Exploration Of Plastic-Degrading Bacteria From Marina Beach, Semarang, Central Java

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Abstract

Plastic waste has threatens the environment and affect to the economic and tourism sectors, marine life, coastal ecosystems and human health. World Wide Fund for Nature (WWF) states that 85% of waste in the oceans is plastic. The Ministry of Environment and Forestry also noted that Indonesia experienced an increase in plastic waste from 14% in 2013 to 16% in 2016. By 2020 the volume of plastic waste in Indonesia predicted to reach 67.8 million tons. Plastic waste takes 100-500 years to completely decompose. An alternative solution is to involve microorganisms to decompose plastic polymers. However, plastic waste reducing bacteria isolated from coastal ecosystem has not been much explored. In this study, an exploration of natural bacteria that degrades plastic waste from coastal ecosystems is carried out. Plastic samples were collected from the Marina Beach Semarang, Central Java. Plastic samples were taken from a depth of 0-10 cm in three coastal ecosystems: coastal sand sediments, rocks and mangroves. Samples then isolated and screened to obtain bacteria that have the potential to degrade polyethylene. Selected bacteria were identified by biochemical physiology according to the method of Cappuccino and Sherman and classified to genus level according to Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology. *The results showed that three genera of bacteria had high polyethylene degradation potential with the speed of degradation: Enterobacteriaceae 0.0091%; Moraxella spp. 0.0066%; and Pseudomonas spp. 0.0076% per week.*

Keywords: *plastic waste, polyethylene, microorganisms, degradation.*

Introduction

An increase in the demand for plastic occurs every year with the use of polyethylene type plastic an average of 12% per year, 85% of waste in the oceans is plastic (WWF, 2018). The Ministry of Environment and Forestry also noted that Indonesia experienced an increase in plastic waste from 14% in 2013 to 16% in 2016. By 2020 the volume of plastic waste in Indonesia predicted to reach 67.8 million tons (KLHK, 2020).

Plastic is a long chain of hydrocarbon polymer that is bonded to each other and has the character of being lightweight, waterproof, durable and practical (Gnanavel *et al*., 2012). One type of plastic that is commonly used is polyethylene (PE). PE is a hydrocarbon polymer consisting of long chains of ethylene monomers formed through a polymerization process. This type of plastic is difficult to decompose naturally because it has stable C-C and C-H covalent bonds. The addition of antioxidants and stabilizers as well as high and dense molecular weight makes PE difficult to degrade (Usha *et al*., 2011).

The increasing of plastic waste on land is a threat in the waters. Plastic carried by river currents will accumulate in the sea and can harm marine ecosystems (Fleming *et al*., 2014). Law and Thompson (2014) stated that almost 10% of the total plastic will be dumped into rivers and ends up in the sea. In other words, plastic waste will continue to increase until it accumulates over a long period of time. Although plastic waste can be decomposed by

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by direct sunlight, hydrolysis, oxidation and abrasion, its non-destructive nature makes plastic take 100-500 years to completely decomposed (Law and Thompson, 2014).

Generally, the way to deal with plastic waste in the environment is done by burning. The combustion process is considered less effective and dangerous because the plastic can release toxic gases such as CO_2 and CO, giving rise to new problems in the environment, alternative solution offered is a biological method by involving microorganisms. The use of microorganisms to overcome plastic waste can avoid the creation of toxic at the final products (Lu *et al*., 2011). Several species of microorganisms capable of reducing or degrading plastic polymers that have been isolated from waste and compost disposal soil (Yoon *et al*., 2012), namely *Bacillus sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Streptomyces sp.*, *Shewanella*, *Moritella*, *Psychrobacter* and the fungus *Fusarium solani*, *Aspergillus sp.*, *Aureobasidium pullulans*, and *Curvularia senegalensis* (Usha *et al*., 2011; Pramila and Ramesh, 2011; Sivan, 2011). However, plastic waste reducing bacteria isolated from coastal ecosystem in Marina Beach has not been much explored. In this study, an exploration of natural bacteria that degrades plastic waste from coastal ecosystems is carried out. Exploration was carried out at Marina Beach Semarang because Marina is the only beach that is officially managed as a tourist spot in Semarang. Furthermore, the population of Semarang City was relatively high, 1.7 million people and the presence of several rivers that directly lead to the sea contributed to the high pollution of plastic waste in the coastal area of Marina Beach (BPS, 2019; Sibero *et al*., 2020).

