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Distribution and biodegradation analysis of polyvinyl chloride microplastic by indigenous bacteria isolated from Supit Urang Landfill, Malang, Indonesia

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Abstract. *Rozana K, Prabaningtyas S, Widyatama DR. 2023. Distribution and biodegradation analysis of polyvinyl chloride microplastic by indigenous bacteria isolated from Supit Urang Landfill, Malang, Indonesia. Biodiversitas 24: 3853-3859.* Microplastic waste is one of the most common forms of environmental pollution. Indonesia is the world's fourth largest contributor of plastic waste. One type of plastic that accumulates in the environment is P*olyvinyl Chloride* (PVC). Various attempts or methods have been developed to reduce PVC plastic waste, one of which is with the help of using indigenous bacteria. This research is essential in determining the best optimization method for PVC biodegradation agents through molecular and bioinformatic approaches. Indigenous bacteria were isolated from Supit Urang Landfill, Malang City, with three dilution levels (10^{-4}, 10^{-5}, and 10^{-6}). The three highest isolates were taken for further biodegradation tests for 30 days and were identified based on the 16S rRNA gene. Then, the BLAST results were made into a phylogenetic tree to determine the kinship of each species. The isolation results obtained 17 bacterial isolates, selected by initial biodegradation screening for 10 days. Isolate K4 has a degradation percentage of 1.61±0.007379%. While isolates K4 and K15 have a biodegradation potential of 3.04±0.001861%. and 1.90±0.005576%. The BLAST results showed that K4 isolate had a 99% similarity to *Staphylococcus capitis*, K14 had a 99% similarity to *Bacillus subtilis*, and K15 had a 100% similarity to *Acinetobacter pittii*.

Keywords: Biodegradation, indigenous bacteria, microplastic, *polyvinyl chloride*, phylogenetic

INTRODUCTION

Microplastic waste is one of the most common forms of pollution in the environment, both terrestrial and aquatic. Around 585 million tons of plastic waste is generated worldwide each year, of which only 15% is recycled, 12% is burned, and 73% is disposed of in the environment or landfilled (Abbasi and Turner 2021). Indonesia is among the major plastic-producing countries and is the fourth largest contributor in producing 20.5 million tons of plastic waste annually (Benson et al. 2021). After being released and accumulated in the environment, plastic waste decomposed into microplastic particles with a size of less than 5 mm (Rozana et al. 2023a). Due to their tiny size, abundance, and nature, microplastics become widespread in the environment (Wong et al. 2020). In terrestrial environments, microplastics can be found abundantly in the soil (Rillig et al. 2017a). They also can be detected in various organisms, including earthworms (Rillig et al. 2017b), snails (Panebianco et al. 2019), pets (Zhang et al. 2019), and livestock (Beriot et al. 2021). Microplastics can also be found in drinking water (Pivokonsky et al. 2018) and human food (Danopoulos et al. 2020). Microplastic content that initially accumulated in water and animal bodies is now also found in the human body, so it is predicted to cause many health problems in the future (Geyer et al. 2017).

Polyvinyl Chloride (PVC) is one of the most widely used types of plastic, both in the world and in Indonesia. The use of PVC every year has increased. PVC ranges from building materials, automotive, and medical devices to daily goods. PVC is known to be a chlorine-based plastic, one of the most dangerous compounds. PVC is a source of dioxins. During the PVC manufacturing process, from the formation of *Vinyl Chloride Monomer* (VCM) to becoming a plastic product, hazardous materials, such as additives, stabilizers, and plasticizers, are used. In Europe, PVC has become a serious concern since the late 1990s. Europe banned the use of PVC until 2020. In Indonesia, no regulations explicitly prohibit the use of PVC. PVC products are feared to harm human health and the environment. According to research results, when compared to *Polyethylene* (PE), the danger of PVC to humans and the environment is higher (Hasanudin 2008).

However, the extent of microplastic contamination in the human body is unclear because studies using human specimens are still limited. Previous studies have reported microplastic contamination of the digestive tract (Wibowo et al. 2021), human stool samples (Zhang et al. 2021), placenta (Ragusa et al. 2021), lungs (Amato-Lourenco et al. 2021), skin surface, scalp hair, and saliva (Abbasi and Turner 2021). The most significant concern regarding human exposure to microplastics is the potential toxicity and effects on human health. Several studies using animal and human cells have reported the potential adverse effects of microplastic contamination on human health. This contamination is reported to induce pro-inflammatory cytokines that cause an immune response (Hwang et al.

2019), inhibit cell proliferation, cause morphological changes in lung cells (Goodman et al. 2021), and causes immunotoxicity in a short time (Han et al. 2020).

