state (S^{2-}, S^{0}) or oxidizing state (S^{4+}, S^{6+}). When plant and animal proteins are degraded, sulfur is released and accumulates in the soil which is then oxidized to sulfate in the presence of oxygen and under anaerobic condition, organic sulfur is decomposed to produce hydrogen sulfite (H_2S). H_2S can also accumulate during the reduction of sulfate under anaerobic conditions which can be further oxidized to sulfate under aerobic conditions (Behera *et al*., 2014).

$$S^{2-} \rightarrow S^{0} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$$

Microbial sulfur transformation is a key process for the biogeochemical sulfur cycle in mangrove. The process comprises of mineralization of organic sulfur to the inorganic forms, conversion of inorganic sulfate into organic forms for systhesis of microbial tissue, oxidation of reduced-state sulfur into oxidized-state sulfur, and reduction of oxidized-state sulfur into reduced-state sulfur (Behere *et al*., 2014; Jorgensen *et al*., 2019).

Microorganisms involved in the oxidation of sulfur have been early recognized as photoautotrophic and chemolithotrophic bacterial groups. Phototrophic oxidation of sulfur is an anaerobic process which is carried out by green sulfur bacteria such as *Chlorobium*, and purple sulfur bacteria such as *Allochromatium*, *Chromatium*, *Thiocystis*, *Thiococcus*, *Thiospirillum*, etc. Chemolithotrophic sulfur oxidation is an aerobic process which is carried by colourless sulfur bacteria such as *Thiobacillus*, *Sulfolobus*, *Thiomicrospira*, *Beggiatoa*, *Thiothrix*, *Desulfuromonas*, etc. (Pfenning & Truper, 1989; Chapman, 1990; Suzuki *et al*., 1999; Ravichandra *et al*., 2007; Kamba *et al*., 2014; Gros *et al*., 2018). Recent research shows that sulfur oxidation is not only performed by the autotrophic bacteria groups but also by heterotrophic bacteria groups such as *Pseudomonas*, *Xanthobacter*, *Bacillus*, *Micrococcus*, *Klebsiella* and *Escherichia*. Some heterotrophic sulfur oxidizing strains play a more important role than *Thiobacilli* (Nakada *et al*., 1999; Schook & Berk, 1979; Sorokin *et al*., 1999; Behera *et al*., 2014; Kour *et al*., 2018; Hassanshahiana *et al*., 2019).

Currently, sulfur is considered as an important nutrient for plants only after N, P and K and is one of 16 essential nutrients for plant growth and development (Shinde *et al*., 1996). However, plants only absorb primarily in the oxidation state (SO_4^{2-}). In addition, sulfur exists as a reducing agent like sulfide which is toxic to aquatic animals. Therefore, the study of sulfur oxidizing bacteria group has high scientific and practical significance. This is the scientific basis to study and apply them in the fields of mangrove restoration and environmental treatment.

MATERIALS AND METHODS

A. Samples

Sediment samples were collected at 06 sites in the coastal mangrove forest along to Hai Phong - Quang Ninh coastal. At each site, the sediments were collected at 5 different points then mixed together and put into sterile zipper bags with proper labelling. During the sampling process, remove the thin surface layer (about 1 cm) and only collect the lower sediment. The samples are stored in an ice box before being brought to the laboratory for processing and analysis.

B. Isolation of sulfur oxidizing bacteria

First, sulfur oxidizing bacteria are enriched in mineral medium. The enrichment medium of sulfur oxidizing bacteria includes: 0.4g of NH_4Cl, 0.2g of $MgCl_2.6H_2O$, 1.2g of KH_2PO_4, 1.2 g of K_2HPO_4, 5.0g of $Na_2S_2O_3.5H_2O$, 0.01g of $MgSO_4.7H_2O$ and 15g of NaCl dissolved in 1 liter of distilled water. The medium is adjusted to pH to 7.5 - 8.0 and sterilized at 121^oC, 1 atm within 30 minutes. Inoculate 10g sediment into a flask containing 90ml of the enrichment medium and culture at 90 rpm for 1 minute for 7-10 days

After the enrichment stage, spread the enrichment fluid onto agar plate with composition similar to the enrichment medium with additional 5.0 g of Pepton and 18.0 g of agar (in 1 liter of medium), kept in an incubator at 30^oC within 72 hours. Select typical colonies, streak on agar plate for purification and preservation of microorganisms.

