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The Bioremediation of Lead from Used Lubricating Oil by Bacillus Subtilis Subspstercoris Strain EG127

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Abstract: **Background and Objective**: ***The heavy metal content of used vehicle lubricating oil waste, particularly Lead (Pb), is a persistent waste that is difficult to decompose naturally. Bioremediation technology is one of the efforts to reduce lead (Pb) content in waste by utilizing the enzymatic activity of indigenous bacteria in the waste. This study aims to find, identify, and test bacteria that have the ability to bioremediate the heavy metal lead found in used vehicle lubricating oil. Methods: This study is a true experimental quantitative study. The rate of discoloration in Bromine Tymol Blue indicates that the original bacteria have been filtered. Using an atomic absorption spectrophotometer, the bacteria were tested for lead (Pb) degradation. The bacteria were then identified using morphology, biochemistry, and molecular genetics. Web-NCBI was used for genetic analysis, and MEGA 11 was used for phylogenetic structure. Result: The study showed that1 strain of bacteria was found to have the greatest ability to degrade lead. Bacillus velezensis is a close relative of this bacterium. Bacillus subtilis subspstercoris strain EG127 was identified as having the ability to bioremediate heavy metal Pb with 32 percent capability, from lead concentration of 1.62 ppm to 1.1 ppmfor 60 hours. Conclusion: This study concluded that bacteria capable of degrading lead from used oil waste can be found, isolated in nature as indigenous bacteria, and proven to be able to degrade lead from used oil waste.***

Keywords: Bioremediation, Vehicle lubricating oil waste, Bacillus subtilis subspstercoris strain EG127, Lead (Pb), Indigenous bacteria

1. Introduction

The increasing activity of motorized transportation in Indonesia has resulted in faster population mobility from one location to another, which has an impact on the dynamics of society, both economically, socially, culturally, and in other aspects of life. However, in addition to having a positive impact, it also has a negative impact due to the waste generated during the motor vehicle maintenance process (1). Motor vehicle maintenance must be carried out regularly to ensure the vehicle remains in good condition; Thus, the activity of motor vehicle maintenance workshops increased significantly (2). Used motor vehicle lubricating oil contains toxic hazardous waste materials and heavy metal content, specifically lead, which has the potential to cause environmental disruptions due to its persistence, difficulty in biodegradation, bioaccumulation, and biomagnification properties (3). Used motor vehicle lubricating oil contains additives, fuel residues, ash, asphalt, and corrosive materials, as well as other deposits that are hazardous to the environment if discarded (4)(5), because of its persistent nature, it can last a long time in nature and cannot decompose in the environment for a long time.(6)(7)

Lead bioremediation technology from used lubricating oil is an attempt to improve polluted environmental conditions into better (non-polluted) conditions by using biological agents (microorganisms) that work enzymatically to reduce lead heavy metals contained in used motor vehicle lubricating oil.(8) Several environmental factors, including optimization of incubation time, optimal concentration of microbes inoculated on the substrate, and the best environmental conditions for pH, water activity (Aw), and relative humidity (RH) on the substrate/media, all have a strong influence on the success of the degradation process. in bioremediation technology

Molds, yeasts, bacteria, and algae are some of the biological agents that can be used to remove lead heavy metal (Pb) from a polluted environment (9). Similarly, bioremediation using the microorganism Sporosarcinapasteurii as a biological agent can recover between 33.3 and 85.9% of lead polluted soil (Pb) (10). Studies on the bioremediation process by *Bacillus sp*. L14 show that the efficiency of bioremediation proceeds through the mechanism of inhibition of ATPase ezymatic activity and demonstrates the superiority of lead (Pb) degradation (11). Another study showed that laboratory bioremediation studies revealed that Bacillus sp. able to reduce the lead (Pb) content by 50%, in this study, the amount of Bacillus sp biomass was able to develop properly and was able to utilize lead (Pb) as an energy source for its life needs (12)

Based on this background, this study aims to discover, isolate, and identify indigenous bacteria as biological agents capable of bioremediation of lead (Pb) in used vehicle lubricating oil

2. Materials and Methods

This study's scientific scope is in the field of environmental microbiology. The research was carried out from September to December 2021 at Dian Nuswantoro University's environmental microbiology laboratory. The bioremediation research aims to find indigenous bacteria through a screening process, identify biochemical and

molecular genetics, and test the ability of these bacteria to degrade lead (Pb) in used motor vehicle lubricating oil.