Indigenous bacteria live naturally in nature, benefiting humans and the environment (Rozana et al. 2023b). Several previous research results have proven that indigenous bacteria can degrade waste, control plant life, and act as antibiotics (Batubara et al. 2015). Indigenous bacteria have a higher efficiency of microplastic degradation than non-indigenous bacteria because they can undergo an adaptation process in their native environment for a relatively long time (Yazid 2014). *Bacillus cereus* (Sharma et al. 2014) and *Pseudomonas citronellolis* (Giacomucci et al. 2019) are some bacteria that can degrade PVC. One of the most potent sources of finding indigenous bacteria is the Final Disposal Site (FDS). The area has much waste, both organic waste that has been buried for a long time and has become rotten and inorganic waste that does not decompose, including plastic. One of the areas in Malang City with high contamination of microplastics is the Supit Urang Landfill, located in the Mulyorejo Village, Sukun District, with an area of 28 hectares.

Determination of the species of bacteria is carried out based on genotyping because bacterial species are easier to identify than eucaryotes. Genotypic identification of bacteria involves the DNA of the bacteria. Ribosomal ribonucleic acid (rRNA) is a molecule that combines with other RNA molecules to form ribosomes which are used in protein synthesis (Rozana et al. 2023b). Besides, phylogenetics was also carried out to understand similar bacteria with the same ability to degrade microplastics. Previous research related to involving indigenous plastic degrading bacteria showed that *Pseudomonas* sp. and *Aspergillus* is a candidate for plastic biodegradation agent, which has the highest proportion of degradation compared to other indigenous bacteria, such as *Streptomyces, Emericella nidulans,* and *Bacillus* (Poonam et al. 2013). The results of these studies are still limited to research on plastic degradation in general.

In contrast, research on the potential for degradation of native bacteria on PVC plastic is still limited. Because of these reasons, the present study has an essential role in giving pieces of information about the molecular structure and characteristics of PVC biodegradation agents. The molecular structure and characteristics of PVC biodegradation agents can support any methods in genetic engineering, including recombination, cloning, and mutation, which impact increasing degradation.

MATERIALS AND METHODS

Study area

The research was carried out from June to October 2022. Soil sampling was carried out at the Supit Urang Landfill, Malang. Soil sampling is carried out at the final waste accumulation site, where it is estimated that the area has a significant bacterial population due to the high carbon source of the waste collection, which can be used for indigenous bacterial metabolism. The high carbon source impact the mass or population of indigenous bacteria in landfills. Bacterial isolation and biodegradation tests were conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang. Meanwhile, DNA isolation, PCR, and sequencing were sent to PT. Indoplus Prima Biotech.

Materials

The materials used during the present study include a micropipette, microtip, microscope, Laminar Air Flow (LAF), autoclave, petri dish, analytical balance, test tube, test tube rack, vortex, stove, shaker, hot plate, Erlenmeyer, spatula, stirring rod, spreader, ose needle, dropper, shovel, styrofoam box, tweezers, preparation glass and cover, support wire, bowl, spray bottle, ruler, scissors, glove. Materials used include samples, NA (*Nutrient Agar*) media, NB (*Nutrient Broth*) media, 70% alcohol, PVC plastic, PVC powder, aluminum foil, cling wrap, matches, spirits, sterile plastic, label paper, tissue, distilled water, crystal violet solution, iodine solution, safranin solution, 95% alcohol, immersion solution, Lysol solution, brown cover paper, thread/rope, cotton rolls, sterile gauze.

Procedures

This quantitative experimental study was carried out by using a Completely Randomized Design (CRD) with control and treatment groups. Along with incubation time 30 days with 3 replications.

Sampling

Sampling was carried out on land where plastic waste had accumulated for a long time, and there were indications of plastic waste starting to degrade. The characteristics of degraded plastics can be seen through changes in the plastic structure which decomposes and forms smaller-sized plastics with faded plastic colors due to soil mixing. Soil samples were taken as much as 10 grams using a shovel at 5 different points with a depth of 5-10 cm, then put in a sterile plastic bag and taken to the laboratory to be stored in the refrigerator. Indigenous bacteria were taken from soil samples around degraded plastic, assuming that soil is suitable for bacteria. Although some indigenous bacteria can use plastic as a carbon source, bacteria prefer to use simple carbon sources found in the soil.

Making isolation medium

The isolation medium used in this research were medium Nutrient Agar and Nutrient Broth, weighed as much as 20 grams and 8 grams, respectively, put into an Erlenmeyer, dissolved with 1 liter of distilled water, and then heated. Then, the medium was sterilized by autoclaving at 121°C in 1 atm for 15 minutes.

