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ISOLATES OF POLYPROPYLENE-DEGRADING BACTERIA FROM A LANDFILL

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Abstract

Polypropylene (PP) is a synthetic, heat-resistant, plastic. This quality has made it the second most commonly used in everyday life, including in disposable medical face masks. While the COVID-19 pandemic has led to an escalation of the face masks use in the last two years, their abundant waste, which is difficult to decompose naturally, can have a negative impact on the environment and human health. The purpose of this study was to obtain isolates of PP decomposing bacteria from the landfill in Ngipik, Gresik. The isolates were obtained from soil samples in the landfill. Bacterial isolates were tested for their ability to degrade PP during the 28 days of the liquid phase. The data obtained were bacterial growth data and PP degradation. There were three isolates that had the potential to decompose PP, namely NG 1, NG 2, and NG 3. It was concluded that the NG2 bacterial isolate is able to decompose PP by 0.47%. Based on the results of 16S sRNA, these three tended to be in the genus of Bacillus. The results of this study are expected to enrich the data of potential local bacteria to decompose PP obtained from Gresik landfill areas.

Keywords: *bacteria; biodegradation; COVID-19; medical face mask; polypropylene*

1. Introduction

Plastic is a synthetic polymer that is widely used for daily needs in industrial, medical, and household environments. World plastic production continues to increase every year. Until 2015, there were around 6300 metric tons (Mt) of plastic waste worldwide, most of which was accumulated in landfills or natural environments including waters (Geyer et al., 2017). The COVID-19 pandemic caused by the SARS Cov-2 virus has been responsible for the increase of plastic production of Personal Protective Equipment (PPE) and disposable face masks, both used by health workers and the public as single-use plastics. This indicates that more than eight million tonnes of pandemic-related plastic waste has been generated globally (Peng et al., 2021). Plastic waste such as disposable face mask components can consists of materials of polypropylene (PP), polyurethane (PU), polyacrylonitrile (PAN), polystyrene (PS), polycarbonate (PC), polyethylene (PE), and polyethylene terephthalate (PET) (Aragaw, 2020).

The world is facing accumulation of microplastics, and plastic waste of this form can

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harm not only the environment but also human health (Shams et al., 2021). Microplastics are plastic particles with a small size of less than 5 mm. Microplastics appear from domestic and industrial activities such as exfoliating facial scrubs, textiles/clothing, and toothpaste. In addition, they can occur from greater damage under ultraviolet (UV) radiation or mechanical abrasion (Auta et al., 2018). Disposable face masks were identified as the source of microplastics during the pandemic. There was an dramatical increase of single-use masks production, which means there is an urgent need to implement a waste management system now (Liang et al., 2022).

In Indonesia, processing of medical waste from the pandemic has been carried out through re-crystallization or recycling. However, when the waste has released microplastics into natural environments, the particles become barely visible and roam the environment to last a long time. The fact that plastics and microplastics are difficult to degrade and last a long time in the environment has drawn huge concern from the world. Their presence in the environment will endanger the health of the environment, biota, and humans. Microplastic exposure to humans through ingestion, inhalation, and skin contact can cause toxicity through oxidative stress, inflammatory lesions, and increased absorption or translocation that can cause metabolic disorders, neurotoxicity, and cancer risk (Rahman et al., 2021).

A previous study before the pandemic in Gresik Regency of East Java has found abundant microplastics in the waters in Banyuurip Subdistrict to be around 57.11 x 10^2 particles/m^3 (Ayuningtyas, 2019). However, the possibility of microplastics surge in the sub district may happen during the pandemic. Microplastic particles can be released from surgical masks into the environment as many as 1136 –2343 particles per day with a particle size of <5 mm (Liang et al., 2022). Medical masks contain a plastic material called polypropylene. A disposable surgical mask consists of 3 layers of which the outermost layer is spunbond polypropylene while the middle layer meltblown polypropylene fabric (Spenneman, 2021).

Succeeded in illustrating a 5-year change in the structure of polypropylene in a landfill, as being degraded on which surface microorganism cells lived (Potrykus et al., 2021). Microplastic particles' surfaces can become new niches for certain bacteria to colonize on. By taking bacterial isolates from plastic-contaminated environments, several studies have revealed the ability of bacteria to decompose plastic. Bacteria known to have the ability to decompose plastics include *Brevibacillus* sp. and *Aneurinibacillus* sp. isolated from sewage treatment plants and landfills (Skariyachan et al., 2018), *Bacillus* sp. from soil contaminated with plastic waste (Kavitha & Bhuvaneswari, 2021), *Bacillus* sp. and *Rhodococcus* sp. from mangrove sediments (Auta et al., 2018).

