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RESEARCH ARTICLE OPEN ACCESS

The Chemical Compounds from Degradation of Profenofos and Malathion by Indigenous Bacterial Consortium

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Abstract

The Indonesian Pesticide Regulations state that Malathion and Profenofos have been restricted in their use for agriculture because of is bioaccumulative in ecological systems. Cleaning technology using microorganisms is an effective solution for cleaning pesticide residues. This study aims to identify the bacteria that degrade and the degradation process of Malathion and Profenophos into non-toxic compounds. The research method was experimental, identification of bacteria by 16S-rRNA gene analysis, degradation ability by GC MS. The results of phylogenetic tree analysis showed that the tested bacteria were closely related to *Oceanobacillus iheyenis* (RPL1) and *Exiquobacterium profundum* (RPL5) with a similarity level of 87% and 99%. The two bacteria are used as a consortium of test bacteria. The results of degradation based on the observation chromatogram T = 0 showed that the Malathion compound $C_{10}H_{19}O_6PS_2$ or butanedioic acid [(dimethoxyphosphinothioyl) thio]) was detected at peak 4, real-time = 19,675, area% = 7.37 and Profenofos compound $C_{11}H_{15}BrClO_3PSO$-(4-Bromo-2-chlorophenyl) o-ethyl s-propyl thiophosphate, peak 8, real-time = 23,957, area% = 6.91. Likewise, the chromatogram results at T = 96 were still detected Malathion ((dimethoxyphosphinothioyl) thio) at peak 14, real-time = 19,675, area% = 2.25, and Profenofos (o- (4-Bromo-2-chlorophenyl)) o - ethyl. s - propyl thiophosphate) peak = 22 real-time = 23,951, area% = 2.2. However, the observation of T = 192 hours, Malathion and Profenofos compounds were not detected. The conclusion showed that the consortium bacteria were able to completely degrade Malathion and Profenophos within 192 hours.

Keywords: Consortium bacteria, *Exiquobacterium profundum, Oceanobacillus iheyenis,* Biodegradation, Malathion, Profenofos

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INTRODUCTION

Pesticides are widely used to increase agricultural yields, plantations, forestry production, but pesticides can have a negative impact on the non-target environment. Pesticide pollution needs to be controlled because it can damage the ecological balance [1]. Malathion and Profenofos are types of pesticides that are widely used by farmers around Lake Rawa Pening in Central Java, Indonesia, even though these pesticides have been banned especially for rice cultivation based on Regulation of the Minister of Agriculture of the Republic of Indonesia Number 39 / Regulation of the Minister of Agriculture / SR.330 / 7 / 2015 [2]. It is proven that Profenofos is widely used by farmers around Rawa Pening (50%), then Carbamate (16%), Deltamethrin (8%), Imidacloprid (6%), Fentoat (5%), Carbosulfan (5%), Carbofuran (5%) and Lamda Sihalotrin (4%). The results of the Profenofos residue analysis in the waters of Rawa Pening ranged from 0.021 ppm - 0.08 ppm, the sediments ranged from 0.12 ppm - 0.28 ppm while the Malathion residue in the waters ranged from 0.0366 ppm - 0.0521 ppm and in sediments ; 0.0567 ppm - 0.12 ppm [3], is above the specified threshold $\leq$ of 0.01 ppm [4]. Therefore, it is necessary to make efforts to clean the residue of Malathion and Profenofos which have long been exposed to the environment. Indigenous bacteria are developed as biological agents in modifying toxic residues into non-toxic compounds [5]

The degradation process of Malathion - Diethyl (dimethoxythiophosphorylthio)succinate ($C_{10}H_{19}O_6PS_2$) in aquatic systems will be degraded to monocarboxylic acid-dimethyl monocarboxylic acid - dicarboxylic acid - dimethyl dicarboxylic acid-dicarboxylic acid-CO_2 [6.] Malathion fragmentation in the environment will become maloxon ($C_{10}H_{19}O_7PS$), malathion monocarboxylic acid ($C8H_{15}O_6PS_2$), and 2-mercaptosuccinic acid ($C_6H_5NO_2S$) are derivative compounds that are no more toxic than the initial compound, Malathion [7].

Malathion-degrading bacteria produce catabolic enzymes-malathion carboxyl esterase and malathion dicarboxy latoxy reductase which are able to convert malathionic compounds into thiophosphates and phosphates[8].

Profenofos pesticides will be broken down by bacteria into mono metabolite compounds and divalent acids through the enzymatic activity of oxidative desulfurization carboxylesterase [6 9] and demethylation processes in mineralization mechanisms that cause minor routes of metabolism, including oxidation, reduction of sulfur and methyl. [10]. The result of enzymatic degradation by consortium bacteria is able to degardate Profenofos into simpler compounds, namely 4-Bromo-2-chlorophenol and 1-phenyl-3-hydroxy-1, 2,4-triazole [11]

This article discusses critical areas regarding the degradation of Malathion and Profenophos residues contained in the water and sediments of the Rawa Pening lake by a consortium of indigenous bacteria *Exiquobacterium profundum - Oceanobacillus iheyenis* which are expected to produce simpler and non-toxic final compounds.

MATERIAL AND METHODS

Genomic DNA (Promega) Wizard Extraction Kit: EDTA, Lytic enzyme, nuclei lysis solution, RNAase solution, protein precipitation solution, DNA rehydration solution. Bact-FI primer. 5'AGAGTT TGATCMTGGCTCAG3 '/ UniB1.5'GGTTACSTTGTTACGACTT3' (Eurogentec AIT), agarose (Vivantis), ethanol 70%, Ethidium bromide, isopropanol, loading dye (Vivantis), marker (Vivantis), and $HgCl_2$. Sediment from Rawa Pening Lake, Profenofos and Malathion Pro Analisys (PA), Sigma Aldrich Laborochemikallen GmbH, Malathion Pestanal Bath SEBC132XV, Profenofos pestanal Bath SZBC132XV, Malathon 96% and Profenofos Curacron 500 EC

Microbial Identification based on 16S-rRNA Gene Analysis

Bacterial identification was carried out using the 16S-rRNA gene analysis method which included DNA extraction, DNA amplification, purification of DNA amplification results, DNA sequencing, and subsequent construction of phylogenetic trees to obtain genetic diversity.

DNA extraction

DNA extraction using the Chelex 100 Kit. Bacterial cells that have been grown for 24 hours are put into a 1.5 ml Eppendorf tube containing 100 µl of aquadest, then add 0.5% saponins and let stand for 24 hours at 4 °C. The samples were centrifuged at 12,000 rpm for 10 minutes, the supernatant from the centrifuge was discarded. A total of 1 ml of Phosphate Buffer Saline (PBS

1x) was added to the Eppendorf tube, then centrifuged again at 12,000 rpm for 15 minutes, the supernatant was removed, 100 µl aquadest and 50 µl Chelex 100 were added to the tube. The samples were boiled for 10 minutes (samples were vortexed in the first 5 minutes), then centrifuged again at 12,000 rpm for 10 minutes. The DNA containing the supernatant is transferred to a new Eppendorf tube which is ready for the DNA amplification process. [12]

DNA amplification

Amplification is a molecular marker using the 16s rDNA Polymerase Chain Reaction (PCR) method. The temperature treatment used in the DNA amplification process is initial denaturation at 95 ^{0}C for 3 minutes, then 30 cycles (denaturation at 95 ^{0}C for 1 minute, annealing process at 55 ^{0}C for 1 minute and extension at 72 ^{0}C for 1 minute), then extension at 72 ^{0}C for 7 minutes [13]. The primers used for PCR 16S rDNA were universal primers for 27F bacteria (5'-AGAGTTTGATCMTGGCTCAG-3') and eubacteria specific primers 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [14]. The mixture of materials used were Promega kit (25 µl) primer 27^0 F (2.5 µl), primer 1492 R (2.5 µl), DNA template (2.5 µl) and aquabides (17.5 µl) so that total volume 50 µl. The ingredients were mixed in a 0.2 ml PCR tube.[15]

Visualization of DNA Amplification Results

Visualization of the results of DNA amplification was carried out through electrophoresis by inserting 5 µl of PCR products into 1% agarose gel wells. Making 1% agarose gel by dissolving 1 gram of agarose in 100 ml of TAE 1x buffer solution, then heating it in an oven until homogeneous. A total of 5.33 µl Ethidium Bromide was put into the gel solution and shaken so that it was homogeneous. The gel solution is poured into a comb-shaped mold that is placed in an upright position so that it passes through the comb to the desired thickness. Then the gel was allowed to stand for a while until it hardened, then the gel was immersed in a 1x TAE buffer solution, the gel was electrophoresed with a voltage of 100 V for ± 30 minutes. The amplified DNA bands were observed using the Gel Documentation tool. [16]

