INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ISOLATION AND CHARACTERIZATION OF POLYSTYRENE-DEGRADING BACTERIA *Bacillus* sp. ITP 10.1.1 FROM SOIL SAMPLE OF JAYAWIJAYA MOUNTAINS, PAPUA, INDONESIA

Rustini Ruslan [1], Muhammad Iqbal [1], Anthonia Y Pekey [1], Asiska Permata Dewi [2] and Akmal Djamaan [1,3]*
[1]Department of Pharmaceutical Chemistry, University of Andalas, Padang, Indonesia
[2]Department of Pharmacy, Faculty of Medicine and Publich Health, University of Abdurrab, Pekanbaru, Indonesia
[3]Laboratory of Biotechnology Biotan Sumatra, AndalasUniversity, Padang, Indonesia
*Corresponding Author Email: akmaldjamaan@phar.unand.ac.id

Article Received on: 05/09/18 Approved for publication: 22/10/18

DOI: 10.7897/2230-8407.0910231

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 todegrading 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 plastic[1]. The most widely circulated plastic material for food packaging is plastic with polyethylene and polystyrene ingredients[2].

Increased consumption of plastic and improper handling of waste can be waste can pollute the soil environment due to the nature of plastics that are not easily degraded naturally[3]. This plastic is single use plastic and then becomes trash[4]. Increasing environmental pollution and waste that cannot be renewed and degraded will encouraged more research and studies in the field of biosynthetics and biodegradation[5]. 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 landfills[6]. 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 Polyetylene (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 atJayawijaya 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 10^{-9}. The results were taken as 1 ml for surface spread plate on NA medium and incubated at 37 °C for 24 hours[8,9].

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 distilledwater, 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[7]:

$$\% \text{ Reduction in plastic weight} = \frac{R1-R2}{R1} X\ 100\%$$

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[7].

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[10].

RESULT AND DISCUSSION

The results of polystyrene plastic-degrading bacteriaisolation 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.

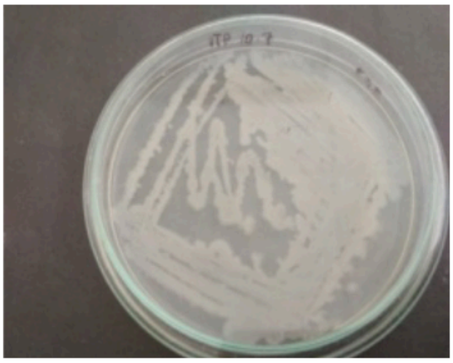


Figure 1: Examples of bacterial isolates from the soil of Jayawijaya Mountains, Papua

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.

Table 1: Result of polystyrene plastic biodegradation test using *Bacillus* sp. ITP 10.1.1 isolates

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

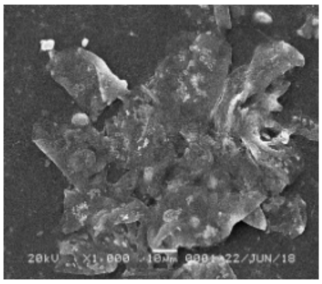
The results of polystyrene plastic film biodegradation testing towards ITP 10.1.1 bacterial isolates showed that this bacterium has high potential to be further developed commercially as synthetic plastic-degrading bacteria. With its ability to degrade polystyrene plastic films up to 29.28% b / b for 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[4,7].

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[11]. Carbon oxidation produces low molecular weight functional groups such as ketones, carboxylic acids, and hydrocarbons[12]. 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[13,14].

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 depolymeraseenzyme [15]. Bacterial colonies attached to the surface of the plastic film will form biofilm[15]. 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[16].

As shown in Figure 2, this is the result of the Scaning 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[15].

(a)



(b)

Figure 2: Scanning electron microscopy image of polystyrene plastic film surface, (a) before biodegradation test, (b) after biodegradation test using bacterial isolates obtained from the soil of Jayawijaya Mountains, Papua in Agar Nutrient medium

Furthermore, the macroscopic, microscopic and biochemical characteristics of bacterial isolates of ITP 10.1.1 are presented in Table 2. These bacterial isolates belong to the group of Gram-positive bacteria and has cell forms such as stems or bacillus (Fig. 3). The differences possessed by each bacterial colony are characteristic of a particular species. The shape and color of the colony, is the colony shiny or not, smooth or rough surface are the characteristics needed for species identification. Most bacteria have a whitish color, gray, yellowish, to clear but, in some species have a bolder color pigment[17,18].

Table 2: Characteristics of *Bacillus* sp. ITP 10.1.1 isolate obtained from the soil of Jayawijaya Mountains, Papua in Agar Nutrient medium

Observation	Result
Macroscopic	
Colony form	Round
Colony coloration	White
Edge	Smooth
Elevation	Flat
Surface	Smooth
Microscopic	
Gram Staining	+
Cell form	Basil
Size (length)	0,4 μm
Size (width)	0,1 μm
Endospore coloration	-
Biochemistry Test	
Motility Test	Motile
Catalase Test	+
Nutrient Agar Test	+
Aerob/Anaerob Test	Aerob
TSIA Test	+
H2S Test	-
Oxidase Test	-
Indole Test	-
Urea Test	+
Citrate Test	-
Lactose Test	-
Glucose Test	-
Sucrose Test	-
Mannitol Test	-
Methyl Red Test	+
VP Test	+
Oxidase Fermentation Test	-
Arabinose Test	-
Xylose Test	-
Nitrate Test	+
Gelatin Test	+

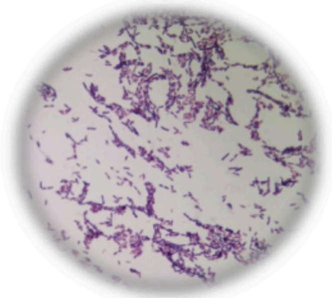


Figure 3: Microscopic profile ITP 10.1.1 bacterial isolate after Gram staining

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.

