ABSTRACT

The use of plastic is considered efficient because it can protect the product, resist impact, has good stretchpower, water resistant, and relatively cheap price. However mass plastic use can lead to serious environmental damage, because after use the plastic is discharged into the environment and not easily decomposed for a long time. Therefore, one effort that can be done is finding bacteria from nature that will be able to degrading the synthetic plastic. This research was conducted to isolate and characterize bacteria samples of the Jayawijaya Mountains soil that potentially able to degrading polystyrene plastics. Isolation of soil bacteria was carried out with the enrichment method and spread plate. Polystyrene plastic that has been made in thin film form, thencut to 1 cm x 1 cm, weighed, aseptically implanted on NA medium which was inoculated with bacterial isolates, and incubated for 4 weeks at 30 °C. After the incubation period, the plastic film was taken, cleaned, dried and weighed again. Reduction of the weight of the plastic film before and after incubation was calculated in percentages by weight / weight. From this experiment, one potential bacterial strain to degrading polystyrene plastic film was obtained up to 29% b / b, namely ITP isolate 10.1.1. Macroscopic, microscopic and biochemical characterization of isolates of ITP 10.1.1 indicated that this bacterium is a Gram positive with bacillus form. The surface profile of the plastic film after 4 weeks of observation was observed with Scanning Electron Microscope (SEM) showing the erosion and damage to the surface of the tested polystyrene plastic film.

Keywords: isolation, characterization, polystyrene-degrading bacteria, plastic, Jayawijaya, Papua

INTRODUCTION

The development of science and technology today, especially in the last two decades, shows the increasing number of synthetic polymers produced throughout the world annually. One synthetic polymer that is often known as artificial polymer is plastic1. The most widely circulated plastic material for food packaging is plastic with polyethylene and polystyrene ingredients2.

Increased consumption of plastic and improper handling of waste can be waste that can pollute the soil environment due to the nature of plastics that are not easily degraded naturally3. This plastic is single use plastic and then becomes trash4. Increasing environmental pollution and waste that cannot be renewed and degraded will encouraged more research and studies in the field of biosynthetics and biodegradation5. Many indigenous bacterial isolates have been reported to be able to degrade plastic. Indigenous plastic-degrading bacteria is plastic polymer-degrading bacteria from native habitats such as soil and landfills6. Previous studies have shown the potential of indigenous bacterial from soil and landfills to degrade plastic, one of which has found 11 bacterial isolates which indicate the degradation of Low Density Polyethylene (LDPE) plastic from soil at Padang City Final Processing Site (TPA)7.

In this paper we report the isolation and characterization of polystyrene plastic-degrading bacteria from soil samples from Jayawijaya Mountains, Papua. Sampling was carried out by one of our researchers namely Anthonia Y Pekey who was a native resident of Papua. The soil samples are interesting to study because the altitude of the area reaches 4,884 m above sea level and has a very cold temperature of around 10 °C. It is suspected that microorganisms from these soil samples can live and adapt to extreme conditions and have enzymes and more ability to maintain its life. Therefore, this study was conducted to isolate and characterize waste soil bacteria that can degrade plastic from the soil at Jayawijaya Mountains, Papua.

MATERIALS AND METHODS

Sample Collection

Samples were taken from 10 location within 2 kilometers area in Tembagapura, Jayawijaya Mountains, Papua, Indonesia. These samples were put into a plastic bag and stored in a refrigerator for preservation purposes.

Isolation of Soil Bacteria

Isolation of soil bacteria was done by enrichment and spread plate methods. As much as 10 g of soil sample was put into 90 ml medium nutrient broth, then incubated for 1 day in a rotary shaker incubator with 200 rpm rotation at 37 °C. Then multilevel dilution was performed using 0.85% NaCl up to 10⁹. The results were taken as 1 ml for surface spread plate on NA medium and incubated at 37 °C for 24 hours8,9.
Polystyrene Plastic Biodegradation Test

Prepared polystyrene plastic was cut for 1 cm x 1 cm, then thin plastic film was washed with 70% alcohol, rinsed with a distilled water, and put into oven at 80 °C until it reaches a constant weight, then the plastic was weighed. Bacterial isolates are inoculated into mineral medium. Then, the thin film of polystyrene plastic was inserted aseptically and incubated for 4 weeks at temperature 30°C. The final weight of the plastic after 4 weeks was weighed, then the weight reduction of the plastic film obtained was calculated by using the following formula:

\[ \% \text{Reduction in plastic weight} = \frac{R_1 - R_2}{R_1} \times 100\% \]

Annotation: \( R_1 \) = Initial weight of plastic films, \( R_2 \) = Plastic film weight after 4 weeks incubation

Overview of Scanning Electron Microscope of Plastic Film Surfaces

The polystyrene plastic film to be tested for biodegradation was observed using Scanning Electron Microscopy (SEM). This test was carried out to obtain a description of the plastic surface before the biodegradation test that would be compared with the surface image after incubation with bacteria for 4 weeks incubation.