Purification of DNA Amplification Result

Purification was carried out to obtain pure DNA from PCR 16S rDNA amplification. The PCR results were centrifuged at a speed of 12,000 rpm for 7 minutes. The supernatant was removed using a micropipette until the DNA was completely pure. A total of 50 µl of sterile aquadest was added to the DNA pellet and the results of the pure DNA were sequenced to determine the sequence of DNA bases.[17]

DNA sequencing

Sequencing was carried out according to the PCR sequencing cycle using Big Dye Terminator v.3.1. The formula for sequencing PCR reactions are 2 µl big dye, 2 µl 10x buffer, 4 µl DNA template, 1 µl primer with a concentration of 3.2 pmol, ddH2O to a final volume of 10 µl. DNA amplification carried out by cycles were initial denaturation (96 °C for 2 minutes), denaturation (96 °C for 10 seconds); annealing (50 °C for 5 seconds); and extension (60 °C for 4 minutes) by 25 cycles. PCR results were purified and sequenced using 27F primer. The sequences were analyzed automatically (ABI 3130XL, Applied Biosystem). [18]

Phylogenetic Tree Construction

The pesticide-degrading bacteria that had successfully amplified their 16S rRNA gene could be seen from their relationship with other prokaryotes in the database based on their 16S-rRNA gene sequences. The results of partial sequences are edited using the Bioedit program. After obtaining data on the results of nucleotide sequence contigs, the homology will be compared with other prokaryotes in the Gene Bank database. [19] Cluster analysis was carried out using a database from the RDP website (Ribosomal Database Project) with the website (http://www.rdp.com). while making phylogenetic trees using the MEGA 5 program [20]

Biodegradation Test of Malathion and Profenofos

The quantitative data analysis was carried out by determining the levels of *Malathion* and *Profenofos* which could be obtained based on the area of the chromatogram produced on Gas chromatography-Mass Spectrometry (GC-MS).[21] The analysis was performed using a Gas Chromatography-Mass Spectrometry (GS-MS) instrument. The GS-MS conditions at the time of the study were injector temperature 250°C, oven temperature 80°C, column temperature 280°C, detector temperature 250 °C, helium gas flow rate 1ml / min, constant rate, sample constant rate 1 ›l splitless, standard mix 1 ›l 100 ppm.[22] To determine the degradation results of the

specimens that had been refused were analyzed using GC MS at 0 hours, 96 hours and 192 hours observations.

RESULTS AND DISCUSSION

Identification of indigenous bacteria

Molecular genetic identification of indigenous bacteria by using genomic-DNA isolation as a template, then the results of genomic-DNA isolation are shown based on the DNA-bands resulting from the 16S-rRNA gene amplification electrophoresis process, shown in the following figure (Fig. 1):

The species identification by polymerase chain reaction technology (PCR product) using gene-16S rDNA / 16S rRNA (PCR-amplified 16S rRNA) of bacterial species, was carried out using agarose gel electrophoresis method[23]. The DNA fragments with a size of 50-20,000 bp are the best sizes that agarose gel can separate [24] Analysis using the 16S rDNA/16S rRNA gene has been carried out experimentally in the laboratory because the 16S rDNA/16 rRNA gene is universal and is part of the ribosomal structural RNA which plays an important role in protein synthesis. Therefore the 16 rRNA gene is always present in prokaryotic organisms, is immortal, and almost never is transferred horizontally. This makes the 16S rRNA gene ideal for the reconstruction of the phylogenetic tree and the identification of prokaryotic organisms [25]

The isolation process of the tested bacterial genome with the code RPL1 and RPL5 was marked by the formation of one band for each genome of the tested bacteria after being observed using Ultra Violete Transluminator, then it was described by the 16S rRNA gene coding band 1.5 kb, then compared with a Marker (1kb DNA ladder). The results of 16 rRNA DNA amplification were sequenced to obtain the nucleotide sequence and analyzed for similarities using the Gen Bank with the BLAST-N (Basic Local Alignment Search Toll-Nucleotode) program so that the homology and species of bacteria tested could be determined. [26], to determine the phylogeny relationship / relationship with other organisms, the 16S rDNA sequencing results of RPL1 and RPL5 isolates were compared with 16S rDNA sequence data from several species obtained from the data bank. The 16S rDNA sequence data was then synchronized with the ClustalX ver 2.0

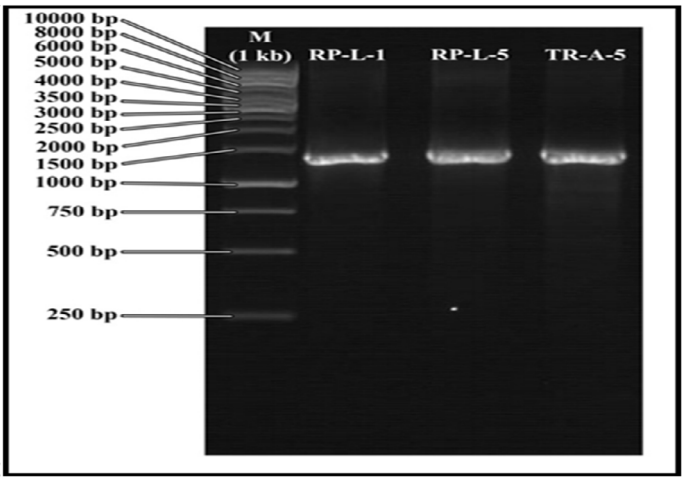


Fig. 1. The Results from the Gel Electrophoresis process - 16S-rRNA Amplification. (M) Marker; (1) Bacteria Identification Code = RPL1 and (2) Bacteria Identification Code = RPL5

Table 1. Sequencing results (primary forward and reverse)

No	Code	Nucleotide base (bp)	Species name	Homology	No accession
1	RPL 1	1071	*Oceanobacillus iheyensis*	87 %	LC10790
2	RPL 5	1238	*Exuquobacterium profundum*	99 %	LC19791

program[27] The next process is the creation of a phylogenetic tree using the MEGA version 5.03 program with the Neighbor-Joining Tree statistical method, 1000 bootstrap level p-distance models[28] The PCR results of the 16S rDNA gene were shown with a single band on the gel electrophoresis with a size of about 1500 bp.

The results of sequencing using forward and reverse primers to determine the sequence of bacterial nucleotide bases are as follows: (Table 1, fig 2 and fig 3)

Results of 16S-rRNA Gene Sequence of RPL-1 Bacterial GGGGTATTGCATCATAATGCAGTC GAGCGCAGGAAGCTATCTGATCCTCTTTTAG AGGTGACGATAATGGAATGAGCGGCGGACGG GTGAGTAACACGTAGGCAACCTGCCTGTAAGAC TGGGATAACTCGTGGAAACGCGAGCTAATA CCGGATAACACTTTTCATCTCCTGATGAGAAGTTG AAAGGCGGCTTTTGCTGTCACTTACAGATG GGCCTGCGGCGCATTAGCTAGTTGGTAAGG TAATGGCTTACCAAGGCGACGATGCG TAGCCGACCTGAGAGGGTGATCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTAGGGAATCTTCCGCAATGGAC GAAAGTCTGACGGAGCAACGCCGCGTGAGTGA TGAAGGTTTTCGGATCGTAAAACTCTGTTGTTA GGGAAGAACAAGTGCCATAGTAACT ATGGCA CCTTG ACGGTACCTAACCAGAAAGCCACGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGTG GCAAGC GTTGTCCGGAATTATTGC GCGTAAAGCGCTCGC AGGCGGTTCTTTAAGTCTGATGTGAAATCT TACGGCTCAACCGTAAACGTGCATTGGAAA CTGGGGAACTTGAGTGCAGAAGAGGAGA GTGCAATTCCACGTGTAGCGGTGAAATGC GTATAGATGTGGAGGAACACCAGTGGCGAAC GCGACTCTCTGG TCTGTAACTGACGCTGAGT AGCCAAGCGTCGGGAGCGACAGGATTAGATACC CTGGTAGCCCCTGCCGTAGACGATGAGCGC TAGTCGTCAGGGGTTTCCGCCCCTTATGCTGAAG TTACTCATTAAGCACTCCACCTGTGACGTCAGA CGCAAGCATCAACTCAAAGGATTTACGCGGAC CACTCAAGCGATGATCACTCGTTTAAT TACAGCACCGCGAGAACTTACCAGGCTTGGATT CCTCTGAACATCTAAAATAGCCTTTCCTT CAGGGAAGAGTTCTCCCGACAAAGATTTTTCAA CCCANACCTAAATTTCAGTAAGCCCGCACGA AGAAATCTTGA

