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Research Paper

Molecular Identification of Poly-3-hydroxulbutyrate-Producing Bacterial Isolates from White Sand Soil and Mud

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ABSTRACT: *Plastic is used as product packaging or wrapping items for easy carrying. The use of plastic is still very high, although it has begun to decline, replaced by carrying bags. Generally, plastic is used sparingly, so plastic waste accumulates. Plastic waste accumulates because it is not easily decomposed. It is because plastic raw materials are generally not easily decomposed naturally. An effort to overcome this is the production of biodegradable plastics. Biodegradable means can decompose biologically. This type of plastic is also called bioplastic. One of the primary materials used for bioplastics is Poly-3-hydroxybutyrate P(3HB). P(3HB) is a readily biodegradable compound. Certain bacteria under nutrient-deficient conditions can produce this compound. Therefore, this study was conducted to identify P(3HB) molecularly-producing bacteria isolates T.11 from sandy soil and T9.2 from mud. The genome DNA of both isolates was isolated and amplified using the 16S rRNA gene. Sequencing of amplification results was analyzed for homology and kinship with bacteria in the databank. The results showed that isolate T1.1 is a group of cereus bacteria and T9.2 is Achromobacter bacteria. The homology level of isolate T1.1 reached 100%, while the highest homology level of isolate T9.2 was 99.66%. Isolate T9.2 has the closest kinship with Achromobacter xylosoxidans.*

KEYWORDS: *Double Pendulum, Numerical Solution, Simulation, Behaviors of the System*

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I. INTRODUCTION

The percentage of plastic use in daily life is increasing. Plastic is a material for wrapping luggage and packaging a product. However, unused plastic will be wasted, where plastic raw materials are generally not easily biodegradable in nature[1]. Plastic waste will pollute the environment and disrupt the life mechanism of organisms. Finally, in 1992, researchers discovered a new innovation in plastic raw materials that are predicted to be able to decompose in nature. This innovation is termed bioplastic. Bioplastics are plastics derived from living things and can degrade well in soil and water. In 2015 the total production for bioplastics reached almost 1% of the total global plastic production \[2].

The visible impact of the use of plastics provides one appropriate solution to deal with the accumulation of plastic waste: making plastic materials from materials easily decomposed by microorganisms. Biodegradable plastics are made from natural polymer materials such as starch, glycerol, and chitosan. One of the biodegradable materials is Poly-3hydroxybutyrate P(3HB). This compound has 100% biodegradable properties within a particular time if discarded[3].

Poly-3-hydroxybutyrate or P(3HB) is a PHA widely found in microorganisms[4]. More than 300 species of bacteria have been reported to produce P(3HB)[5]. Although many bacterial species can produce P(3HB), the potential to discover and identify new species with superior and widespread production capabilities has yet to be discovered[4]. Bacterial identification studies have been conducted in West Sumatra province's forest soils and mountain peaks[6,7]. The production of P(3HB) by bacteria indicates that the living environment of bacteria is nutritionally deficient[4,8]. Therefore, this study aims to molecularly identify P(3HB) producing bacteria living in

nutrient-deficient environments such as sand and mud soil. Bacteria were isolated from white sand soil in the Gunung Sarik Kuranji area, Padang city, and mud soil of Batang Harau river, Padang city.

II. MATERIALS AND METHODS

Screening of P(3HB) bioplastic-producing bacteria using crude palm oil media. The primary materials of the study were bacterial isolates of Air Dingin limestone soil in Padang City and Batang Harau river mud in Padang City that have been tested to produce P(3HB) (data not shown). The research method is experimental-descriptive. Samples from sand soil were coded T1.1 and mud soil T9.2.

2.1 Genomic Isolation of Bacterial DNA and Amplification of 16S rRNA Gene

Bacterial cells from CPO-Bakto Agar media were cultured on specific mineral liquid media[8]. Then DNA was isolated using a Thermo scientific kit. The isolation results were measured using electrophoresis and viewed with a UV transilluminator.

Amplification of 16S rRNA gene using PCR technique in Thermocycler Mupid-Exu. The primers used are forward primer 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and reversal 1525R (5' AAG GAG GTG WTC CAR CC 3'). The composition of the PCR cocktail is Master Mix 25 uL, Primer 27F 2 uL, primer 1525R 2 uL, bacterial genomic DNA 2 uL, and nuclease-free water 19 uL. The PCR reaction program can be seen in Table 1. Visualization of PCR results using electrophoresis and viewing with a UV transilluminator.

Table 1. PCR Program

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Analysis

2.2 Sequencing Data Analysis

Sequencing data analysis was conducted using the sequencing method, according to Depson[9]. Sequencing data were analyzed using DNA star software to see peaks in the sequencing results. For sequence alignment analysis, it was done by comparing the sequences obtained (query) with those already in the Gene Bank with the NCBI database (http//www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) (Depson, 2012). Then the data were aligned using BioEdit 7.2 software (https://bioedit.software.informer.com/7.2/).

2.3 Phylogenetic Tree Analysis

Phylogenetic tree analysis was performed by the procedure of Mustopa[10]. The sequencing data obtained were analyzed using offline software (DNA Star) in FASTA format. The sequences that have been edited by equalizing the sequences read on the forward with the reverse. To determine the homology of the sequence, BLAST was performed. This analysis will process and align the sequences deposited in the database (NCBI) with the sequences analyzed. The percentage of homology will be calculated based on the similarity of bases compared with the assumption that the sequence with the highest percentage has a significant similarity with the sequence being analyzed. The phylogenetic tree will be constructed based on the BLAST results. This study used the neighbor-joining method to construct a phylogenetic tree.

