BIOREMEDIATION ACTIVITY OF ISOLATED CHROMIUM-RESISTANT BACTERIA FROM ESTUARY SEDIMENTS IN LA UNION, PHILIPPINES

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Abstract: Chromium contamination in sediments is contributed by industrialization. Elevated levels of Cr(VI) have crucial effects on living organisms' health and are detrimental to the environment. Bioremediation is an innovative and sustainable way of reducing hazardous substances to lesser ones. In this study, Cr-resistant bacteria were isolated from Maragayap Estuary sediments and its bioremediation activity was assessed. Soil samples were collected from the study site and were serially diluted and grown in Luria-Bertani (LB) medium. Soil microcosm was amended with 60 ppm Cr(VI) and its heavy metal levels were evaluated in four weeks through US EPA 3060A and 7196A. Bacterial isolates were characterized and identified by 16s rRNA gene sequencing and their phylogenetic trees were constructed. Based on heavy metal analyses, Maragayap Estuary was found to be polluted with chromium. Isolates, designated as LG-01 and LG-03, were identified as *Staphylococcus sciuri* and *Bacillus cereus*, and LG-02 as the novel bacteria, *Bacillus aerius*. It was observed that all isolates can tolerate up to 300 ppm of chromium. The two Bacillus species produced putative carotenoids due to heavy metal stress. Results revealed that there was a reduction of Cr(VI), as bioremediation time progresses. The bacterial consortium reduced chromium faster than pure cultures at a rate of 0.8931 ppm/week. Individually, *S. sciuri* had the highest reduction of 1.65 ppm in four weeks. The results of this study may inform the community on possible human pathogens thriving in estuaries and may provide a possible microbial source of bioremediation agent to address environmental concerns in the freshwater ecosystem.

Keywords: *bioremediation, chromium-resistant bacteria, molecular identification, estuary*

1. INTRODUCTION

Estuaries are one of the most biologically prolific environments on the planet. It serves as a crucial natural habitat among numerous animals and a catch basin of sediments and run-off from other bodies of water (United States Environmental Protection Agency [US EPA], 2015). Due to its limited absorption capacity, nutrients and pollutants are being confined in the water system, which is a potential biological risk in the ecosystem (Pinet, 2011; Lakshmanasenthil et al., 2013). Thus, dealing with pollution on estuarine sediments imposes a great concern in the scientific community (Caeiro et al., 2005).

Heavy metals are naturally present in bodies of water in very low concentrations. However, as a by-product of industrial activities, its levels intensely increased, which enforce a threat in natural ecosystems (Ansari et al., 2004; Naser, 2013; Saranraj & Sujitha, 2013). Chromium is a heavy metal considered an essential element in trace amounts of Cr(III). However, Cr(VI) a highly toxic form of chromium causes severe health issues when exposed for a long time (Cervantes et al., 2000; Silver et al., 2001; Saranaj & Sujitha,

2013). The trivalent form is less toxic and immobile compared to the hexavalent form that is naturally mutagenic and carcinogenic. Thus, the absence of reducing agents that can convert chromium(VI) to lesser toxic ones imposes a major threat to the environment (Kamaludeen et al., 2003).

Bioremediation is a technology of utilizing microorganisms to degrade contaminants in the environment. Intrinsic bioremediation is a widely used approach to detoxify environments using organisms thriving within the polluted site (US EPA, 2006).

In this study, molecular characterization, and bioremediation ability of isolated chromium(VI) resistant bacteria from the sediments in the estuary of Maragayap River in Bacnotan, La Union was evaluated.

2. METHODOLOGY

2.1 Collection of soil samples

Soil sediments were collected from the topsoil horizon (0-20 centimeters) (Chai et al., 2009) in an estuary of Maragayap River located at Luna, Bacnotan, La Union, Philippines (16º45.658' N and 120°20.226' E). Sediments were placed in plastic zipper bags and air-dried for 24 hours and homogenized using a two-millimeter plastic mesh wire. Samples were submitted to Benguet State University, in La Trinidad, Benguet for chemical analysis such as nitrogen, phosphorus, potassium, pH, soil moisture, and organic matter.

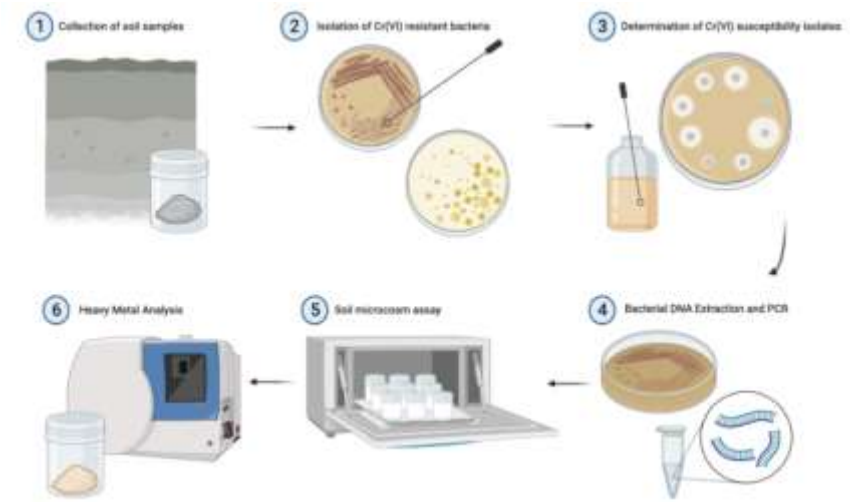

Figure 1. Flowchart of activities.

2.2 Isolation of Cr (VI) resistant bacteria

Ten grams of soil sample were added to three 250 mL Erlenmeyer flasks containing 150 mL of modified Luria-Bertani (LB) broth (Hi-media, Mumbai, India) comprising (g/L) peptone (10.0), yeast extract (5.0), beef extract (5.0), and NaCl (5.0), and 60 mg/L of chromium(VI) in potassium dichromate or $K_2Cr_2O_7$ (99% purity). Bacterial cultures were grown at room temperature for 24 hours (Chrysochoou et al., 2013).

