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Research Article
Identification of Bacteria Indigenous Selected Rawa Pening Lake Owns Best Degradation Capability of the Organophosphate Pesticides (Malathion and Profenofos)

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Abstract

Background and Objectives: The process of selection and identification is a very important thing to get the names and types of bacteria that have the best bioremediation capabilities so that the bioremediation process can run optimally. Identification of selected bacteria which has the best degradation ability on (malathion and profenofos) is one way to get the ultimate bioremediation agent. **Methodology:** This study successfully selecting three best bacteria based on their ability to degradation of the malathion and profenofos perfectly is a bacterium with a code RPL-1, RPL-5 and TRA-5. The selected bacteria are then identified by molecular biology. **Results:** Results Identification of molecular genetics and phylogenetic analysis of bacteria that the level of equality test with the maximum bacterial identification RPL-1 is approximately 87%, with the results of phylogenetic tree analysis has the closest kinship relations as a bacterium with the name *Oceanobacillus iheyensis*. While the results of the analysis of the level of similarity with the bacterial maximum identity RPL-5 is approximately 99% with the results of phylogenetic tree analysis test showed the closest kinship with bacteria *Exiguobacterium profundum*. **Conclusion:** The results of the analysis of the maximum levels of bacterial identity TRA-5 is equal to 98% with the results of phylogenetic tree analysis test against bacterial isolates TRA-5 has addressed a very close kinship with *Bacillus firmus*. Bacteria have been registered in the DNA Data Bank of Japan, DDBJ Center, National Institute of Genetics, Shizuoka, Japan.

Key words: Pollution of pesticide, malathion, profenofos, bioremediation, molecular biology degradation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bioremediation of pesticides is a most effective way in tackling the pesticide contamination in the waters Rawa Pening. Concerns about pesticide contamination that are bioaccumulative and persistent in the lake Rawa Pening concern to the Ministry of Environment of Indonesia, in this case the concentration of malathion and profenofos Rawa Pening waters[1].

Bioremediation is a process that involves the reactions of catabolism of organic compound and inorganic transformation processes involving contaminants and biological agents in their ability to detoxify pollutants either singly or concortia. The process of degradation of pesticides by bacteria determined by strains of bacteria as biological agents that have the ability of degradation. The process of screening bacteria very determine to get the superior quality of the bacteria which will then be used in bioremediation technology of pesticide compounds[2].

Indigenous bacteria are ecologically settled at a certain place and experienced an adjustment environment long enough, so that it is become stable bacteria. The utilization of indigenous bacteria in bioremediation technology has developed a lot since the success of indigenous bacteria in modifying compound impurities which are compounds into simple compounds are not toxic. The study of bioremediation of pesticide by making use of indigenous bacteria would be easier and safer, because indigenous bacteria live in a normal and genetically modified environment of the place of its life. Therefore the stages of screening and identification of degrading bacteria are very important to get the good bacteria[3].

Identification of bacteria can be done in several ways as follows: Observation of cell morphology, Gram staining, test the physiological, biochemical and molecular biology. Identification of bacteria in the morphology and biochemistry is done to determine the strain of the bacteria test. The methods of identification and classification of bacteria can be carried by means of morphological characteristics include differential staining, biochemical test that includes test detection of lactose fermentation and H_2 production test, serology test agglutination, the test includes serology, ELISA assay and molecular tests. Test the molecular biology of DNA base covers composition, plasmid finger printing, sequencing, hybridization, nucleic acid polymerization chain reaction. Identification with physiological or biochemical properties[4].

Identification of bacteria can be done in a phenotypic analysis and genotypic. Phenotypic analysis is the identification with physiological or biochemical properties (Bergey's manual of determinative), while the genetic analysis study of the characterization of the strains in one species of molecular biology to know that it can be known to the phylogenetic affinities of the test strains of bacteria. Phenotypic analysis and genotypic analysis needs to be done on this case bacteria identification process to complete and to get results with good validity. Bacterial identification by analysis of genotypic gene, 6S rRNA gene was used as a target for analyzing genomic diversity, 16S rRNA because it universally distributed on living beings, so the analysis of kinship and 16s rRNA was one of the compilers of the subunits of the 30S that are essential for translation consisting of 1542 base pairs so that the 16S rRNA, used as molecular markers which have accurate validity. The 16S rRNA molecular markers used because this molecule is ubiquitous with identical functions of the entire organism. It can be used as a chronometer of the evolution of the organism because it can reconstruct the tree phylogenetic. It can be used to track the diversity and puts strain-strain in one species. The 16S rRNA can be changed according to the distance to its evolution. It has some areas which have a relatively conservative base sequence and some areas that have been catalyzed by many sequence variations. In molecular biology, using a phylogenetic approach is the analysis of 16S rRNA gene sequences that do not depend on the growth conditions and media used[5]. Ribosomal RNA (rRNA) is a macromolecule most important, because this is the skeleton of the molecule that plays an important role in ribosomal translation mechanism. The rRNA is functionally involved in the production of protein synthesis but sequences in certain parts continues to evolve and change at the level of primary structure while maintaining secondary and tertiary structures[6]. Some segments of DNA can be reconstructed. The rRNA segment spread in every living thing varied from higher organisms to lower organisms level so that the classification of the species to a higher level (genus) will be more easy. The 16SrRNA molecules and 23S rRNA were evolved independently in nature, so that it can provide data to track the relationship phylogenetic organisms[5]. The molecular identification method by using rRNA more practical and more valid because of the availability of rRNA that is universal and wide spread in nature, making it possible to synthesize a universal primer. In the PCR process capable attached to the rRNA gene sequences of the three domain is phylogenetic as in archaea, bacteria and eukaryotic. Conserved regions could be used as an area where the adhesion primer on the 16S rRNA gene amplification *in vitro* of template isolated

from the environment[7]. The principle of 16S rRNA gene amplification by PCR technique is the use of DNA template isolated from the environment as the test gene clone libraries. Sequences of genes 16S rRNA can be used to predict the properties of the organism before culturing. Identification model of cultivation organisms closely related. Classification based on morphology and detection of the growth of a specific organism in mixed cultures, monitoring the distribution of organisms in nature so it can be used to determine the biodiversity accurately and quickly[8].

