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

Research Article

CHARACTERIZATION OF *Bacillus* sp. ITP 10.2.1 AS DEGRADING-BACTERIA OF POLYETHYLENE TEREPHTHALATE (PET) SYNTHETIC PLASTIC

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

Article Received on: 25/10/18 Approved for publication: 22/11/18

DOI: 10.7897/2230-8407.0911258

ABSTRACT

The negative effects of the use of synthetic plastics lately have been very worrying, such as serious environmental damage because plastics are difficult to decompose over a long period of time. Therefore, one effort that can be done is to find the bacteria from nature that are able to degrade the synthetic plastic. In this paper we report macroscopic, microscopic and biochemical characterization of the bacterium *Bacillus* sp. ITP.10.2.1 as a new bacterial from soil isolate which capable of degrading the synthetic polyethylene terephthalate (PET) plastics. In this paper, 1 (one) bacterial strain has the potential to degrading polyethylene terephthalate plastic films up to 4.77 b/b for 4 weeks, namely ITP 10.2.1 isolates. Macroscopic characterization of bacteria ITP 10.2.1; white coloration of the colonies, round shape, flat edges, smooth surfaces and raised elevations. Microscopic characterization, these bacteria has bacil form and belong to the Gram Negative group. Biochemically the bacteria ITP 10.2.1 is aerobic, the TSIA test: red/yellow, does not produce H_2S gas, catalase test: positive, oxidase test: negative, motility test: positive, indole test: negative, urea test: citric positive: positive, lactose: negative, glucose: positive, sucrose: positive, mannitol: positive, methyl-red: negative, fermentation oxidation: negative, Voges-Proskauer: negative, xylose: positive, arabinose: negative, nitrate: positive, gelatin: positive. The characterization results showed that the bacterial isolate ITP 10.2.1 was included in the *Bacillus* sp. group.

Keywords: characterization, degrading-bacteria, synthetic plastic, Jayawijaya, Papua

INTRODUCTION

Every year more than 260 million tons of plastic are produced in various countries. The development of science and technology recently, especially in the past two decades, shows the increasing number of synthetic polymers known as plastics which produced worldwide each year. One of the synthetic polymers often known as artificial polymers is plastic[1]. The most widely distributed plastic material for food packaging is plastic with polystyrene and polyethylene terephthalate[2].

Increased consumption of plastic and improper handling of waste can pollute the soil environment due to the nature of plastics that are not easily degraded naturally[3]. This plastic is a type of plastic that is used once and then becomes trash[4]. Increased environmental pollution and waste that cannot be renewed and degraded thus encourages 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 bacteria degrading plastic is a plastic polymers-degrading bacteria originating from original habitats such as land and landfills[6]. Several previous studies have proven the potential of indigenous bacteria from land and landfills[7].

In this paper we report the isolation and characterization of polyethylene terephthalate plastic-degrading bacteria from soil samples from Jayawijaya Mountains, Papua, Indonesia. Sampling was carried out by one of our researchers (Anthonia Y Pekey) who is a native of Papua. This soil samples were very interesting to investigated because the altitude of the area reached 4,884 m above sea level and had a very cold temperature of around 10 °C. It was suspected that microorganisms from the soil samples could live and adapt to extreme conditions will having more enzymes and ability to maintain its life. Therefore, this study was conducted to isolate and characterize the synthetic plastic-degrading soil bacteria from the Jayawijaya Mountains, Papua.

MATERIALS AND METHODS

Samples collection

Samples was collected from 10 location points within 2 kilometers in one area in Tembagapura, Jayawijaya Mountains, Papua, Indonesia. The sample then put in a plastic bag and stored in the refrigerator for preservation purposes before analysis.

Isolation of PET degrading-bacteria from soil sample

The process of soil sample bacteria was done by *Enrichment* and *Spread plate* methods. As much as 10 g of soil sample was put into 90 ml *Nutrient Broth* medium, then incubated for 1 day in *rotary shaker incubator* with 200 rpm at 37°C. Then serial dilution was done using NaCl 0.85% until 10^{-9}. From the results were taken 1 ml for surface spread plate on NA medium and incubated at 37 °C for 24 hours[8,9].

