Role of *Lactobacillus sakei* strain pro7 to reduce dichloro diphenyl trichloroethane level

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**Abstract.** Several species of bacteria can be utilized to degrade the residual insecticides. One of them was chosen from the genus Lactobacillus. The purposes of soils in Merdeka Village, Karo Regency, North Sumatra, Indonesia as well as to investigate bacteria which is capable of surviving in DDT-contaminated soil. Method for the residual analysis of the soil utilizes Quechers tube, and the bacterial propagation were performed in the medium mineral 4 (MM4). Purification to bacterial isolates was carried out in the Plate Count Agar (PCA) medium and subsequently followed by morphological identification. The results of this study show that the soil were contaminated by O'P-DDT ranging from 2.2 μgKg⁻¹ to 9.0 μgKg⁻¹. Phylogenetic analysis proves that the Lactobacillus sakei Strain PRO7 bacteria was identified. The growth rate of DDT biodegradation by Lactobacillus sakei Strain PRO7 bacteria is noted the highest at 7.89 observation and reaches 95.1% of biodegradation percentage at the 20 ppm DDT concentration.

1. **Introduction**

Intensive use of insecticides can cause to remain residues under the soils and in the plants as well as to bring bereavement for some beneficiary types of microorganisms; such insectisides are even found in the fish, animals and aquatic biota [1]. Some important effects the insecticides have are related to the changes in the ecological balance of soil microflora [2]. The use of insecticides has intensively transpired since twenty years ago. The preliminary research was aimed at obtaining the type of residual insecticides existing in the research locations which become the vegetable producers and farmers here generally use insecticides to control plant pests.

DDT is an organochloric insecticide, crystalline solid, and hydrophobic, and contains oils and it is almost insoluble in water but has excellent solubility in organic solvents, fats and oils. DDT has two isomers, namely p, p and o, p isomers. In general, the two isomeric components are known as "pp DDT" and "op DDT" [3]. DDT can be transformed by several types of bacteria under aerobic and anaerobic conditions [4]. In the first condition the DDT might be converted into DDE (1,1-bis (p-chlorophenyl) -2-dichloroethylene) then it changes into 4-chloro benzoic acid; meanwhile, in the second condition DDT can switch into DDD (1,1-dichloro-2, 2-bis (p-chlorophenyl) ethane), into DDA (bis (p-chlorophenyl) acetic acid), and into DBP (p, p-dichlorobenzophenone).
2. Materials and Methods

2.1. Isolation of Bacteria from DDT Residue Contaminated-Soils

We used forty sample points from leek vegetables planting areas, covering an approximately area of ± 1000 m², make use of soil core tool having 130 cm in length, 2.5 cm in diameter, and 0-20 cm in depth. The sample points were composited, put into aseptic sterile plastics, and taken to the Microbiology Laboratory which belongs to Faculty of Medicine, University of Sumatera Utara, Medan. Then 10 grams was weighed and diluted gradually in 10 ml of sterile distilled water to reach a concentration of 10-8. Furthermore, bacterial plating on the medium of Plat Count Agar (PCA) [5] was carried out.

2.2. Morphological and Biochemical Characteristics of DDT Residue-Reducer Indigenous Bacteria

Bacteria which grows in PCA were then vegetated in MM4 media containing yeast extract. The bacteria were subsequently subcultured to obtain two pure isolates of aerobic bacteria and one pure anaerobic isolate. The results of the subcultures of the three isolates were observed with magnification of 10X40 and 10X1000 using Japanese Olympus stereo microscope and equipped with bacterial gram staining method and colony color observation, colony shape, colony edge and bacterial colony elevation. Furthermore, the bacteria is tinted with Gram staining to distinguish groups of positive and negative Grams. Biochemical tests were carried out using the Vitec 2 Compact tool.

2.3. Phylogenetic Analysis of DDT Residue-Reducer Indigenous Bacteria

Isolation of DNA bacterial genome was done through freeze and thaw process. The 1.5 ml eppendorf tube was filled with 100 μl of labidest in aseptic condition, then an ose of a 24-hour pure bacterial culture was taken and inoculated into the eppendorf tube. The cell suspension was then frozen at -10 °C until the crystallized solution was then melted at 90 °C for 10 minutes. Cycle repetition was undertaken seven times for cell-breaking efficiency. The DNA from isolated results was used to amplify the 16S rRNA gene and this was performed by a Polymerase Chain Reaction (PCR) machine (Veriti® 96-Well Thermal Cycler 4375786, Applied Biosystems, Singapore) with a 16S rRNA-specific universal prokaryot, 63f (5’-CAG GCC TAA CAC ATG CAA GTC-3’) and 1387r (5’-GGG CGG WGT GTA CAA GGC-3’) [6]. The sequential process was worked using the Automated DNA sequencer (ABI 3730xl DNA Analyzer, Applied Biosystems). Data sequence was then compared with the one in GenBank which was owned by the National Center for Biotechnology Information (NCBI) database and the comparation used Basic Local Alignment Search Tool (BLAST) program.