RESEARCH METHODS

The scope of this study includes the scientific field of environmental sciences, environmental microbiology, ecology and biochemistry aimed isolation and selection of indigenous bacteria selected from Rawa Pening waters which indicated contamination organophosphate (prefenofos and malathion).

Isolation and identification of bacteria indigenous to the test methods morphology and biochemistry (Bergey's manual systematic method) held at the Integrated Laboratory of the University of Diponegoro in Semarang, Central Java and Research Center of Aquaculture, Brackish Water, Jepara, Central Java and the identification of molecular tests Polymerase Chain Reaction (PCR) done Integrated Laboratory Biotechnology, Laboratory at University of Diponegoro.

EQUIPMENT AND MATERIALS RESEARCH

Equipment: Micro Centrifuge equipped cooling (Sorvall Fresco), incubator (Memmert), autoclave (Hirayama, Japan), laminar air flow cabinet (ESCO), pH meter (Eutech), digital cameras (HP Photosmart R607), analytical balance (Scout and Acculab), deep freezer -20°C (GEA), oven (lab-line instruments and WTB binder), vortex mixer (25 mL PCR Master Mix (0.05 U mL^{-1} of *Taq* DNA polymerase; 0.4 mM each dNTP, 4 mM $MgCl_2$), 2 mL primer 16E1, 2 mL 16E2 primer, 1 mL of MilliQ and 10 mL health genomic DNA template, mini gel electrophoresis (Mupid-ex Advance), UV transluminator (BDA IT Biometra 1), PCR thermal cycler (MJ Mini Biorad), 25 mL of PCR Master Mix (0.05 U mL^{-1} of *Taq* DNA polymerase, 0.4 mM each dNTP, 4 mM $MgCl_2$), 2 mL primer 16E1, 2 mL 16E2 primer, 1 mL of MilliQ, 10 and 25 mL of genomic DNA template PCR Master Mix (0.05 U mL^{-1} of *Taq* DNA polymerase; 0.4 mM each dNTP, 4 mM $MgCl_2$), 2 mL of primer 16E1, 2 mL 16E2 primer, 1 and 10 mL MilliQ genomic DNA template microcentrifuges minispin (eppendorf) and a glass beaker.

Material: Materials used are lysozyme (Sigma), sodium dodecyl sulfate/SDS (Sigma), proteinase-K (Usb), sodium chloride (Merck); distilled water (BRATACO), aquabidest (Otsuka), aquabidest free DNAse and RNAse (ddH_2O), tris base (Merck), Etylene Diamine Tetra Acetic Acid (EDTA) (Sigma); chloroform (Merck), isoamyl alcohol (Sigma), PCR master mix (Fermentas), primary 16E1: GGG AGT TTT AAA ACC GCT AAT GTT C (Biotech) [4], primary 16E2: TTC CCG AAG GCA CAT TCT (Biotech) [4], agarose ultrapure (Invitrogen), loading buffer, ethidium bromide (Sentra BD); 1 kb plus DNA ladder (Invitrogen), Ehrlich reagent, methyl red, potassium hydroxide, α-naphthol. Nutrient Broth (NB) media solution (Pronadisa) pH 6.8±0.2, nutrient media order (NA) (Difco) pH 6.8±0.2, lactose monohydrate (Merck), media Brilliant Green Lactose Bile (BGLB) broth 2% (Pronadisa) pH 7.2±0.2, media Eosin Methylene Blue (EMB) agar (Merck) pH 7.3, peptone (Difco), media Methyl Red Voges-Proskauer (MRVP) (Merck), media simmons citrate (Difco), Tris Acetate EDTA (TAE) buffer 1% PVP, chloroform, isopropanol, NaCl, ethanol 100%, TAE buffer, agarose, buffer TAE1x, DNA extraction, EtBr. Specific primers forward and reverse, Green Go *Taq* Master Mix.

METHODS

Bacterial isolation methods of water and sediment Rawa Pening lakes: Water samples were taken from water incorporated into the sample bottle, then put in a cool box and taken to the laboratory to isolate the bacteria. A total of 1 mL samples of lake water Rawa Pening put in 9 mL of sterile distilled water, to obtain 10^{-1} dilution, the sample is shaken with used vortex until homogeneous. A total of 1 mL of 10^{-1} transferred into 9 mL of sterile distilled water to obtain a 10^{-2} dilution, the sample is shaken/divortex until homogeneous. Step number 6 was repeated to obtain 10^{-5} dilution. From each dilution was taken 35 mL, then implanted into the surface of the agar Zobell 2216 E. Results Isolation incubated at 35°C for 2×24 h. Colonies, which grew observed by their morphology (shape, color and texture).

Extraction of DNA by using Chelex 100 Kit: Bacterial cells that had been grown for 24 h put into a 1.5 mL eppendorf tube containing 100 mL aquabidest, then added 0.5% saponin and allowed to stand for 24 h at 4°C. Samples centrifuged at 12,000 rpm for 10 min then centrifuged supernatant was discarded result. A total of 1 mL phosphate buffer saline (1×PBS) was added to the eppendorf tube, then centrifuged again with the speed of 12,000 rpm for 15 min,

the supernatant was discarded then as many as 100 and 50 mL akuabides Chelex 100 was added to the tube. Samples were boiled for 10 min (the sample was homogenized using a vortex in the first 5 min). Centrifugation back with a speed of 12,000 rpm for 10 min. Supernatant containing the DNA was transferred into a new eppendorf tube and is ready for the process of DNA amplification.