Results of 16S-rRNA Gene Sequence of RPL-5 Bacterial Samples CAATTGCG CGGCTATAATGCAGTCGAGCGCAGGAAACCG TCTGAACCCTTCGGGGGGGACGACGGCGGA ATGAGCGGGGGACGGGTGAGTAAC

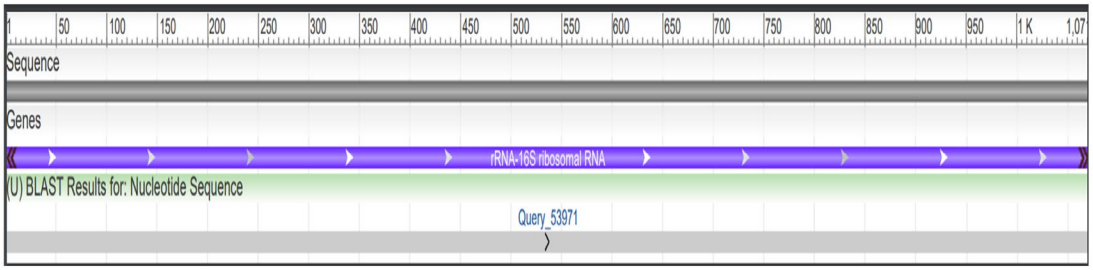


Fig. 2. *Exiguobacterium profundum* gene for 16S rRNA, partial sequence, strain: RP-L-5 1,238 bp linear DNA GenBank: LC019791.1, species, firmicutes

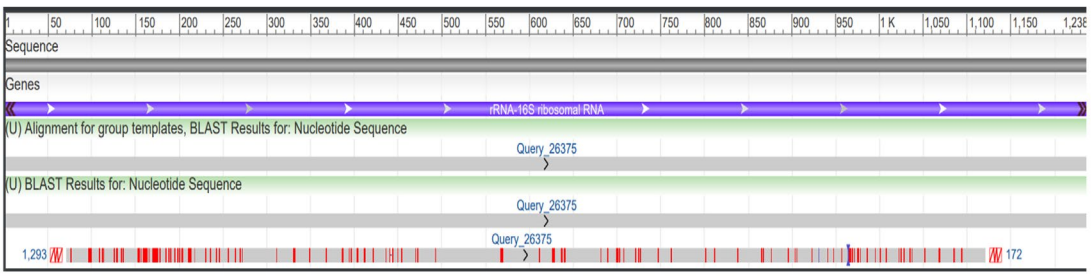
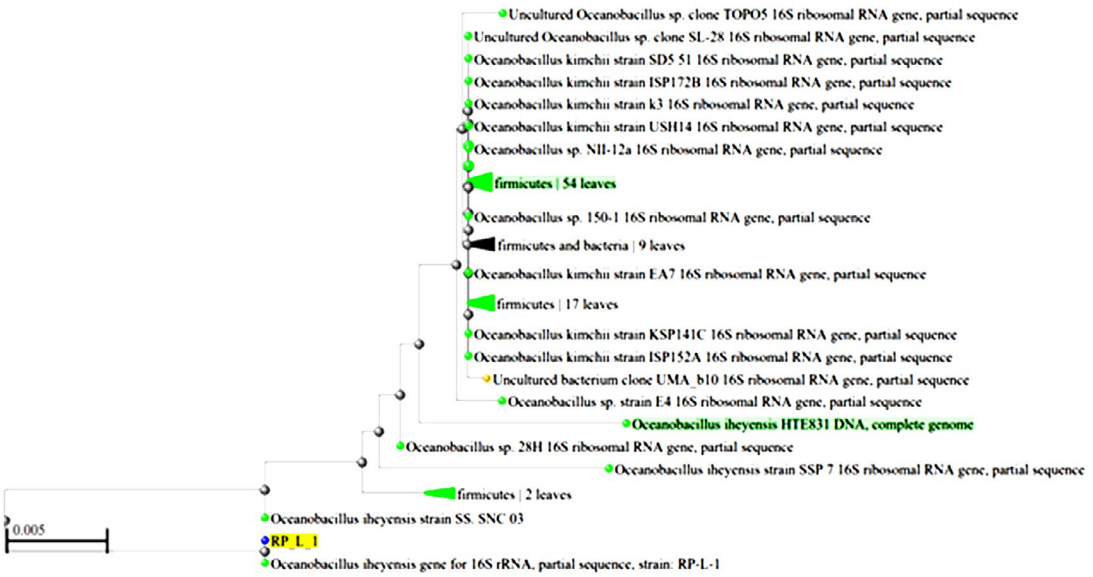
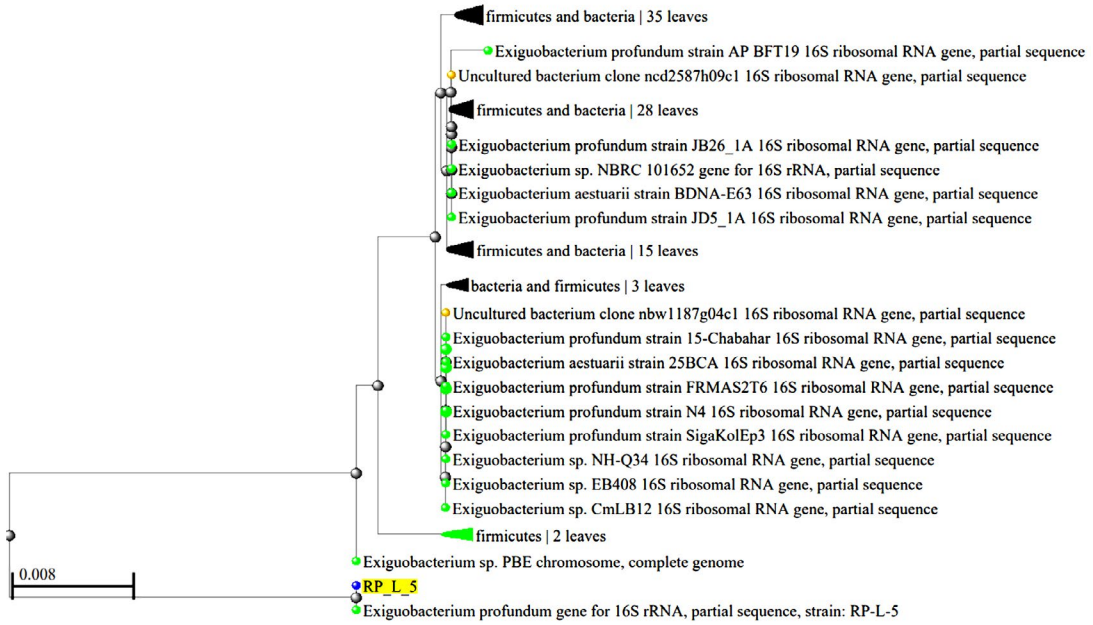


Fig. 3. *Oceanobacillus iheyensis* gene for 16S rRNA, partial sequence, strain: RP- L-1 1,071 bp linear DNA. species, firmicutes

ACGTAAAGAACCTGCCCATAGGTCTGGGATAAC CCGCGTGAACGATGAAAGCTTTCGGGGCGTAA
CACAAGAAATCC GGGCTAATACCGGATGTGTCAT AGTTCTGTTGTAAGGGAAGAACAAGTGCCGCAC
CGGACCGCATGGTCCGCTGATGAAAGGGGCTCCG G C A A T G G C G G C G C C T T G A C G G T A C C T
GCGTCTCCCATGGATGGCTTTGCGGTGCATTAGC T G C G A G A A A G C C A C G G C T A A C T A C A
TAGGTGGTGGGGTAAAGGCCCACCAAGGCG T G C C A G C A G C C G C G G T A A T A C G T A G G
ACGATGCATAGCCCAGCTGAGAGGGTGATCGGCCAC T G G C A A G C G T T G T C C G G A A T T A T T G G G
A C T G G G A C T G A G A C A C G G G C C A G A C T C C CGTAAAGCGCGCGCAGGCGGCCTCTTAAGT
T A C G G G A G G G G G C A G T A G G G A A T C T T C T G A T G T G A A A G C C C C C G G C T C A A C C
CCCCAATGGACGAAAGTCTGATGGAGCAACG G G G G A G G G C C A T T G G A A A C T G G G A