The research population is made up of Indigenous bacteria isolated from waste motor vehicle lubricating oil that can degrade lead (Pb), whereas the research sample is made up of waste motor vehicle lubricating oil suspected of containing Indigenous bacteria. The inclusion criteria were samples of used lubricating oil that were contaminated with lead and indigenous bacteria that had lead degrading abilities, while the exclusion criteria were samples of used lubricating oil that were not contaminated with lead and indigenous bacteria that did not have lead degrading abilities. Bioremediation research was conducted exploratively and experimentally in the laboratory with the variable degradative ability of lead-degrading bacteria at various concentrations in used motor vehicle lubricating oil samples by testing the bioremediative ability of selected indigenous bacteria as indicated by the decreasing speed of lead concentration in the final results of research observations.

The study was conducted in four stages, which were as follows: isolation of indigenous bacteria, screening of selected bacteria, degradation activity test, and identification of selected indegenous bacteria.

Isolation of indigenous bacteria: Isolation of indigenous bacteria from waste used lubricating oil with Strike plate method (13). Bacteria were purified by incubating them at 37 degrees Celsius for 48 hoursonHeartInfusion media (HIB) enriched with waste lubricating oil. This purification process is repeated until a colony of bacteria grows independently or singly. For naming purposes, each bacterial isolate was assigned an isolate code.(14)

Screening for selected bacteria: The rate of color change in Heart Infusion (HIB) media enriched with used lubricating oil and given thymol blue bromine as an indicator, the rate of color change from blue to yellow, and then the isolates were transferred to slanted media as stock culture.(15)

Degradation Activity Test: Medium Agar: Bromothymol Blue (BTB) was added to Heart Infusion Agar (HiB) as an indicator and sterilized in an autoclave. The sterilized medium is supplemented or enriched with 50, 75, and 100 ppm concentrations of used motor vehicle lubricating oil. The isolated bacteria were inoculated on the surface of the Heart Infusion Agar (HIB) using the scratch method. Incubate for 6 x 24 hours at 37 0C and observe the color changes that occur, if the color of the medium around the bacteria changes to yellow, it indicates that the bacterial isolate can degrade Lead and the speed of color change is calculated. Liquid Medium: Medium Heart Infusion broth (HIB) was added with Bromine Thymol Blue (BTB) which was used as an indicator, then sterilized using an autoclave. The sterilized medium was made with concentration variations of 50, 75, and 100 ppm, then one loop of isolated bacteria was inoculated into the liquid medium, then the sample was shaken at 150 rpm for 2x24 hours and the color changes that occurred were observed, if the medium's color was liquid. The color of the bacterial isolate changes from blue to yellow, indicating that it can degrade lead, and the rate of color change is calculated.(16)

Identification. Identification of morphology, biochemistry, and molecular genetics Morphological, biochemicaland molecular genetic tests were used to identify bacteria capable of degrading Pb from used motor vehicle lubricants (17) The test bacteria that have known the ability to degrade the lead contained in used motor vehicle lubricating oil are then identified morphologically and biochemically to determine the initial screening and morphological and biochemical properties of the test bacteria, as well as further microbial identification based on 16S-rRNA Gene Analysis, including DNA extraction. , DNA amplification, and visualization of DNA amplification. . Following the purification of DNA amplification results, 16 phylogenetic trees were built using the MEGA ver 5.03 program, the Neighbor-Joined Tree statistical method, and a p-distance model with 1000 bootstrap levels.(18)