DNA amplification: The treatment temperature used in the amplification of DNA were: initial denaturation at 95°C for 3 min, then 30 cycles (denaturation at 95°C for 1 min. Annealing at a temperature of 55°C for 1 min and extension at 72°C for 1 min and extension at 72°C for 7 min and the last 4°C. Primers used for PCR 16S rDNA is a universal primer for bacteria 27f (5'-agagtttgatcmtggctcag-3') and eubacteria 1492r-specific primer (5'-tacggytaccttgttacgactt-3') blend the ingredients used that kit promega (25 mL) primer 270f (2.5 mL), the primary 1492 r (2.5 mL), DNA template (2.5 mL) and aquabidest (17.5 mL), so that the total volume was 50 mL. The materials were mixed in a 0.2 mL PCR tube.

Visualization of DNA amplification results: Visualize results of DNA amplification are done through electrophoresis by inserting 5 mL PCR products into the well 1% agarose gel. Making 1% agarose gel by dissolving 1 g of agarose in 100 mL of 1×TAE buffer and then heated using the oven until homogeneous mixture (clear). A total of 5.33 mL ethidium bromide gel incorporated into the solution, then rocked-shake the homogeneous mixture. The gel solution was poured into the mold with comb mold mounted in an upright position to pass a comb in accordance with the desired thickness. Gel left for some time to harden. The next step gel soaked in 1×TAE buffer solution, gel and then electrophoresed with a voltage of 100 V for ±30 min. The band DNA amplification product was observed by using gel documentation.

Purification of DNA amplification results: Purification performed to obtain pure DNA results of 16S rDNA PCR amplification. Results of PCR centrifuged at 12,000 rpm for 7 min. Supernatant was removed by using a micropipette, make sure the correct DNA-pure (no primer is left behind). A total of 50 mL of sterile akuabides added to the DNA pellet, let stand for 5 min. Outcome of pure DNA can be sequenced to determine the sequence of DNA bases.

DNA sequencing: Sequencing was carried out by PCR cycle using the Big Dye Terminator v.3.1. The formula for sequencing PCR are: 2 big dye, 2 mL buffer 10×, 4 mL of template DNA, 1 mL with a concentration of 3.2 pmol primer, ddH_2O to a final volume of 10 mL. Amplification of DNA is carried out with a cycle as follows: initial denaturation at 96°C for 2 min, then denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec and extension at 60°C for 4 min in 25 cycles. The results of the PCR purified and sequenced using the primers 27f. Sequences were analyzed automatically (ABI 3130XL, Applied Biosystems).

Construction of phylogenetic trees: Degrading bacteria pesticides that have been successfully amplified 16S rRNA gene of its visible kinship with other prokaryotes that exist in the database based on 16S rRNA gene sequences here. Sequences were performed at the University Integrated Laboratory Dipenogoro. Partial sequences obtained by going through the editing process using Bioedit program. Having obtained the data of contiq nucleotide sequence based amplification with the universal, the homology will be compared with other prokaryotes that exist in the database at the GeneBank. Cluster analysis was performed using the data base of the website Ribosomal Database Project (RDP) with the site (http://www.rdp.com). While the manufacture of phylogenetic trees using MEGA program 5.

RESULTS AND DISCUSSION

Identification carried out to find the name and type of bacteria that has the best degradation capability towards malathion and profenofos with the code RPL-1, RPL-5 and TRA-5.

Biochemistry test: Bacterial identification test done in biochemistry to find out the initial screening and morphological properties and biochemical test bacteria. Table 1 and Fig. 1 shows the biochemical tests.

Figure 1 and 2 shows bio-chemical tests that indicate the test bacteria with code RPL-1, RPL-5 and TRA-5 is a Gram-positive and has the ability to hydrolyze to strach. Photo below shows one biochemical tests on bacteria test.

Bacillus is a genus of bacteria that have a rod shape, Gram (+), motile spores can produce a single but most species do not produce spores, dispersed in nature and will withstand extreme conditions. Genera Bacillus bacteria usually is catalase positive, oxidase positive capable of metabolizing carbohydrates through fermentation, is not able to produce acids from mannitol and in part from the genera Bacillus are