Isolation and purification of bacteria

Isolation was started by dissolving 10 grams of soil sample with 90 mL of Nutrient Broth medium and homogenizing using a vortex. This solution is a stock solution that will be analyzed further. As much as 1 mL of the stock solution is put into a test tube containing 9 mL of Nutrient Broth media. The mixed solution is called a 10^{-1}

dilution. Then take 1 mL of solution from dilution 10^{-1} to be added to the second test tube containing 9 mL of Nutrient Broth media. The mixed solution is called dilution 10^{-2}. The process was repeated sequentially until a 10^{-6} dilution was obtained. The multilevel dissolution process was carried out to obtain a single colony of bacteria. After dilution process is complete, then each dilution 10^{-1}, dilution 10^{-2}, dilution 10^{-3}, until dilution 10^{-6} is taken 1 mL to grow in 6 pieces of Nutrient Agar media which have been added PVC in a petri dish using the spread plate method. Each Nutrient Agar medium was then incubated at 37°C for 1x24 hours from 00.00 p.m. After the incubation period, the isolates were purified by taking 1 ose of every single colony found and grown on new Nutrient Agar media using the streak plate method and then incubated for 24 hours at 37°C. After incubation, pure cultures were obtained from each indigen bacterial isolate. The work was carried out aseptically in a laminar airflow to prevent contamination by other microorganisms.

Initial screening Biodegradation Test

Initial screening was carried out to select bacteria to be used for biodegradation tests. All single bacteria colonies found were tested to degrade PVC plastic for 10 days with a shaker at 120 rpm. As many as 3 bacteria with the highest degradation ability were taken, indicated by the reduced weight of PVC. The three bacteria will be used for the biodegradation test.

Biodegradation effectivity test

PVC plastic was cut 1x1 cm, and then the initial weight was weighed with an analytical balance, washed with sterile distilled water, and sprayed with 70% alcohol. The plastic is put into an Erlenmeyer flask containing 50 mL of NB media. Pure bacterial isolates were inoculated into the media and then incubated with a shaker at 120 rpm at room temperature for 30 days. After 30 days, the PVC plastic samples were washed with sterile distilled water and sprayed with 70% alcohol, and then they were dried and weighed using an analytical balance. Determination of the percentage of degradation of microplastic samples by indigenous bacteria is calculated using the formula:

$$\% \text{ degradation} = \frac{(\text{Initial weight} - \text{final weight})}{\text{Initial weight}} \times 100\%$$

Identification of bacteria based on 16s rRNA gene and phylogenetic analysis

Sequencing results received in present study were analyzed using the online BLAST (*Basic Local Alignment Search Tools*) program. Then the data were analyzed by aligning the isolate sequences obtained with the comparator bacterial sequences obtained from Gene-Bank NCBI. Then the sequences were aligned with the Mega program. The alignment result fasta file is then imported into the MEGA X program to create a phylogenetic tree using the *Neighbor-Joining* (NJ) algorithm.

RESULTS AND DISCUSSION

Indigenous bacteria isolation

Indigenous bacterial isolates were obtained from soil samples at the Supit Urang Landfill in Malang City. The sampling location point was on the ground where there was a pile of plastic waste that was old enough, and there was plastic that had begun to degrade. The soil dilution process obtained 17 bacterial colonies and was part of the isolation process to obtain pure isolates. The streak plate method used four-way streak lines to purify the 17 bacterial colonies obtained. According to Irianto (2012), the streak plate method aimed to separate colonies from mixed cultures from obtaining pure isolates. To ensure that no other bacterial isolates grew after the first purification, all isolates were purified repeatedly on NA medium plates. The isolated indigenous bacteria profile can be seen in Table 1.

Biodegradation test initial screening

The screening was performed to determine or select bacterial isolates for biodegradation tests. Preliminary screening results showed that not all bacterial isolates could degrade PVC plastic within a 10-day incubation. Initial screening data for bacterial isolates can be seen in Table 2.

Based on the results of the initial screening data, the formation of a biofilm layer on the plastic's surface and the reduced plastic weight were two indicators of the ability of bacteria to degrade microplastics. According to Mardiana (2003), one of the characteristics of bacteria that has the potential for biodegradation is the formation of biofilms on plastic surfaces. Three bacterial isolates with the highest percentage of weight loss were selected, namely K4 (0.98%), K14 (1.21%), and K15 (0.95%).