Laboratory equipment and materials: laboratory equipment and materials used in the research are: Microcentrifuge Equipped With Cooler (Sorvall Fresco), Incubator (Memmert); Autoclave (Hirayama, Japan), Laminar Air Flow Cabinet (Esco); Ph Meter (Eutech), Digital Camera (HP Photosmart R607), Analytical Balance (Scout And Acculab), Deep Freezer -20 ^{O}c (GEA), Oven (Lab-Line Instruments And WTB Binder); Vortex Mixer (25 L PCR Master Mix (0.05 U/Ml Taq DNA Polymerase; 0.4 Mm Each Dntp; 4 Mm Mgcl2), 2 L Primer 16E1, 2 L Primer 16E2, 1 L Milliq, And 10 L Health Genomic DNA Template, Mini Gel Electrophoresis (Mupid-Ex Advance), UV Transluminator (BDA Biometra TI 1), PCR Thermal Cycler (MJ Mini Biorad), 25 L PCR Master Mix (0.05 U/Ml Taq DNA Polymerase; 0 ,4 Mm Dntp Each; 4 Mm Mgcl2), 2 L Primer 16E1, 2 L Primer 16E2, 1 L Milliq, And 10 L Genomic DNA Template 25 L PCR Master Mix (0.05 U/Ml Taq DNA Polymerase; 0 ,4 Mm Each of Dntp; 4 Mm Mgcl2), 2 L Primer 16E1, 2 L Primer 16E2, 1 L Milliq and 10 L Minispin Microcentrifuge Genomic DNA Template (Eppendorf), and Glass Beakers

The materials: the materials used in the research are Lysozyme (Sigma), Sodium Dodecyl Sulfate/SDS(Merck), Proteinase-K (USB), Sodium Chloride (Merck); Aquadest (local), Aquabidest (Otsuka), Aquabidest Free ofDNAse And RNAse (DDH_2O), Tris Base (Merck), Etylene Diamine Tetra Acetic Acid/EDTA (Sigma); Chloroform (Merck), Isoamil Alcohol (Sigma), PCR Master Mix (Fermentas), Primer 16E1: GGG AGT AAA GTT AAT ACC TTT GCT C (Biotech) [4]; Primer 16E2: TTC CCG AAG GCA CAT TCT (Biotech) [4], Agarose ultrapure (Invitrogen), Loading Buffer, Etidium bromide (BD); 1 kb plus DNA ladder (Invitrogen), Ehrlich Reagent, Methyl Red, Potassium Hydroxide, Naphthol. Nutrient Broth/NB (local/Pro-analysa) media solution PH 6.8 ± 0.2; medium Nutrient Agar/NA (DIFCO) pH 6.8 ± 0.2; Lactose Monohydrate (Merck); Media Brilliant Green Lactose Bile Broth/BGLB 2% (Merck) pH 7.2 ± 0.2; Media Eosin Methylene Blue/EMB agar (Merck) Ph 7.3; Peptone (Difco); Media Methyl Red Voges- Proskauer/MRVP (Merck); Simmons Citrate Media (Merck); Tris Acetate Buffer EDTA/TAE, PVP 1%, Chloroform, Isopropanol, NaCl, Ethanol 100%, Buffer TE,

Agarose, Buffer TAE1x, DNA Extraction, EtBr. Forward and reverse specific primer, Green Go Taq Master Mix (Merck);

3. Results

Selected bacteria screening: Used motor vehicle lubricating oil was obtained for this study from one of Semarang's automotive maintenance workshops. The discovery of indigenous bacteria is a stage in the exploration and screening process. The ability of selected bacteria to degrade Pb in used lubricating oil is a test of their lead-reduction ability. Indigenous bacteria were chosen because they have the best ability to degrade the lead content in used motor vehicle lubricants, and they were then identified using morphology, biochemistry, and molecular biology.

The isolation results were grown on Heart Infusion media (HIB) media with used lubricating oil and Brom Thymol Blue (BTB) indicator added (0.5 ml). Based on these findings, bacteria that change color more quickly will be used as test materials in future studies during the bioremediation test stage (Table 1. And Fig. 1.). The screening results revealed six bacteria with the fastest rate of color change after three days of incubation on solid media. Table 1 shows the rate of color change per day as follows:

Table 1: The rate of color change in isolate observations on Heart Infusion agar medium

Heart Infusion agar	Week 1	Week 2	Week 3	Week 4
Isolate 1		+	++	+++
Isolate 2			+	++
Isolate 3				-
Isolate 4			+	++
Isolate 5				+
Isolate 6				-
Heart Infusion Broth	Day 1	Day 2	Day 3	Day 4
Isolate 1		+	++	+++
Isolate 2			+	++
Isolate 3				-
Isolate 4			+	++
Isolate 5				+
Isolate 6				-

Information : (+) indicates a color shift from blue to yellowish green.