One milliliter of the inoculum underwent six serial fold dilutions until 10^{-6} and one mL aliquots of each dilution was cultured on a solid medium containing the enriched broth with 15 grams of agar for every liter of distilled water. The plates were stored in an incubator at 37°C for 72 hours, and each morphologically distinct colony was transferred to fresh agar plates for purification with the same incubation conditions. Each isolated colony was grown in LB broth for the disk diffusion test and soil microcosm.

2.3 Determination of Cr(VI) susceptibility of bacterial isolates

One milliliter of 48-hour old bacterium grown in LB broth was poured in a spread plate with LB agar. The potassium dichromate was obtained from the University of the Philippines-Baguio Chemistry Laboratory, Baguio City, Philippines. It was dissolved in distilled water with the following concentrations: 60 mg/L, 120 mg/L, 180 mg/L, 240 mg/L, and 300 mg/L. Sterile Whatman® Grade 1 paper disks (Sigma-Aldrich, Darmstadt, Germany) were soaked in each metal solution overnight and were dried at 37°C for one hour (Luli et al., 1983). The filter papers were placed on the plates of each isolate together with the control containing distilled water only.

The plates were incubated at 37ºC for 48 hours and the zone of inhibition (ZOI) of each disk was measured using a Vernier caliper (Mitituyo, Kanagawa, Japan). Small disc diameter indicated that culture is more resistant to heavy metals than larger ones. Moreover, Molina et al. (2010) designated considerations in assessing the activity of the isolates. Cultures were deliberated resistant if they have less than five mm diameter ZOI; intermediate ones have 6 to 16 mm and sensitive to the metal with greater than 16 mm diameter.

2.4 Bacterial DNA extraction and polymerase chain reaction

The cell plates of the bacterial cultures were sent to Macrogen, Inc. in Seoul, South Korea for bacterial sequencing service. Polymerase chain reaction (PCR) products were amplified using the universal bacterial 16S rRNA primers, 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' TAC GGY TAC CTT GTT ACG ACT T 3') (Lane, 1991). The genomic sequence was analyzed using the known sequences in BLAST (Basic Local Alignment Search Tool) using the universal bacterial sequencing primers 785F (5' GGA TTA GAT ACC CTG GTA 3') and 907R (5' CCG TCA ATT CMT TTR AGT TT 3') (Lane et al., 1985) and the phylogenetic trees of the isolates were constructed by Macrogen, Inc. in Seoul, South Korea.

2.5 Soil microcosm assay

Uncontaminated soil samples were collected in Don Mariano Marcos Memorial State University-Fisheries Research and Training Institute (DMMMSU-FRTI) in Paraoir, Balaoan, La Union (16°47'53.4" N and 120°19'34.1" E). The site has no history of industrial activity to ensure that only the natural components of the sediment are present on the samples. The sediments were placed in plastic zipper bags and were kept for 48 hours at room temperature. Air-dried samples were filtered using two mm plastic mesh wire. One hundred grams of soil was placed in glass jars sealed with aluminum foil, polyethylene bag, and rubber band. It was sterilized thrice in an autoclave at 121°C for one hour with an interval of 24 hours. A sterilized solution of $K_2Cr_2O_7$ was added to the soil with a final concentration of 60 mg/L, soil moisture was adjusted according to the laboratory results (Bahafid et al., 2013).

2.6 Heavy metal analysis

Bacterial cultures incubated for 48 hours in LB broth were inoculated in the soil microcosm following the treatments below which was patterned after the methods described by Chrysochoou et al. (2013).

The treatments were incubated at 37°C for 28 days at 120 rotations per minute (rpm) in a shaking incubator. Sampling was done during Day 7, 14, 21, and 28. Levels of soil-bound chromium(VI) were determined using US EPA methods 3060A (alkaline digestion) and 7196A (colorimetric test using 1,5-diphenylcarbazide) by Société Générale de Surveillance (SGS) Philippines in Makati, Metro Manila (Chrysochoou et al., 2013). All treatments were done in triplicates. Sample disposal of the sediments is done by SGS Philippines as a part of their service. Isolated bacterial cultures are preserved through stab cultures and are deposited in a standard freezer in the university microbiology laboratory.

Table 1. Treatments for chromium reduction used in the study.

TREATMENTS	SOIL MICROCOSM COMPOSITION
1	Control Group: 50 g soil, distilled water
2	Test Group: 1 mL LG-01, 50 g soil, distilled water
3	Test Group: 1 mL LG-02, 50 g soil, distilled water
4	Test Group: 1 mL LG-03, 50 g soil, distilled water
5	Test Group: 1mL of LG-01, LG-02, & LG-03, 50 g soil, distilled water

*LG refers to the last name of the researchers.

3. RESULTS AND DISCUSSION

3.1 Soil characteristics of Maragayap estuarine sediments

The collected sediment samples were made up of sand particles and silt, with gray to black color. Heavy metals chromium, zinc, and manganese were found to be present in the Maragayap site sediments. Furthermore, chromium levels present in the sample were above the Threshold Effect Concentration (43 ppm) in sediments compared to zinc (121 ppm), and manganese, an essential element, are within the acceptable levels in the environment (US EPA, 2002).

3.2 Isolation and identification of chromium resistant bacteria

3.2.1 Bacterial classification and phylogeny

Based on the distinct morphological types observed growing on the plates, three colonies were selected and designated as LG-01, LG-02, and LG-03. Molecular identification of the isolates through comparative analysis of its genomic sequences from NCBI databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that the isolates belong to the family Bacillaceae and Staphylococcaceae.

Table 3 shows the isolates LG-01, LG-02, and LG-03 were identified as *Staphylococcus sciuri* (92% similarity), *Bacillus cereus* (99% similarity) and *Bacillus aerius* (99% similarity), respectively.

Table 2. Chemical properties of sediments from La Union, Philippines.

	Maragayap Sediments	Paraoir Sediments
pH	8.03	8.14
Moisture Content (%)	2.81	0.75
Organic Matter (%)	0.89	0.64
Nitrogen (ppm)	450	320
Phosphorus (ppm)	13	13
Chromium (ppm)	60	n.d.*
Zinc (ppm)	97	-
Manganese (ppm)	455	-

*n.d. means not detected; below the detection limit of 0.05 ppm.