C. Initial screening of sulfur oxidizing bacteria

For qualitative screening of sulfur oxidizing strains, the isolates will be transferred to enrichment medium with pH phenol red indicator, pH adjusted medium to 8.0. Sulfur-oxidizing strains convert $S_2O_3^{2-}$ to SO_4^{2-} which acidifies the environment, which changes the color from orange to yellow (Behera *et al*., 2016).

D. Determination of sulfate production activity

The amount of sulfate ions (SO_4^{2-}) generated during growth on thiosulfate medium is determined by spectrophotometic method. Cultures of microorganisms are screened into 10 ml of thiosulfate solution. The test tubes were cultured at 30°C for 7 days. After 7 days of enrichment, the suspension was centrifuged 7,000 rpm for 10 minutes to eliminate bacterial biomass.

The supernatant is supplemented with sulfate reagent in proportion of 5 ml of clear solution: 1ml of buffer solution and mix well (dissolve 30g of $MgCl_2.6H_2O$, 5g of $CH_3COONa.3H_2O$, 1g of KNO_3 and 20ml of CH_3COOH in 500ml of distilled water and dilute to 1000ml), 0,1 g of $BaCl_2$ crystals, shake well for 1 minute.

The white turbidity due to $BaSO_4$ formation is measured at 450 nm using a spectrophotometer. The received value will be referenced with the sulfate standard curve using Na_2SO_4 as the standard for constructing the calibration curve (Kolmert *et al*., 2000).

E. Analysis of biological characteristics

Active bacterial strains will be analyzed for morphological, physiological and biochemical characteristics. The basic morphological characteristics are colony morphology, cell shape and size. Biochemical characteristics analyzed include Gram staining, mobility, catalase, oxidase, urease resolution, Voges-Proskauer reaction (VP), Indole production, nitrification reduction, citrate anabolism, starch hydrolysis, hydrolysis Gelatin stool, hydrolyzed sugars. In addition, several physiological characteristics such as the ability to grow in environments with different temperatures, pH and salinity were analyzed.

F. Analysis of 16S rRNA

Four of 9 sulfur oxidizing bacterial strains (SO1, SO2, SO3 and SO7) are chosen to sequence of 16S rRNA. Chromosomal DNA was extracted by using a Cell SV mini (GenAll, Korea). The 16S rRNA genes were amplified using PCR with OneTaq® Hot Start 2X Master Mix (NEB, USA) and the universal primer pair of 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1527r (5'-AGGAGGTGATCCAGCCGCA-3') described by Lane (1991).

The PCR products purified with Gen JETTM PCR Purification Kit (Thermo Scientific) were sequenced directly using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems). Sequence reaction mixtures were electrophoresed and analysed with an Applied Biosystems^{TM} 3500 Genetic Analyzer. Subsequently, the nucleotide sequence was calibrated based on fluorescence signals using BioEdit software in combination with comparing 16S rRNA data of bacterial strains on GenBank. Based on the sequence of 16S rRNA gene segments of 4 bacteria strains, we proceeded to build the phylogenetic tree based on MEGA X software by the Neighbor-Joining Tree method with Bootstrap 1000.

RESULTS AND DISCUSSIONS

A. Isolation of sulfur oxidizing bacteria

After enrichment in the mineral broth medium, 15 strains of bacteria were isolated and purified on agar plate with $S_2O_3^{2-}$ substrate. These bacterial strains are cultured in a broth medium with a phenol red indicator, sulfur-oxidizing bacteria will acidify the environment by converting $S_2O_3^{2-}$ to SO_4^{2-} and causing the environment to change from orange to yellow. 09 strains that changed the color of the broth medium were re-examined on agar plates using phenol red indicator for confirmation. The isolates were considered as sulfur oxidising bacteria and named as SO1-SO9.

B. Sulfate ion production of sulfur oxidizing bacteria

The strains changed the color of the medium are inoculated into mineral broth media to assess the ability to produce sulfate ion. Of the 09 bacterial strains tested, the SO7 strain can produce sulfate ion in highest - 86.60 mg/ml within 10 days of culture, followed by SO9 - 63.95 mg/ml, SO8 - 28, 29 mg/ml, SO1 - 20.92 mg/ml, SO4 - 20.02 mg/ml, SO5 - 11.0 mg/ml, SO2 - 10.01 mg/ml, SO3 - 10.0 mg/ml, and the lowest is SO6 with sulfate ion production of 8.0 mg/ml after 10 days of culture (Fig. 1).