2.4. Utilization of Bacillus cereus Indigenous Bacteria To Degrade DDT Residue with Invitro

Soil samples of 10 grams were diluted on the test tube until it reached a dilution value of 10-8. In a container of erlenmeyer flasks, 9 ml of sterile 0.85% NaCl were weighed then poured into a minimal salt medium known as MM4 medium, but no naphthoquinone, casamino acid, 7-vitamin solution, folic acid, riboflavin and lipoic acid are added. Bacterial isolate was propagated by using MM4 mineral media [7]. The pure bacteria which grow at dilution 10⁸ was further adapted with MM4 mixture of phosphate buffer and DDT solution on a 250 ml erlenmeyer. The bacteria was shaked and incubated for 24 hours at 300 °C in dark conditions until bacterial growth became fixed. Then, two bacterial isolates were observed using UP-LC tools at 0, 24, 48, 72, 96 hours (for four observations) at 10 ppm, 20 ppm and 30 ppm DDT concentrations [3]. The observations were conducted at the Fish Quarantine Laboratory of Kuala Namo, Medan.

3. Results and Discussion

3.1. Isolation of Indigenous Bacteria from DDT Residue contaminated-soils

DDT degradation in the soil depends on the persistence, ability and quantity of microbial degradation in the contaminated-soil. Strains of Bacillus bacteria species are capable of producing DDT, DDE and DDD phenol metabolites but algae has a metabolic and relatively small ability to lower DDT compared to bacterial species. In this condition, the isolation of indigenous bacteria from the soil can
be used to degrade DDT residues in the soil. The results show that there is a significant relationship between the use of insecticides in agricultural products [8]. The isolation of bacteria originated from pure dishaker culture is possible by using shaker orbitals at 350 rpm for 24 hours. Then, the extraction is fed into a tube containing 200 ml aquabidest in which the extraction results are continued by analyzing the DDT residues using a gas chromatography-mass spectrometry (GC-MS) tool. Results of bacterial isolation for the soils contaminated by DDT pesticide residues indicate that these bacteria can survive and are expected to degrade DDT in the soils.

![Figure 1. Pure Isolate of Lactobacillus sakei Strain PRO7 From DDT contaminated soils](image)

Visually, the color tends to become creamy white, the stems are growing and including positive gram bacteria. Meanwhile, based on the test using sequencing analysis with 94% homologous, the bacteria were identified, such as the bacteria from Lactobacillus sakei Strain PRO7 type. These bacteria are expected to serve as one of the DDT biodegradation agents.

3.2. Morphological and Biochemical Characteristics of DDT Residue-Reducer Indigenous Bacteria

Results from gram test indicate the availability of gram-positive reaction to produce purple stem cell shape as shown in Figure 2.

![Figure 2. Gram Staining to Lactobacillus sakei Strain PRO7 Bacteria (10X40 zoom to bacterial isolates of Lactobacillus sakei Strain PRO7)](image)

Furthermore, bacterial isolates were identified molecularly by taking into account the sequence of 16S rRNA gene which is adjusted by using bioedit software package of allignment editor sequences to provide a full sequence of about 1500 nucleotide bases. The sequence is then compared to the one in the general database by using the Basic Local Local Allignment Search Tools (BLAST) search program issued by the National Center for Biotechnology Information (NCBI) website (see
http://www.ncbi.nlm.nih.gov/) to find bacteria with a 16S rRNA gene sequence which has the closest association [3]. Results obtained from analysis show that it is found Lactobacillus sakei Strain PRO7 species with DNA sequence the following:

3.3. Sequencing Results of Lactobacillus sakei Strain PRO 7 Bacteria

> Lactobacillus sakei Strain PRO7

AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAAGTCAACGC
GACACTCTCCTAGATATGGAAGAGTGCTTCTGATATTGAATAACATTTGAGTGGAGCG
GACGGGTGAGTACACCTGGTTAGTTAATTACCTTGCCCTAAAGTGGGATACATTTGGAACAG
ATGCTAATAAGCCTAAACCTAAAACACCCGATGTTGAGTGGTTGAAGATGAGGTTTCGGCT
ATCAGTTAGATGGACCGCGGTCGATTAGTTAGATTGGTGAGGTAAAGGCTCACCAAGA
CCGTGATGCATAGCGACCTGAGGCTGTTGAAATACGACCGAGCACTGAGCATGGAGG
CGACGCGCGTCGGATCAGGAAGGAAATGGAATATCCACAAATGCGAAGGAAAGTCTGATGGAG
CAACGCGCAGGGATGTAAGAAGGCTTCTCAGCTGATAGGATGAGTGGAGCG

The ARB database software package was also used to adjust and identify bacteria with 16S rRNA gene sequences that have the closest connection. The 16S rRNA gene sequences in the closest isolates and bacteria are also confirmed and processed by phylogenetic trees using the Molecular Evolutionary Genetics Analysis (MEGA) software package. The history of evolution is evaluated using the Neighbor-Joining method [3]. Equivalent results in the gene bank will show some individuals who have 99% resemblance to the samples. The kinship analysis is performed by using phylogenetics software. The results can be seen in Figure 3.