Table 3**.** Summary of bacterial molecular identification.

Sample	Description	E-value	Similarity **%**	Accession Number
LG-01	*Staphylococcus sciuri*	0.0	92%	KT270573.1
LG-02	*Bacillus aerius*	0.0	99%	NR_118439.1
LG-03	*Bacillus cereus*	0.0	99%	NR_074540.1

Studies by Branco et al. (2005) validated the findings of the study that all the isolates are gram-positive bacteria belonging to Phylum Firmicutes. The majority of them are from the Bacillaceae family, which dominates the CRB community isolated from soils contaminated with potassium dichromate (Camargo et al., 2005). Moreover, Cr-resistant bacteria species such as *Bacillus* sp. and *Staphylococcus* sp. are also present in leather tannery effluents polluted primarily with high levels of chromium (Nazeema & Nirmala, 2017).

A phylogenetic tree was constructed to exhibit the phylogenetic neighbors of the identified bacterial species. However, there was no constructed relationship in the *S. sciuri* due to an insertion/deletion (indel) event in the gene. In evolutionary genetics, indels are considered as universal mutators in the genome of prokaryotes and eukaryotes (McDonald et al., 2011), thus, it may be inferred that mutations occurred in the genome of LG-01.

S. sciuri is one of the most primitive species in their genus. It belongs to the *Staphylococcus sciuri* species group together with *Staphylococcus lentus, Staphylococcus vitulinus, Staphylococcus fleurettii*, and *Staphylococcus stepanovicii*. They belong to the same clade with the *Staphylococcus saprophyticus* group comprising of *Staphylococcus saprophyticus, Staphylococcus cohnii*, and *Staphylococcus xylosus* (Nemeghaire et al., 2014). Moreover, the phylogenetic trees of Sample 2 and 3 namely, *B. aerius* and *B. cereus* are displayed below.

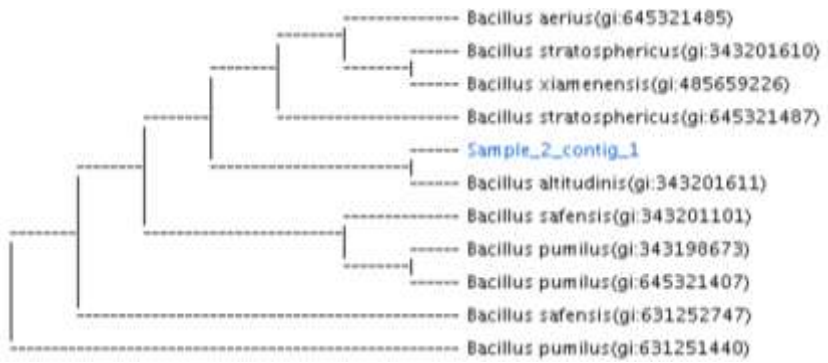

Figure 2. Phylogenetic tree based on 16S rRNA gene sequences of LG-02.

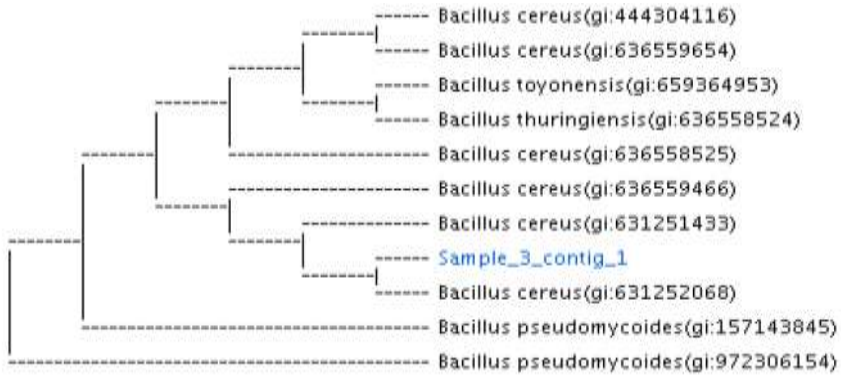

Figure 3. Phylogenetic tree based on 16S rRNA gene sequences of LG-03.

3.2.2 Bacterial characteristics

Staphylococcus sciuri is an aerobic, cream-colored coccus, non-spore-forming, and nonmotile bacterium. After 5-7 days of incubation, it exhibits cell colonies with a hollow center or crateriform (Figure 4) (Kloos et al., 1976). It can be found in freshwater environments such as water samples of a eutrophic lake (Kalwasińska et al., 2008), estuary (Wu et al., 2004), copper contaminated river (Robidillo et al., 2014), and on the shell of estuarine clams (Jalal et al., 2009).

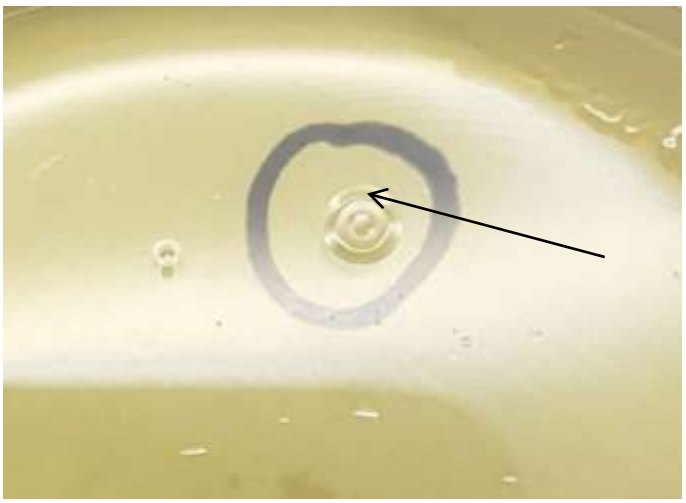

Figure 4. The hollow center of *Staphylococcus sciuri* in a plate with Luria-Bertani Agar amended with 60 ppm chromium.