However, research on bacterial degrading ability on plastic is facing a challenge. Rapid and efficient degradation of plastic depend on bacterial isolates. Luckily, isolation and identification of potential microbes can be made possible by looking directly to the locations contaminated with plastic (Maity et al., 2021). In Gresik Regency, there are several Final Disposal Sites (FDS) that have the potential to be a location for searching for plastic decomposing bacteria. This study aims to obtain local isolates of polypropylene decomposer bacteria from Gresik FDS, determine their growth and degradation ability on polypropylene, and identify the best bacteria. This is meant to be one of the basic research that can help solve environmental problems which have indirect impacts on human health.

2. Method

Determination of location points and sampling

Sampling was carried out at the largest FDS in Gresik Regency. The location of the landfill is in Ngipik, Gresik Regency, East Java with coordinates 7°08'58.9"S 112°37'58.1"E. Sampling was carried out in April 2022 with an air temperature of 33°C and humidity of 80%. Five soil points were randomly chosen from the entire FDS area. Soil samples about 1-5 cm depth from the point surface were dug out in as much as 100 g for each point. These soil samples full of waste were taken from passive cells consisting of inorganic waste in the form of soil that has been buried or mixed with plastic waste. The composited soil was brought to the laboratory for further testing.

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Material preparation

The plastic used in this study is polypropylene (PP) in the form of plastic pellets obtained from the plastic production industry in East Java, Indonesia.

Isolation of potential bacteria

Soil samples from each sampling point were composited. A total of 25 g of sample was put into 225 mL of physiological saline solution and then homogenized in an orbital shaker at 120 rpm for 30 minutes. 1 mL of the suspension was poured into a petri dish and grown on nutrient agar for 24-48 hours in an incubator at 30°C. Bacterial isolates that grew were characterized macroscopically, then isolates that were different from the others were coded and transferred to new petri dishes for bacterial purification.

Bacteria identification

Bacteria that have activity in degrading PP were then identified through macroscopic, microscopic, and 16S sRNA characterization. Macroscopic character observations include color, size, shape, margin, and elevation of colony bacteria. Microscopic character observations include shape, Gram staining, and endospore existence of bacteria cells under microscope. For 16S sRNA characterization using a Presto^{TM} Mini gDNA Bacteria Kit according to the instructions from the company. Molecular analysis data were analyzed using the BLAST website.

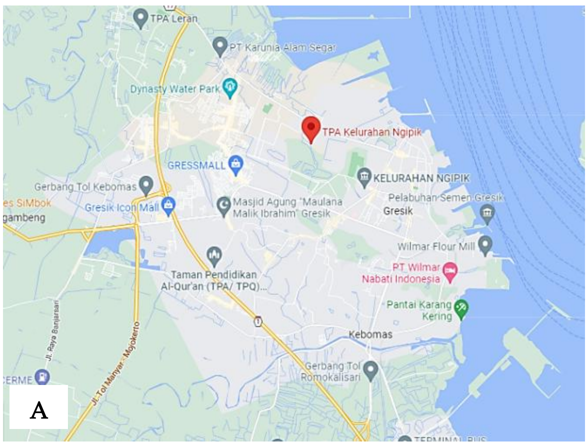

Figure 1. *The sampling location is at the Ngipik landfill, Gresik, East Java (A) The condition of that landfill (B)*

PP biodegradability test

One loop of pure bacterial isolates was inoculated on Nutrient Broth and incubated overnight until the $OD_{\lambda 600\ nm}$ 0.5. A total of 2% (v/v) culture of bacteria were tested for their ability to degrade PP. The treatment groups were differentiated based on the type of bacteria, namely NG 1, NG 2, and NG 3, also negative control (without culture) and positive control (without plastic pellets). A total of 0.2 g of PP substrate was added to the liquid mineral media until the total volume was 30 mL. The mineral medium was Bushnell Haas (BH) medium consist of 0.20 g $MgSO_4$, 0.02 g, $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3 and 0.05 g $FeCl_3$ per litre of distilled water. The PP biodegradability test was carried out on a 250 mL culture bottle on an orbital shaker at 120 rpm for 28 days of incubation at room temperature.

Every week, the growth of the bacteria culture was determined among others optical density (OD) and pH. On the last day of incubation, the percentage of degradation was calculated following the procedure (Auta et al., 2018). The percentage of degradation is calculated using the formula:

$$\text{Percent biodegradation} = \left(1 - \frac{\text{final weight}}{\text{initial weight}}\right) x\ 100\%$$

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Data analysis

The data obtained from this study were bacterial growth, pH, biodegradation percentage, and names of bacterial isolates. Data on bacterial growth, pH, and biodegradation were analyzed using descriptive statistics.

