Selection of Plastic Degradation Indigenous bacteria Isolated from Tamangapa Landfill Macassar City

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Abstract. Plastic is the most widely used polymers in all aspects of human life, including food packaging and pharmaco-cosmetics. The complex structures with long and repetitive chains make plastic difficult to be degraded naturally. Consequently, it will accumulate in landfills and the soil. One solution that can be done to overcome this problem is by biodegradation using microorganisms. This study aims to select bacterial isolates that are able to degrade plastics which are isolated from soil samples at Tamangapa landfill in Makassar City. Sampling of soil containing plastic waste used purposive sampling method at 5 different points at Tamangapa Landfill. Isolation of bacteria was conducted using Nutrient Agar (NA) which added 2% Polyethylene Glycol (PEG) and selection of degradation ability was carried out with liquid Mineral Salt media containing HDPE (High Density Polyethylene) and LDPE (Low Density Polyethylene) media. The degradation ability was observed based on the optical density value (OD) at a wavelength of 600 nm and loss of plastic dry weight for 14 days of incubation. The results of isolation and selection obtained 6 bacterial isolates that were able to degrade HDPE (High Density Polyethylene) and LDPE (Low Density Polyethylene) plastics. T7 isolates showed the highest degradation ability of 6% for 8% HDPE (High Density Polyethylene) plastic and LDPE (Low Density Polyethylene) compared to the other five bacteria isolates.

Introduction

The plastic could be classified as one of a complex polymer that has degraded for a long time period. The range of the period time because plastic has a long structure polymer that recurrent so it takes quite a lot times to cut the chains become a short chain molecule [1,2]. Plastic is one of the products that commonly used by the community as a packaging, household products up to the office equipment and public facilities. According to [3], the plastic needs from 220 million Indonesian peoples in 2003 approximately 1.35 million tons of waste while the ability of waste management by the government about 20% - 30% [3]. As increasing of the world population, the use of plastic materials has been up increasing. Increasing the number of request plastic it is because plastic has more advantages than the other material. The materials which are made from raw plastic generally lighter, is an insulator, and the manufacturing process is cheaper [4,5,6]. Increasing the consumption of plastic has a chance to waste and it can pollute the land. The characteristics of plastic that is not easily degraded as naturally will accumulate in waste ground and heaped up in the ground. It will affect the biological activity in the ground. A proscession which is done by the government limited only by accumulation in the landfill area [2,7]. One of the solutions is high on discussed is the biodegraded plastic by using microorganisms which is cheap and very environmentally friendly [2,8,9].
Biodegradation is a process where the microorganisms can be degraded or break up the nature polymer (such as lignin, cellulose) and synthetic polymer (such as polyethylene, polistrin) [1,8,10]. Every microorganism having characteristics of each in degrades polymer. According to [11] that microorganism such as fungi and bacteria include the main component from biosphere which is contributing in a break up the organic compounds. Microorganisms having endoenzime and eksoenzime which can degrade a substrate into the simpler component [4,6,12,13]. The components can be used as a carbon source and energy by microorganisms. Microorganisms degrade a polymer with a formation of biofilms on the surface of a polymer [8,10]. The research was conducted to selection bacteria from the Final Disposal Place of garbage which can be used for degrades the polymer of plastic. This research was conducted to select an indigenous bacterial degrading plastic which was isolated from the Tamangapa landfill Makassar city.

1. Materials and Methods

Collecting of Samples

Soil samples taken from TPA Tamangapa at 5 points which is contained plastic garbage with the purposive sampling method. The upper soil samples taken as much as 100 gram for every point use a trowel and then put into steril and labeled with the sterile ziplock. Then soil samples stored in container sterile with the temperature 4ºC and take into laboratories for analysis.

Isolation and Characterization of Bacteria

Isolation

As many as 1 mL of each samples dissolved use aquades sterile and diluted with the high-rise dilution. The last three of dilution planted in a media NA (Nutrient Agar) which is added by 2% of PEG (Polyethylene Glycol) with the pour plate method in order to test the ability of growing isolates in environments that are containing a basic source of some plastic. After that incubate at a temperature of 37ºC during 48 hours.