![Phylogenetic Tree of Lactobacillus sakei Strain PRO7](image)

Figure 3. Phylogenetic Tree of Lactobacillus sakei Strain PRO7

In China, Lactobacillus sakei Strain C2 bacteria is traditionally isolated from chilies which is traditionally fermented, pure bacterial cultures are developed in the MRS medium at 30°C for 24 hours to get rapid fermentation of sauce and such bacteria is also effective to control pathogenic microorganisms and to reduce the content of Malondialdehyde (MDA) and nitrites [9]. While in Argentina, the Lb. sakei undergoes characterization in order to have its ability to grow at a temperature of 15°C, pH 4.5 and 5% NaCl (95%) [10]. Some microbial cultures in food are predominantly dominated by bacterial strains Lactobacillus sakei, Lb. curvatus and Weissella cibaria. The human digestive tract consists of a large number of microorganisms from groups bacteria groups that have an impact on metabolic diseases such as obesity, type II diabetes and atherosclerosis. The types of
Microorganisms include the Lactobacillus rhamnosus GG, L. Sakei NR28, Firmicutes, Bacteroidetes and Clostridium [11].

Lactobacillus plantarum MBSa4 comes from Brazil and is capable of producing more than 1 peptide with antibacterial effectiveness [12]. The results from [13] who examined the ability of Lb. pentosus Strain K2N7 and analyzed the 16s rDNA is proved to produce bacteriocin. The maximum production of bacteriocin is noted at the beginning of the bacterial growth phase. The Lb. pentosus Strain K2N7 bacteria is stable at 100 °C for 2 hours and pH between 2.0-12.

3.4. DDT Biodegradation Rate of Lactobacillus sakei Strain PRO7 Aerob Indigenous Bacteria

The biodegradation rate of Lactobacillus sakei Strain PRO7 aerob indigenous bacteria in relation to the incubation time can be seen in Figure 4.

![Graph of Effects of the Incubation Time in Relation to the DDT Biodegradation Rate by Lactobacillus sakei Strain PRO7 at Concentrations 10, 20 and 30 ppm.](image)

From Fig. 4 above, it can be seen that the highest concentration of DDT degradation is recorded on the Lactobacillus sakei Strain PRO7 biodegradation at 10 ppm is 96 hours at 4.77 ppm. The optimum value of DDT biodegradation rate in MM4 medium shows degradation of 90.8%. The amount of degradation concentration of DDT has increased quite high when seen from the incubation period of 72 hours to 96 hours.

In relation to biodegradation at 20 ppm, it can be seen that the highest degradation concentration is also available at 96 hours, that is 7.89 ppm. The optimum value of DDT biodegradation rate in MM4 medium showed degradation result amounting to 95.1%. The medium MSM + DDT with pH between 4-10 can degrade pH 5-8 and optimum pH 7 [14].

In contrast to Lactobacillus sakei Strain PRO7 biodegradation at 30 ppm, the Fig. 4 also shows that DDT degradation concentrations increases with a high enough amount at each incubation period and the highest degradation concentration is still high remaining at 96 hours of observation, namely 10.77. The optimum value of DDT biodegradation rate in MM4 medium showed degradation of 96.2%. From these three figures, it can be seen that the concentration of DDT degradation continues to increase every time when the incubation is observed. [15] explains that there is a link between time (0.6, 10, 14, 16, 20, 28 days) and the fermentation process (anaerobes and aerobes) in DDT, the longer the period is, the DDT, DDE and DDD is more degraded by different fermentation processes. The DDT...
concentrations decrease at the highest anaerobic conditions while the DDE and DDT are degrade at the highest aerobic conditions.

The DDT biodegradation of Lactobacillus sakei Strain PRO7 which was observed at 10, 20 and 30 ppm shows a considerable increase in the concentration of 30 ppm DDT degradation with a value of 10.77. The optimum value of DDT biodegradation rate in MM4 medium points out the degradation of 96.2% at 30 ppm concentration compared to concentration at 10 ppm and 20 ppm. The rate of DDT biodegradation of Lactobacillus sakei Strain PRO7 at 24 hours achieving 13.5-21.3%, at 72 hours gaining 23-31%, and at 96 hours getting 36-47%.

Some bacteria are able to convert DDT into DDD in the pure breeding cultures, such as Escherichia coli, Enterobacter aerogenes, E. cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. putida, Bacillus cereus species "Hydrogenomonas" and Saccharomyces cerevisiae, Phanerochaete chrysosporium, and Trichoderma viridae [16, 17, 18].

4. Conclusions
Aerob indigenous bacteria which is obtained from morphological characterization and DNA sequencing is named Lactobacillus sakei Strain PRO7. The biodegradation rate was carried out by Lactobacillus sakei Strain PRO7 bacteria using biodegradation rate of 7.89 and reached 95.1% biodegradation percentage at 20 ppm DDT concentration.

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