3. Result and Discussion

Isolation of potential plastic degrading bacteria

The results of bacterial isolation showed the number of bacteria per gram of sample was 1.22 x 106 CFU/g. A number of bacteria have been isolated from these samples. There were three dominant bacteria which were characterized macroscopically and microscopically. The macroscopic and microscopic data of the bacteria found are shown in Table 1 and visualized in Figure 2.

Table 1. Macroscopic and microscopic data of landfill bacteria

Character	Isolate code		
	NG 1	NG 2	NG 3
Macroscopic			
Color	Yellow	White	White
Size	Moderate	Moderate	Moderate
Shape	Circular	Circular	Circular
Margin	Serrate	Entire	Entire
Elevation	Flat	Flat	Convex
Microscopic			
Shape	Rod	Rod	Rod
Gram	+	+	+
Endospora	+	+	+

Landfill is a potential location to obtain potential microbes that decompose plastic because landfill accommodates all types of organic and inorganic waste, including plastic waste. Generally, the role of bacteria in nature is as a decomposer, so that bacteria will be easily found in the area. Bacteria can be used as potential agents in breaking down plastics. *Brevibacillus* sp. and *Aneurinibacillus* sp. isolated from landfills and sewage treatment plants can decompose polypropylene (Auta et al., 2018). Other studies have also tested bacteria isolated from landfills, *Pseudomonas aeruginosa* can decompose polypropylene (Wang et al., 2022).

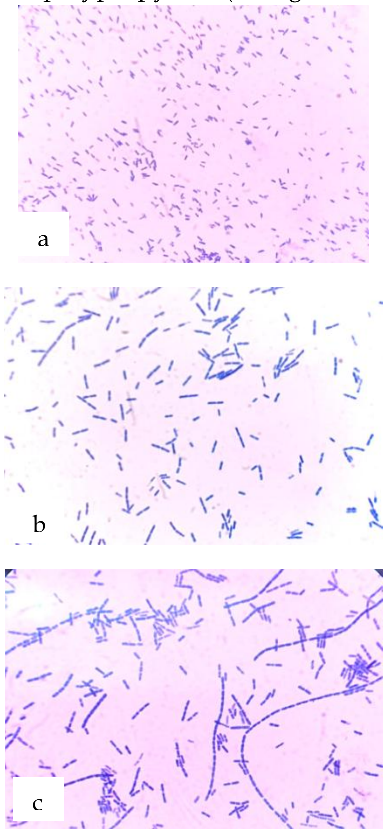

Figure 2. *Bacterial cells at 1000x magnification using Gram staining (a) Isolate NG 1; (b) Isolate NG 2; (c) Isolate NG 3*

Identification of potential PP decomposing bacteria

The results of temporary identification using macroscopic and microscopic characteristic data showed that the three isolates belonged to the genus *Bacillus*. This genus has special characteristics such as positive gram and endospores. Based on the 16S sRNA identification, the isolate NG 1 was *Bacillus velezensis* (97%), NG 2 *Bacillus amyloliquefaciens* (98.7%), and NG 3 *Bacillus wiedmannii* (99,8 %) decompose polypropylene (Auta et al., 2018). Other studies have also tested bacteria isolated from landfills, *Pseudomonas aeruginosa* can decompose polypropylene (Wang et al., 2022).

PP biodegradation

The biodegradation process was carried out for 28 days in closed liquid culture. In the biodegradation process, bacterial growth and

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culture pH were observed. Positive control was cultured without plastic substrate.

Compared with the control, the test culture was given the plastic substrate and observed to be cloudier as a result of bacterial growth. The bacterial growth was seen from the first week with average multiplication of doubled turbidity in the culture. Turbidity indicates the presence of multiplication of bacterial cells in culture. The highest turbidity was found in the NG 3 isolate culture in the first week. The difference in the growth of the three cultures was that the NG 3 culture had decreased the number of cells from the third week onwards. Meanwhile, the NG 1 and NG 2 cultures increased until the third week and decreased at the fourth week. The pH of the culture was observed to remain constant at 6 from week zero to the fourth. The absence of pH change in the medium indicated that there was no significant chemical change during incubation. The growth of bacterial cultures in plastic substrates is visualized in Figure 3.