Test Biodegradation Capability of PET film Plastics by separate bacterial isolates

Plastic films were made by dissolving them using xylem organic solvents and poured into Petri dishes and allowed to dry (solvent casting methods). The plastic polyethylene terephthalate then cut into a size of 1 cm x 1 cm. Then the plastic film was washed with 70% alcohol, then rinsed with sterile distilled water, dried until

the constant weight by put it into an oven with a temperature of 80 °C then the plastic was weighed as the initial weight. Bacterial isolates were inoculated into mineral medium. Then, thin films of polyethylene terephthalate plastic were added aseptically and incubated for 4 weeks in 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]:

$$\% \text{ Plastic weight reduction} = \frac{R1-R2}{R1} X\ 100\%$$

Annotation : R_1 = Initial weight of plastic film, R_2 = Plastic film weight after 4 weeks experiment

Overview of Scanning Electron Microscopy of PET plastic film surfaces

The polyethylene terephthalate plastic film which was tested for biodegradation was carried out using Scanning Electron Microscope (SEM). This test was carried out to obtain a plastic surface image before and after testing with bacteria during ITP 10.2.1 for 4 weeks[7].

Macroscopic, Microscopic and Biochemical Characterization of Isolated Bacteria

Isolates in NA medium were observed for shape, color, edge/side, texture and surface of bacterial colonies. For microscopic observations for bacteria was observed from Gram staining and biochemical tests[10].

RESULTS AND DISCUSSION

In this study, sampling was carried out in ten different locations within a radius of approximately 5 km² in the area of Tembaga Pura, Jayawijaya Mountains, Papua, Indonesia. In the initial stage, the isolation of the degrading bacteria from polyethylene terephthalate from soil samples obtained was successfully isolated and purified by 16 (sixteen) bacterial isolates. The soil samples were previously grown in nutrient agar (NA) as shown in Figure 1. Sixteen of these bacterial isolates were purified by quadrant methods. Of the sixteen bacteria that could potentially degrading the polyethylene terephthalate plastic, the characterization process was carried out macroscopically, microscopically, biochemical tests and genetics, what we report in this article is bacterial isolate ITP 10.2.1.

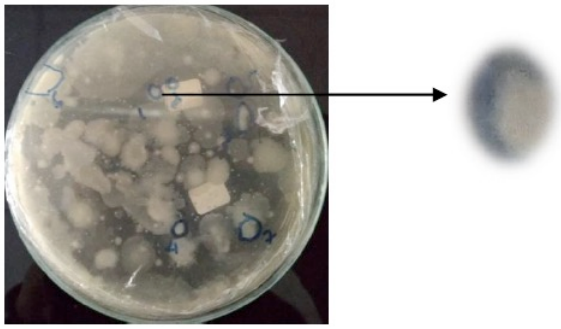


Figure 1: Examples of soil bacteria that have been isolated from the Jayawijaya mountain, Papua which have the ability to degrading PET synthetic plastic

In the next stage, purified bacterial isolates were tested for the biodegradation ability of plastic films made from synthetic PET plastic. The test was carried out by incubating the PET plastic film in a medium containing *Bacillus* sp. ITP 10.2.1 for 4 weeks at 30 °C. The potential or degrading ability of bacteria was determined by calculating the percentage of the initial weight difference and the final weight of the test sample (weight loss percentage). The results of biodegradation testing of polyethylene terephthalate plastic films using bacterial isolates ITP 10.2.1 is shown in Table 1.

Table 1: The result of in-vitro biodegradation of polyethylene terephthalate plastic film using *Bacillus* sp. ITP 10.2.1

Number of PET plastic film tested	Initial weight of PET plastic film (g)	Weight of PET plastic film after 4 weeks (g)	Reduction of PET plastic film weight (%b/b)	Average±SD Weight reduction (%b/b)
1	0.025	0.024	4.00	4.77±1.461
2	0.031	0.029	6.45	
3	0.026	0.025	3.84	

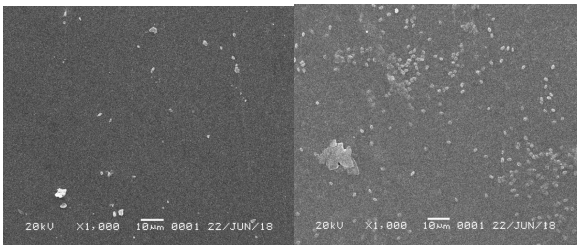
Table 1 presents the results of biodegradation test of polyethylene terephthalate plastic film against bacterial isolates ITP 10.2.1. Information obtained were that this bacterium has the potential to be further developed as a commercially synthetic plastic-degrading bacteria in industrial rank. With its ability to decompose polyethylene terephthalate plastic film to reach 4.77% b/b over a period of 4 weeks, it can be said a significant capability. The significant decrease in the weight percentage of polyethylene terephthalate plastic showed depolymerase enzyme activity possessed by isolates from Jayawijaya Mountain soil, Papua, were relatively strong. It is reported that each bacterium may produce the same depolymerase enzyme but the strength of its enzymes can differ from one bacterium to another[4,7].