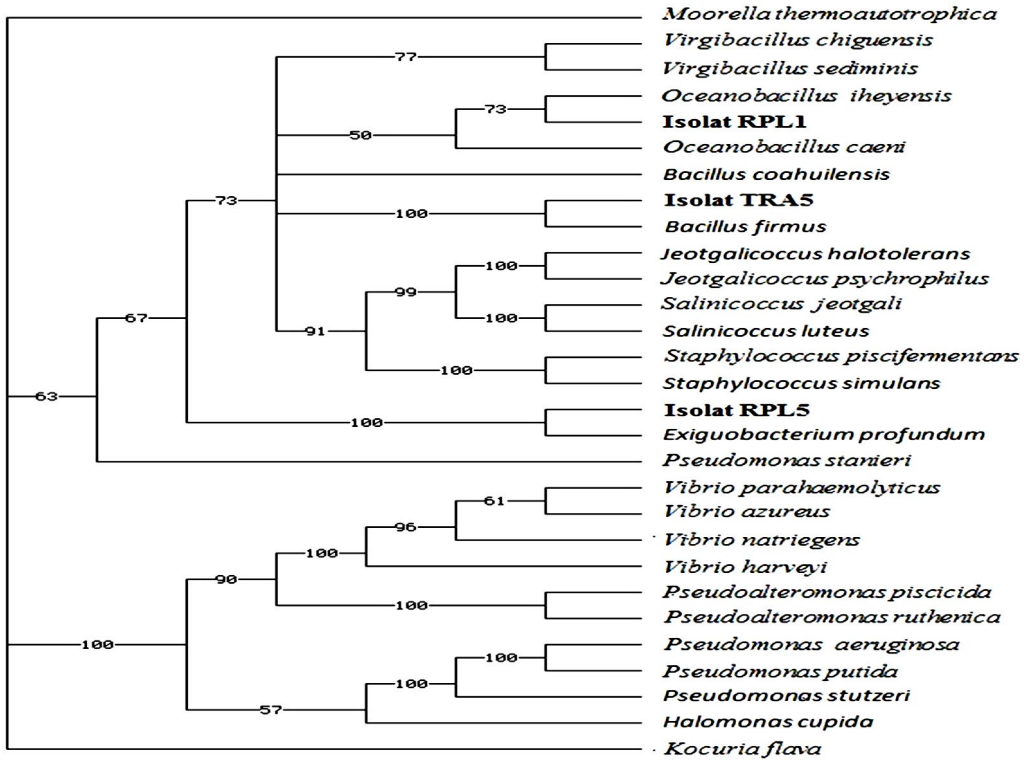


Fig. 4. Phylogenetic tree reconstruction results

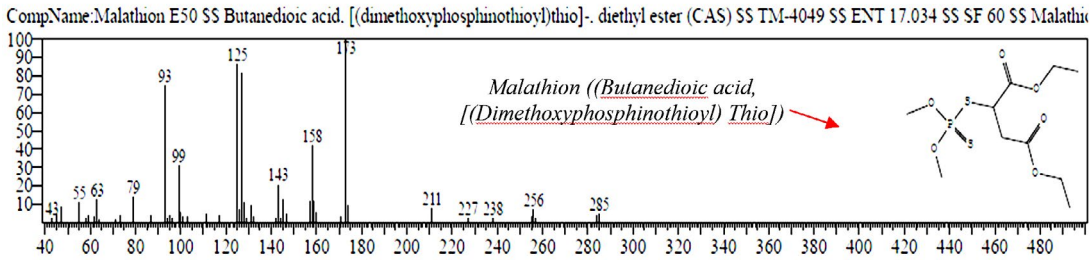


Fig. 5. The chromatogram of the chemical compound Malathion ($C_{10}H_{19}O_6PS_2$ or Butanedioic acid, ((dimethoxyphosphinothioyl) thio) -, monoethyl ester

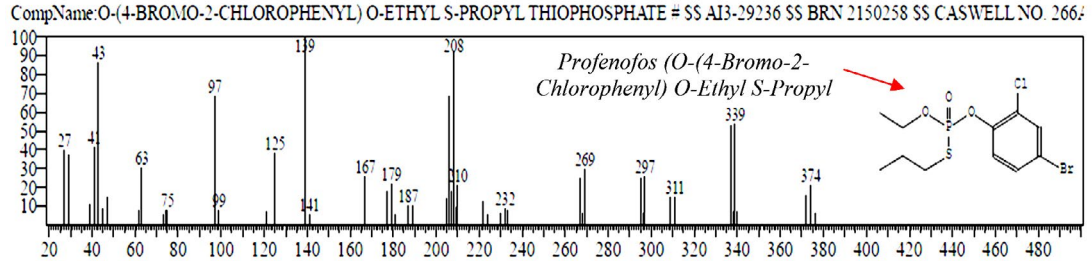


Fig. 6. Chromatogram and chemical structure of Profenofos (O- (4-Bromo-2-Chlorophenyl) O- Ethyl S- Propyl Thiophosphate))

GGCTTGAGTATATGAGAGAAGAGTGGA
ATTCCACGTGTAGCGGTGAAATG
CGTACAGATGTGAAGGAACA
CCCTTGTCGAAAGCGACTCTTTGGCCTATA
TCTGACGCTGAGGCGCGAAAACGTGGG
GAGCAACACGATTAGATACCCTGGTAGTC
CACGCCGTAAACGATGAGAGCTAA
GTGTTGGAGGGTTCCGCCCTTTGTGCT
CAGCTAAGCATTAACACTCCCCTGGGGA
GACAGTCGCAGGCTCAACTCAAGGATTG
ACGGGACCCCACACCAGTGGAGCATGTGGTT
TATTTGAGCACACGGAAAACTTTCCACTCTTG
AATCCCCTGACCGGAAAAAATGTACCTTCC
CTCTGGGGCAGGGTGACAAGTGTGGATG
GTTGCGTCAGCCCCGTCCGAGAGATGCG
TTAATCCCCAACAAGGCAACCTTGTCTTTTTTGC
ACATTCGTTGGCCCCCTAGGAAATGCCG
TGACAACCGAAGAAGGGGGATAA
CCAAATTCATGCCCTTAAAGTGGGTACACGT
GTCAATGGAGGGCAAGGGACCCAAC CCCAGTGGAC
CATCCCAAACGTTTCNTTGGATGGGGGGCACC
CCCGTAGACCGAATCTGGCGGGTGC
TATACATGCAGTCGAGCGGACAGATGGGA
GCTTGCTCCCTGAAGTCAGCGGCGGACGGGT
GAGTAACACGTGGGCAACCTGCCTGTA
AGACTGGGATAACTCCGGGAAACCGGG
GCTAATACCGGATAATTCTTTCCCTCACATGAGGGA
AAGCTGAAAGATGGTTTCGGCTATCACTT
ACAGATGGGCCCGCGGCGCATTAGCTAGT
TGGTGAGGTAACGGCTCACCAAGGCAAC
GATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCG CAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAGTGATGAAGGT
TTTCGGATCGTAAAACTCTGTTGTTAG
GGAAGAACAAGTACCGGAGTAA
CTGCCGGTACCTTGACGGTACCTAACCA
GAAAGCCACGGCTAACTACGTGCCAGCA
GCCGCGGTAATA CGTAGGTGGCAAGCGTTGT
CCGGAATTATTGGGCGTAAAGCGCGCGCA
GGCGGTTCCTTAAGTCTGATGTGAA
AGCCCCCGGCTCAACCGGGGA
GGGTCATTGGAAACTGGGGAACTTGAGT
GCAGAAGAGAAGAGTGGAATTCCACGTGTA
GCGGTGAAATGCGTAGAGATGTGG

Table 2. Chemical compounds from the biodegradation of the consortium bacteria (observation t = 0 hours)

Peak	R.Time	Area	Area%	Height	Name
1	4.034	163.635	6,7	21025	(R,S)-2-Butanol, (3R)-3-[(Benzyloxycarbonyl)Amino]-
2	4.240	57.279	2,4	12654	T-Butyl (R)-3-(Benzyloxy)-Butanoate
3	7.957	59.397	2,4	14450	4,6-Dimethyl-4-Hydroxyhept-5-Enoic Acid
4	13.113	98.397	4,0	39793	Pentadecanenitrile(CAS)
5	17.833	427.673	17,6	141327	Hexadecanenitrile(CAS)
6	18.384	171.142	7,0	61700	Hexadecanoic Acid,Methyl Ester(CAS)
7	19.675	179.194	7,4	61739	Malathion E50
8	21.927	145.766	6,0	45806	9-Octadecenal,(Z)-(CAS)
9	22.345	85.071	3,5	30227	9-Octadecenoic Acid (Z)-,Methyl Ester(CAS)
10	22.481	126.591	5,2	42298	Hexadecanenitrile(CAS)
11	22.958	65.369	2,7	21510	Heptadecanoic Acid,16-Methyl-,Methyl Ester(CAS)
12	23.914	87.158	3,6	43370	Hexadecanamide(CAS)
13	23.957	168.200	6,9	72947	O-(4-Bromo-2-Chlorophenyl)O-Ethyl S-Propyl Thiophosphate #
14	25.481	79.450	3,3	27975	9-Octadecenamide(CAS)
15	25.635	147.922	6,1	29660	1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethylheptasiloxane
16	26.930	91.252	3,8	17443	1-Piperazinepropanamide, N-(4-Fluorophenyl)-4-Methyl-
17	27.164	60.743	2,5	17767	3-Octadecene-1,2-Diol(CAS)
18	27.258	58.346	2,4	20271	14.Alpha-Cheilanth-12-Enic Methyl Ester
19	28.801	82.966	3,4	15704	Silicone Grease,Siliconfett
20	29.705	77.285	3,2	19635	Silicone Grease,Siliconfett
		2432836	100	757301	