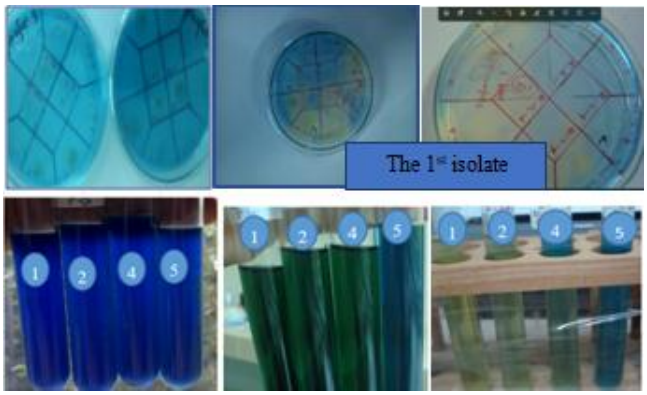

Figure 1: (a) Isolate 1's color changed from blue to yellowish green, on Heart Infusion Agar media, (b) The comparison of the color changes of isolates 1, 2, 4, and 5 on Heart Infusion broth media.

Lead (Pb) parameters are used in the bioremediation of used lubricating oil waste. The selected bacteria (isolate 1) was inoculated 1 ose on the Heart Infusion media (HIB) test medium (250 ml) containing 75 ppm used lubricating oil. Every 24 hours, measurements were taken for four study days. Pb levels in used lubricating oil waste media were determined using atomic absorption spectroscopy. Table 2 shows the result of measuring the concentration of Lead (Pb) based on the time of observation.

Table 2: The result of measuring the concentration of Lead (Pb) based on the time of observation

Parameter	Time to 0 hours	Time to 24 hours	Time to 48 hours	Time to 60 hours
Pb	1,62 ppm	1,43 ppm	1,35 ppm	1,10 ppm

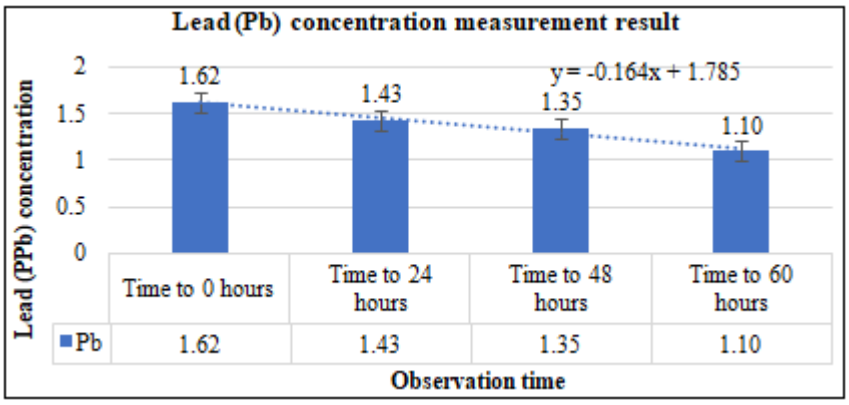

Figure 2: Decrease in the content (lead) of Pb in used lubricating oil
Noted: The linear equation y = -0.164x + 1.785, the degradation percentage is 32%

Identification of Lead (Pb) Degrading Bacteria. Bacterial identification based on morphology and biochemistry. The test bacteria were identified morphologically and biochemically to determine the initial screening at the identification stage. Macroscopic identification of morphological structures includes observations of colony shape, elevation, color, edges, and surfaces, whereas microscopic identification includes observations of cell shape, shoots, and cell sizeusing a microscope stained with methylene blue.(19). Table 3 shows the results of the morphology and biochemical analysis of bacteria, which resulted in the identification of bacteria at the genus level