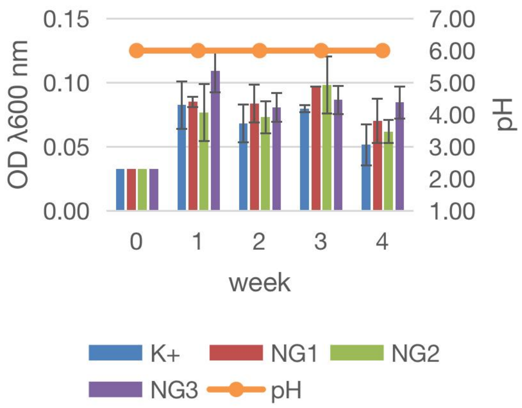

Figure 3. *Growth of bacterial culture in PP substrate (OD) and pH of culture during incubation*

After 4 weeks of incubation in microbial culture, the plastic weight reduced. Although the percentage of plastic biodegradation is very low, even less than 1%, there is a significant difference between negative control, treatment without bacterial culture and cultures containing NG 1, NG 2, and NG 3 bacteria. The highest percentage was achieved by NG 2 isolates of 0.47%. The percentage of plastic degradation is visualized in Figure 4.

This research has succeeded in discovering three potential isolates to decompose polypropylene from the Gresik landfill. The isolates found tended to resemble the genus Bacillus based on their macroscopic and microscopic characteristics. In this study, the highest polypropylene biodegradation was 0.47% as calculated from the plastic weight lost during 28 days of incubation at 30°C. The very low rate of plastic weight reduction, as compared to other studies, is probably due to the media used in the plastic degradation process and the incubation time. *Bacillus cereus* can degrade polypropylene granules in mineral salt (MSM) media by 12% during 40 days of incubation (Helen et al., 2017). The addition of yeast extract in mineral media can stimulate the growth of Bacillus subtilis in hydrocarbon degradation and stimulate the production of biosurfactants (Ni'Matuzahroh et al., 2017). They are active ingredients produced by living things such as amphiphilic bacteria and thus dissolve hydrocarbon materials in the aqueous phase. Biosurfactants help increase access to plastic materials and contribute with the process of bacterial attachment on to plastic materials. The addition of biosurfactant (surfactin) to the media has been proven to be effective in increasing the ability of *Bacillus subtilis* to biodegrade polyethylene and plastic (Vimala & Mathew, 2016).

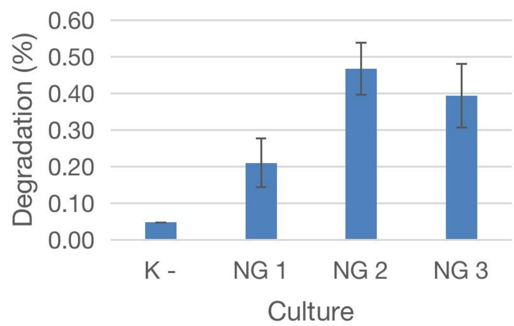

Figure 4. *Percentage of PP biodegradation after 4 weeks incobation*

Plastic biodegradation mechanisms can be biotic and/or abiotic. Biotic mechanisms employ microbes' abilities to break down plastics, such as disintegrating hydrocarbon bonds in plastics through extracellular enzymes, producing biosurfactants, and attaching bacterial cells to plastic materials. *Bacillus* sp. is known to be able to degrade polyethylene plastics during which process ligninolytic enzymes such as laccase, manganese peroxidase, lignin peroxidase, lipopeptide, and biosurfactant products are produced (Kavitha & Bhuvaneswari, 2021). Cell attachment to the plastic material is usually

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accompanied by the formation of a biofilm on the plastic surface which makes the plastic material a habitat for microorganisms. Indicators of the ability of microorganisms to decompose plastic can be seen from the increase in the number of cells and microbial biomass, the destruction of the structure and the reduction of plastic material. Meanwhile, in the abiotic mechanism the environmental factors include temperature, the presence of UV rays, growth media/supplements, pH, and the contact time between plastic materials and microorganisms.

4. Conclusion and Suggestion

The bacterial isolates of NG 1, NG 2, and NG 3 from Ngipik FDS have the potential to decompose PP. They were able to grow and reproduce on mineral media with plastic substrates as indicated by the presence of turbidity. The highest plastic degradation results were indicated by NG 2 isolates at 0.47%. Macroscopically and microscopically, NG 1, NG 2, and NG 3 isolates had characteristically resemblance to the Bacillus genus.

However, it is necessary to test for optimization of biodegradation using the addition of supplements for bacterial growth such as yeast extract. In addition, one may want to increase the incubation time of the polypropylene degradation process to obtain a higher level of plastic biodegradation.

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