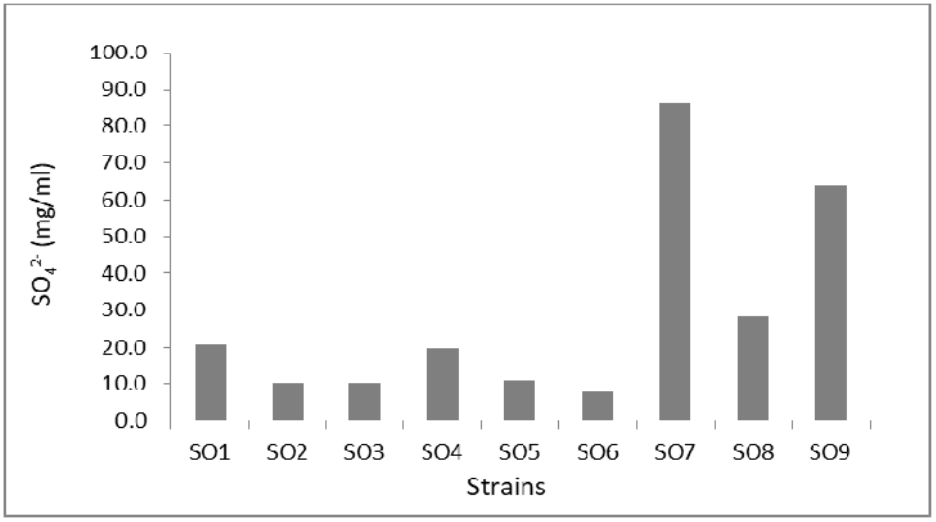

Fig. 1. Sulfate producing potential of sulfur oxidizing isolates.

Other studies showed that sulfate ion production ranged from 14-245 mg/ml. Ravichandra *et al.* (2007) reported that the maximum sulfate ion production from 14-150 mg/ml by a *Thiobacillus spp.* Babana *et al.* (2011) reported that the sulfate ion production was the highest in strain ATTC55128 and followed by AHB436 with sulfate ion concentration of 230 mg/ml. Behera *at al.* (2014) reported that the sulfate ion production was the highest in SOB-7 (245 mg/ml) and the lowest in SOB-4 (125 mg/ml).

C. Morphological and biochemical characteristics

Almost of isolates are rod-shaped cells exception of SO9 with spherical shape. Cell size ranged from 0.6-1.4 × 1.6 - 4.0 µm.

All strains are mobile and aerobic. The 6 isolates are Gram-positive cells, 2 isolates are Gram-negative cells, 1 isolate has changing Gram reactions - SO3. 6 isolates are capable of spore-forming comprising of SO1-SO5 and SO8, 03 remaind isolates of SO6, SO7 and SO9 are unable to produce spores.

Most strains have a positive activity of catalase, gelatin hydrolyzate and using glucose and mantose. However, most strains did not have activity of citrate using, LDC was negative. All strains were unable to generate gas from glucose and could not assimilate lactose. The remaining biochemical indicators were equally positive and negative activities among 09 studied strains (Table 1).

Table 1: Morphological and biochemical characteristics of sulfur oxidizing bacteria.

Characteristics	SO1	SO2	SO3	SO4	SO5	SO6	SO7	SO8	SO9
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Cocci
Gram stain	+	+	-/+	+	-/+	-	-	+	+
Size (µm)	0.8-1 x 2.6-4	0.8-1.2 x2.4-3.6	0.9-1.2 x2-3.2	0.8-1x 2.4-4	0.6-0.8 x2-2.4	1-1.4x 2.8-3.2	0.6-0.8 x1.6-2.4	1-1.2 x1.8-2	0.2-0.3
Spore	+	+	+	+	+	-	-	+	-
Growth on NA	+	+	+	+	+	+	+	+	+
Mobility	+	+	+	+	+	+	+	+	+
Aerobic growth	+	+	+	+	+	+	+	+	+
Oxidase	-	+	+	-	+	-	+	-	-
Catalase	+	+	-	+	-	+	+	+	+
MR	+	+	-	+	+	-	-	-	-
Citrate	-	-	-	-	-	-	+	-	-
Urease	-	-	+	+/-	+/-	-	+	+	-
H_2S	-	-	-	-	-	-	-	-	-
ADH	+	+	-	-	+	-	+	+	-
LDC	-	-	-	-	-	-	+	-	-
ONPG	-	-	+	-	-	+	-	+	+
Nitrate reduction	+	+	-	+	+	+	-	-	-
Protease:									
Gelatin	+	+	+	+	+	-	-	+	+
Gas production									
Glucose	-	-	-	-	-	-	+	-	-
Acid from:									
Glucose	+	+	+	+	+	+	-	+	+
Lactose	-	-	-	-	-	-	-	-	-
Mantose	+	+	+	+	+	+	-	+	+
Saccarose	-	-	-	+	-	-	-	+	+