Table 3. Chemical compounds from the biodegradation of the consortium bacteria (observation t = 96 hours)

Peak	R.Time	Area	Area%	Height	Name
1	3.975	44718	1.25	9359	Phenol,3,5-Dimethyl-(CAS)
2	4.477	31093	0.87	10563	2-Butenedioic Acid (E)-,Diethyl Ester
3	6.706	36954	1.04	20894	Phenol,2-methoxy-4-(2-propenyl)-(CAS)
4	7.965	72138	2.02	16400	1-(3,3-dimethyl-bicyclo[2.2.1]hept-2-yl)pentan-2-one
5	10.485	34744	0.97	8757	Pentanedioic acid,2,2-dimethyl-,bis(1-methylpropyl)ester (CAS)
6	12.989	54577	1.53	23184	Acethydrazide,2-(2-naphthylamino)-N2-(2,6-dichlorobenzylideno)-
7	13.105	121792	3.42	43652	Dodecanenitrile(CAS)
8	15.995	31813	0.89	13964	Cyclo(-L-Pro-L-Val-)
9	16.575	62669	1.76	17072	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
10	17.827	401025	11.25	140342	Hexadecanenitrile(CAS)
11	18.384	139427	3.91	47978	Hexadecanoic acid, methyl ester (CAS)
12	18.891	72297	2.03	15832	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a
13	19.493	57249	1.61	18294	Tetradecanamide
14	19.670	80070	2.25	30221	Malathion E50
15	21.914	121504	3.41	37368	Hexadecenenitrile
16	22.025	46680	1.31	15990	9-Octadecenal, (Z)-(Cas)
17	22.336	98016	2.75	34032	14-Octadecenoic Acid,Methyl Ester(CAS)
18	22.469	150614	4.22	42639	Hexadecanenitrile (CAS)
19	22.946	75605	2.12	23171	Octadecanoic Acid,Methyl Ester(CAS)
20	23.770	26632	0.75	7619	4-(4-Bromo-3-Nitro-Benzylidene)-1-(4-Chloro-Phenyl)-Pyrazolidine-3,5-Dione
21	23.913	90583	2.54	42273	Hexadecanamide(CAS)
22	23.951	78877	2.21	34703	O-(4-Bromo-2-Chlorophenyl)-O'-Ethyl Ester of Propylthio-Phosphoric Acid
23	24.527	41166	1.15	10099	Acetamide,N,N'-[(3.beta.)-18-hydroxypregn-5-ene-3,20-diyl]bis- (CAS)
24	24.615	33053	0.93	11041	3-(4-Hydroxy-3-methoxyphenyl)-2-isothiocyanatopropionic acid, ethyl ester,TMS
25	25.225	65384	1.83	13189	1,3,5,7,9-Pentaethyl-1,9-Dibutoxypentasiloxane
26	25.475	28709	0.81	13917	1-(Cyanomethyl)-3-Piperidinecarboxamide
27	25.550	34399	0.96	14017	N-(2-Adamantan-1-Yl-Ethyl)-4-(Piperidine-1-Sulfonyl)-Benzamide
28	25.620	33752	0.95	7609	(3E)-4-(1,2-Methoxycarbonylepimino-2,6,6-Trimethylcyclohexyl)-3-Buten-2-One
29	25.863	32630	0.92	20625	Hexasiloxane, Tetradecamethyl-(CAS)
30	25.936	28321	0.79	14045	Silikonfett
31	26.010	58329	1.64	19251	Cyclotetrasiloxane, Octamethyl(CAS)
32	26.055	50380	1.41	19742	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decamethyl-

33	26.120	26494	0.74	11753	(2,2-Dibromo-1-Propylcyclopropane)Carboxylic Acid
34	26.150	70222	1.97	17378	Pentasiloxane, Dodecamethyl- (CAS)
35	26.316	58923	1.65	17078	1-Pentene, 1,3-Diphenyl-1-(Trimethylsilyloxy)-
36	26.345	86119	2.42	17965	14.Alpha.-Cheilanth-12-Enic Methyl Ester
37	26.430	35483	1.00	16504	4-Acetyloxyimino-6,6-Dimethyl-3-Methylsulfanyl-4,5,6,7-Tetrahydro-Benzo
38	26.480	63149	1.77	18989	Tartronic Acid, 4-(Dimethylethylsilyl)Phenyl-, Dimethyl Ester
39	26.560	59074	1.66	15147	Cyclotetrasiloxane, Octamethyl- (CAS)
40	26.730	34307	0.96	14017	Cyclopentasiloxane, Decamethyl- (CAS)
41	26.786	31297	0.88	19358	Silikonfett
42	26.882	51321	1.44	13561	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-Decamethyl-
43	26.920	25776	0.72	12505	Sarpagan-17-Ol, 16-[(Acetyloxy)Methyl]-, Acetate (Ester) (CAS)
44	26.985	30442	0.85	12869	1,2-Bis(Trimethylsilyl)Benzene
45	27.712	47255	1.33	17719	Silikonfett
46	27.760	30689	0.86	14958	Silane, [[4-(2-Isothiocyanatoethyl)-1,2-Phenylene]Bis(Oxy)]Bis[Trimethyl- (CAS)
47	28.085	39623	1.11	20936	3-Ethoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsilyloxy)Trisiloxane
48	28.268	28903	0.81	12857	Tetradecamethylcycloheptasiloxane
49	28.530	36573	1.03	16310	Silikonfett
50	28.585	96854	2.72	18658	Silane, Trimethyl[5-Methyl-2-(1-Methylethyl)Phenoxy]- (CAS)
51	28.690	40740	1.14	22872	Cyclotetrasiloxane, Octamethyl- (CAS)
52	28.747	38641	1.08	23116	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-Decamethyl-
53	28.855	53826	1.51	16658	3,4-Isopropylenedioxy-10b(S)-Pancratistatin-1,2-Cyclic Sulfate
54	28.938	39942	1.12	15193	3,3-Diethoxy-1,1,1,5,5,5-Hexamethyltrisiloxane
55	29.022	35906	1.01	19894	Silikonfett
56	29.128	28055	0.79	15987	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-Decamethyl-
57	29.375	31829	0.89	9676	N-(Cyclohexyl)-3-Ethyl-3-Methyl-1,3-Dihydropyrrol-2-One Alpha.Methyl Ester
58	29.610	32848	0.92	10645	14.Alpha.-Cheilanth-12-Enic Methyl Ester
59	29.755	29380	0.82	1E+06	1H-Pyrrole-2,4-Dicarboxylic Acid,3,5-Dimethyl-,Diethyl Ester (CAS)
60	29.853	45965	1.29		Cyclotetrasiloxane,Octamethyl-(CAS)
		3564836	100.00		

AGGAACACCAGTGGCGAAGGCGACTCTTTGGTC
TGTAACTGACGCTGAGGCGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTA AGTGT
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CGCATTAAGCACTCCGCCTGGGGAGT
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GACGGGGGCCCGCACAAGCGGTGGAGCAT
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GCGTTCCCCTTCGGGGGA CAGGATG ACAGGTGGTG
CATGGTTGTC GTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGC AAC CCTTGAT CTTAGTT
GCCAGCATTCAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAAGGAAGGTGGG
GGATGACGGTCAAATCATCATGGCCCCTTA
AGGACCTGGGGCTAACNCACGTGCTACAATGGGA
TGGGAACAAAGGGGTTCGAAGACCC
GCAAGGTTAANCGGAATCCCCATAAAACATTT
TTCAAGTTCN GAATTG CAGGGTTGAAACTCTCC
TTGTTTGAAACCCGGATT

Based on the phylogenetic tree analysis, the test bacterial isolate with code RPL1 has the closest relationship with the *Oceanobacillus*