Materials and Methods

Plastic samples were collected from a pile of sand (6°57'03.6"S 110°23'25.6"E), rock (6°56'49.1"S 110°23'28.0"E) and mud (6°57'35.0"S 110°23'12.0"E) from Marina Beach, in a depth of 0-10 cm by random sampling method, then soaked in 0.9% physiological salt (Asadi *et al*., 2019). The liquid from plastic and physiological salt mixture were taken 0.1 ml then diluted by serial dilution (10-2–10-6). After that, the dilution was spread on selective growth medium (2% NA; 2% PE powder; 0.1% Tween 80; 50% sterile seawater; 50% aquadest) and incubated for 48 h at 37ºC (Mukamto *et al*., 2015). Furthermore, bacterial isolates were purified, then colony and cell morphology were identified (Leboffe and Pierce, 2011; Usaha *et al*., 2011). After obtained pure cultures, polyethylene degrading bacteria were screened on selective medium added with 0.02% Congo Red dye, then incubated for 24-48 h at 37ºC (Mukamto *et al.,* 2015). The diameter of the clear zone around the colony was observed and measured (Usha *et al*., 2011).

Three isolates had the largest clear zone diameter were identified by biochemical physiology according to the method of Cappuccino and Sherman in Madigan *et al*. (2015) and classified to genus level according to Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology. Three strain of bacteria were cultured in MSM medium ($MgSO_4$ 0.2 $g.L^{-1}$; CaCl 0.02 $g.L^{-1}$; KH_2PO_4 1 $g.L^{-1}$; K_2HPO_4 1 $g.L^{-1}$; NH_4NO_3 1 $g.L^{-1}$; $FeCl_3$ 0.05 $g.L^{-1}$) contained polyethylene, and the growth was measured with spectrophotometric at 600 nm every 24 h (Aziz *et al.,* 2019). Absorbance results were visualized with a growth graph.

Sterilized 5x3 cm plastic pieces which had been dried in an oven at 40ºC for 24 h were weighed as the initial dry weight (Ainiyah and Shovitri, 2014). Plastic pieces were inoculated in MSM medium and three pure bacterial isolates were cultured. Every 2 weeks of 3 months incubation, the plastic was taken to measure the dry weight. Then, the percentage of plastic weight loss was calculated using this formula % weight loss = dry weight (initial) – dry weight (final) / dry weight (final) x 100%

Results and Discussion

Based on the observations that have been made, each bacteria isolated from coastal sand, rock and mangrove ecosystems has a diversity of colony morphology and the number of types of bacterial isolates. Each ecosystem found variations in the number and appearance of different morphology, among others: mangrove ecosystem there are 7 isolates with color appearance to diverse colony forms, coastal sand ecosystem 4 isolates and rock 6 isolates where the two ecosystems have a color appearance to almost similar shape (Table 1.).

Furthermore, the isolates were screened to confirm degradation ability of polyethylene in minimum mineral medium containing polyethylene and Congo Red Eight of the twelve isolates formed a bright zone around the colony (MDP1; MDP2; MDP5; MDP6; MDP7; MDP9; MDP10 and MDP12) (Figure 1.) with an average light zone diameter of 0.6–2.0 cm (Table 2.). Three of the bacterial isolates had the largest bright zone diameter, namely MDP9 2.0 cm; MDP12 1.9 cm; MDP6 1.7 cm. The isolate is thought to have the ability to utilize polyethylene as the main carbon source.

The number of bacterial isolates in the mangrove ecosystem is higher than sandy and rocky sedimentary ecosystems because the mangrove ecosystem contains a lot of nutrients and carbon sources that come from the decay of other organic materials such as mangrove leaf fall (Andrianto *et al*, 2015). In addition, according to Simanjuntak (2018), muddy sediment conditions can accumulate organic matter carried by water flows. However, there were only 3 bacterial isolates with polyethylene degrading activity in the mangrove ecosystem. It is suspected that bacteria prefer to use organic carbon sources that are abundantly available and easily digestible compared to using carbon sources from plastic to survive and reproduce. Meanwhile, the ecosystem with organic material and carbon sources is relatively less because the ecosystem is an open area and is directly affected by the waves causing organic material to be carried along with the waves and sea water (Rani, 2017). Although few bacterial isolates were found in sand and rock ecosystems, these bacteria had a greater potential to degrade plastics than mangrove ecosystem isolates (Table 2.). Furthermore, the results of this bacterial exploration were coded Microbial Degradation of Polyethylene (MDP).