Purification

Purification performed on media NA (Nutrien Agar) which has been added 2% of PEG. Isolates bacteria grown in a media NA by the streak plate method. The treatment will be carried out until obtained the pure isolates. The pure isolates grown on MSM (Mineral Salt Medium) solid and liquid which is already added 2% of PEG then incubate at temperature of 37ºC for 48 hours.

Morphological Characterization of Colonies and Cells

The morphology of each single colony formed after purification was then characterized by a colonial morphology and Gram staining in the form of colonies, edge shape, color and colony surface.

Selection of Bacteria Which Can Degrades Plastic

Rejuvenation of isolates

Bacterial isolates tested for their ability to degrade rejuvenated plastics by inoculating one ose in NA medium for 24 hours at 37ºC. Isolates were replanted in NB medium and incubated for 24 hours at 37ºC and the density was 10^6 CFU.
Preparation of Inoculum

One mL of inoculum bacteria was inoculated into Mineral Salt Medium which is have been added by glucose and 2% of PEG (Polyethylene Glycol) then incubated at 37°C for 48 hours.

Selection in Liquid Media

100 mL of liquid Mineral Salt Media that has been made previously poured on an Erlenmeyer flask measuring 250 mL and added with 0.1% each powder HDPE (High Density Polyethylene) and plastic LDPE (Low Density Polyethylene), than sterilisation in autoclave. Aseptically, 10% bacterial inoculums were poured and then incubated at shaker incubator 150 rpm at room temperature for 14 days. Then growth measurements were carried out based on the value of OD (Optical Density) with a spectrophotometer at a wavelength of 600 nm and a percentage of the ability of plastic degradation. The formula of percentage for the lost of dry weight based on the following:

\[
\text{Percentage the lost of dry weight} = \frac{W_i - W_f}{W_i} \times 100\%
\]

Where:
- \(W_i\) = Dry weight before degradation (g)
- \(W_f\) = Dry weight after degradation (g)

2. Result and Discussion

Isolation of Bacteria

The results of isolation by using the NA (Nutrient Agar) media, which is adding 2% of PEG with the SPC methods obtained the number of colonies which 1,4 x 10^7 CFU. The colonies that grow obtained 10 isolates bacteria showing a difference based on the color, the edge, the form of colonies, and elevation next purified by using the same media with incubation as long as 48 hours. PEG (Polyethylene Glycol) is monomers of plastic used as a proxy for to know the ability of the bacteria use plastic. When the bacteria grow in a medium that is contained PEG it is assumed that the bacteria is capable to degrade plastic. The result of the observation of morphology colonies isolates bacteria shown in Table 1.