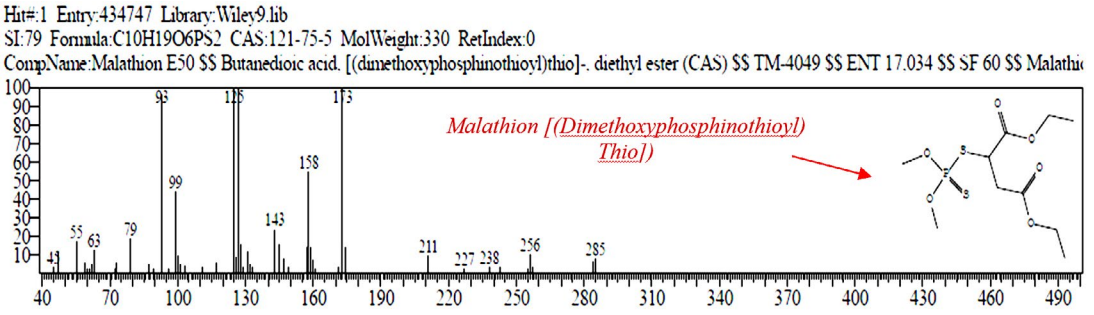

Fig. 7. The chromatogram and chemical structure of Butanedioic Acid Malathion [(Dimethoxyphosphinothioyl) Thio] at observation t = 96 hours

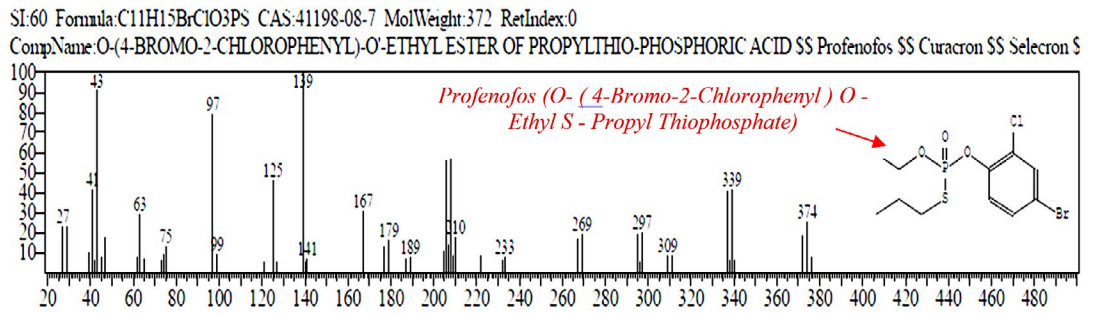

Fig. 8. The chromoatogram and chemical structure of Profenofos[O-(4-Bromo-2-Chlorophenyl) -O'-Ethyl Ester Of Propylthio-Phosphoric Acid] at observation t = 96 hours

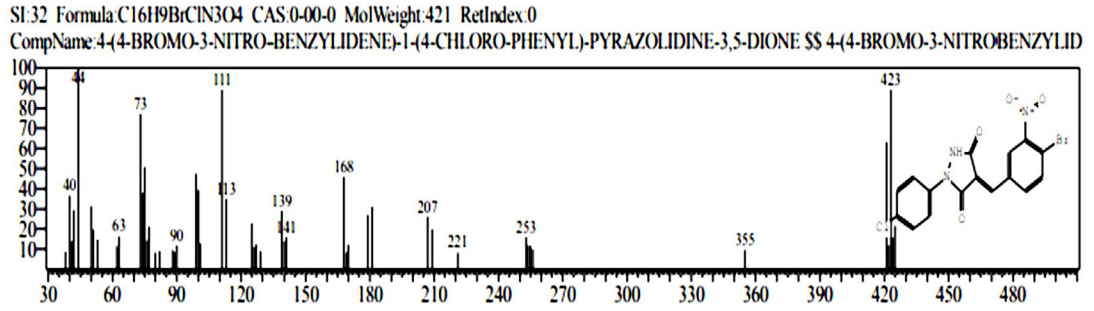

Fig. 9. Chemical compounds (*4 - Bromo - 3 -Nitro - Benzylidene) -1- (4- Chloro - Phenyl) -Pyrazolidine-3,5-dione*

iheyenis bacteria with a maximum similarity rate of 87%, while the tested bacterial isolates with code RPL5 had the closest relationship with *Exiquobacterium profundum* with a maximum similarity level of 99%. as follows (Fig 4)

Biodegradation of Profenofos and Malathion by indigenous bacterial consortium

Oceanobacillus iheyenis and *Exiquobacterium profundum* are indigenous bacteria isolated from the Rawa Pening lake. Both of these bacteria have the ability to degrade against Malathion and profenofos, therefore these bacteria are used as consortium bacteria for research on the biodegradation process of Malathion and Profenofos. The results of Isworo, Purwanto and Sabdono (2016) The results of the test of the degradation ability of selected bacteria in the form of a consortium showed a better ability than the degradation ability of a single isolate. The bacterial consortium *Exuquobacterium profundum* and *Oceanobacillus iheyen*sis had the best degradation ability of 83.23% while the bacteria consortium Exuquobacterium profundum and bacillus formis had the best degradation ability with a value of 68.75% on the Profenofos substrate [29]. The detected biodegradation chemical compounds will be translated into a chromatogram that represents the compound being analyzed. Analysis of the test sample was carried out by observing the retention time and chemical structure of Malathion and Profenofos due to degradation from the bacterial consortium.[30] Observations

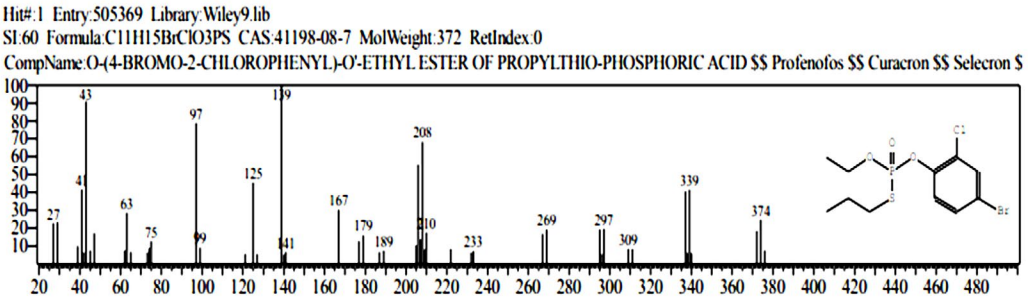

Fig. 10. The chemical compounds O-(4–Bromo–2-Chlorophenyl)-O'-Ethyl Ester of Propylthio-Phosphoric Acid

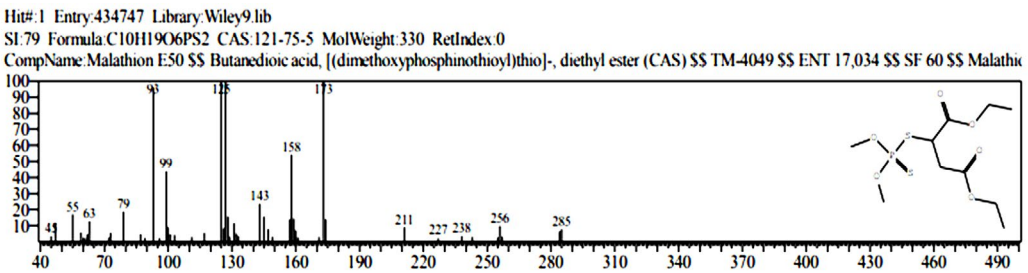

Fig. 11. Chemical compounds of Butanedioic Acid, [(Dimethyl Phosphinothioyl) Thio])

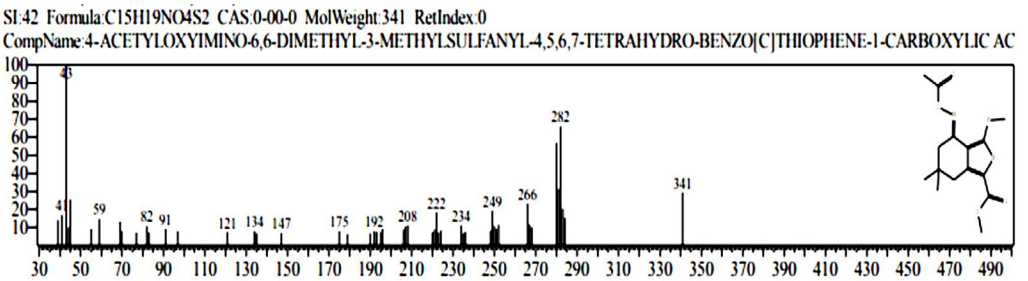

Fig. 12. The chemical compounds 4-Acetyloxyimino-6,6-Dimethyl-3-Methylsulfanyl - 4,5,6,7-Tetra hydro-Benzo [C] Thiophene-1-Carboxylic Acid

Table 4. Degradation results of chemical compounds (the observation t = 192 hours)