Three bacteria which formed the largest bright zone then grew in Mineral Salt Minimum (MSM) with the addition of polyethylene powder to measure the growth. Optical density (OD) was analyzed by UV-Vis spectrophotometer and showed relatively significant growth at a certain incubation time. It was suspected that the three bacterial isolates could utilize polyethylene powder maximally as the main carbon source for their survival during the incubation time (72 h for MDP6 and MDP12 isolates and 96 h for MDP9 isolates).

Table 1. Colony morphology of twelve isolates that were isolated from three points in the Marina Beach Area in selective medium

Isolates	Location			Size	Shape	Edge	Elevation	Pigmentation
	Sand	Rock	Mangrove					
MDP1	√			point	circular	smooth	convex	transparent white
MDP2		√	√	small	circular	slick	signage	white milk
MDP3			√	small	circular	slick	droplets	beige transparent
MDP4			√	small	circular	slick	droplets	yellow
MDP5		√		being	circular	smooth	convex	milky white
MDP6	√	√		small	circular	smooth	convex	dark beige transparent
MDP7		√		dots	irregular	wavy	embossed	white-beige transparent
MDP8			√	small	circular	smooth	convex	orange
MDP9	√	√		medium	circular	wavy	flat	dark beige transparent
MDP10			√	small	circular	slick	droplets	beige transparent
MDP11			√	small	circular	slick	signage	white-yellow
MDP12	√	√	√	small	circular	slippery	slimy	beige-brown

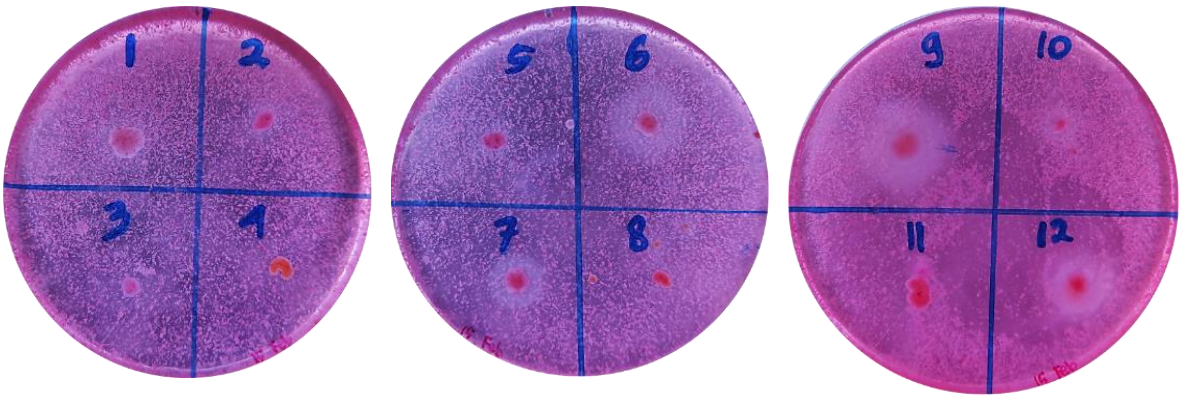

Figure 1. Confirmation test for polyethylene degradation using the clear zone method on 8 competent isolates.

The third growth measurement graph of the polyethylene degrading bacteria show that all isolates were unable to grow and develop on growth media without carbon content (Figure 2a.), while significant growth was shown on growth media with polyethylene addition (Figure 2b.). This proves that the three isolates are able to use polyethylene as a source of carbon for survival. This statement is also supported by Prabhat *et al.* (2013) in their research that polyethylene degrading bacteria grown in media containing only mineral salts without a carbon source will be in a choked condition, so when polyethylene is added as a carbon source then the bacteria will adhere and form a biofilm on the polyethylene surface and use it as energy.