On the other hand, *Bacillus aerius* is an aerobic, white rod, spore-forming, motile bacterium with an irregular margin (De Guzman et al., 2016). Due to its recent discovery, the nature of the environment where it thrives is not yet established. Some studies reported that it was isolated in the sediment samples from the rice ecosystem (Vardhan et al., 2013; Jhala et al., 2014), hot springs, and salt marshes (Aanniz et al., 2015). In the Philippines, the species were first isolated from an industrial site soil in Marikina (de Guzman et al., 2016). Lastly, *Bacillus cereus* is a facultative anaerobe rod, endospore-forming, and a motile bacterium that grows well in anaerobic conditions (Granum & Lund, 1997). It is present in mangrove estuarine ecosystems through sediments and water in Thailand (Chantarasiri, 2016), Malaysia (Ghaderpour et al., 2014), and the Philippines (Tabao & Monsalud, 2010). It can be deduced that the ubiquitous characteristics of bacteria are the major factor of its existence in the sediments of the Maragayap river estuary. To our knowledge, this is also the pilot study that describes the isolation of *B. aerius* from an estuarine ecosystem.

3.2.3 Bacterial chromium resistance mechanism

All of the isolates did not form inhibition in the heavy metal disk diffusion assay, thus, they are resistant to up to 300 ppm of chromium. Among the isolates, only *B. cereus* has previous literature on its chromium resistance that it can tolerate as high as 75,000 ppm (Mollania et al., 2013). Contrarily, there is no existing literature on the chromium resistance of *B. aerius* and *S. sciuri*.

Heavy metal resistance mechanism among bacteria is species-specific, but due to a limited number of studies, the assumed mechanistic behaviors (related to their reduction process) of the isolates are based on genus level comparison. Various adaptations are performed by *Bacillus* species in tolerating chromium, and some of these are the intercellular reduction of Cr(VI) via accumulation of electron-dense precipitates (EDP) (Upadhyay et al., 2017), and the presence of soluble chromate reductase that can detoxify Cr(VI) to lesser one (Mangaiyarkarasi & Geetharamani, 2014). Similarly, *Staphylococcus* sp. has chromate reductase enzyme (Mangaiyarkarasi & Geetharamani, 2014), apart from that they have nitrate reduction activity, which is involved in Cr-resistance and transformation (Kouadjo & Zeze, 2011).

Microorganisms present in the metal-polluted environment are adapted to toxic heavy metal contaminants and become resistant. Isolates from hexavalent chromium-contaminated environments are reported to have a highly resistant mechanism against the pollutants (Narayani & Shetty, 2013). And the utilization of indigenous heavy metal-resistant bacteria from its environment is more favored in bioremediation due to its tolerance and adapted mechanisms for its removal (Gonzales et al., 2014). Natural gene transformation through horizontal gene transfer happens in soil microbial communities to adapt and evolve in stressful environments. Thus, the bacterial Cr-resistance and reduction capacity are accounted for from selective stress in areas contaminated with Chromium (Camargo et al., 2005). Some bacteria exhibit mechanisms against resistance; it was observed that the two *Bacillus* species isolated in the study changed in color from white to yellow-orange after a few days of storage.

Marine bacterial species can synthesize unique secondary metabolites (such as carotenoids), which are utilized in various biological activities. Carotenoids are pigments with red, yellow, or orange coloration present in plants and microorganisms. One significant source of carotenoids is marine bacterial species, but not all of them can synthesize one. The production of yellow to orange spores among marine *Bacillus* species is evidence that they can produce their carotenoids (Abdul et al., 2013). Natural carotenoids have significance in marine bacterial membrane stability and anti-oxidant activity (Sy et al., 2015). And some of the factors associated with the stimulation of carotenoid production in bacteria are influenced by temperature and the presence of chemical compounds in the environment (Baranska & Kaczor, 2016).

During storage, the plates were placed inside a refrigerator with a temperature ranging from four to 13°C. *B. aerius* and *B. cereus* might have produced putative carotenoids to cope with the thermal stress in cold environments. Bacteria at low temperatures produce more carotenoids to stabilize their membrane for its survival. Carotenoids are added to membrane vesicles resulting in increasing the girth of the membrane lipid bilayer. This mechanism enhances bacterial membrane rigidity against low-temperature exposures (Fong et al., 2001). Moreover, carotenoids synthesized under low temperatures are present in extremophilic organisms to cope with various environmental stresses (Baranska & Kaczor, 2016).

Additionally, heavy metal stress harms bacteria due to the production of Reactive Oxygen Species (ROS) that causes oxidative damage to living cells. These affect the cell's normal physiology and as a response, microorganisms form enzymatic systems to adapt. Particularly, chromium stress influences an organism's metabolic activity which results in either the increase in metabolite production or synthesis of new metabolites to resist or tolerate the heavy metal (Batool et al., 2014). Elevated levels of heavy metals in the environment elicit carotenogenesis (the biosynthesis of carotenoids), which counteracts ROS due to its antioxidant property (Duc et al., 2016). *Bacillus* isolates might produce carotenoids to survive in the stress of 60 ppm of Cr(VI) in the LB agar. Chromium in the form of $K_2Cr_2O_7$ is a strong oxidation agent and can easily penetrate across membranes. Its toxicity affects the bacteria intracellularly and its action to intolerable chromium levels is the conversion of Cr(VI) to lower oxidation states, which is less toxic to the bacteria (Batool et al., 2014). Moreover, the yellow to orange colonies of *Bacillus* species are hypothesized to indicate the presence of chromium in a particular area. These species might serve as a bioindicator or biomonitoring organism in chromium-contaminated environments (Kirti et al., 2014). Also, the production of carotenoids exhibits the potential of these species in the bioremediation process.

Figure 5. Formation of putative carotenoids of *Bacillus* sp. in an LB Agar after a few days of incubation.

3.3 Bioremediation performance of chromium resistant bacteria (CRB)

In the soil microcosm, the sediment was amended with 60 ppm of Cr(VI) but its level was reduced to 12.71 ppm in one week and lowers further as time progresses. Cr(VI) can be converted into Cr(III) in the sediments because of organic matter that serves as electron donors. The hydrolysis of reduced chromium results in its precipitation as chromium hydroxide in water or bounded in the organic carbon in the sediment (Palmer & Puls, 1994). Thus, from 60 ppm base concentration of hexavalent chromium, its levels on each week were adjusted to the Cr(VI) present in the control from Week 1 to 4 with 12.71 ppm, 8.28 ppm, 9.55ppm, and 7.69 ppm, respectively.

