Screening Polyethylene Synthetic Plastic Degrading-Bacteria from Soil

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ABSTRACT

Screening polyethylene synthetic plastic degrading-bacteria from soil sample has been conducted. Soil samples that been used in this research taken from The Final Waste Process Area, Padang City, West Sumatra, Indonesia. The purpose of this research is to know bacterial isolates that have potential to degrading polyethylene synthetic plastic. Soil bacterial isolation has been done with serial dilution then inoculated with pour plate technique in Nutrient Agar medium. Results showed that there is 24 different bacterial isolated. From 24 bacterial isolated there is 11 isolates indicating degrading bacteria of polyethylene synthetic plastic. Bacterial isolates with code BTS-5 has highest potential in degrading polyethylene plastic (11.7% w/w) and BTS-9, BTS-12 has lowest potential (0.9% w/w).

Keywords: bacteria, polyethylene, degradation, screening

INTRODUCTION

These days plastic use is increasing in all kind of business, including small business until industrial scale use plastic as packaging materials, wrapping materials, sheathing materials, stationery materials, automotive industry, building tools etc. Use of plastic considered as efficient because can protect the products. Some excellence from plastic are: flexible, economic, not easy to crack, don’t get rotten, not easy to break, strong, can be combined in many color and form and some kind of plastic can be design to be heat resistant.

Plastic materials that most used for food wrapping is plastic with polyethylene basic material. Polyethylene plastic is one use plastic that can be wastes. Polyethylene plastic waste can’t be degraded in soil, so it can be big threat for life [1,2]. Increased consumption of plastic can become wastes and can contaminate the soil environment because plastic nature that not easy to degraded naturally [3]. One method to overcome environmental issues from plastic wastes is to use biological agent like bacteria, where the bacteria will breaking polymer bond from plastic into monomer chain and create environmental-friendly plastic.

One most potential source for degrading bacteria of polyethylene synthetic plastic is where in those place we can discover many wastes, both organic and inorganic waste such as plastic.

MATERIALS AND METHODS

Taking of Soil Samples
Soil sample taken with purposive sampling technique, soil taken must contained pile of food wrapping plastic wastes that already buried in soils. Sample taken put into plastic and measurement of temperature and soil pH done directly.
Soil Bacterial Isolation
Soil sample weighed until 20 g then diluted until 100 ml sterile distilled water in Erlenmeyer, then it was vortex until homogenous. Serial dilutions done until $10^{-7}$. The results taken 1 ml and put into Petri dish and poured into medium NA with pour plate technique. Then it’s incubated at room temperature for 24 hours.

Screening of Degrading Bacteria of Polyethylene Synthetic Plastic
Bacterial isolates inoculated into mineral medium added with polyethylene plastic polymer, then incubated in room temperature. From the results can be observed the formation of clear zone formed by bacteria. If there’s no clear zone but bacteria can grow in the medium, then it continued with biodegradation test of polyethylene plastic [5].

Biodegradation Test of Polyethylene Plastic
Bacterial isolates inoculated into mineral medium. Then, thin film of polyethylene plastic inserted aseptically, and incubated in room temperature for 4 weeks, then thin plastic film washed with alcohol 70%, then rinsed with sterile distilled water, and put into oven with $80^\circ C$ temperature until it reached constant weight. Plastic film final weight is weighed. Percentage of decreasing of plastic film weight counted with those formula [1]:

$$\text{% decrease of plastic weight} = \frac{R_1 - R_2}{R_1} \times 100\%$$

whereas:

$R_1$ = Initial Weight of Plastic Film  
$R_2$ = Final Weight of Plastic Film

Macroscopic and Microscopic Characterization
Isolates in NA medium then observed form, color, edge/side, texture and surface of bacteria colony. For microscopic observation for bacteria can be observed from gram coloration.

RESULTS AND DISCUSSION
Isolation of degrading bacteria of polyethylene synthetic plastic in The Final Wastes Process, Padang City’s soil with NA medium some bacteria isolates has been found. Bacteria obtained live in environment condition with soil pH about of 8.2, those are bacteria live in low alkali condition. This can be proved with the result that so many bacteria grew in this kind of medium. Bacteria grew in medium also influenced with mineral contents in soil. Condition of pH alkali in soil will affect different organisms diversity with acid soil.

Acid pH tend to has poor mineral contents, in contrast to alkali pH which rich in mineral contents. This condition will resulted in difference of organisms diversity. Bacteria obtained live in 28°C-33°C temperature. This temperature will also affected microorganisms growth in the soil. Variance in microorganisms population in soil caused by mineral contents in soil [4].