Table 1. Profile of indigenous bacteria isolates

Bacteria Isolates	Shape	Gram
Isolat K1	Coccus	-
Isolat K2	Coccus	-
Isolat K3	Coccus	+
Isolat K4	Coccus	+
Isolat K5	Rod	+
Isolat K6	Rod	+
Isolat K7	Coccus	+
Isolat K8	Rod	+
Isolat K9	Rod	+
Isolat K10	Rod	+
Isolat K11	Rod	+
Isolat K12	Coccus	+
Isolat K13	Coccus	-
Isolat K14	Rod	+
Isolat K15	Coccus	-
Isolat K16	Coccus	+
Isolat K17	Coccus	+

The results of biodegradation screening for 10 days of incubation period showed that the first day was an adaptation period for bacteria, so the bacteria did not use PVC in the medium as a carbon source. Meanwhile, during the next incubation period, all isolates showed a gradual increase in degradation percentage until the end. From these results, there has not been found a stagnant point indicating the degradation process has reached the saturation limit, so for further research, it is necessary to increase the incubation time to know the incubation time limit which reaches the degradation saturation point. The three bacterial isolates were then used for biodegradation tests and molecular species identification based on the 16S rRNA gene.

Biodegradation Test

The biodegradation test was carried out to determine the potential of indigenous bacterial isolates in degrading PVC plastic with an incubation period of 30 days so that the isolated bacteria could adapt well. Natural and synthetic polymers can be degraded chemically or mechanically by microbes. The adaptation of microorganisms to new substrates is related to polymer biodegradation (Rozana et al. 2023a). Microbes use carbon as a source of nutrition, and the PVC plastic used in the research is expected to be used as the primary carbon source for the isolated bacteria. The biodegradation test results were determined by calculating the weight loss percentage in PVC plastic. This test method is used because it is easy and does not require special tools in microplastic biodegradation (Agustien et al. 2016). The results of the biodegradation test by the three bacterial isolates can be seen in Table 3.

The results obtained revealed that the isolates of bacteria with the highest biodegradation potential, indicated by isolate K14 with a percentage of 3.04 ± 0.001861%. While isolates K4 and K15 have biodegradation potential of 1.61 ± 0.007379% and 1.90 ± 0.005576% respectively.

Based on the results of the biodegradation test above, the treatment group showed a loss of plastic weight, which means that PVC plastic biodegradation had occurred and can use plastic as a carbon source for growth for 30 days. This result was supported by the control group in which there was no weight loss in the plastic because there were no bacterial isolates. When bacteria are grown in media with a nitrogen source and test plastic, the test plastic experienced a decrease in weight because the bacteria use the test plastic as a carbon source during the growth process (Agustien et al. 2016). In this study, the nitrogen source came from NB media, a universal growth medium with a very high nitrogen content.

According to Hasanah (2015), bacteria and other microorganisms can survive after moving from their original habitat. These changes can result in tense conditions for microbes. Microbes can survive, stop growing, or even experience a lag phase increase depending on the extent of the changes. If microbial cells grow in the lowest settings, these microbes can be tolerated in extreme conditions up to the maximum time limit. Bacteria will form a biofilm layer when in an environment that lacks nutrients. Microorganisms produce energy when there is a shortage of nutrients due to biofilms. The growth of bacteria that degrade plastic produce biofilms. One factor that accelerates biodegradation is biofilms' appearance (Fadlilah and Shovitri 2014). During biodegradation, microbes form biofilms on surfaces that break down high molecular weight polymers into smaller sizes or produce short chains such as oligomers and monomers through enzymatic processes (Bhone et al. 2012). Enzymes produced by microbes to degrade plastics are very effective without causing environmental damage (Bhardwaj et al. 2012).

Table 2. Biodegradation initial screening data with an incubation period of 10 days

Bacteria isolates	Weight loss percentage (day)										Biofilm formation
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Control	0	0	0	0	0	0	0	0	0	0	-
Isolat K1	0	0.1	0.1	0.15	0.18	0.23	0.28	0.28	0.3	0.33	-
Isolat K2	0	0	0	0	0	0	0	0	0	0	-
Isolat K3	0	0.03	0.03	0.05	0.1	0.16	0.19	0.26	0.29	0.31	-
Isolat K4	**0**	**0.3**	**0.36**	**0.42**	**0.45**	**0.51**	**0.63**	**0.79**	**0.87**	**0.98**	+
Isolat K5	0	0.15	0.19	0.21	0.25	0.32	0.44	0.51	0.58	0.60	-
Isolat K6	0	0	0	0	0	0	0	0	0	0	-
Isolat K7	0	0.05	0.09	0.13	0.16	0.22	0.25	0.27	0.29	0.30	-
Isolat K8	0	0.02	0.05	0.09	0.13	0.16	0.24	0.26	0.27	0.30	-
Isolat K9	0	0	0	0	0	0	0	0	0	0	-
Isolat K10	0	0.03	0.08	0.11	0.19	0.21	0.25	0.26	0.29	0.30	-
Isolat K11	0	0.02	0.07	0.09	0.12	0.18	0.21	0.24	0.27	0.30	-
Isolat K12	0	0	0	0	0	0	0	0	0	0	-
Isolat K13	0	0.05	0.09	0.13	0.16	0.23	0.25	0.27	0.27	0.30	-
Isolat K14	**0**	**0.22**	**0.28**	**0.35**	**0.43**	**0.67**	**0.76**	**0.83**	**0.96**	**1.21**	+
Isolat K15	**0**	**0.1**	**0.16**	**0.21**	**0.39**	**0.47**	**0.67**	**0.77**	**0.90**	**0.95**	+
Isolat K16	0	0	0	0	0	0	0	0	0	0	-
Isolat K17	0	0.1	0.13	0.15	0.19	0.22	0.24	0.26	0.29	0.31	-