The bacterial isolates can develop on environments with salinity of 1 - 6%, the best range is from 1% - 3%, corresponding to the study area, growing well in high alkaline environment with pH 7 - 9, almost them does not grow or is destroyed very quickly in an acidic environment. All studied strains were anaerobic bacteria and warm-loving bacteria, growing in the range of 20-30^{o}C with the optimal temperature of 30°C strains (Table 2).

Table 2: Some physiological characters of the sulfur oxidizing bacteria.

Strains	Temperature (^{o}C)					Salinity (‰)						pH			
	5	10	20	30	40	0	1	3	6	8	10	3	5	7	9
SO1	-	-	++	+++	+	+	++	++	+	-	-	-	++	++	+
SO2	-	-	++	+++	+	+	++	+++	++	+	-	-	+	+++	+
SO3	-	+	++	+++	+	+	++	+++	+	-	-	-	+	+++	+
SO4	-	-	++	+++	+	+	++	+++	++	+	-	-	+	+++	++
SO5	-	+	++	+++	+	+	++	++	+	-	-	-	+	+++	+
SO6	-	+	++	+++	+	+	++	+++	+	-	-	-	-	+++	++
SO7	-	-	++	+++	+	+	++	+++	++	+	-	-	+	+++	+
SO8	-	-	++	+++	+	+	++	++	+	-	-	-	+	+++	+
SO9	-	+	++	+++	+	+	++	+++	++	+	-	-	+	+++	++

** (-): No grow; (+): Weak grow; (++): Medium grow; (+++): good grow*

According to Bergey's Manual of Systematic Bacteriology and on the basis of phenotypic characteristics, the isolates are tentatively identified as following:

SO1, SO2, SO3, SO4, SO5 and SO8 are rod-shaped, Gram-positive, spore-forming cells, the isolates are tentatively assigned to the genus *Bacillus*.

SO9 is Gram-positive spheres, nonmoblie and nonsporeforming, strict aerobic and catalase positive. The isolate is tentatively assigned to the genus *Micrococcus*, named *Micrococcus spp*. SO9.

SO6 is Gram-negative, oxidase-negative, rod-shaped bacteria, the isolate is placed into the genus Klebsiella, named *Klebsiella spp*. SO6.

SO7 is Gram-negative, oxidase-positive, aerobic, rod-shaped bacteria, mobile, oxidize but do not ferment glucose. The isolate is placed into the genus Pseudomonas, named *Pseudomonas spp.* SO7.

C. 16S rRNA gene sequencing analysis

For molecular identification of the isolates, we used the pair of primers 16S rRNA. Four bacterial strains (SO1, SO2, SO3 and SO7) were selected to determine the gene sequence. After amplification and sequencing, the 16S rRNA gene segment was compared with the 16S rRNA gene segments on Genbank by BLAST tool. The analysis results showed that the level of similarity of the studied bacterial strains with the strains available on the Genebank ranges from 90.83 to 99.31%.

The highest similarity is 99.31% between SO7 and *Pseudomonas plecoglossicida* (KY880958, LT718471, LT718463, LT718459). The SO3 isolate has 99.17% similarity against *Bacillus cereus* (MN421119, MN420730, MN232160, MN232137). Strain SO1 has 98.43% homogeneity compared to *Bacillus spp.* (MG733579, MG733513, KX641570, KF641920, AY842872). The SO2 strain had 90.83% similarity to the *Bacillus megaterium* (MF083056, MF076231, MF076557, KY616664) (Table 3). This result can initially confirm that the isolate SO1 is *Bacillus* spp., SO3 is *Bacillus cereus*, SO7 is *Pseudomonas plecoglossicida*. However, SO2 is not classified into available isolate because of the similarity is lower than 97%, named *Bacillus* spp. SO2.

Based on the sequence of 16S rRNA gene segments of 4 bacterial strains, we proceeded to build a phylogenetic tree based on MEGA X software by the Neighbor-Joining Tree method. The result is shown in Fig. 2.