The mechanism of polymers biodegradation by depolymerase enzymes usually starts with an abiotic degradation process through photodegradation which can alter the main chain groups 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 formed will cause the hydrophobic polymer properties which are initially hydrophobic to become hydrophilic, so the polymer surface is hydrophilic and makes it easier for microorganisms (bacteria) to do degradation process [13,14].

According to the American Society for Testing Materials, the process of plastic biodegradation can be carried out by microorganisms, such as bacteria, fungi and algae by water hydrolysis process[4]. A hydrophilic plastic surface will make it easier for bacteria attachment and colonization also to release depolymerase enzymes[15]. Theoretically, bacterial colonies that attached to the plastic films surface will form biofilms[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 these compounds are easily transported into bacterial cells as a source of carbon and energy[16].

The Scanning Electron Microscope (SEM) profile of the surface of polyethylene terephthalate plastic film before and after biodegradation test using bacterial isolate ITP 10.2.1 is shown in Figure 2. This figure confirms that the tested depolymerase enzyme from bacterial isolates has been able to break or erode the

surface the polyethylene terephthalate polymer film tested. The presence of grooves, scrapings and cracks shows the occurrence of damage to sheets of plastic film in sheet testing with bacterial culture[15].



(a) **(b)**

Figure 2: *Scanning Electron Microscope* (SEM) profile of the surface of PET plastic, (a) before biodegradation test, (b) after biodegradation test in NA medium which inoculated by ITP 10.2.1 bacteria.

The Scanning Electron Microscope (SEM) profile of the surface of polyethylene terephthalate plastic film before and after biodegradation testing using bacterial isolate ITP 10.2.1 is shown in Figure 2. This figure confirms that the tested depolymerase enzyme from bacterial isolates has been able to break or erode the surface the polyethylene terephthalate polymer film tested. The presence of grooves, scrapings and cracks shows the occurrence of damage to sheets of plastic film in sheet testing with bacterial isolated in this study.

Table 2: Macroscopic, Microscopic and Biochemistry Test Characteristics of *Bacillus* sp. ITP 10.2.1 isolate used in this study.

Observation	Result
Macroscopic	
Colony form	Round
Colony coloration	White
Edge	Smooth
Elevation	Flat
Surface	Smooth
Microscopic	
Gram Stain	+
Cell form	Bacil
Size (length)	0,4 µm
Size (width)	0,1 µm
Endospore coloration	-
Biochemistry Test	
Motility Test	Motil
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	+
Voges-Proskauer Test	-
Oxydase Fermentation Test	-
Arabinose Test	-
Xylose Test	+
Nitrate Test	+
Gelatine Test	+

In Table 2, the macroscopic, microscopic characteristics and biochemical tests of ITP 10.2.1 bacterial isolates is showed. It was found that these bacterial isolates belonged to the Gram positive group and has stems or bacil cell forms (Figure 3). The differences possessed by each bacterial colony are characteristic of a particular species. It is known that the differences of each colonies of microbes are characteristic of a particular species. The form of colonies, coloration, is the surface shiny or not, smooth or rough are the characteristics needed for the identification of a species. Most bacteria have whitish, gray, yellowish, to clear colors but, in some species, have more pronounced color pigments[7,17,18].

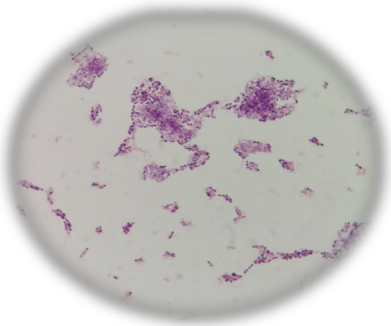


Figure 3: The observation result of bacteria under microscope was enlarged 1,000 times where the result of bacterial staining was purple which was confirmed to be gram positive bacteria in the sample with ITP code 10.2.1.