Table 3. Data on PVC plastic Biodegradation Test results

Isolates	Percentage
Control	0 ±0.000000%
K4	1.61 ±0.007379%
K14	3.04 ±0.001861%
K15	1.90 ±0.005576%

Molecular identification of indigenous bacteria (16S rRNA Gene)

The 16S rRNA gene is about 1550 base pairs long, and there is a region known as the hypervariable region at the end of the sequence, which is about 500 bases long. It is this region that differentiates between organisms (Rinanda 2011). Bacterial species names were determined using the BLAST algorithm in the NCBI database (Table 4).

Based on the BLAST test results, all isolated bacterial DNA sequences were compared with sequence data in the NCBI database to measure the degree of similarity. BLAST results showed that K4 isolates had 99% similarity to *Staphylococcus capitis*, K14 isolates had 99% similarity to *Bacillus subtilis*, and K15 isolates had 100% similarity to *Acinetobacter pittii*. According to Montazer et al. (2018), the degree of similarity or similarity of the base sequence of the 16S rRNA encoding gene is more than 97% not a new species or still within the same species. Research conducted by Miloloza et al. (2021) proved that *Bacillus subtilis* could degrade PVC, but choosing the right conditions for the highest efficiency is significant. Previous studies have reported bacteria that can degrade PVC plastic (Table 5).

Compared to several previous studies, *Bacillus subtilis* (K14) in this study had a biodegradation percentage of 3.04% which was lower than *Bacillus cereus* in Sharma's study et al. (2014) of 22.22% but higher when compared to *Bacillus* sp. in Kumari's et al. study (2019). Then, the *Staphylococcus capitis* (K4) bacteria in this study also had a lower biodegradation percentage of 1.61% with an incubation time of 30 days than *Staphylococcus* sp. in Kumar's (2017) study of 11%, but with a longer incubation time of 10 months. Several factors affect biodegradation: the type of bacteria, polymer, and the incubation time. The differences in the characters of the genus can also affect the results of biodegradation (Rozana et al. 2023b).

As for the *Acinetobacter pittii* bacteria, this possibility is the first biodegradation reported by this bacteria, although there are reports about its ability to degrade LDPE plastic with a degradation percentage of 26.8%. Other research by Giacomucci et al. (2019) also reported that *Pseudomonas citronellolis* bacteria could degrade PVC with a percentage of 18.58% with an incubation period of 30 days.

The enzymes involved in the PVC biodegradation process are still unknown. However, the study of Sharma et al. (2014) reported positive results of chitinase and glucanase enzyme tests on *Bacillus cereus* that degrade PVC. Several groups of enzymes are claimed to degrade polymers into their monomer form. Oxidases, amidases, laccases, hydrolases, and peroxidases are enzymes involved in polymer degradation. Furthermore, the BLAST test results made a phylogenetic tree using the MEGA program to see the kinship of the species.

The phylogenetic tree of K4 bacterial isolates shows a close relationship with the species *Staphylococcus capitis* and a distant relationship with *Staphylococcus epidermidis*. In isolate K14, bacteria showed closeness to the species *Bacillus subtilis*. They had a distant relationship with *Bacillus spezizenii*, and in isolate, K15 showed closeness to the species *Acinetobacter pittii* and had a distant relationship with *Acinetobacter calcoaceticus*. It is possible to know other bacteria with a similar ability to degrade microplastics from the phylogenetic trees, although it needs more research, especially on transcriptomics and proteomics analysis. The limitation of this research is that the ideal culture conditions in the laboratory cannot be adapted to nature, so it is possible to reduce the percentage of degradation if applied in nature. Besides, for further research, it is necessary to increase the incubation time to know the limit that reaches the degradation saturation point.