Bacteria have several mechanisms in utilizing polyethylene as a source of carbon: first the bacteria adhere to the surface of the polyethylene and form a biofilm. Biofilm is a natural strategy of polyethylene degrading bacteria to form a defense in unfavorable environments (Shemesh *et al.,* 2011). It is thought to be strong in causing the polyethylene powder to appear to coalesce to form clumps, thus affecting the measurement to be unreadable (Figure 2a.). The second mechanism is depolymerization by breaking C–C bonds into short chains so that the compound can dissolve in water and pass through a semi-permeable membrane. In this mechanism, bacteria utilize polyethylene that has been synthesized into a simple form as a source of carbon and energy (biodegradation stage). The third mechanism is assimilation and mineralization (Andreas *et al.,* 2017).

The three characteristics of polyethylene degrading bacteria used a method of physiology biochemical known to resemble the genus *Pseudomonas*, *Enterobacteriaceae*, and *Moraxella*. Raziyafathima *et al.* (2016) and Urbanek *et al.* (2017) stated that the genus *Pseudomonas*, *Enterobacteriaceae* and *Moraxella* are capable of degrading plastic polymers and are widely found in aquatic areas.

Table 2. Clear zone test on bacterial isolates from the Marina Beach area which has the ability to degrade plastic

Isolate	Test Results (+/-)	Zone diameter (cm)	Zone rating
MDP1	+	1	5
MDP2	+	0.6	6
MDP3	-	-	-
MDP4	-	-	-
MDP5	+	0.8	7
MDP6	+	1.7	3
MDP7	+	1.2	4
MDP8	-	-	-
MDP9	+	2	1
MDP10	+	0.8	8
MDP11	-	-	-
MDP12	+	1.9	2

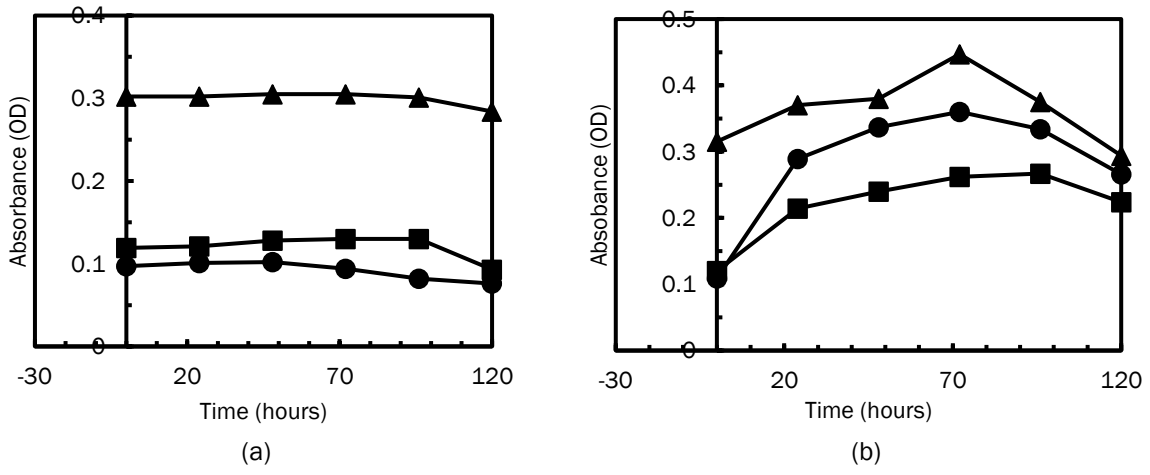

Figure 2. Growth of polyethylene degrading bacteria MDP6 (triangle), MDP9 (box) and MDP12 (circle) a) without the addition of polyethylene powder b) addition of polyethylene powder

Table 3. Characterization of bacterial isolates with the greatest ability to degrade polyethylene

Characteristics of	Bacterial Isolates		
	MDP6	MDP9	MDP12
The colony morphology			
Shape	Circular	Irregular	circular
Edge	Slick	Wavy	Slick
Elevation	Droplets	Flat	Droplets
Optical	Transparent	Transparent	Opaque
Pigmentation	Creamy	Beige	Beige-brown
clear zone diameter (cm)	1.70	2.0	1.90
Cell Morphology			
Shape	Cocci	Basil	Basil
Cell chain	Monococcus	Diplobasil	Monobasil
Gram	Negative	Negative	Negative
Size (µm)	1	2.5	2
Test Physiology Biochemistry			
Catalase	Positive	Negative	Positive
oxidase	Positive	Negative	Positive
Needs O_2	Aerobic	Aerobic	Aerobic
KOH Yarn Forming	Positive	Positive	Positive
Glucose Fermentation	Negative	Positive	Negative
Hydrolysis of fats	Positive	Positive	Positive
Bacteria Isolate Location			
Soil samples	Sand & rock	Sand & rock	Sand, rock, mangrove soil
Possible genus	*Moraxella spp.*	*Enterobacteriaceae*	*Pseudomonas spp.*