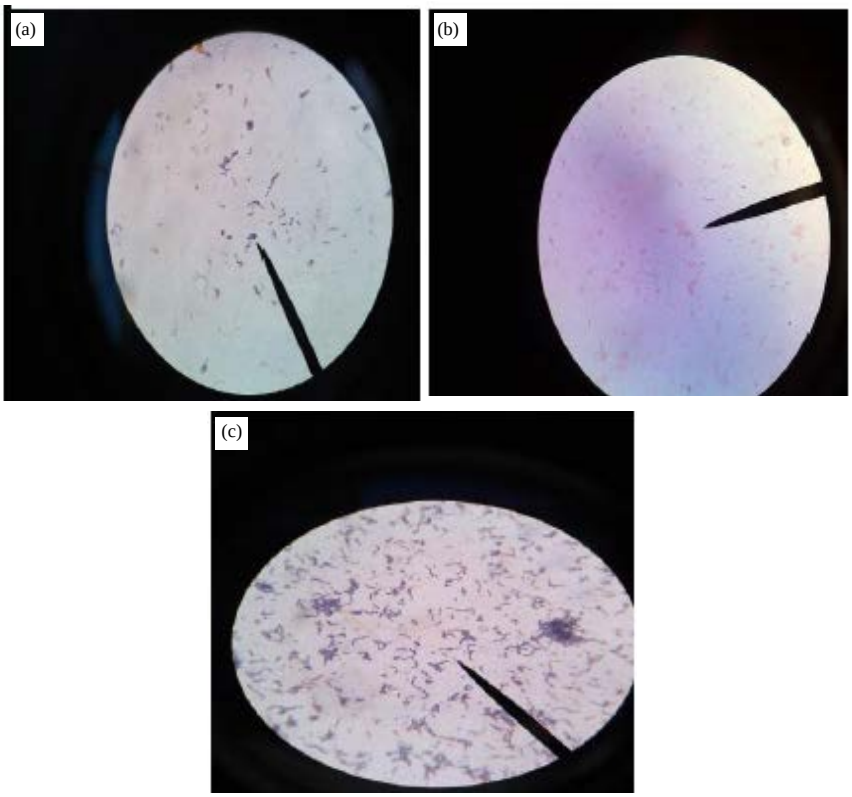

Fig. 1(a-c): Shape isolate test and Gram staining, bacteria code (a) RPL-1, (b) RPL-5 and (c) TRA-5 are Gram positive with bacterial form of rods

Table 1: Biochemical tests in bacteria test with code RPL-1, TRA-5 and RPL-5

	Isolates codes				Isolates codes		
Biochemical tests	RPL-1	TRA-5	RPL-1	Biochemical tests	RPL-1	TRA-5	RPL-1
Shape isolates	+	+	-	Acid from phenol red medium			
Gram staining	+	+	+	Glucose	×	×	-
Motility	+	+	+	Celibiose	×	×	-
Length of bacteria 3>mm	-	-	-	Galactose	×	×	-
Position and length spores	vx	vx	-	Mannose	×	×	-
Spore	+	+	-	Melibiose	×	×	-
Growth on 50°a	+	+	-	Rafinose	×	×	-
Growth on 37°C	+	+	×	Salicin	×	×	-
Growth on 47°C	+	+	-	Rylase	×	×	-
Growth with 10% NaCl	+	-	×	ONPG	+	-	-
Anaerobic	+	+	+	Utilization of citrat	+	-	-
Aerobic	+	+	+	Urease	-	-	-
Acid from ASS medium				YP	+	+	+
Glucose	+	+	+	Nitrate reduced	-	+	+
Celibiose	+	+	+	Starch hydrolysis	+	+	(-/×)
Galactose	+	+	+	Oxidase	+	+	+
Mannose	+	+	+	Katase	+	+	+
Melibiose	+	+	+	Acid fast	-	-	+
Rafinose	+	+	+	Tween 20 hydrolysis	×	×	+
Salicin	+	+	+	Growth on centrimide agar	×	×	+
Rylase	+	+	+	Yellow pigmen	-	-	-

VX: Central/Oval, X: Not on testing, -: Facultative anaerobic and +: Aerobic

hetero fermentative anaerobic. Bacillus bacteria are also able to metabolize carbohydrates, proteins, amino acids and can reduce nitrate to nitrite. Based on the analysis of the test bacteria, the bacteria RPL-1 and TRA-5 included in cypress that

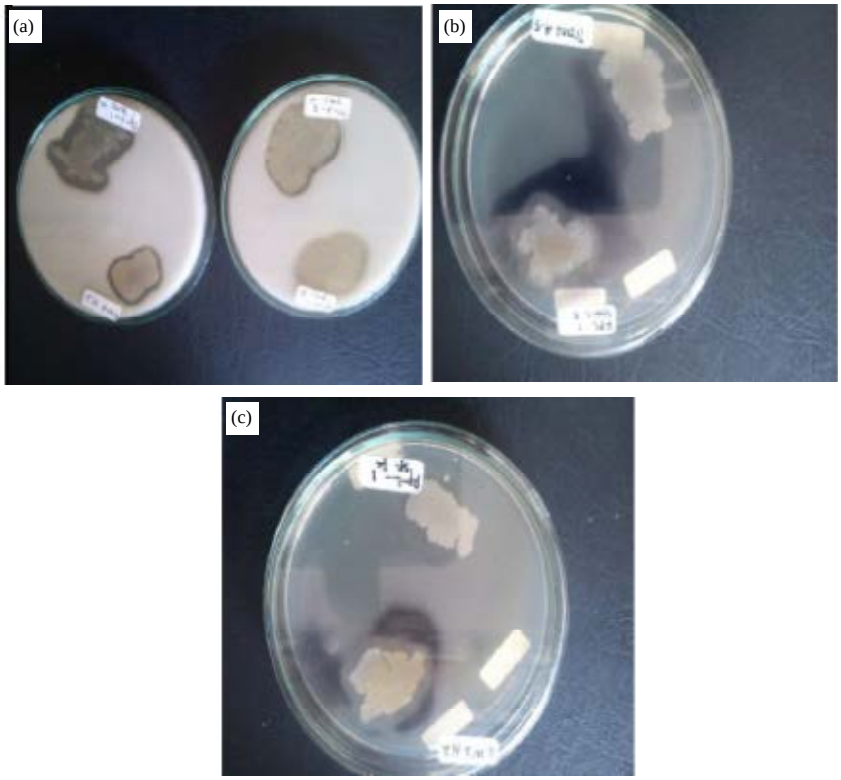

Fig. 2(a-c): Starch hydrolysis test, bacteria code (a) RPL-1, (b) and RPL-5 and (c) TRA-5 can hydrolyze starch

is Bacillus genera[9]. Exiguobacterium bacteria capable of living in extreme conditions alkaline (pH 9.5) and halotolerant, areobic and facultative anaerobic, fermentative to glucose homolactic, some species are heterolactic Exiguobacterium against lactic fermentative, catalase-positive, Gram-positive, produces spores, motile, chemo-organotrophic and heterofermentat if anaerobic. Catalase-positive and oxidase negative. Based on morphological and biochemical test results of the test bacteria TRA-5 it is possible bacteria from the genera Exiguobacterium[10].