Table 4. Data on BLAST results of indigenous bacterial isolates

Isolates	Species	Max. identity	Seq id
K4	*Staphylococcus capitis*	99%	NR_113348.1
K14	*Bacillus subtilis*	99%	CP020102.1
K15	*Acinetobacter pittii*	100%	NR_117621.1

Table 5. Percentage of PVC plastic biodegradation based on previous research

Species	Incubation period	Degradation percentage	References
Bacillus cereus	45 days	22.22%	Sharma et al. (2014)
Bacillus sp.	90 days	0.26%	Kumari et al. (2019)
Staphylococcus sp.	10 months	11%	Kumar et al. (2017)
Pseudomonas citronellolis	30 days	18.58%	Giacomucci et al. (2019)

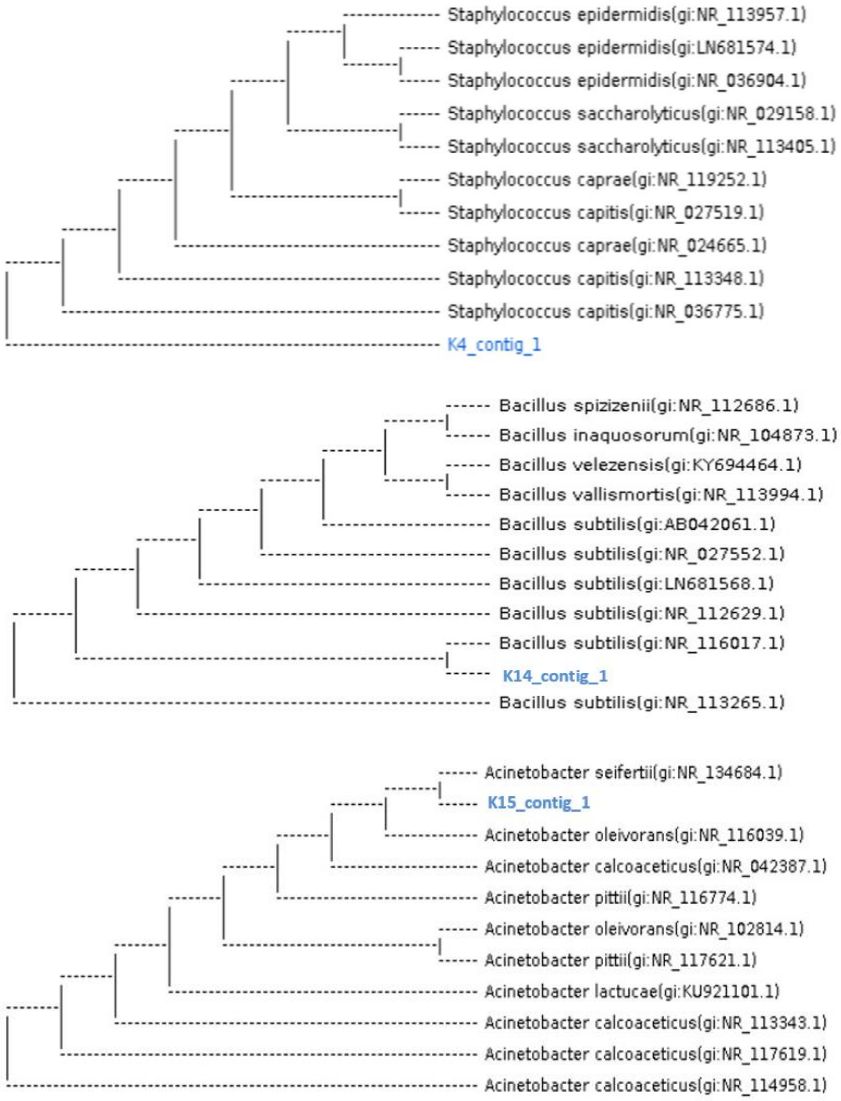

Figure 1. Phylogenetic tree of indigenous bacterial species: K4 (top), K14 (middle), and K15 (bottom)

In conclusion, isolates K4, K14, and K15 have a higher potential for biodegradation of PVC plastic than other isolates based on initial biodegradation screening. In the 30-day biodegradation test, isolate K14 had a 3.04 ± 0.001861% degradation percentage. While isolates K4 and K15 have biodegradation potential of 1.61 ± 0.007379% and 1.90 ± 0.005576%. The identification results of the bacterial isolates based on the 16S rRNA gene showed that isolate K4 had a similarity percentage of 99% with the *Staphylococcus capitis*, isolate K14 had a similarity percentage of 99% with *Bacillus subtilis*, and isolate K15 had a percentage similarity of 100% with *Acinetobacter pittii*. From these results, some bacteria known as PVC biodegradation agents can explore and identify more organisms to increase the possibility of PVC biodegradation in nature. Besides, much future research related to the optimization and genetic engineering of PVC biodegradation agents can be done to solve many problems of plastic in real life.