Table 1. Morphology of the colony and cell characterization

| Isolate | Colony Morphology | Cell Morphology |
|---------|------------------|----------------|
|         | Color | Edge | Form | Elevation | Form | Color | Gram | Endospora |
| T1      | pink   | entire | circular | flat | bacil | red | negative | - |
| T2      | white milk | entire | circular | raised | bacil | red | negative | - |
| T3      | beige | entire | circular | convex | cocci | red | negative | - |
| T4      | yellow | labote | irregular | raised | bacil | red | negative | - |
| T5      | beige | serrate | filamentous | convex | bacil | red | negative | - |
| T6      | white milk | entire | circular | flat | cocci | purple | positive | - |
| T7      | beige | entire | circular | convex | bacil | purple | positive | - |
| T8      | beige | entire | circular | flat | cocci | purple | positive | - |
| T9      | beige | entire | circular | flat | bacil | purple | positive | - |
| T10     | beige | entire | circular | flat | bacil | purple | positive | - |
Based on the observation of morphology colony by using medium NA shows that 10 isolates have some similarities. The isolates of T3 and T7, which is the same in color of the colony is beige, the edge of the colony is entire, the form of a circular, and the elevation of the colony is convex. In common morphology colonies are also same as for isolating T8, T9 and T10, which is the color is beige, the edge of the colony is entire, the form of a circular, and the elevation of the colony is flat. The Isolates of T1 colony is pink, the edge of the colony entire, the form of a circular, and the elevation of the colony flat. Isolates T2 and T6 there are similarities on the color of the colony, milky white, the edge of the colony is entire, but isolates T2 have a circular elevation which is raised and on isolated T6 which is flat. Isolates of T4 colony have the yellow color, the edge of the colony labote, the form of the colony irregular, and the elevation is raised. The Isolates of T5 colony have a beige color, the edge of the colony serrate, the form of the colony is filamentous, and elevation of the colony is convex. The difference in the form of growth shows that isolates on medium NA are different bacteria. In addition to the observation of macroscopic, is also done on microscopic observation of morphology, cell to find out the cell shape and gram staining to distinguish bacteria into gram positive and gram negative. Isolates of gram positive bacteria characterized by the purple color and isolates gram-negative bacteria characterized with the red color, in endospores staining all isolates showed negative results.

The result of observation to 10 isolates of bacteria which is can degrades can be obtained into 7 isolates (T1, T2, T4, T5, T7, T9, T10) were rod, while 3 isolates (T3, T6, T8) were a coccus or round. The 10 isolates bacteria observed showed that is 5 isolates the bacteria is gram-positive bacteria and 5 isolates bacteria is gram-negative bacteria. Gram negative and gram positive is a clarification of bacteria which is distinguished from the physical characteristics of these bacteria. The fundamental difference is in peptidoglycan contained in the cell walls of the two bacteria. In gram-positive bacteria the peptidoglycan layer is thicker, whereas in gram-negative the peptidoglycan layer is thinner. Gram-positive bacteria will maintain dark purple after washing with alcohol, while gram-negative bacteria do not [1,14].

Selection of Bacteria Which Is Can Degrades Plastic

The results of 10 isolates bacteria planted in the media MSM (Mineral Salt Medium) which is added 2% of PEG (Polyethylene Glycol) showed only 6 isolates bacteria which is showing the growth optimal, thera are T1, T6, T7, T8, T9, and T10 that will be used for the next testing. From 6 isolates bacteria obtained, then measured the values of OD (Optical Density) from observation day 1 until day 14 on the type of plastic HDPE (High Density Polyethylene) and plastic LDPE (Low Density Polyethylene). The measurement result of OD (Optical Density) shown in Figure 1.

A type of plastic HDPE (High Density Polyethylene) obtained isolates T6 and T9 shows the growth maximum on day 6, isolates bacteria T7, T8, T9 shows the growth maximum on day 4. A type of plastic LDPE (Low Density Polyethylene) obtained isolates bacteria T6 shows the maximum growth on the day 8, isolates bacteria T7 show isolates maximum bacteria growth on the day 8, isolates bacteria growth T8 shows isolates maximum bacteria swirling caused by the media formed flok, which causes bacterial growth is not optimal, isolates bacteria T9 and T10 shows whether the bacterial isolates the maximum occurs on the day 6 but in isolates bacteria T1 does not showed the growth of bacterial.
According to [1] normal bacterial growth in media containing plastic polymers and some elements of nitrogen can indicate that bacteria are able to use carbon elements from polymers to meet their carbon requirements in their metabolic processes and microorganisms will secrete a catalytic enzyme that can damage the structure of the polymer [1, 15].

**The percentage ability of plastic degradation**

The percentage the ability of degradation of the 2 types of plastics isolates bacteria on HDPE (*High Density Polyethylene*) and plastic LDPE (*Low Density Polyethylene*) showed in Table 3.