Bioremediation performance is quantified using bioremediation rate, and hexavalent chromium levels in four weeks. The table below displays the bioremediation performance of the treatments in terms of the bioremediation rate of bacterial consortium against pure cultures in four weeks.

The bacterial consortium exhibited the fastest bioremediation rate compared to individual isolates. A consortium can continuously reduce chromium(VI) without any replenishment of nutrients since it can use a wide variety of carbon and energy sources to reduce chromate (Bahafid et al., 2013).

As the week progresses, there are decreasing levels of Cr(VI) present in the microcosm, from the trend, it may be deduced that Cr(VI) levels reduce as the bioremediation time increases.

Table 4. Comparison of bioremediation rate between the pure cultures and bacterial consortium.

Group		Bioremediation Rate (ppm/week)	Rank	Overall rating
Pure cultures	*Staphylococcus sciuri*	0.718125	II	II
	Bacillus aerius	0.65375	III	
	Bacillus cereus	0.721875	I	
Consortium	*S. sciuri*, *B. aerius*, and *B. cereus*	0.893125	-	I

In terms of chromium reduction, the bacterial consortium has the best performance in reducing 26.07% of 9.55 ppm control (2.49 ppm) in a span of three weeks. Additionally, in four weeks, *S. sciuri* yielded a reduction of 21.45% (1.65 ppm) and followed by *B. cereus* with 17.03% of 7.69 ppm base concentration (1.31 ppm). *B. aerius*, however, had a slight increase in hexavalent chromium levels in Week 4, yet among others, it has the lowest reduction of 12.88% of 9.55 ppm (1.23 ppm) in Week 3.

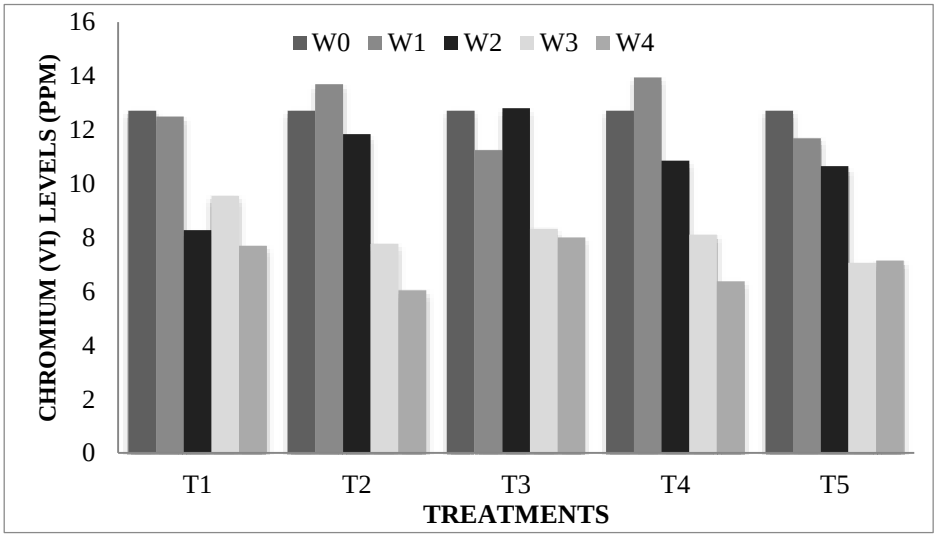

Figure 6. Chromium reduction of *Staphylococcus sciuri*, *Bacillus aerius, Bacillus cereus,* and consortium per week.

Gram-positive bacteria like *S. sciuri, B. aerius,* and *B. cereus* can reduce Cr(VI) by utilizing it as the final electron acceptor aerobically or anaerobically (Kamaludeen, 2003). This may be due to the adaptive property of Gram-positive bacteria, they have the presence of a thick peptidoglycan layer and wall with enhanced metal chelating activity (Gadd, 1990). Moreover, the mechanism of chromate-resistant bacteria correlates with the plasmid or chromosomal genes (Cervantes & Campos-Garcia, 2007). Some of these gene-controlled reductions of chromium include the presence of chromate efflux in the membrane transporter from the cytoplasm, ATPases, chemiosmotic ion or proton pumps, and the reduction mechanism of chromate through free-radical detoxification, and specific and unspecific hexavalent chromium reduction (Lloyd & Lovley, 2001; Pimentel et al., 2002). Thus, these mechanisms may explain the observed chromium-resistant properties of the three isolates in this present study.

In terms of individual species level, *B. cereus* can uptake intracellular deposition of different metals (Sakurai & Haung, 1995). Additionally, reports also state that *B. cereus* can exhibit heavy metal transport by bioaccumulation, biosorption, and intracellular Cr(VI) reduction because of the presence of a localized hexavalent chromate reductase in the cytoplasmic fraction (Rani & Goel, 2007; Saranraj & Sujitha, 2013; Joutey et al., 2015).

However, due to its great impact in terms of antibiotic resistance and health hazards, there is little knowledge about the properties and activities *S. sciuri* can perform such as its bioremediation mechanism. Also, studies of Robidillo et al. (2014) mentioned that the bioremediation ability of this species has been set aside due to its pathogenicity. Additionally, the mechanism of *B. aerius* and *S. sciuri* is also unknown due to limited literature available to comprehensively explain the characteristics of the species and the activities they can perform since it is the first study in assessing their chromium resistance and bioremediation activity in estuarine sediments.

4. CONCLUSIONS

Three isolates were found to be resistant to up to 300 ppm of chromium and were identified as *Staphylococcus sciuri, Bacillus cereus,* and the novel bacteria *Bacillus aerius* using 16s rRNA sequencing. The three pure isolates and the bacterial consortium were compared in terms of their capacity to reduce hexavalent chromium to a trivalent chromium, and the bioremediation rate of each treatment. In terms of chromium reduction, *S. sciuri* yielded the highest reduction followed by *B. cereus* and *B. aerius*. On the other hand, results showed that the bacterial consortium has the highest bioremediation rate in the four-week experiment. However, the efficiency of *S. sciuri* and *B. cereus* in terms of their potential as bioremediation agents were precluded due to their pathogenicity to animals and humans. Thus, only *B. aerius* has the potential to be used in Cr(VI) bioremediation, since it is non-pathogenic. This study may provide information to the community about the possible risks of serious pathogens thriving in the Maragayap estuary. Also, the study provides microbial resources of bioremediation agents in addressing environmental concerns in the freshwater ecosystem.