Molecular identification: Molecular identification by using sequences of 16S rRNA by utilizing the isolation of genomic DNA as a template and address the ribbon of DNA is based on the results of gel electrophoresis of 16S-rRNA gene amplification as in Fig. 3.

The PCR product identification is using 16S rRNA gene/16S rRNA (PCR-amplified 16S rRNA) from bacterial species through agarose gel electrophoresis method. The 50-20000 bp DNA fragment is the best measure that can be separated by agarose gel. Analysis using 16S rDNA gene/16S rRNA because gen16S rDNA/rRNA had 16 sustainable properties (conserved) and is a structural part of the ribosomal RNA (for a structural part of the RNA of the ribosome), which plays an important role in protein synthesis. Therefore, the 16S rRNA gene is always there and is owned by prokaryotic organisms, sustainable and almost never transferred horizontally, causing the 16S rRNA gene is ideal for phylogenetic tree reconstruction and identification of prokaryotes[11].

Process-1 genome isolation RPL-1, RPL-5 and TRA-5 marked the formation of the band for each of the genomes of bacteria after the test was observed using UV transluminator with tape encoding 16S rRNA gene and 1.5 kb compared to the marker (1kb DNA ladder). The 16S rRNA amplification product DNA sequencing to obtain the nucleotide sequence similarity and analyzed using the GeneBank with Basic Local Alignment Search Toll Nucleotode (BLAST-N program) which can find homology and tested bacterial species. To determine the phylogeny/kinship with other organisms, the results of 16S rDNA sequencing-1 isolates RPL, RPL-5 and TRA-5 were compared with 16S rDNA sequence data obtained from the Data Bank of some species. The 16S rDNA sequence data then dialignment ClustalX program ver 2.0. Then the phylogenetic tree created using MEGA ver program 05:03 with statistical methods Neighbor-Joining tree with a model P-1000

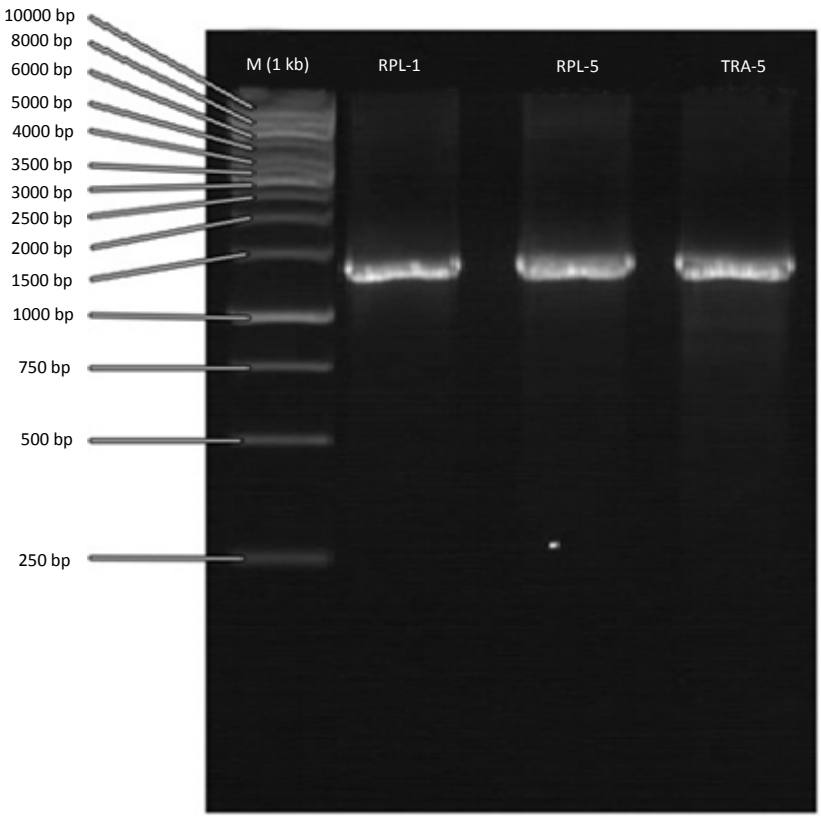

Fig. 3: Results gel electrophoresis of 16S-rRNA gene amplification

Table 2: Sequencing using the forward and reverse primer

Code isolates	Nucleotides base pair (bp)	Name of bacteria	Homology (%)	Accession No.
RPL-1	1071	*Oceanobacillus iheyensis*	87	LC019790
RPL-5	1238	*Exiqoubacterium profundum*	99	LC019791
TRA-5	1326	*Bacillus firmus*	89	LC019792

levels within the bootstrap. The 16S rDNA gene PCR results indicated by a single band on gel electrophoresis with size of about 1500 bp.

Sequencing using the forward and reverse primer determine the nucleotide sequences of bacteria are as in Table 2.