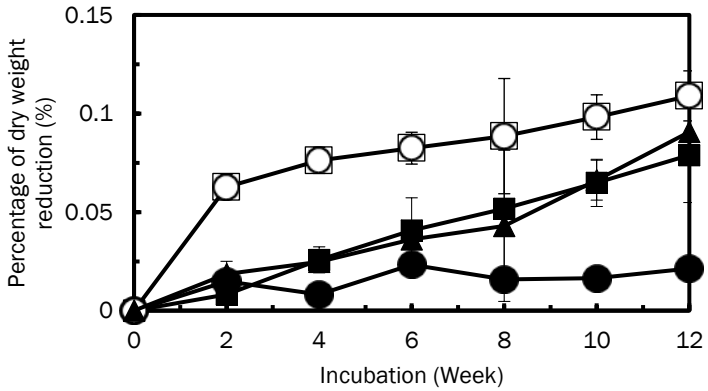

Figure 3. Plastic dry weight reduction by polyethylene degrading bacteria MDP6 (square), MDP9 (white circle), MDP12 (triangle) and negative control (black circle)

The biodegradation process is generally influenced by the interaction of enzymes secreted by microorganisms in the form of enzymes that catalyze hydrolysis reactions and non-enzymatic molecules derived from the environment or from microorganisms that can damage the structure of the polymer. In a study conducted by Efendi (2016) stated that most microorganisms, especially bacteria and fungi are known to produce alkane hydroxylase (AHs) which is a key enzyme involved in the aerobic degradation of alkanes by bacteria. AHs form key enzymes in the alkane hydroxylase system pathway that are involved in PE degradation in the beta oxidation pathway and are known to degrade linear alkanes. The most important enzyme in the alkane hydroxylase system is monooxygenase. The biodegradation process involves the hydroxylation of C-C bonds to release primary or secondary alcohols that are oxidized to ketones or aldehydes and subsequently to hydrophilic carboxylic acids. Microbial oxidation in reducing the number of carbonyl groups is a result of the formation of carboxylic acids. The products of microbial oxidation carried out through this beta oxidation pathway and tricarboxylate cycle can then be absorbed by microbial cells (Bhardwaj *et al*., 2012; Ameen *et al*., 2015; Sowmya *et al*., 2015; Skariyachan, 2015). Polyethylene degrading bacteria, especially

Pseudomonas, *Enterobacteriaceae* and *Moraxella* can be found scattered in aquatic and terrestrial environments with varying pH, temperature, oxygen content and mineral nutrients. These bacteria were also found in several studies by Urbanek *et al.* (2018) and Usha *et al.* (2011); which were successfully isolated from seawater, final waste disposal sediments, mangrove sediments and roots.

There was a significant reduction in dry weight of plastic sheets as much as 0.0789% for MDP6; 0.1089% for MDP9; 0.0906% for MDP12 while the control showed a reduction of dry weight of 0.0214% after 0-12 weeks incubation. The reduction in dry weight of plastics caused by the addition of bacterial isolates MDP6, MDP9 and MDP12 can occur presumably due to the communication between bacterial cells, specifically polyethylene degrading bacterial cells called quorum-sensing. Waters & Basler (2015) stated that bacterial cells build appropriate interactions with other bacterial cells by involving signals in the form of chemical molecules as autoinducers. This process allows bacterial cells to monitor the environment for other bacteria so that they can change the number of bacterial cells on a population and community scale. The reduction in dry weight that occurred in the control was likely due to non -maximal sterilization treatment, and the process of implanting the plastic into a less aseptic medium.

Conclusion

Based on a series of exploration and identification of polyethylene-degrading bacteria from Marina Beach, three of the eight isolates obtained the highest average polyethylene degradation rate, namely MDP6 0.0066%; MDP9 0.0091%; and MDP12 0.0076% per week. This isolate was thought to have similarities with the genus *Moraxella spp., Enterobacteriaceae,* and *Pseudomonas spp.*

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