This study suggests the discovery of the molecular and genetic mechanisms on the chromium resistance and reduction activity of the isolates, or the exploration of their bioremediation activity in other pollutants.

5. ACKNOWLEDGMENT

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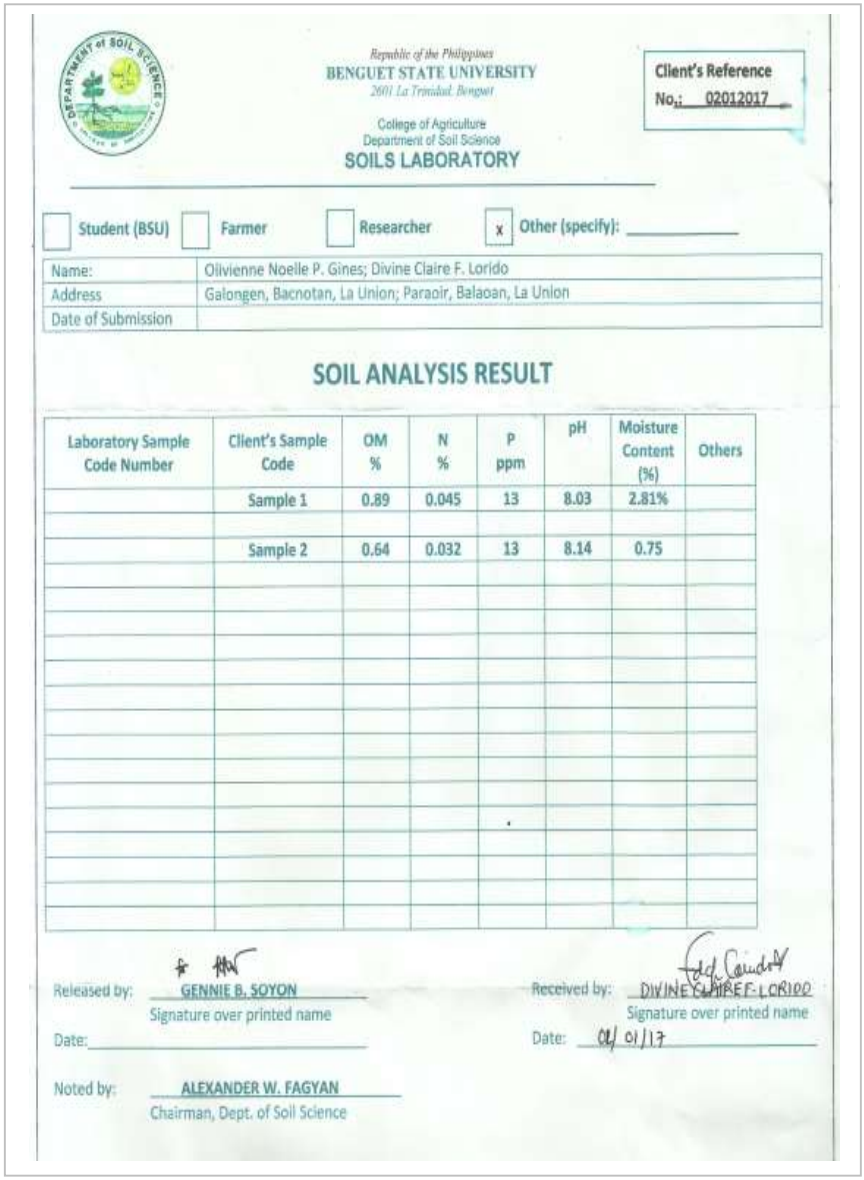
Appendices



Republic of the Philippines
BENGUET STATE UNIVERSITY
2601 La Trinidad, Benguet

College of Agriculture
Department of Soil Science
SOILS LABORATORY

Client's Reference No.: 02012017

☐ Student (BSU) ☐ Farmer ☐ Researcher ☒ Other (specify): _____

Name:	Olivienne Noelle P. Gines; Divine Claire F. Lorido
Address	Galongen, Bacnotan, La Union; Paraoir, Balaoan, La Union
Date of Submission	