Bacteria that have been registered in the DNA Data Bank of Japan, DDBJ Center, National Institute of Genetics. Research Organization of Information and Systems Mishima, Shizuoka 411-8540, Japan (web http//Update www.ddj.nig.ac.jp/updt-from-e.html). The Table 3 below shows selected regristration indigenous bacteria degrading pesticide malathion and profenofos best of Rawa Pening, as follows:

Results of 16S-rRNA gene sequences of bacteria samples

RPL-1: GGGGTATTGCATCATAATGCAGTCGAGCGCAGGAAGC TATCT GATCCTCTTTTAGAGGTGACGATAATGGAATGAGCGGCG GACGGGTGAGTAACACGTAGGCAACCTGCCTGTAAGACTGGG ATAACTCGTGGAAACGCGAGCTAATACCGGATAACACTTTTCA TCTCCTGATGAGAAGTTGAAAGGCGGCTTTTGCTGTCACTTAC AGATGGGCCTGCGGCGCATTAGCTAGTTGGTAAGGTAATGGC TTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCG GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG AGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAAC TCTGTTGTTAGGGAAGAACAAGTGCCATAGTAACTGATGGCAC CTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGCGCGTAAAGCGCTCGCAGGCGGTTCTTTAAGTCTGATG TGAAATCTTACGGCTCAACCGTAAACGTGCATTGGAAACTGGG GAACTTGAGTGCAGAAGAGGAGAGTGCAATTCCACGTGTAGC GTGAAATGCGTATAGATGTGGAGGAACACCAGTGGCGAACGC GACTCTCTGGTCTGTAACTGACGCTGAGTAGCCAAGCGTCGG GAGCGACAGGATTAGATACCCTGGTAGCCCCTGCCGTAGACG

Table 3: Registration bacteria (RPL-1, PRL-5 and TRA-5) in the DNA Data Bank of Japan, National Institute of Genetics. Research Organization of Information and systems Mishima, Shizuoka 411-8540, Japan

***Oceanobacillus iheyensis* gene for 16S rRNA, partial sequence, strain: RP-L-1**	
GenBank	LC019790.1
LOCUS	LC019790 1071 bp DNA linear BCT 17–JAN-2015
Definition	*Oceanobacillus iheyensis* gene for 16S rRNA, partial sequence, Strain: RP-L-1
Accession	LC019790
Version	LC019790.1 GI: 748585160
Key words	
Source	*Oceanobacillus iheyensis*
Organism	*Oceanobacillus iheyensis*
	Bacteria, Firmicutes, Bacilli, Bacillales, Bacillaceae, Oceanobacillus
Reference 1	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Organophospate (malathion and profenofos) Bioremidiation by selected indigenous bacteria from Rawa pening lake, central Java, Indonesia
Journal	Unpublished
Reference 2 (Bases 1 to 1071)	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Direct submission
Journal	Submitted (16-Jan-2015) contact: Slamet Isworo diponegoro University, Doctorate Enviromental Science, Prof. Soedarto, SH., Semarang Central Java 50275, Indonesia
***Exiguobacterium profundum* gene for 16S rRNA, partial sequence, strain: RP-L-5**	
GenBank	LC019791.1
LOCUS	LC019791 1238 bp DNA linear BCT 17-Jan-2015
Definition	*Exiguobacterium profundum* gene for 16S rRNA, partial sequence, strain: RP-L-5
Accession	LC019791
Version	LC019791.1 GI: 748585161
Key words	
Source	*Exiguobacterium profundum*
Organism	*Exiguobacterium profundum*
	Bacteria, Firmicutes, Bacilli, Bacillales, Bacillaceae family XII
	Increate sedis, Exiguobacterium
Reference 1	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Organophospate (malathion and profenofos) Bioremidiation by selected indigenous bacteria from Rawa Pening lake, Central Java, Indonesia
Journal	Unpublished
Reference 2 (Bases 1 to 1238)	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Direct submission
Journal	Submitted (16-Jan-2015) contact: Slamet Isworo diponegoro University, Doctorate Enviromental Science, Prof. Soedarto, SH., Semarang Central Java 50275, Indonesia
***Bacillus firmus* gene for 16S Rrna, partial sequence, strain: TR-A-5**	
GenBank	LC019792.1
LOCUS	LC019792 1326 bp DNA linear BCT 17–JAN-2015
Definition	*Bacillus firmus* gene for 16S Rrna, partial sequence, strain: TR-A-5
Accession	LC019792
Version	LC019792.1 GI: 748585162
Key words	
Source	*Bacillus firmus*
Organism	*Bacillus firmus*
	Bacteria, Firmicutes, Bacilli, Bacillales, Bacillaceae, Bacillus
Reference 1	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Organophospate (malathion and profenofos) Bioremidiation by selected indigenous bacteria from Rawa pening lake, central Java, Indonesia
Journal	Unpublished
Reference 2 (Bases 1 to 1326)	
Authors	Isworo, S., Purwanto and Sabdono, A.
Title	Direct submission
Journal	Submitted (16-Jan-2015) contact: Slamet Isworo diponegoro University, Doctorate Enviromental Science, Prof. Soedarto, SH., Semarang Central Java 50275, Indonesia

ATGAGCGCTAGTCGTCAGGGGTTTCCGCCCCTTATGCTGAAGT
TACTCATTAAGCACTCCACCTGTGACGTCAGACGCAAGCATCA
ACTCAAAGGATTTACGCGGACCACTCAAGCGATGATCACTCGT
TTAATTACAGCACCGCGAGAACTTACCAGGCTTGGATTCCTCT
GAACATCTAAAATAGCCTTTCCTTCAGGGAAGAGTTCTCCCGA
CAAAGATTTTTCAACCCANACCTAAATTTCAGTAAGCCCGCAC
GAAGAAATCTTGA.