Table 3: Bacterial Morphological and Biochemical Analysis Results

Morphological and Biochemical Test	Isolation code	Morphological and Biochemical Test	Isolation code
	Isolate 1		Isolate 1
Shape	rod	**Acid Form ASS Medium**	
gram	+	a. Glucose	+
Motility	+	b. Celibiose	+
Length 3>mm	-	c. Galactose	+
Spore Position and Length	VX	d. Mannose	+
Spores	+	e. Melibiose	+
Growth on 50 ^{o}	+	f. Rafinose	+
Growth on 37 ^{o}	+	g. Sucrose	+
Growth on 42 ^{o}	X	h. Xylose	-
Growth on 42^{o}	X	**Acid Form Phenol Red Meduim**	
Growth with 10% NaCl	+	a. Glucose	X
Anaerobic		b. Celibiose	X
Aerobics	+	c. Galactose	X
O-Nitrophenyl-β-D-Galactopyranoside (ONPG) test	+	d. Mannose	X
Utilization Of Citrate	+	e. Melibiose	X
Urease	-	f. Rafinose	X
Indo	-	g. Sucrose	X
Voges–Proskauer (VP) Test	-	h. Xylose	X
Nitrate Reduced	-	i. Growth on Cetrimide Agar	X
Starch Hydrolysis	+	J.Yellow Pigment	-
Oxidase	+		
Catalase	+		
Acid Fast	-		
Tween 20 Hydrolysis	x		

Note: V = Central/Oval, + = Facultative aerobics, x = not in the test

Bacterial identification results isresults positive : *Bacillus sp*, with methods (morphology and biochemistry (19)

Molecular Biology test: Molecular identification using the 16S rRNAsequencebyisolatinggenomic DNA as a template and displaying DNA bandsbasedon 16S-rRNA geneamplification gel electrophoresisresults as follows (Fig. 3.):

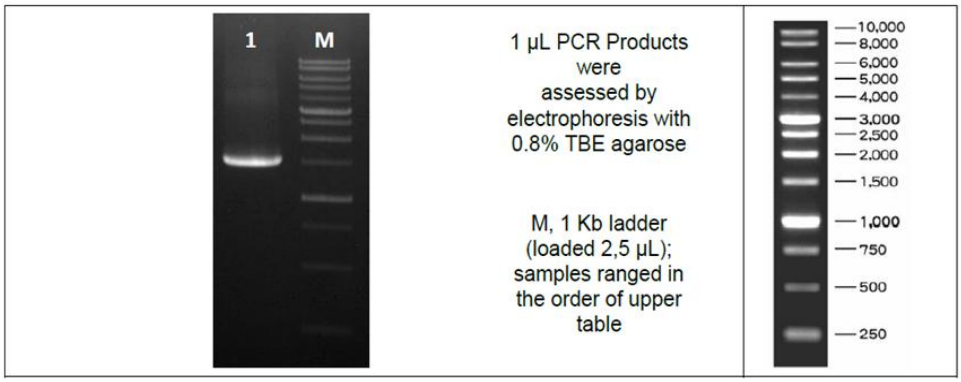

Figure 3: Results of 16S-rRNA Gene Amplification Gel Electrophoresis. (M) Markers; (1) Isolate 1

4. Discussion

Pollution problems in the environment that are thought to be caused by lead (Pb) from the disposal of motorized vehicle lubricating oil can disrupt the ecological balance, causing bioaccumulation and biomagnification processes that are harmful to human life and the environment in the long run (20). Bioremediation is an attempt to reduce lead compounds from polluted environments and modify toxic compounds into non-toxic compounds. This process involves many enzymatic, biochemical, and biotransformation processes that use microorganisms as remediation agents. The use of indigenous bacteria in bioremediation technology is the most efficient, cost-effective, and environmentally friendly way to address lead pollution issues (21) Bioremediation regulations in Indonesia are governed by the Decree of the Minister of the Environment No.128 of 2003, Concerning procedures and technical requirements and management of biological waste (bioremediation), which requires that indigenous microorganisms be used (22), Indigenous bacteria are bacteria that are found in nature and have genetically adapted to their living environment. Indigenous bacteria will be more stable because they are bacteria that live in the research location, and modification of indigenous bacteria is more controlled and not invasive for other organisms (23).