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REFERENCES

Abbasi S, Turner A. 2021. Human exposure to microplastics: A study in Iran. J Hazard Mater 403: 123799. DOI: 10.1016/j.jhazmat.2020.123799.

Agustien A, Jannah M, Djamaan A. 2016. Screening synthetic polyethylene plastic degrading bacteria from soil. Der Pharmacia Lettre 8 (7): 183-187.

Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, dos Santos Galvão L, Ando RA, Mauad T. 2021. Presence of airborne microplastics in human lung tissue. J Hazard Mater 416: 126124. DOI: 10.1016/j.jhazmat.2021.126124.

Batubara UM, Susilawati IO, Riany H. 2015. Isolasi dan karakterisasi bakteri indigenous tanah di kawasan kampus Universitas Jambi

isolation and characterization of indigenous soil bacteria. SEMIRATA 2015 4 (1). [Indonesian]
Benson NU, Bassey DE, Palanisami T. 2021. COVID Pollution: Impact of COVID-19 Pandemic on Global Plastic Waste Footprint. Heliyon 7 (2). DOI: 10.1016/j.heliyon.2021.e06343.
Beriot N, Peek J, Zornoza R, Geissen V, Lwanga EH. 2021. Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain. Sci Total Environ (755): 142653. DOI: 10.1016/j.scitotenv.2020.142653.
Bhardwaj H, Gupta R, Tiwari A. 2012. microbial population associated with plastic degradation. Open Access Sci Rep 1 (5). DOI: 10.4172/scientificreports.272.
Bhone MK, Ravi C, Meena KS, Chu SL, Kishore RS. 2012. Biodegradation of Low-Density Polyethylene (LDPE) by *Pseudomonas* species. Indian J Microbiol 52 (3): 411-419. DOI: 10.1007/s12088-012-0250-6.
Danopoulos E, Jenner LC, Twiddy M, Rotchell JM. 2020. Microplastic contamination of seafood intended for human consumption: A systematic review and meta-analysis. Environ Health Perspect (128): 126002. DOI: 10.1289/EHP7171.
Fadlilah M, Shovitri M. 2014. Potensi isolat bakteri Bacillus dalam mendegradasi plastik dengan metode kolom winogradsky. Jurnal Sains dan Seni ITS 3 (2): E40-E43. DOI: 10.12962/j23373520.v3i2.6730. [Indonesian]
Geyer R, Jambeck JR, Law KL. 2017. Production, use, and fate of all plas-tics ever made. Sci Adv 3 (7): e1700782. DOI: 10.1126/sciadv.1700782.
Giacomucci L, Raddadi N, Soccio M, Lotti N, Fava F. 2019. Polyvinyl chlo-ride biodegradation by *Pseudomonas citronellolis* and *Bacillus flexus*. New Biotechnol 52: 35-41. DOI: 10.1016/j.nbt.2019.04.005.
Goodman KE, Hare JT, Khamis ZI, Hua T, Sang QXA. 2021. Exposure of human lung cells to polystyrene microplastics significantly retards cell proliferation and triggers morphological changes. Chem Res Toxicol (34): 1069-1081. DOI: 10.1021/acs.chemrestox.0c00486.
Han S, Bang J, Choi D, Hwang J, Kim T, Oh Y, Hwang Y, Choi J, Hong J. 2020. Surface pattern analysis of microplastics and their impact on human-derived cells. ACS Appl Polym Mater (2): 4541-4550. DOI: 10.1021/acsapm.0c00645.
Hasanah NU, Shovitri M. 2015. Potensi mikroorganisme air sampah mangrove untuk mendegradasi plastik hitam. Jurnal Sains dan Seni ITS 4 (2): 45-49. [Indonesian]
Hasanudin MI. 2008. Kajian Dampak Penggunaan Plastik PVC Terhadap Lingkungan dan Alternatifnya di Indonesia. [Thesis]. Magister Program Studi Ilmu Lingkungan, Universitas Indonesia, Depok. [Indonesian]
Hwang J, Choi D, Han S, Choi J, Hong J. 2019. An Assessment of the Toxicity of Polypropylene Microplastics in Human Derived Cells. Sci Total Environ 684: 657-669. DOI: 10.1016/j.scitotenv.2019.05.071.
Irianto K. 2012. Bakteriologi, Mikologi, Virologi. Alfabeta, Bandung. [Indonesian]
Kumar S, Teotia UVS, Singh Y. 2017. Screening poly vinyl chloride degrading bacteria from plastic contaminated area of baddi. J Appl Pharm Res 5 (3): 34-37. DOI: 10.18231/2348-0335.2017.0005.