Macroscopic, Microscopic Characterization and Biochemical Bacteria from Isolation Results

Isolation was done in NA medium then the shape, color, edge / side, texture and surface of bacterial colonies was observed. For microscopic observations was observed from Gram staining and biochemical tests.

<table>
<thead>
<tr>
<th>Polystyrene plastic film number</th>
<th>Initial weight of plastic film (g)</th>
<th>Weight of plastic film after 4 weeks (g)</th>
<th>Weight reduction potential (%b/b)</th>
<th>Average+SD Reduction weight (%b/b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.004</td>
<td>0.003</td>
<td>25</td>
<td>29.28±12.01</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>0.004</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.007</td>
<td>0.004</td>
<td>42.8571</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.004</td>
<td>0.003</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.004</td>
<td>0.004</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

RESULT AND DISCUSSION

The results of polystyrene plastic-degrading bacteriasiation from soil samples taken in the mountains of Jayawijaya Mountains, Papua was obtained 16 (sixteen) bacterial isolates using nutrient agar (NA) media. Sixteen bacterial isolates were purified by quadrant method (Fig. 1). Of the sixteen bacteria that have the potential to decompose the polystyrene plastic, the characterization process was carried out macroscopically, microscopically and the biochemical test that we reported in this article was bacterial isolate ITP 10.1.1.

Purified bacterial isolates were tested for biodegradability on plastic films made from polystyrene synthetic plastics. The test was carried out by incubating the polystyrene plastic in a medium containing Bacillus sp. ITP 10.1.1 for 4 weeks at 30°C. Decomposition potential was obtained by calculating the percentage difference in initial weight and final weight of the test plastic sample (weight loss percentage). The test results of the biodegradation of polystyrene plastic films using ITP 10.1.1 bacterial isolates are shown in Table 1.

The results of polystyrene plastic film biodegradation testing towards ITP 10.1.1 bacterial isolates showed that this bacterium has high potential to be further developed commercially as synthetic plastic-degrading bacteria. With its ability to degrade polystyrene plastic films up to 29.28% b / b for 4 weeks, it is extraordinary. This significant decrease in the percentage of polystyrene plastic weight showed that the activity of the depolymerase enzyme possessed by isolates from the soil at Jayawijaya Mountains, Papua were very strong. It was reported that each bacterium might produce the same depolymerase enzyme, but the enzyme strength could differ from one bacterium to another.

The mechanism of polymer biodegradation by the enzyme depolymerase usually begins with the process of abiotic degradation through photodegradation which can change the main chain group in the presence of carbonyl groups (C = O), so that carbon oxidation occurs in the polymer chain. Carbon oxidation produces low molecular weight functional groups such as ketones, carboxylic acids, and hydrocarbons. The functional group that is formed will cause the hydrocarbon polymer properties which are initially hydrophobic turned into hydrophilic, so that the polymer surface is hydrophilic and facilitates microorganisms (bacteria) to carry out the degradation process.

The next process is biotic degradation which is referred as biodegradation. Biodegradation is carried out by microorganisms, one of which is bacteria. The hydrophilic surface of the plastic will make it easier for bacteria to attach to the plastic surface and will colonize and release the depolymerase enzyme. Bacterial colonies attached to the surface of the plastic film will form biofilm. Then the bacteria will break down plastic complex polymers into simpler compounds (oligomers, dimers, and monomers) with the help of intracellular and extracellular depolymerase enzymes so that they are easily transported into bacterial cells as carbon and energy sources.

As shown in Figure 2, this is the result of the Scanning Electron Microscopy of the surface of a polystyrene plastic film after biodegradation testing using bacterial isolates obtained from the soil of Jayawijaya Mountains, Papua. This picture confirms that
the depolymerase enzyme from bacterial isolates tested has been able to break or erode the surface of the polystyrene polymer film tested. The presence of grooves, scrapes and cracks shows the appearance of brittleness on plastic film sheets on testing sheets with bacterial culture\textsuperscript{15}.

**CONCLUSION**

From this experiment, one potential bacterial strain was obtained which able to degrade polystyrene plastic film up to 29\% b/b, namely ITP isolate 10.1.1. Macroscopic, microscopic and biochemical test of isolates of ITP 10.1.1 indicated that this bacterium was a Gram positive with bacillus form. The surface profile of the plastic film after 4 weeks of observation was observed by SEM showing the erosion and damage to the surface of the polystyrene plastic film tested.

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