Bacteria test with RPL code-I am a nucleic acid molecule with a type-length 1071 bp query. Results of bacterial lineage test report RPL-1 by category taxonomy is as follows:

Kingdom : Prokaryotae
Domain : Bacteria
Phylum : Firmicutes
Class : Bacilli
Order : Bacillales
Family : Bacillaceae
Genus : Oceanobacillus
Species : *Oceanobacillus Iheyensis*

The level of maximum 87% identity similaristas RPL-1 is *Oceanobacillus iheyenis* and based on phylogenetic tree analysis, bacterial isolates RPL-1 has the closest kinship with *Oceanobacillus iheyenis*.

Results of 16S-rRNA gene sequences of bacteria samples RPL-5: CAATTGCGCGGCTATAATGCAGTCGAGCGCAGGAAAC
CGTCTGAACCCTTCGGGGGGACGACGGCGGAATGAGCGGGG
GACGGGTGAGTAACACGTAAAGAACCTGCCCATAGGTCTGGG
ATAACCACAAGAAATCCGGGCTAATACCGGATGTGTCATCGG
ACCGCATGGTCCGCTGATGAAAGGGGCTCCGGCGTCTCCCAT
GGATGGCTTTGCGGTGCATTAGCTAGGTGGTGGGGTAAAGGC
CCACCAAGGCGACGATGCATAGCCCAGCTGAGAGGGTGATCG
GCCACACTGGGACTGAGACACGGGCCAGACTCCTACGGGAG
GGGGCAGTAGGGAATCTTCCCCAATGGACGAAAGTCTGATGG
AGCAACGCCGCGTGAACGATGAAAGCTTTCGGGGCGTAAAGT
TCTGTTGTAAGGGAAGAACAAGTGCCGCACGCAATGGCGGCG
CCTTGACGGTACCTTGCGAGAAAGCCACGGCTAACTACATGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAA
TTATTGGGCGTAAAGCGCGCGCAGGCGGCCTCTTAAGTCTGAT
GTGAAAGCCCCCGGCTCAACCGGGGAGGGCCATTGGAAACT
GGGAGGCTTGAGTATATGAGAGAAGAGTGGAATTCCACGTGT
AGCGGTGAAATGCGTACAGATGTGAAGGAACACCCTTGTCGA
AAGCGACTCTTTGGCCTATATCTGACGCTGAGGCGCGAAAAC
GTGGGGAGCAACACGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAGAGCTAAGTGTTGGAGGGTTCCGCCCTTTGTGCT
CAGCTAAGCATTAACACTCCCCTGGGGAGACAGTCGCAGGCT
CAACTCAAGGATTGACGGGACCCCACACCAGTGGAGCATGTG
GTTTATTTGAGCACACGGAAAACTTTCCACTCTTGAATCCCCTG
ACCGGAAAAAATGTACCTTCCCTCTGGGGCAGGGTGACAAGT
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CAACAAGGCAACCTTGTCTTTTTTGCACATTCGTTGGCCCCCTA
GGAAATGCCGTGACAACCGAAGAAGGGGGATAACCAAATTC
ATGCCCTTAAAGTGGGTACACGTGTCAATGGAGGGCAAGGGA
CCCAACCCCAGTGGACCATCCCAAACGTTTCNTTGGATGGGG
GGCACCCCCGTAGACCGAATCTGGCGGGTGCTATACATGCAG
TCGAGCGGACAGATGGGAGCTTGCTCCCTGAAGTCAGCGGCG
GACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGG
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TCACATGAGGGAAAGCTGAAAGATGGTTTCGGCTATCACTTAC
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TCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCG
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AGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAAC
TCTGTTGTTAGGGAAGAACAAGTACCGGAGTAACTGCCGGTA
CCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGC
CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAA
TTATTGGGCGTAAAGCGCGCGCAGGCGGTTCCTTAAGTCTGAT
GTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTG
GGGAACTTGAGTGCAGAAGAGAAGAGTGGAATTCCACGTGTA
GCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGA
AGGCGACTCTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGT
GCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGC
CGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCTCCTGACAACCCTAGAGATAGGGC
GTTCCCCTTCGGGGGACAGGATGACAGGTGGTGCATGGTTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTC
TAAGGTGACTGCCGGTGACAAACCGGAAGGAAGGTGGGGGA
TGACGGTCAAATCATCATGGCCCCTTAAGGACCTGGGGCTAAC
NCACGTGCTACAATGGGATGGGAACAAAGGGGTTCGAAGAC
CCGCAAGGTTAANCGGAATCCCCATAAAACATTTTTCAAGTTC
NGAATTGCAGGGTTGAAACTCTCCTTGTTTGAAACCCGGATT.

The RPL-5 Molecular type-nucleic acid with a length query with Lineage 1238 report as follows:

Kingdom : Prokaryotae
Domain : Bacteria
Phylum : Firmicutes
Class : Bacilli
Order : Bacillales
Family : Bacillaceae

Genus : Exiguobacterium
Species : *Exiguobacterium profundum*

Similarity maximum level identity test bacteria with RPL-5 is 99% of bacteria *Exiguobacterium profundum* and based on phylogenetic tree analysis, bacterial isolates RPL-5 has the closest kinship with bacteria *Exiquobacterium profundum*.

Results of 16S-rRNA gene sequences of bacteria samples

TRA-5: GGCGGGTGCTATACATGCAGTCGAGCGGACAGATGGGA GCTTGCTCCCTGAAGTCAGCGGCGGACGGGTGAGTAACACGT GGGCAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGG GGCTAATACCGGATAATTCTTTCCCTCACATGAGGGAAAGCTG AAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGCGC ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATG CGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGT GATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGAA CAAGTACCGGAGTAACTGCCGGTACCTTGACGGTACCTAACC AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGC GCGCAGGCGGTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTC AACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG AAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAG AGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGGTCT GTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATT AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT CTCCTGACAACCCTAGAGATAGGGCGTTCCCCTTCGGGGGAC AGGATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTT AGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTG ACAAACCGGAAGGAAGGTGGGGGATGACGGTCAAATCATCA TGGCCCCTTAAGGACCTGGGGCTAACNCACGTGCTACAATGG GATGGGAACAAAGGGGTTCGAAGACCCGCAAGGTTAANCGG AATCCCCATAAAACATTTTTCAAGTTCNGAATTGCAGGGTTGA AACTCTCCTTGTTTGAAACCCGGATT.