SOIL ANALYSIS RESULT

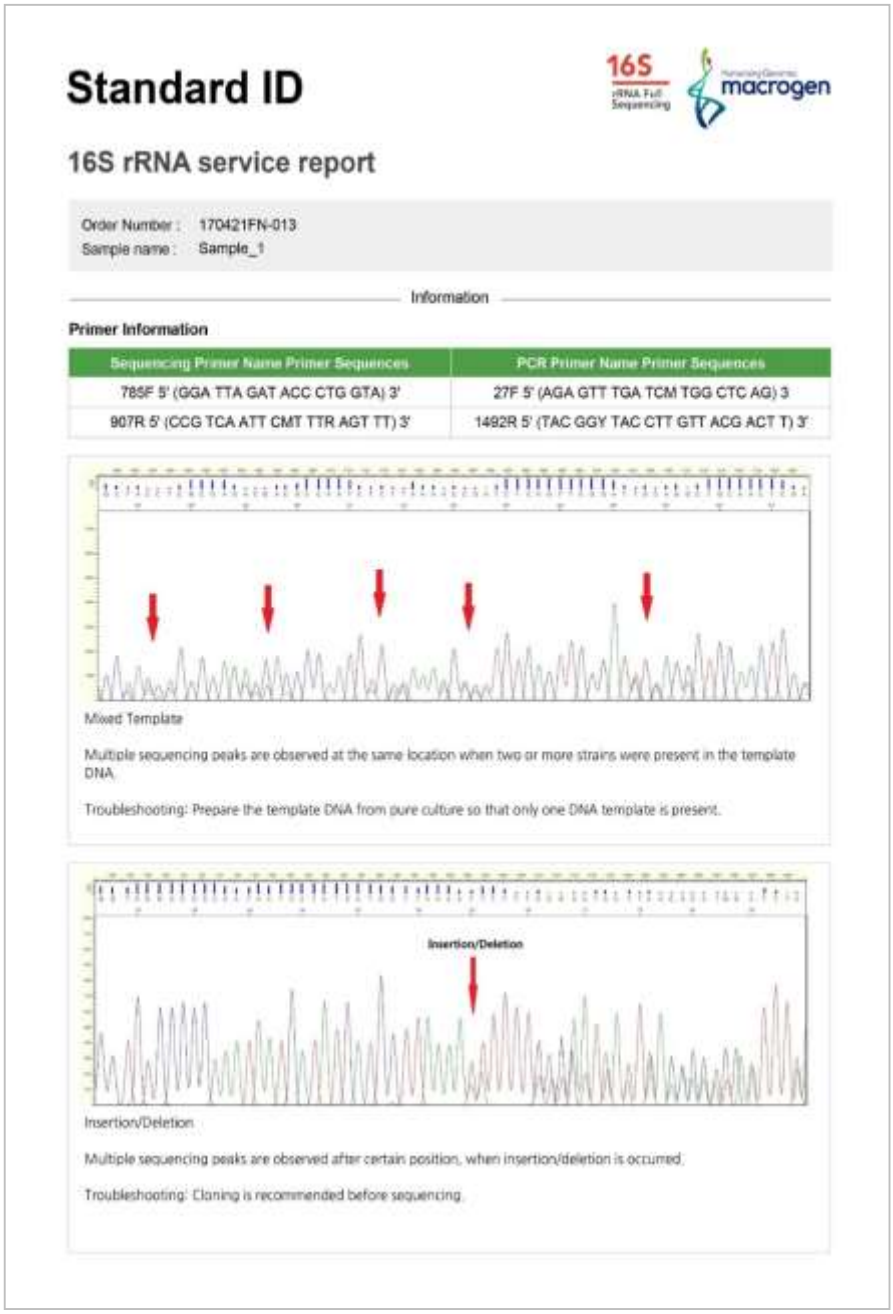
Laboratory Sample Code Number	Client's Sample Code	OM %	N %	P ppm	pH	Moisture Content (%)	Others
	Sample 1	0.89	0.045	13	8.03	2.81%	
	Sample 2	0.64	0.032	13	8.14	0.75	

Released by: GENNIE B. SOYON
Signature over printed name
Date: _____

Received by: DIVINE CLAIRE F. LORIDO
Signature over printed name
Date: 01/01/17

Noted by: ALEXANDER W. FAGYAN
Chairman, Dept. of Soil Science

Figure 7. Soil Laboratory Analysis Result of Experimental Soil Samples.



Standard ID

16S rRNA Full Sequencing

macrogen

16S rRNA service report

Order Number : 170421FN-013
Sample name : Sample_1

Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Multiple sequencing peaks are observed at the same location when two or more strains were present in the template DNA.

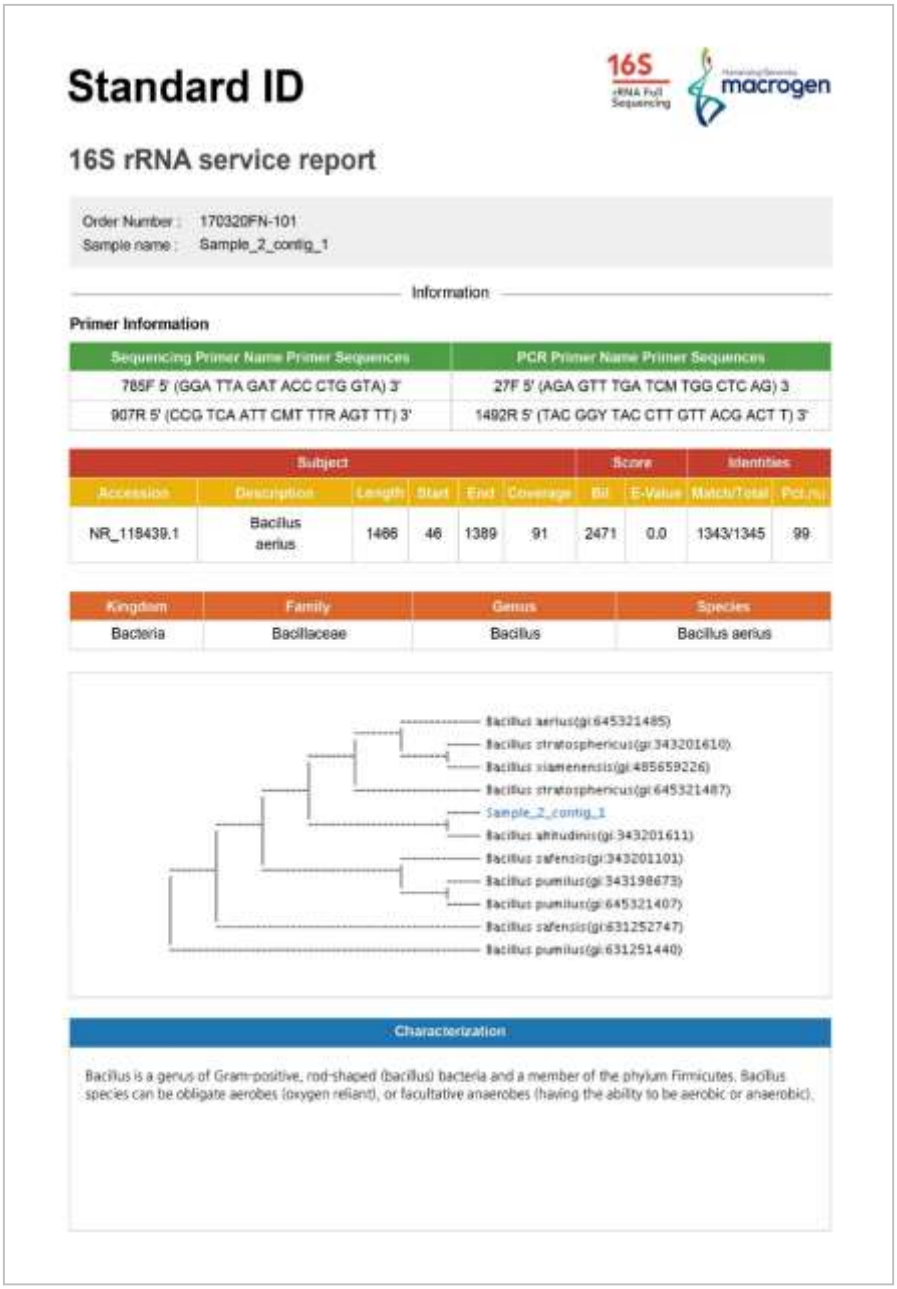
Troubleshooting: Prepare the template DNA from pure culture so that only one DNA template is present.