The results of comparative analysis of the 16S rRNA gene sequence showed that SO1 belongs to the species *Bacillus cereus* or *Bacillus anthracis* with similarities of 98.43%. However, according to Bergey's Manual of Systematic Bacteriology, the phenotypic characteristics of SO1 is more similar to *Bacillus anthracis* than *Bacillus cereus*. SO1 can not utilize citrate while *Bacillus cereus* can utilize citrate.

The results of comparative analysis of the 16S rRNA gene sequence showed that SO2 had the highest similarity to *Bacillus megaterim* at 90.83%. It is placed in genus Bacillus, named as *Bacillus spp.* SO2.

The results of comparative analysis of the 16S rRNA gene sequence showed that SO3 belongs to the species *Bacillus cereus* with the similarity of 99.17%. However, there are some phenotypic characteristics different from those of *Bacillus cereus* in Bergey's (1979) such as oxidase, catalase and citrate utilization. On basis of phenotypic and molecular characteristics, it is named as *Bacillus cereus* SO3.

Table 3: Similarity of 16S rRNA among the isolates and available strains on Genbank.

Clone	Accession no.	Description	Identities
SO1	MG733579.1	*Bacillus* sp. (in: Bacteria) strain APBSWPTB106 16S ribosomal RNA gene, partial sequence	98.43%
	MG733513.1	*Bacillus anthracis* strain APBSWPTB40 16S ribosomal RNA gene, partial sequence	98.43%
	KX641570.1	*Bacillus cereus* strain BacI87 16S ribosomal RNA gene, partial sequence	98.43%
	KF641920.1	*Bacillus anthracis* strain WY2 16S ribosomal RNA gene, partial sequence	98.43%
	AY842872.1	*Bacillus cereus* strain CICC10185 16S ribosomal RNA gene, partial sequence	98.43%
	MH643889.1	*Bacillus* sp. (in: Bacteria) strain RP6 16S ribosomal RNA gene, partial sequence	98.24%
	MN428211.1	*Bacillus cereus* strain PK2-18 16S ribosomal RNA gene, partial sequence	98.24%
	MN422007.1	*Bacillus anthracis* strain DFRL.BHE_12 16S ribosomal RNA gene, partial sequence	98.24%
SO2	MF083056.1	*Bacillus megaterium* strain USS-CAP-2 16S ribosomal RNA gene, partial sequence	90.83%
	MF076231.1	*Bacillus megaterium* strain DOS-CAP-3 16S ribosomal RNA gene, partial sequence	90.83%
	MF076557.1	*Bacillus megaterium* strain DSS-ERY-6 16S ribosomal RNA gene, partial sequence	90.83%
	KY616664.1	*Bacillus megaterium* strain DOW-CTC-4 16S ribosomal RNA gene, partial sequence	90.83%
	KY312763.1	*Bacillus meqaterium* strain GT21 16S ribosomal RNA gene, partial sequence	90.83%
SO3	MN421119.1	*Bacillus cereus* strain SK4-6.2 16S ribosomal RNA gene, partial sequence	99.17%
	MN420730.1	*Bacillus cereus* strain B34 16S ribosomal RNA gene, partial sequence	99.17%
	MN232160.1	*Bacillus cereus* strain PR58 16S ribosomal RNA gene, partial sequence	99.17%
	MN232137.1	*Bacillus cereus* strain PR10 16S ribosomal RNA gene, partial sequence	99.17%
SO7	KY880958.1	*Pseudomonas plecoglossicida* strain SitB431 16S ribosomal RNA gene, partial sequence	99.31%
	LT718471.1	Uncultured *Pseudomonas* sp. partial 16S rRNA gene, isolate VM10	99.31%
	LT718463.1	Uncultured *Pseudomonas* sp. partial 16S rRNA gene, isolate VKh14	99.31%
	LT718459.1	Uncultured *Pseudomonas* sp. partial 16S rRNA gene, isolate VKh7	99.31%
	JF688587.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03G59VV 16S ribosomal RNA gene, partial sequence	99.31%
	JF688535.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03G4ZON 16S ribosomal RNA gene, partial sequence	99.31%
	JF688309.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03G7QBY 16S ribosomal RNA gene, partial sequence	99.31%
	JF687910.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03HB4YA 16S ribosomal RNA gene, partial sequence	99.31%
	JF687807.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03GGMYY 16S ribosomal RNA gene, partial sequence	99.31%
	JF687449.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03HCARB 16S ribosomal RNA gene, partial sequence	99.31%
	JF687392.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03F706Y 16S ribosomal RNA gene, partial sequence	99.31%
	JF687344.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03GAOAX 16S ribosomal RNA gene, partial sequence	99.31%
	JF686511.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03G0KGL 16S ribosomal RNA gene, partial sequence	99.31%
	JF686442.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03FXJL7 16S ribosomal RNA gene, partial sequence	99.31%
	JF685734.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03G5K29 16S ribosomal RNA gene, partial sequence	99.31%
	JF685611.1	*Bacterium* enrichment culture clone ALO1_GLFRUDD03HCHGR 16S ribosomal RNA gene, partial sequence	99.31%
	AB247233.1	*Pseudomonas* sp. Hugh2773 gene for 16S rRNA, partial sequence, strain: Hugh2773	99.31%
	MN429134.1	*Pseudomonas* sp. strain RABA8 16S ribosomal RNA gene, partial sequence	99.08%
	MN227542.2	*Pseudomonas mosselii* strain NG1 16S ribosomal RNA gene, partial sequence	99.08%
	MK519202.1	*Pseudomonas plecoglossicida* strain pgE 16S ribosomal RNA gene, partial sequence	99.08%
	MK519182.1	*Pseudomonas plecoglossicida* strain Pg2-1 16S ribosomal RNA gene, partial sequence	99.08%
	MK519152.1	*Pseudomonas plecoglossicida* strain NK21 16S ribosomal RNA gene, partial sequence	99.08%
	MN380226.1	*Pseudomonas plecoglossicida* strain ATRS 618 16S ribosomal RNA gene, partial sequence	99.08%