Bacteria test with the code TRA-5 is a nucleic acid molecule with a type-length query lineage 1326, with the report as follows:

Kingdom : Prokaryotae
Division : Bacteria
Class : Firmicutes
Order : Bacillales
Family : Bacillaceae
Genus : Bacillus
Species : *Bacillus Firmus*

The maximum rate of 98% identity TRA-5 is *Bacillus firmus*, based on the phylogenetic tree analysis, bacterial isolates TRA-5 has a very close kinship with *Bacillus firmus* (Fig. 4).

Oceanobacillus iheyensis HTE 831 were isolated from marine sediments at depths ranging 1050-10897 m in the area of Okinawa, Japan. *Oceanobacillus iheyensis* HTE 831 had optimum growth at a pressure >0.1 MPa at a temperature of <15°C, even able to grow at a growth temperature at 0°C or lower, *Oceanobacillus iheyesis* capable of living in extreme conditions, very tolerant of salt content, grown under high hydrostatic pressure and have the least there are 29 proteolytic enzyme[12]. *Oceanobacillus iheyenis* are bacteria capable of breaking the bonds CP enzymatic complex, including CP lyase complex, phosphonatase, phosphonoacetate hydrolase and hydrolase phosphonopyruvate[13].

A study and succeeded in isolating and identifying the facultative anaerobic bacteria, gram-positive, motile rod and grows optimally at 45.6°C (range 12-49.6°C), pH 7.0 (pH range from 5.5-9.5) and 0-2% NaCl (range 0-11%), is halotoleran, thermophilic and not berspora, isolated from the sea in the East Pacific at a depth of about 2600 m has kinship with *Exiguobacterium aestuarii* TF-16T and *Exiguobacterium marinum* TF-80T (16S rRNA gene sequence similarity>99%). *Exiguobacterium profundum* identified by name[10]. *Exiguobacterium* spp., including gram-positive, non-spore, facultative anaerobic habitat extensive, scattered in places of extreme, life at low temperatures, including in the area of Siberia, an area hydrothermal sea and can be symbiotic with the plant, applications *Exiguobacterium* spp. bacteria to the environment is that it can be used as a bioremediation agent of organic material including pesticides and heavy metals[14].

The composition of the lipid membrane cytochromes and *Bacillus firmus* considered to have a mechanism of electron transport chain Na^+ on the specific cell membrane causing *Bacillus firmus* is halotolerant and alkalotolerant. *Bacillus firmus* able to survive on environmental pH changes from acidic to alkaline environment or Inversely through the mechanism of changes in the composition of lipids in cell membranes. By activation of fatty acid biosynthesis thereby affecting the proton conductivity of the cell membrane[15].

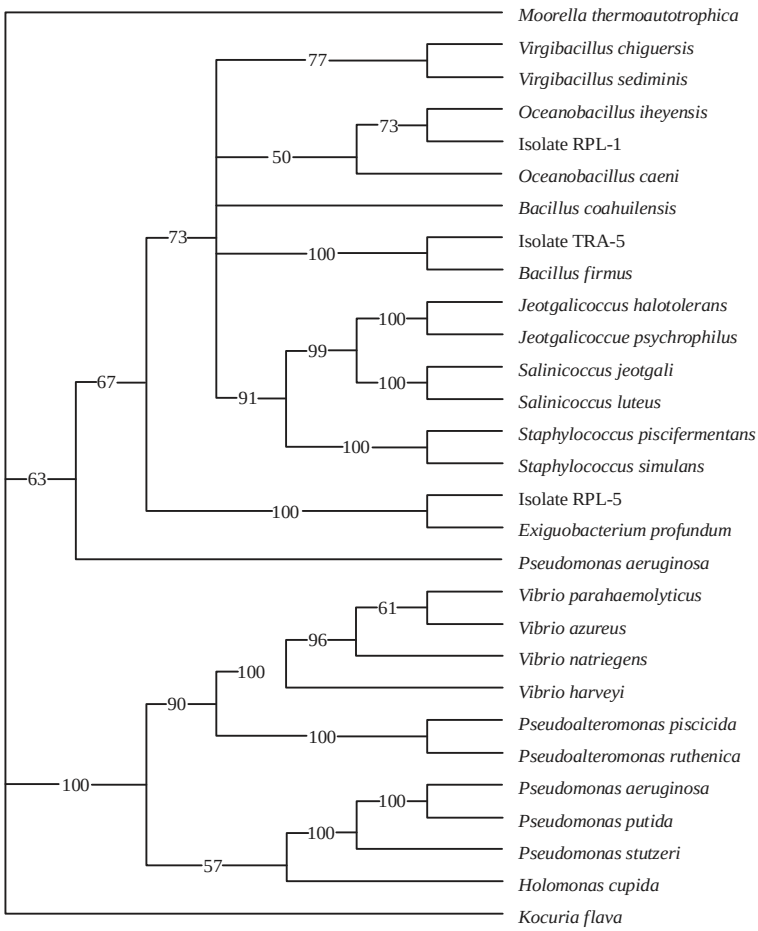

Fig. 4: Phylogenetic tree construction

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