AKNOWLEDGMENT

The author would like to say special thanks to The Rector University of Andalas, Padang, Indonesia for his support this research under Basic Research Grant, BOPTN, Fiscal Year 2018, with Contract Number: 29/UN.16.17/PP.RD/LPPM/2018.

REFERENCES

1. Shimao M. Biodegradation of plastics. *Curr. Opinion Biotechnol*. 2001;12:242-247.
2. Rivai H, Asia A, Rina W, Alen Y, Handayani D, Aldi Y, Marlina, and Djamaan A. Isolation of endophytic bacteria from bark, leaf, and pericarp of Mangosteen (*Garcinia mangostana* L.) and testing of the antimicrobial activity. *Res. J. Pharm. Biol. Chem. Sci,* 2016; 7(1): 1910-1920.
3. Thompson R C, Swan S H, Moore C J, vom Saal F. Ourplastic age. *Philosophical Transactions of the Royal Society BiologicalSciences*. 2009;364: 1973-1976.
4. Akmal D, Asiska P D, Wangi Q A, Rivai H, Agustien A. Biosynthesis of copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) from palm oil and n-pentanol in a10L bioreactor. *Rasayan J. Chem*, 2015; 8 (3): 389-395.

5. Lee M K, Gimore D F, Huss M J. Fungal degradation of the bioplastic PHB (Poly-3-hydroxy-butyric acid). *J. Polym. Environ,* 2005;13(3):213-219.
6. Djamaan A, Rustini R, Andini P, Lalfari R S, Dewi A P, Suci R P. Biosynthesis of poly(3-hydroxybutyrate) from crude palm oil by using *Bacillus* sp. UAAC 21501. *Int. Res. J. Pharm*, 2018; 9 (9): 95-104.
7. Agustien A, Jannah M,Djamaan A. Screening polyethylene synthetic plastic degrading-bacteria from soil.*Der Pharmacia Lett.* 2016;8(7):183-187.
8. Djamaan A, Marjoni M R and Ismed F, The influence of pretreatment time, type and the concentration of yeast on ethanol production from rice straw. *Res. J. Pharm. Biol. and Chem. Sci,* 2015; 6(3): 583-591.
9. Sayuti I, Siregar Y I, Amin B, Agustien A, Djamaan A. Identification of bacterial hydrocarbonoclastic in waste tanks, Petapahan, Riau, Indonesia, using 16sr RNA, *J. Pure Appl. Microbiol*, 2017; 12(2), 671-677.
10. Zam S I, Samsuardi, Agustien A, Jannah M, Aldi Y, Djamaan A. Isolation, characterization of endophytic bacteria from *Citrus aurantifolia* Swingle leaves and testing of antifungal activity towards *Fusarium oxysporum*. *Der Pharmacia Lett*, 2016; 8 (11): 83-89.
11. Leja K, Lewandowicz G. Polymer biodegradation and biodegradable polymers: a review. *Polish J. Environ. Study*, 2009;19(2):225-226.
12. Chiellini E, Corti A, and D'Antone S. Oxobiodegradable full carbon backbone polymers biodegradation behaviour of thermallyoxidized polyethylene in aqueous medium. *Polym. Degrad. Stab.* 2007;92:1378-1383.
13. Gilan I, Hadar Y, Sivan A, Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *J. Appl. Microbiol. Biotechnol*, 2005;65:97-104.
14. Hadad D, Geresh S, Sivan A. Biodegradationof polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol*, 2005;98:1093-1100.
15. Artasastra M A, Yanwirasti, Djamaan A, Cytotoxic activity screening of ethyl acetate fungal extract derived from the marine sponge *Neopetrosia chaliniformis* AR-01. *J. Appl. Pharm. Sci,* 2017;7(12): 174-178.
16. Das M P and Kumar S. Influence of cell surface hydrophobicity incolonization and biofilm formation on LDPE biodegradation. *J. Pharm. Pharmaceut. Sci*, 2013;5(4):690-694.
17. Mohan, S K, Srivastava T. Microbial Deterioration and Degradation of PolymericMaterials. *J. Biochem Tech.* 2010;2(4):210-215.
18. Octavianda F T, Asri M T, Lisdiana L. Potential of oxo-degradable polyethylene-degrading bacteria of Benowo Landfill soil Surabaya. *Lentera Bio*. 2016;5(1):32–35.

Cite this article as:

Rustini Ruslan *et al*. Isolation and characterization of polystyrene-degrading bacteria *Bacillus* sp. ITP 10.1.1 from soil sample of Jayawijaya mountains, Papua, Indonesia. Int. Res. J. Pharm. 2018;9(10):85-88 http://dx.doi.org/10.7897/2230-8407.0910231

Source of support: University of Andalas, Padang, Indonesia, Conflict of interest: None Declared