Insertion/Deletion

Multiple sequencing peaks are observed after certain position, when insertion/deletion is occurred.

Troubleshooting: Cloning is recommended before sequencing.

Figure 8. 16s rRNA Sequence Report of Bacterial Sample 1.



Standard ID

16S rRNA service report

Order Number : 170320FN-101
Sample name : Sample_2_contig_1

Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

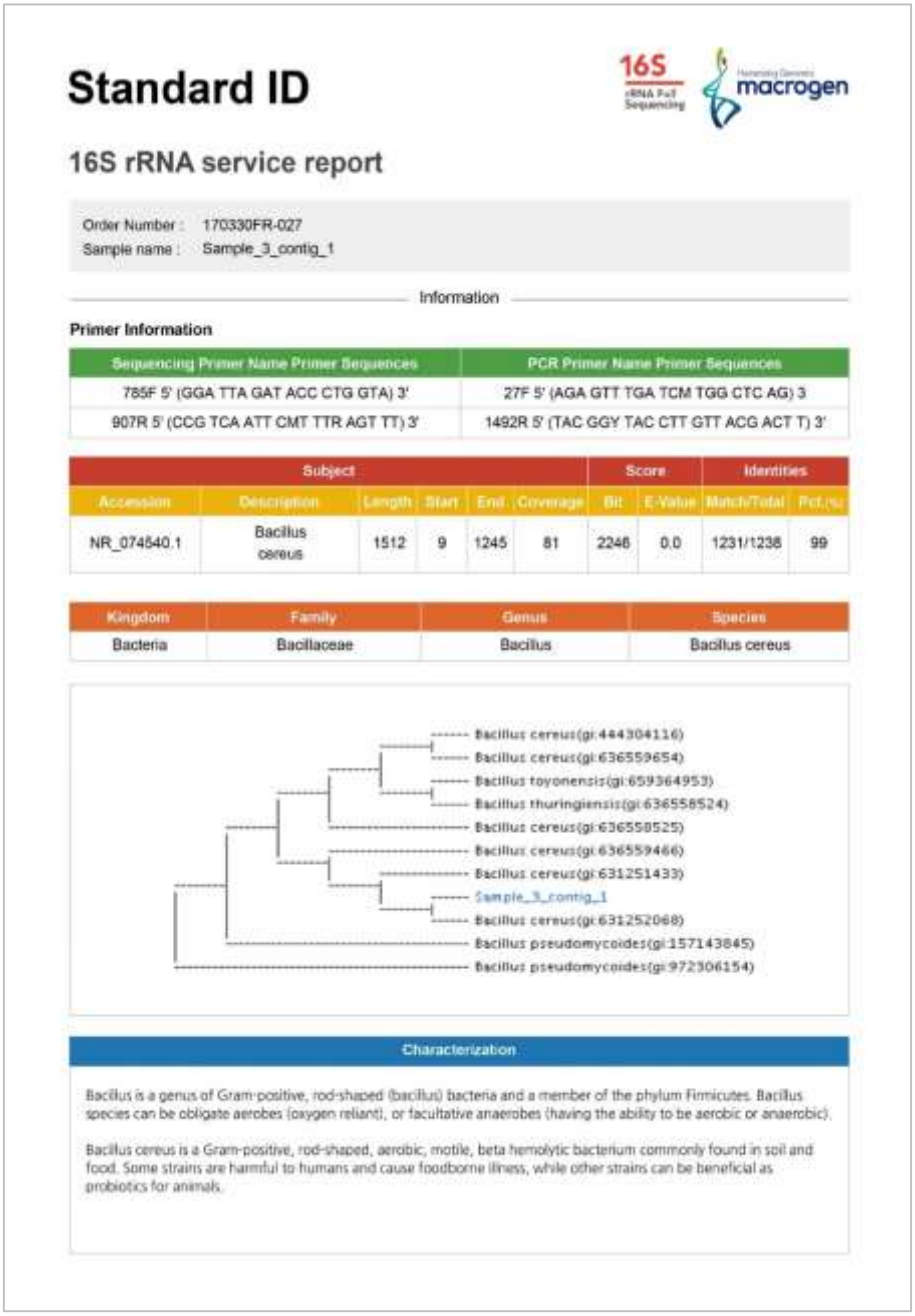
Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_118439.1	Bacillus aerius	1486	46	1389	91	2471	0.0	1343/1345	99

Kingdom	Family	Genus	Species
Bacteria	Bacillaceae	Bacillus	Bacillus aerius

Characterization

Bacillus is a genus of Gram-positive, rod-shaped (bacillus) bacteria and a member of the phylum Firmicutes. Bacillus species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic).

Figure 9**.** 16s rRNA Sequence Report of Bacterial Sample 2.



Standard ID

16S rRNA Full Sequencing

macrogen

16S rRNA service report

Order Number : 170330FR-027
Sample name : Sample_3_contig_1

Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_074540.1	Bacillus cereus	1512	9	1245	81	2246	0.0	1231/1238	99

Kingdom	Family	Genus	Species
Bacteria	Bacillaceae	Bacillus	Bacillus cereus

Characterization

Bacillus is a genus of Gram-positive, rod-shaped (bacillus) bacteria and a member of the phylum Firmicutes. Bacillus species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic).

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals.

Figure 10. 16s rRNA Sequence Report of Bacterial Sample 3