**Table 2.** The results of testing the ability degradation a plastic polymer of HDPE and LDPE

| Isolate | HDPE (High Density Polyethylene) | LDPE (Low Density Polyethylene) |
|---------|---------------------------------|---------------------------------|
|         | Dry weight (Gram) | Difference (Gram) | Degradation (%) | Dry weight (Gram) | Difference (Gram) | Degradation (%) |
| T1      | 1 | 1 | 0 | 0% | 1 | 1 | 0 | 0% |
| T6      | 1 | 0.94 | 0.06 | 6% | 1 | 0.96 | 0.04 | 4% |
| T7      | 1 | 0.92 | 0.06 | 6% | 1 | 0.94 | 0.08 | 8% |
| T8      | 1 | 0.98 | 0.02 | 2% | 1 | 0.99 | 0.01 | 1% |
| T9      | 1 | 0.94 | 0.06 | 6% | 1 | 0.98 | 0.02 | 2% |
| T10     | 1 | 0.96 | 0.04 | 4% | 1 | 0.98 | 0.02 | 2% |

The result of observation, a type of plastic HDPE (*High Density Polyethylene*) have found that isolate bacteria T7 having obtained isolates the percentage ability largest degradation of 6%, then isolates T6 and T9 bacteria having the percentage ability degradation of 8%, bacteria and isolates T10 having the percentage ability degradation of 4% and isolates bacteria T8 having 2% the percentage ability of degradation, isolates bacteria T1 have not experienced a decrease a dry weight about 0% and a kind of plastic LDPE, bacteria T7 having 6% obtained the largest of percentage degradation ability, then isolates bacteria T6 having 4% the percentage ability degradation, and isolates T9, T10 having the percentage of 2%, isolates T8 having 1% the percentage degradation ability, the isolate of bacteria T1 show 0% and it has not experienced a decrease a dry weight.
There are several factors influencing on the environmental degradation, such as the temperature, pH, the chemical structure of plastic, the availability of oxygen, and supply the different nutrients. This is a significant effect on the population and activity of microorganisms in degrades a plastic polymer [4,16,17]. The mechanism of biodegradation begins with abiotic degradation, namely photodegradation which converts major chain groups in the presence of carbonyl groups (C = O), resulting in carbon oxidation in the polyethylene polymer chain [6,18]. This carbon oxidation produces low molecular weight functional groups such as ketones, carboxylic acids, and hydrocarbons [6]. The functional group formed will cause the hydrophobic polymers that are initially hydrophobic to become hydrophilic, so that the surface of the polymer can absorb water and facilitate microorganisms (bacteria) to process degradation [11,12,19]. The next process is biotic degradation or commonly referred to as biodgradation. Biodegradation is carried out by microorganisms, called bacteria. The hydrophilic plastic surface will make it easier for bacteria to attach to the plastic surface and will colonize [6]. The bacterial colonies that attach to the plastic surface will form biofilms [11,20,21], then bacteria break down plastic complex polymers into simpler compounds (oligomers, dimers and monomers) with the help of intracellular and extracellular depolymerase enzymes so that the compounds are easily transported into bacterial cells as a source of carbon and energy [9,22]. In this study showed that the highest ability of plastic degradation was shown by T7 isolates of 8% for LDPE plastic and 6% for HDPE for 14 days of incubation.

Several previous studies have also reported the ability of the bacterium Staphylococcus sp, Pseudomonas sp, Bacillus sp and Ochrobactrum sp, isolated from the soil capable of degrading some plastics [2,23,24,25]. The result of the plastic degradation mechanism will produce CO2, H2O, CH4 and other products [1,24].

3. Conclusions

The results of selection of bacteria in TPA (Garbage Disposal) Tamangapa landfill obtained 6 isolates, the bacteria which have the capability to degrade plastic. From 6 isolates bacteria obtained that isolates bacteria T10 shows the best growth in the type of plastic HDPE (High Density Polyethylene) and LDPE (Low Density Polyethylene) in the selection of the media solidly. Isolates bacteria T7 which have the most percentage of ability degradation on the greatest type of plastic HDPE (High Density Polyethylene) as much as 6% and plastic LDPE (Low Density Polyethylene) as much as 8%.

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