Peak	R.Time	Area	Area%	Height	Name
1	4.989	48189	1.26	9539	Ethanamine,1-(2,4-Cyclopentadien-1-Ylidene)-N,N-Dimethyl-(CAS)
2	5.866	63142	1.65	14298	5h-1-Pyrindine
3	8.732	64651	1.69	16861	1,3,3-Trideuterio-Endo-6-Hydroxy-9-Oxabicyclo(3.3.1)Nonan-2-One
4	12.985	36128	0.94	14142	Acethydrazide,2-(2-Naphthylamino)-N2-(2,6-Dichlorobenzylideno)-
5	13.101	81069	2.11	36269	Tetradecanenitrile
6	15.998	57762	1.51	17709	1,4-Diaza-2,5-Dioxo-3-Isobutyl Bicyclo[4.3.0]Nonane
7	17.826	353665	9.22	122031	Hexadecanenitrile(CAS)
8	18.372	98932	2.58	42608	Hexadecanoic Acid,Methyl Ester(CAS)
9	18.606	35870	0.94	12399	1,4-Diaza-2,5-Dioxo-3-Isobutyl Bicyclo[4.3.0]Nonane
10	19.486	38666	1.01	13905	9-Octadecenamide,(Z)-(Cas)
11	21.917	119642	3.12	40310	Hexadecenenitrile
12	22.020	57378	1.50	17685	1H-Fluorene,Dodecahydro-(CAS)
13	22.329	57101	1.49	22640	6-Octadecenoic Acid,Methyl Ester,(Z)-(CAS)
14	22.472	106596	2.78	37955	Hexadecanenitrile(CAS)
15	22.941	51171	1.33	20237	Tetradecanoic Acid,Methyl Ester(CAS)
16	23.914	63356	1.65	26794	N-Tetradecanoic Acid Amide
17	24.915	30091	0.78	9222	Sydnone, 4-Bromo-3-(Dimethylamino)-(CAS)
18	24.975	34098	0.89	12093	Caprolactone Oxime,(NB)-O-[(Diethylboryloxy)(Ethyl)Boryl]-
19	25.040	79169	2.06	12035	3,3-Diethoxy-1,1,1,5,5,5-Hexamethyltrisiloxane
20	25.190	50875	1.33	14698	1h-Indole-2-Carboxylic Acid,6-(4-Fluorophenyl)-3-Methyl-4-Oxo-4,5,6,7-Tetra
21	25.280	99698	2.60	20663	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decamethyl
22	25.381	46788	1.22	28603	3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane
23	25.415	41195	1.07	22263	3,6-Dioxa-2,7-Disilaoctane,2,2,4,7,7-Pentamethyl-(CAS)
24	25.440	50152	1.31	21294	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-Hexasiloxane
25	25.485	75004	1.96	32286	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-Hexasiloxane
26	25.536	54901	1.43	30545	3-Ethoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsilyloxy)Trisiloxane
27	25.585	90159	2.35	21884	3,7-dibromo-6-ethyl-2-(pent-2'-en-4'-ynyl)octahydropyrano[3,2-b]pyran
28	25.641	58196	1.52	35794	Phenol,2-(4-diethylaminophenyliminomethyl)-
29	25.715	170080	4.43	29568	Silicone Grease,Siliconfett
30	25.854	130310	3.40	29240	(E)-1-[(1',1'-Dimethylethyl)Diphenylsilyl]-2-(Trimethylsilyl)Ethylene
31	25.896	62480	1.63	26179	Silikonfett

32	25.947	121754	3.17	23743	Silikonfett
33	26.134	113921	2.97	20351	3-Ethoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsilyloxy)Trisiloxane
34	26.198	35139	0.92	23472	Silicone Grease,Siliconfett
35	26.303	50603	1.32	21563	1,5-Dimethyl-3-(4-Nitrophenyl)-1,3-Dihydro-2,1-Benzisothiazole 2,2-Dioxide
36	26.705	56983	1.49	15843	1,1,3,3,5,5,7,7-Octamethyl-Tetrasiloxane
37	27.031	55118	1.44	15602	Silikonfett
38	27.130	75329	1.96	14331	Cyclopentasiloxane, Decamethyl-(CAS)
39	27.210	39833	1.04	17390	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decamethyl-
40	27.378	53927	1.41	15526	Hydroperoxide,9,10-Dihydro-9,10,10-Triphenyl-9-Anthryl(CAS)
41	27.440	32994	0.86	12840	Silikonfett
42	27.510	45836	1.20	20107	Tetracosamethylcyclododecasiloxane
43	27.540	41921	1.09	23431	1,3,5,7-Tetraethyl-1-Butoxycyclotetrasiloxane
44	27.674	59065	1.54	14410	Silikonfett
45	27.730	30912	0.81	13975	3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane
46	27.804	46229	1.21	14254	Silicone Grease,Siliconfett
47	27.858	39969	1.04	17706	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-Hexasiloxane
48	28.599	32475	0.85	14537	Cyclotrisiloxane,Hexamethyl-(CAS)
49	28.741	82232	2.14	19951	Silicone Grease, Siliconfett
50	28.830	43445	1.13	19371	3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane
51	28.904	51224	1.34	16427	Tetrakis(Dimethylsilyl)-[18-O]-Dioxide
52	29.064	40249	1.05	19899	1h-Pyrrole-3,4-Diacetic Acid, 2-Acetoxymethyl-5-Methoxycarbonyl-,Dimethyl Ester
53	29.140	38332	1.00	15524	2-{4-[2-(4-Methoxymethylphenyl)Vinyl]Phenyl}Propan-2-Ol
54	29.285	30528	0.80	15811	Anthracene-9-Ol, 9,10-Dihydro-10-(4-Nitrobenzylideno)-
55	29.355	38555	1.01	16368	2,5-Dichloro-N,N-Diethyl-Benzenesulfonamide
56	29.440	44942	1.17	13947	Benzoic Acid, 3-[(Trimethylsilyl)Oxy]-,Trimethylsilyl Ester
57	29.565	34341	0.90	9740	Silane, Trimethyl[[1-[(Trimethylsilyl) Ethynyl] Cyclohexyl]Oxy]
58	29.690	30557	0.80	12757	1,1,1,3,5,7,9,9,9-Nonamethylpentasiloxane18591 Hexasiloxane,Tetradecamethyl-(CAS)
59	29.751	30337	0.79	17289	Hexasiloxane,Tetradecamethyl-(CAS)
60	29.800	31960	0.83	1306505	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decamethyl-
		3835224	100		

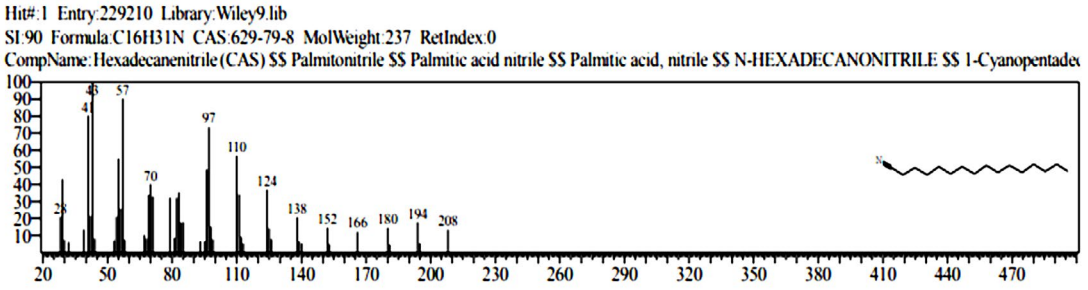

Fig. 13. The chromatogram of chemical compounds *Hexadecanenitrile(CAS) Palmitonitrile, Palmitic acid nitrile, N-Hexadecanonitrile, 1-Cyanopentadecane*

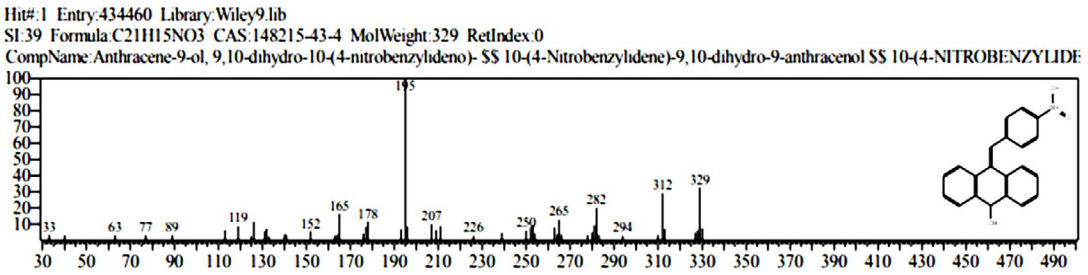

Fig. 14. Chromatogram of chemical compounds Anthracene -9-Ol, 9,10-Dihydro-10- (4-Nitrobenzylideno) - (Functional Group - Ol / Alcohol)