This research yielded the following novelties: Detection of lead-containing compounds in used motor vehicle

lubricating oil. Based on table 1, the 1st isolate changed color from blue to yellowish green on the 8th day (2nd week, on solid agar media), which was faster than the other isolates. However, it will be more obvious the color change in the liquid medium (on day 2 on the 1st isolate). The color change of the medium to yellow is caused by the catabolic process of bacteria(24)

Indigenous bacteria that grow in used motor vehicle lubricating oil are bacteria that live well in their environment and have adapted to use the substrate in the lubricating oil as an energy source.The ability of indigenous bacteria to degrade varies depending on the bacterial species, so a screening stage is required to obtain the best bacteria. Bromothymol blue was used as an indicator to determine the ability of bacterial degradation as visual,bacteria that are able to degrade Lead (Pb) will show a yellowish green color(25). The medium' scolor changes to yellow due to anaerobiccatabolism process that produces acidic compounds that turn the Bromotymol BlueindicatorBrass-Green.(26)

Based on these results (table 2 and figure 2), isolate 1 was able to reduce the lead (Pb) content at the 60th-hour measurement by 32%. According to Gaur et al (2018), several factors, including the incubation time and the initial concentration of microbes inoculated on the media, have a strong influence on the success of the bioremediation process (substrate).(27). The ability of these bacteria to assimilate diesel fuel as the sole carbon source was demonstrated in a bioremediation study on psychrotolerant phenols degraded by *Rhodococcussp* strain AQ5-07. Initial concentration, concentration and type of nitrogen source, temperature, and pH have all been studied as factors that can affect the degradation efficiency of diesel. (28)Because the inoculation of bacteria was only 1 ose, the optimization in this study could still be improved to accelerate the Pb degradation process, this was in accordance with two types of literature which showed that the initial concentration of bacteria inoculated on the substrate had a significant effect on the substrate degradation process.

Research into the Bioremediation Capabilities of the Best Indigenous Bacteria in Degrading Lead from Used Motor Vehicle Lubricating Oil. The results of morphological and biochemical identification showed that the bacteria were rod-shaped, gram +, motile, produced sopra (29), grew at 10% NaCl, were aerobic, ONPG test and scratch test showed positive results. capable of hydrolyzing starch, has oxidase and catalase enzymes, positive hydrolysis tween test (30), test results of medium assay: glucose, cellobiose, galactose The results of the analysis showed that the bacteria identified were the genus *Bacillus sp*. Luang-In et al (2019), *that Bacillus is a genus of bacteria that has a rod shape, gram (+), motile* (31) and can produce a single spore; however, some species do not produce spores, spread naturally, and are resistant to extreme conditions, while most Bacillus bacteria are catalase positive, oxidase positive bacteria can metabolize carbohydrates through fermentation. (32), Some Bacillusgenera are an aerobic heterofermentative and are unable to produce acid from mannitol. Bacillus bacteria can also metabolize carbohydrates, proteins, and amino acids, as well as reducenitratetonitrite. These criteria include genera *Bacillus* characteristics. (33)

PCR products containing 16S rDNA/16S rRNA (PCR-amplified 16S rRNA) genes of bacterial species were identified using the agarose gel electrophoresis method. (34) DNA fragments with a size range of 50-20,000 bp are the most easily separated on agarose gel (35), because the 16S rDNA/16 rRNA gene has conserved properties and is a structural RNA part of the ribosome that plays an important role in protein synthesis, the 16 rRNA gene is always present and owned by prokaryotic organisms, is sustainable, and almost never horizontally transferred in the protein synthesis process, resulting in identification using the 16S rRNA gene, which is ideal for phylogenetic tree analysis (36).

The process of isolating the genome of bacteria 1 was marked by the formation of one band for each genome of the tested bacteria after being observed using a UV Transilluminator with the gene encoding band 16S rRNA-1.5 kb, then compared with a Marker (1kb- DNA ladder) (37). The outcome of DNA amplification 16 rRNA was sequenced to obtain nucleotide sequences, which were then compared using the Gene Bank and the BLAST-N (Basic Local Alignment Search Toll-Nucleotode) program to determine bacterial homology and species. The results of 16S rDNA Isolate 1 sequenci ng were compared with the 16S rDNA sequence data of several species obtained from the data bank to determine phylogeny/kinship with other organisms. The data from the 16S rDNA sequence was then aligned using the clustalX ver 2.0 program (38). The phylogenetic tree was constructed with the MEGA ver 5.03 program, the Neighbor-Joining Tree statistical method, and a 1000 bootstrap level p-distances model. (39)