Kumari A, Chaudhary DR, Jha B. 2019. Destabilization of polyethylene and polyvinylchloride structure by marine bacterial strain. Environ Sci Pollut Res 26: 1507-1516. DOI: 10.1007/s11356-018-3465-1.
Mardiana DNM, Surdia CL, Radiman E, Ratnaningsih A, Rosdiana. 2003. Gliserol: Sumber Monomer Terbaharui dan Pemanfaatannya sebagai Bahan Baku Polimer Ramah Lingkungan. Prosiding Seminar Sehari 70 tahun Noer Mansdjoeriah Surdia. Institut Teknologi Bandung, Bandung. [Indonesian]
Miloloza M, Bule K, Prevaric V, Ruzaj L, Patrcevic P, Cvetnic M, Ukic S, Bolanca T, Kucic Grgic D. 2021. Biodegradation of PS and PVC microplastics by *Bacillus subtilis*. Food Clim Changes 75-75.
Montazer Z, Habibi-Najafi MB, Mohebbi M, Oromiehei A. 2018. Microbial degradation of UV-pretreated low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil. J Polym Environ 26: 3613-3625. DOI: 10.1007/s10924-018-1245-0.
Panebianco A, Nalbone L, Giarratana F, Ziino G. 2019. First discoveries of microplastics in terrestrial snails. Food Control (106): 106722. DOI: 10.1016/j.foodcont.2019.106722.
Pivokonsky M, Cermakova L, Novotna K, Peer P, Cajthaml T, Janda V. 2018. Occurrence of microplastics in raw and treated drinking water. Sci Total Environ 643: 1644-1651. DOI: 10.1016/j.scitotenv.2018.08.102.
Poonam K, Rajababu V, Yogeshwari J, Patel H. 2013. Diversity of plastic degrading microorganisms and their appraisal on biodegradable plastic. Appl Ecol Environ Res 11 (3): 441-449. DOI: 10.15666/aeer/1103_441449.
Ragusa A, Svelato A, Santacroce C, Catalano P. 2021. Plasticenta: First evidence of microplastics in human placenta. Environ Intl (146): 106274. DOI: 10.1016/j.envint.2020.106274.
Rillig MC, Ingraffia R, Machado AADS. 2017a. Microplastic incorporation into soil in agroecosystems. Front Plant Sci (8). DOI: 10.3389/fpls.2017.01805.
Rillig MC, Ziersch L, Hempel S. 2017b. Microplastic transport in soil by earthworms. Sci Rep 7. DOI: 10.1038/s41598-017-01594-7.
Rinanda T. 2011. Analisis sekuensing 16S rRNA di bidang mikrobiologi. Jurnal Kedokteran Syiah Kuala 11 (3): 172-177. [Indonesian]
Rozana K, Haryono NY, Kartikasari NA, Utomo MA. 2023. Molecular analysis of *Brevibacillus brevis* as polyethylene biodegradation agents. AIP Conf Proc 2634 (1): 020090. DOI: 10.1063/5.0111492.
Rozana K, Haryono NY, Kartikasari NA, Utomo MA. 2023. Genomic and proteomic analysis of *Bacillus subtilis* as microplastic bioremediation agents. AIP Conf Proc 2634 (1): 020033. DOI: 10.1063/5.0111489.
Sharma J, Gurung T, Upadhyay A, Nandy K, Agnihotri P, Mitra AK. 2014. Isolation and characterization of plastic degrading bacteria from soil collected from the dumping grounds of an industrial area. Intl J Adv Innovative Res 3 (3): 225-232.
Wibowo AT, Nugrahapraja H, Wahyuono RA, Islami I. 2021. Microplastic contamination in the human gastrointestinal tract and daily consumables as-sociated with an Indonesian Farming Community. Sustainability (13): 12840. DOI: 10.3390/su132212840.
Wong JKH, Lee KK, Tang KHD, Yap PS. 2020. Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. Sci Total Environ 719: 137512. DOI: 10.1016/j.scitotenv.2020.137512.
Yazid M. 2014. Peranan isolat bakteri indigenous sebagai agen bioremediasi perairan yang terkontaminasi uranium. Jurnal Iptek Nuklir Ganendra 17 (1): 35-44. DOI: 10.17146/gnd.2014.17.1.1300. [Indonesian]
Zhang J, Wang L, Kannan K. 2019. Polyethylene terephthalate and polycarbonate microplastics in pet food and feces from the United States. Environ Sci Technol 53: 12035-12042. DOI: 10.1021/acs.est.9b03912.
Zhang N, Li YB, He HR, Zhang JF, Ma GS. 2021. You are what you eat: Microplastics in the feces of young men living in Beijing. Sci Total Environ 767: 144345. DOI: 10.1016/j.scitotenv.2020.144345.