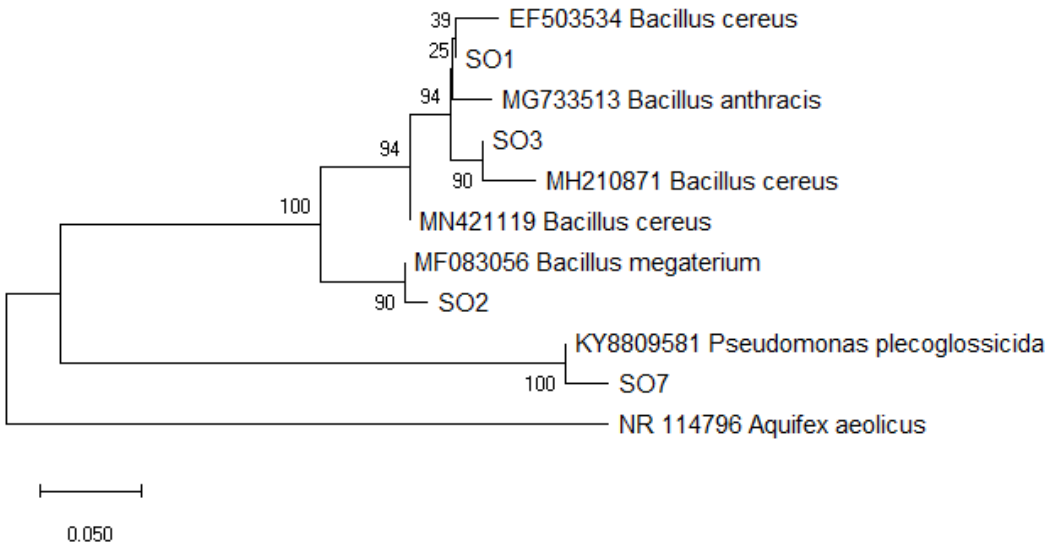

Fig. 2. Phylogentic tree of isolates using 16S rRNA.

The results of comparative analysis of the 16S rRNA gene sequence showed that SO7 belongs to the species *Pseudomonas plecoglossicida* with the similarity of 99.31%. Although there is the high similarity in molecular level, the SO7 was clearly different in phenotypic charaterization. *Pseudomonas plecoglossicida* can reduce nitrate to nitrite while SO7 can not reduce. It is named as *Pseudomonas plecoglossicida* SO7.

Heterotrophic bacteria oxidised reduced sulfur compounds into sulfate are revealed in different ecosystems such as mangrove, coal mines, wastewater, soil. In mangrove environment, the bacteria are identified into the genus *Bacillus, Pseudomonas, Micrococcus, Klebsiella, Xanthobacter and Escherichia* (Sorokin *et al.,* 1999; Thaitoi *et al.,* 2012; Behera *et al.,* 2014; Behera *et al.,* 2016).

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