The following are the results of the sequencing, which used forward and reverse primers to determine the sequence of bacterial nucleotide bases: sample name : bacteria 1, PCR primer : 1 6 s (27F and 1492R) PCR products : species barcoding (~1400 bp). MethodsGenomic DNA extraction with Quick-DNA bacterial Miniprep Kit (Zymo Research, D6005). PCR amplification with (2x) My Taq HS Red Mix (Bioline, BIO-25048) 3. Bi-directional Sequencing. Nucleic Acid (Genomic DNA) Quantification (Nanodrop) No Sample Name Conc. (ng/µl) A260/280 A260/230 Volume (µl) 1 Bacteria 1 34.5 2.21 0.09 35. Sequence Assembly 1422bp : CAAGTCGAGC GGACAGATGG GAGCTTGCTC CCTGATGTTA GCGGCGGACG GGTGAGTAAC ACGTGGGTAA CCTGCCTGTA AGACTGGGAT AACTCCGGGA AACCGGGGCT AATACCGGAT GGTTGTTTGA ACCGCATGGT TCAAACATAA AAGGTGGCTT CGGCTACCAC TTACAGATGG ACCCGCGGCG CATTAGCTAG TTGGTGAGGT AACGGCTCAC CAAGGCAACG ATGCGTAGCC GACCTGAGAGGGTGATCGGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTAGG GAATCTTCCG CAATGGACGA AAGTCTGACG GAGCAACGCC GCGTGAGTGA TGAAGGTTTT CGGATCGTAA AGCTCTGTTG TTAGGGAAGA ACAAGTACCG TTCGAATAGG GCGGTACCTT GACGGTACCT AACCAGAAAG CCACGGCTAA CTACGTGCCA

GCAGCCGCGG TAATACGTAG GTGGCAAGCG TTATGACCTG GGCTACACAC GTGCTACAAT
TTGTCCGGAA TTATTGGGCG TAAAGGGCTC GGACAGAACA AAGGGCAGCG AAACCGCGAG
GCAGGCGGTT TCTTAAGTCT GATGTGAAAG GTTAAGCCAA TCCCACAAAT CTGTTCTCAG
CCCCCGGCTC AACCGGGGAG GGTCATTGGA TTCGGATCGC AGTCTGCAAC TCGACTGCGT
AACTGGGGAA CTTGAGTGCA GAAGAGGAGA GAAGCTGGAA TCGCTAGTAA TCGCGGATCA
GTGGAATTCC ACGTGTAGCG GTGAAATGCG GCATGCCGCG GTGAATACGT TCCCGGGCCT
TAGAGATGTG GAGGAACACC AGTGGCGAAG TGTACACACC GCCCGTCACA CCACGAGAGT
GCGACTCTCT GGTCTGTAAC TGACGCTGAG TTGTAACACC CGAAGTCGGT GAGGTAACCT
GAGCGAAAGC GTGGGGAGCG AACAGGATTA TTTAGGAGCC AGCCGCCGAA AG
GATACCCTGG TAGTCCACGC CGTAAACGAT
GAGTGCTAAG TGTTAGGGGG TTTCCGCCCC
TTAGTGCTGC AGCTAACGCA TTAAGCACTC
CGCCTGGGGA GTACGGTCG AAGACTGAAA
CTCAAAGGAA TTGACGGGGG CCCGCACAAG
CGGTGGAGCA TGTGGTTTAA TTCGAAGCAA
CGCGAAGAAC CTTACCAGGT CTTGACATCC
TCTGACAATC CTAGAGATAG GACGTCCCCT
TCGGGGGCAG AGTGACAGGT GGTGCATGGT
TGTCGTCAGC TCGTGTCGTG AGATGTTGGG
TTAAGTCCCG CAACGAGCGC AACCCTTGAT
CTTAGTTGCC AGCATTCAGT TGGGCACTCT
AAGGTGACTG CCGGTGACAA ACCGGAGGAA
GGTGGGGATG ACGTCAAATC ATCATGCCCC