From results of bacteria isolation obtained has been done bacteria purification and obtained 24 different bacteria isolates. From this 24 bacteria isolates, 11 bacteria able to grew in mineral medium with polyethylene plastic polymer powder, whereas 13 bacteria isolates unable to grew in those medium. This showed that kind of bacteria utilizing plastic as carbon source, and this indicates that those bacteria can degrading polyethylene plastic. Polymer biodegradation process by microbe connected with microbe ability to adapt with new substrates [5].

Isolation of plastic-degrading bacteria from soil that marked with bacteria growth in medium added polyester polyethylene and those bacteria utilizing the only carbon and nitrogen source [6]. Those complex molecules broken into simpler component and will be used in metabolism to produces energy sources. This commonly available carbon source expected to be utilized by microorganism in limited condition [7,8].
As shown in Figure 1, it can be known that 11 bacteria isolates went through a test of polyethylene plastic degradation, showing a decrease in polyethylene plastic. The decrement of polyethylene plastic by bacteria showed different decrement results for each bacteria. The highest decrement of plastic film weight found in bacteria isolates BTS-5 with a decrement percentage of 11.7%, whereas the lowest decrement of film weight was shown by bacteria isolates BTS-9 and BTS-12 with a value of 0.9%. This condition is caused by the different activity of depolymerase enzyme for each bacteria isolate.

Plastic microbe degradation caused by enzyme activity turned to a chain splitting from polymer to oligomer and monomer that metabolism done by microbes cells. Depolymerization results caused by physical strength or biological that produced aerobic metabolism those are carbon dioxide and water whereas anaerobic metabolism produced carbon dioxide, water, and methane as the final product [6].

Each microbe has different characteristics, so the degradation ability possessed will be varied between one microbe with another. Microbe different characteristic includes type of enzyme produced for the biodegradation process that helped in polymer degradation [8].

The results for microscopic observation of degrading bacteria are shown in Figure 2.
Figure 2 showed that microscopic characteristic from 11 isolates of polyethylene plastic degrading-bacteria which observed under microscope with 1000 times magnification. From 11 bacteria isolates obtained 2 bacteria isolates classified as gram-negative group and showed bacil cell form. Whereas, 9 bacteria isolates are gram-positive group. Bacteria isolates BTS-18, BTS-9 has diplo bacil cell formation, BTS-1 has streptobasil cell formation, and BTS-2, BTS-12, BTS-15, BTS-16, BTS-22, BTS-5, BTS-14 has bacil cell formation.

Table 1. Characterization of Polyethylene Plastic Degrading-Bacteria Biochemical Testing

<table>
<thead>
<tr>
<th>Code of Bacteria</th>
<th>Agar Nutrient</th>
<th>Colony Coloration</th>
<th>Gram</th>
<th>TSA Gas</th>
<th>H2S</th>
<th>Ketone</th>
<th>Oxalacetic</th>
<th>Meglitt</th>
<th>Indol</th>
<th>Uracil</th>
<th>Citrate</th>
<th>Glucose</th>
<th>Salicyle</th>
<th>Mannitol</th>
<th>Methyl Red (MR)</th>
<th>Voges Proskauer (VP)</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTS-1</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-2</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-5</td>
<td>+</td>
<td>Greenish white bacil</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Pseudomonas sp.</td>
<td></td>
</tr>
<tr>
<td>BTS-7</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-9</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-12</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-14</td>
<td>+</td>
<td>Clear, bacil</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Klebsiella sp.</td>
<td></td>
</tr>
<tr>
<td>BTS-15</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.2</td>
<td></td>
</tr>
<tr>
<td>BTS-16</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-18</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
<tr>
<td>BTS-22</td>
<td>+</td>
<td>White, bacil</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Bacillus sp.1</td>
<td></td>
</tr>
</tbody>
</table>

To be able to decided the kind of polyethylene plastic degrading-bacteria, biochemistry test that consist several stage of reaction like what shown in Table 1 has been done. The results from those chemical reaction is four bacteria species that has been characterized, those are Bacillus sp.1, Bacillus sp.2, Pseudomonas sp., dan Klebsiella sp. (Table 1). Bacteria isolates BTS-1, BTS-2, BTS-7, BTS-9, BTS-12, BTS-16, BTS-18, BTS-22 showed bacteria Bacillus sp.1, isolates BTS-15 is Bacillus sp.2, isolates BTS-5 refer to Pseudomonas sp., and bacteria isolates BTS-14 refer to Klebsiella sp.

CONCLUSION

About 24 bacteria have been isolated from The Final Wastes Process Area, Padang City, West Sumatra, Indonesia, beyond that are 11 bacteria indicating polyethylene plastic degradation. Bacteria isolates with code BTS-5 has highest ability to degrading plastic, with 11.7% w/w, whereas lowest ability of bacteria isolates in degrading polyethylene plastic found in BTS-9, BTS-12 with value 0.9% w/w.

Acknowledgement

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REFERENCES