III. RESULT AND DISCUSS

3.1 Genomic Isolation of Bacterial DNA and Amplification of 16S rRNA Gene

The 16s rRNA gene is a gene that has conserved regions, so it is used to determine taxonomy, phylogeny, and diversity between species. Amplifying the 16s rRNA gene in bacterial isolates was done to determine the type of species of bacterial isolates that had been tested previously (Figure 1.). The amplification results showed the band size, as estimated, around 1500 bp.

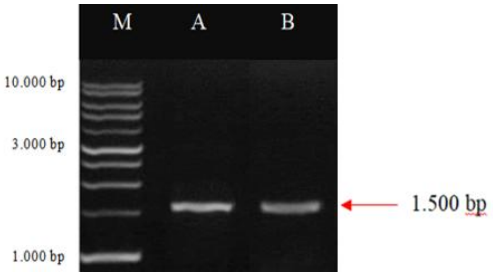

Figure 1. 16s rRNA gene amplification products of bacterial isolates A = T 1.1, B = T 9.2 (M = Marker)

3.2 Sequencing Analysis

PCR products that 16S rRNA gene primers have confirmed were sequenced to determine the exact size and sequence of the base. BLAST analysis shows the level of similarity between the sequences of bacterial isolates obtained with existing species data (Tables 2 and 3).

Table 2. BLAST of 16s rRNA isolat T 1.1

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MW530458100100.008.
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7.Bacillus cereus strain Cr22-MW530458100100.008.
Bacillus cereus strain Cr22-MW530458100100.008.
100100.008.Bacillus cereus strain OM02-MW888398100100.009
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Table 3. BLAST of 16s
rRNA isolat T 9.2
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Based on this theory, it can be seen that isolate T1.1 has a kinship at the 100% similarity level with Bacillus cereus. The most closely related strain is B31-MZ675431. The bootstraps value for phylogenetic analysis of isolate T1.1 with selected Bacillus cereus species is 100, which means that from all repetitions, the results are the same, so the analysis can be said to be precise on Bacillus cereus species (Figure 2).

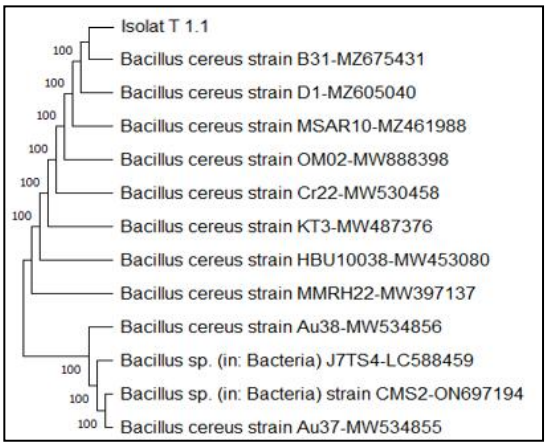

Figure 2. Phylogenetic tree of isolate T 1.1 with Bacillus cereus

Blast results show that sample T9.2 belongs to the group Achromobacter sp. the highest level of similarity is Achromobacter xylosoxidans. Figure 3. shows that the kinship distance of isolate T9.2 is indeed closest to Achromobacter xylosoxidans. The resulting bootstrap values varied. The highest bootstraps value was with the closest kinship distance, and the lowest was 54% with Achromobacter sp. strain BYT-3-MW674664.

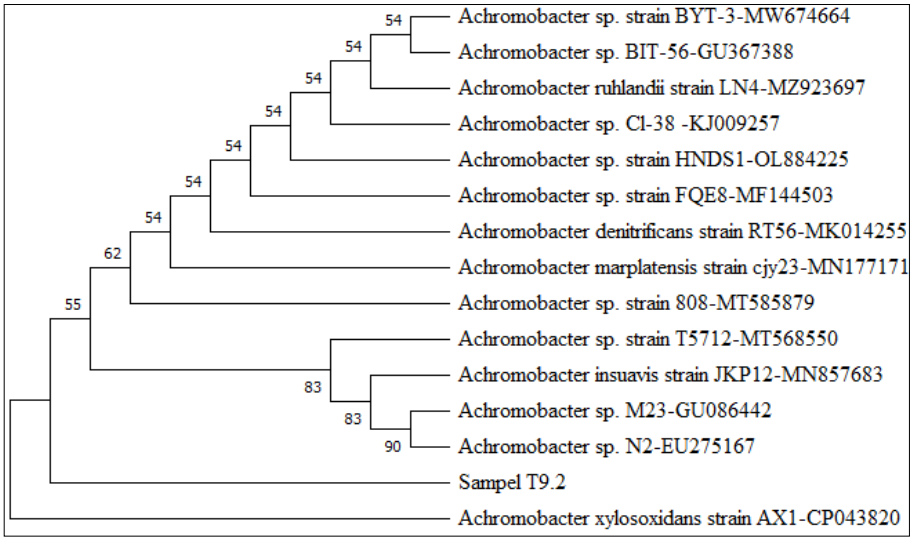

Figure 3. Phylogenetic tree of isolate T 9.2 with Achromobacter

IV. CONCLUSION

The results of molecular tests of bacterial characteristics of sand and sludge soil samples belong to the different bacterial genera. Isolate T1.1 from sand soil is Bacillus cereus species with a 100% homology level. At the same time, isolate T9.2 from mud soil is Achromobacter bacteria with the highest homology level of 99.66%, Achromobacter xylosoxidans. Both isolates are bacteria that can produce P(3HB) bioplastics.

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