MN704467.1 genetic analysis code (bacteria test 1) with a sequence assembly of 1422 bp is identical or has a 100 percent closest relationship with MN704400.1: Bacillus velezensis strain KLP21 16S ribosomal RNA gene, 1445bp Sequence Assembly. **T**he test bacteria with the code bacteria 1 is a molecule type-nucleic acid with a query length of 1422 bp and the following lineage report is Kingdom: *Procaryotae*, Division : *Bacteria*, Class : *Firmicutes*, Family: *Bacillales,Ordo: Bacillaceae*, Genus: *Bacillus*, Spesies: ***Bacillus subtilis subspstercoris strain EG127.*** Based on phylogenetic tree analysis, isolate bacteria 1 ***(Bacillus subtilis subspstercoris strain EG127***) is very similar to Bacillus velezensis, with a maximum identification rate of 100 percent, as follow (Fig. 4.):

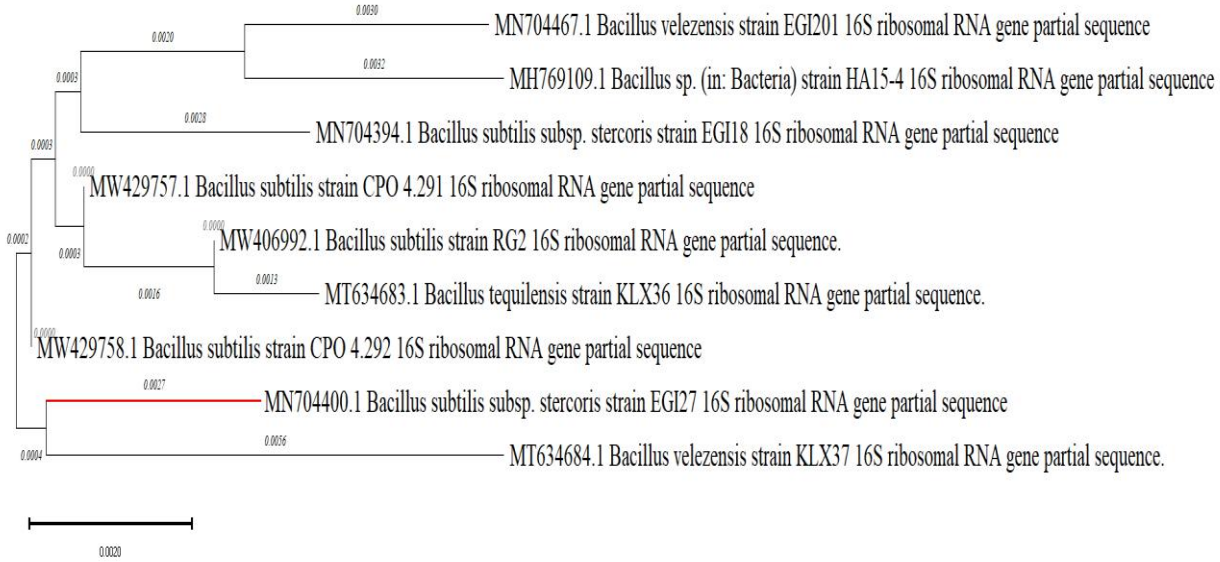

Figure 4: Phylogenetic tree ***Bacillus subtilis subspstercoris strain EG127***

5. Conclusion

The strain Bacillus subtilis subspstercoris strain EG127, an indigenous bacterial strain, was discovered to have the ability to degrade the lead contained in motor vehicle lubricants with the model. According to the linear equation y = -0.164x + 1.785, the degradation percentage is 32% after 60 hours.

Authors' Contribution

This study was carried out in collaboration with three authors: SI, HC, and DAR. SI and DAR authors designed the study, wrote the research findings, and wrote the first draft of the manuscript. The research analysis was administered by SI, HC, and DAR authors in collaboration. S and DAR supervise the literature and revise the final draft. The final draft was read and approved by three authors.

Funding

None

Ethics Statement

Not applicable

Availability of Data

The article contains all relevant research data as well as supporting information. This research aids researcher in identifying critical areas involving indigenous lead-degrading bacteria (Pb) in used lubricating oil in motor vehicles.

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