Gram positive bacteria are the dominant group of bacteria in nature. The dominance of Gram positive bacteria was caused by the structure of the bacterial cell wall of this group which contains a lot of peptidoglycan. Peptidoglycan causes Gram positive bacteria to be more resistant to environmental physical factors, for example extreme temperatures, so Gram positive bacteria are more able to survive and abundant in the environment compared to Gram negative bacteria. Previous researchers also reported that the *Bacillus* group is the genus that can mostly degrade plastic waste from the environment[7]. They found several strains of *Bacillus* which can degrade polystyrene and polypropylene synthetic plastic from the location of the garbage disposal site in Padang city, West Sumatra, Indonesia.

Generally, this research is able to characterize the bacteria that able to degrade the PET synthetic plastic with in-vitro condition. The further research that we are currently doing is the genetic characterization of the bacteria. Characterization of bacterial isolate by 16S rRNA method was done at the Laboratory of Biomedic, Faculty of Medicine, University of Andalas, Indonesia. The base order was checked and edited using the Bioedit Sequence Alignment Editor (*http://www.mbio.ncsu.edu /BioEdit/bioedit.html*). Equality analysis was done by using the Basic Local Alignment Tool at the National Center for Biotechnology Information (*http://www.ncbi.nlm.nih.gov*). While the evolution analysis was done by using ClustalW2 Phylogenetic Tree (*www.ebi.ac.uk*).

So, with the known character of bacterial isolates that have the potential to decompose this synthetic plastic, this bacterium can be further developed, we are in an up-scale process of degradation in a bioreactor using liquid media. It is hoped that in the end this bacterium can be commercialized as a synthetic plastic degrading agent, especially PET at the industrial level. Thus household and industrial plastic waste is expected to be processed into organic materials using degrading bacteria before being discharged into the environment.

CONCLUSION

In this paper one strain of bacteria that has the potential to degrading polyethylene terephthalate (PET) plastic films to reach 4.77% b/b, i.e. ITP 10.2.1 isolates were isolated from soil samples of Jayawijaya Mountain, Papua, Indonesia. Macroscopic,

microscopic characterization and biochemical test of ITP 10.2.1 isolates indicated that these bacteria is classified into Gram positive bacteria with bacill forms. The surface profile of plastic film after 4 weeks of observation which was observed by Scanning Electron Microscopy (SEM) showed the erosion and damage to the surface of the tested polyethylene terephthalate plastic film. This bacterium has the potential to be used for processing plastic waste in the future to reduce environmental damage.

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: 07/UN.16.10/DPPKM/FFARMASI/ 2018.

REFERENCES

1. Shimao M. Biodegradation of plastics. *Current Opinion Biotechnology*. 2001;12:242-247.
2. Rivai H, Asia A, Rina W, Alen Y, Handayani D, Aldi Y, Marlina, 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 Biological Sciences*. 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, Huss M J. Fungal degradation of the bioplastik PHB (Poly-3-hydroxybutyric acid. *J. Polym. Environ,* 2005;13(3):213-219.
6. Majid M I A, Akmal D, Few L L, Agustien A, Toh M S, Samian M R, Najimudin N Azizan M N. Production of poly(3-hydroxybutyrate) and its copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by *Erwinia* sp. USMI-20. *Int. J. Biol. Macromol*, 1999; 25: 95-104.
7. Agustien A, Jannah M, Djamaan A. Screening polyethylene synthetic plastic degrading-bacteria from soil. *Der Pharmacia Lettre*. 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 16S rRNA, *J. Pure Appl. Microbiol*, 2017; 12(2): 671-677.
10. Zam S I, Samsyuardi, 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*. *J. Der Pharmachia Lettre*, 2016; 8 (11): 83-89.
11. Leja K and 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 thermally oxidized polyethylene in aqueous medium. *Polym. Degrad. Stab*. 2007;92:1378-1383.
13. Gilan I, Hadar Y, Sivan A, 2004. 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. Biodegradation of 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 Neopetrosiachaliniformis AR-01. *J. Appl. Pharmaceut. Sci*, 2017; 7(12): 174-178.
16. Das M P, Kumar S. Influence of cell surface hydrophobicity in colonization and biofilm formation on LDPE biodegradation. *J. Pharm. Pharmaceutic. Sci*. 2013;5(4):690-694.
17. Mohan S K, Srivastava T. Microbial deterioration and degradation of polymeric materials. *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*. Characterization of *Bacillus sp*. ITP 10.2.1 as degrading-bacteria of polyethylene terephthalate (PET) synthetic plastic. Int. Res. J. Pharm. 2018;9(11):56-59 http://dx.doi.org/10.7897/2230-8407.0911258

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