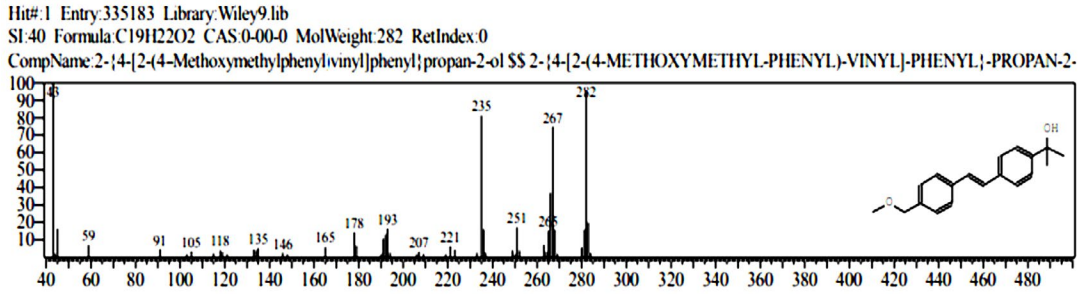

Fig. 15. The chromatogram of chemical compounds 2- {4-[2-(4-Methoxymethylphenyl) vinyl] phenyl} propan-2-ol (functional group –ol/alhohol)

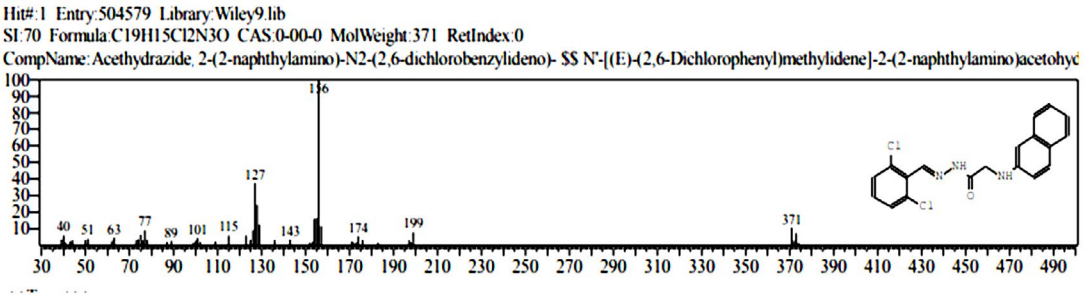

Fig. 16. The chromatogram of chemical compounds Acethydrazide compound, 2- (2-naphthylamino) - N2 - (2 , 6 - dichloro benzylideno)

and sampling were carried out at 0 hours, 96 hours, and 192 hours. The chromatogram of chemical compounds biodegradation of the bacterial consortium *Exiquobacterium profundum - Oceanobacillus iheyenis* at T = 0 hours then detected the malathion compound $C_{10}H_{19}O_6PS_2$ or Butanedioic acid, ((dimethoxyphosphinothioyl) thio) -, monoethyl ester*er* [31], detected on peak 4 with real time = 19,675, area% = 7.37, is follow : (fig 5)

While the cromatogram for the chemical compound Profenofos with the chemical formula $C_{11}H_{15}BrClO_3PSO$- (4-bromo-2-chlorophenyl) o-ethyl s-propyl thiophosphate [32] was detected at peak 8, real time = 23,957, area% = 6.91, as follow : (fig 6)

The complete data on the chemical compounds resulting from degradation is explained based on observations on GC MS with the parameters Peak, Real-Time, Area%, as follows (table 2):

In the observation time of t = 0 hours that the tested Malathion and Profenofos compounds were still detected, this indicates that the Malathion and Profenofos compounds have not been completely degraded into simpler compounds.

The chromatogram data of chemical compounds biodegradation results from the bacterial consortium *Exiquobacterium profundum-Oceanobacillus iheyenis* at T = 96 hours observations, are completely shown in table 3, which is the result of staging on GC MS with parameters peak, real-time, area%, as follow :

Based on table 3, it shows that the compound Malathion [(Dimethoxy-phosphinothioyl) Thio)] was detected at peak 14, real time = 19.675, area% = 2.25 while Profenofos (O-(4-Bromo-2-Chlorophenyl)O-Ethyl S-Propyl Thiophosphate) detected at peak = 22, real time = 23,951 and area% = 2.2, as follows (Fig 7 and fig 8):

Also detected a chemical compound (4-Bromo-3-Nitro-Benzylidene) -1- (4-Chloro-Phenyl) -Pyrazolidine-3,5-dione which is the result of degradation of the profenofos compound, at peak = 20, real-time = 23,770 , area% = 0.75, as follows: (Fig 9)

Likewise the chemical compound O- (4-Bromo-2-Chlorophenyl) -O'-Ethyl Ester from Propylthio-Phosphoric Acid resulted from the enzymatic degradation of Profenofos by bacteria, this compound was detected based on a chromatogram at peak = 22, real-time = 24.525 and area % = 2.21, as follow : (Fig 10)

The chemical compound resulting from the degradation of Profenofos (Profenofos O- (4-Bromo-2-Chlorophenyl) O-Ethyl S-Propyl Thiophosphate) will become a compound of phosphorus and phosphate groups which are degradation compounds that are not toxic. [33]

In table 3 also detected compounds resulting from enzymatic malathion degradation by the bacterial consortium, is Chemical compounds of Malathion degraded into Butanedioic Acid, [(Dimethoxyphosphinothioyl) Thio]) detected at peak = 2, real-time = 4,477 , area %. = 0.87 (fig 11)

The chemical compound Butanedioic Acid, [(Dimethoxyphosphinothioyl) Thio] -, Diethyl Ester is a synonym for Malathion Dicarboxylic Acid or Mercapto-O, O-Dimethyl Phosphorodithioate Succinic Acid which is the result of aerobic degradation of Malathion. Butanedioic Acid, [(Dimethoxyphosphinothioyl) Thio] -, Diethyl Ester will be degraded into a compound with this carboxylate group, namely 4-Acetyloxyimino-6,6-Dimethyl-3-Methylsulfanyl-4,5,6,7-Tetra hydro-Benzo [C] Thiophene-1-Carboxylic Acid. the compound was detected at peak = 37. real time == 26.430, area% = 1.00, as follows: [34 35]. (fig 12)

Based on these data, Malathion and Profenofos compounds have been degraded into simpler compounds, this can be compared with the decrease in peak values, real time and% area of Malathion and Profenofos compounds.[36]

The chromatogram of chemical compounds degradation of Malathion and Profenofos by the bacterial consortium *Exiquobacterium profundum-Oceanobacillus iheyenis* at observation t = 192 hours (table 4), as follows:

Based on table 4, at the observation t = 92 hours, the chemical compounds of Malathion and Profenofos were not detected. This shows that the concentration disturbance and Profenofos in the sample have broken down completely into simple compounds which are not contaminants. Prediction of Biodegradation of Malathion and Profenofos compounds according to the

EAWAG-Biocatalysis and Biodegradation Pathway Prediction System that Malathion and Profenofos compounds will be degraded into simpler compounds, namely Hexadecanenitrile (CAS) chemical compounds Palmitonitrile, Palmitic acid nitrile, N-Hexadecanon 1- Cyanopentadecane which is the result of degradation of Profenofos. these compounds were detected at peak = 7 and real time = 17,826 and Hexadecanenitrile (CAS) at peak = 14, real-time = 22,472 (fig 13), chemical compound Anthracene-9-Ol,9,10-Dihydro-10-(4-Nitrobenzylideno)-(Functional Group-Ol/ Alcohol) was detected at peak 54, real time = 29,285 (fig 14), whereas chemical compound 2-{4- [2- (4-Methoxymethylphenyl) vinyl] phenyl} propan-2-ol (functional group-ol / alhohol) with peak = 53 and real time = 29,140 (fig 15) and chemical compounds Acethydrazide compound, 2 -(2-naphthylamino) -N2- (2,6-dichloro benzylideno) is a decomposed benzyl aldehyde group, is a compound resulting from Malathion degradation, detected with peak 4 and real time = 12,985, as follow [33] (fig 16) :

At the observation of t = 192 hours, the chemical compounds of Malathion and Profenofos have been degraded into simpler and non-toxic compounds.[37]

CONCLUSION

The indigenous bacterial consortium *Exiquobacterium profundum - Oceanobacillus iheyenis* was able to completely degrade Malathion and Profenofos at observation t = 4 (96 hours observation) based on a decrease in the area % of Malathion from 7.37 to 2.25 and a decrease in area % of Profenofos from 6.91 to 2, 21. At the observation t = 8 (192 hours) Malathion and *Profenofos* compounds were not detected (area% = 0)

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SI does the research design, wrote the research results, wrote the initial draft of the manuscript. SI and PSO worked together to manage the research analysis. SI manages the literature and makes final draft corrections. Both authors read and approved the manuscript for publication.

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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