RESEARCH ARTICLE

Plastic-degrading bacteria isolated from contaminated mangrove sediment in Wonorejo, Surabaya

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Abstract: Plastics have become inevitable needs in modern society due to their attractive properties, including thermostability, lightweight, flexibility, superior insulation, and low cost, which have led to massive production. Their persistence and challenges in disposal have detrimental effects on the environment leading to the development of a promising degradation process which is efficient, time-saving, and cost-effective. This study focused on discovering the potential plastic-degrading bacteria isolated from plastic-contaminated mangrove sediment in the Wonorejo area. We buried a commercially available plastic bag in the polluted mangrove sediment for 16 weeks. Our results showed that indigenous bacteria formed a biofilm on the plastic surface leading to a plastic dry weight loss of up to 12%. FTIR analysis revealed obvious transmittance attenuations in the buried plastic polymer, suggesting that their chemical properties may have been interrupted due to bacterial activity. Further, bacteria isolation and biochemical screening revealed that they were primarily dominated by Bacillus. According to 16S rRNA sequencing, they were identified as Brevibacilllus (BIO-B), Stenotrophomonas (BIO-G), and Lysinibacillus (SOI-C). The three genera mentioned earlier exhibited a detectable level of plastic-degrading activity and possessed lipolytic, ligninolytic, and alkane-degrading activities. Stenotrophomonas (BIO-G) showed a degradation activity on low-density polyethylene (LDPE) represented by a plastic dry weight loss of up to 8.9% within 4 weeks. As expected, plastic treated with BIO-G showed transmittance attenuation in FTIR analysis, albeit with a lower percentage than that treated with indigenous bacteria. Moreover, SEM analysis reveals changes in the morphological surface of plastic. Together, FTIR and SEM analysis indicated that bacteria disrupt both the chemical structure and morphological appearance of plastic polymer upon degradation process. These results denote that BIO-G indeed composes the aforementioned indigenous bacteria from polluted mangrove sediment. Thus, our study suggests the indigenous bacteria isolated from contaminated areas produced plastic-degrading enzymes and secreted to the environment to break down plastic compounds.

Keywords: Plastic-degrading bacteria, indigenous bacteria, bacillus, plastic-contaminated areas, mangrove sediment

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1 Introduction

Plastics are widely used in various industries such as transportation, telecommunications, clothing, footwear, and packaging materials, due to their alluring properties, including durability, lightweight, flexibility, and cost-effective (Andrady and Neal, 2009). Thus, plastic production has continuously increased and become invincible. In 2019, it reached 368 million tons field (Plastics Europe, 2019) and possibly double within 20 years (Lebreton and Andrady, 2019). Unfortunately, none of the commercially available (especially single-use) plastic is easily degraded in nature, leading to a substantial number of plastic accumulation (Barnes et al., 2009). For these reasons, considerable efforts, such as chemical recycling and incineration, have been developed. However, it was reported that only 18% and 24% of plastic waste are recycled and incinerated, respectively, while the remaining 58% ends up either in landfills or in the natural environment (Geyer et al., 2017). Besides, chemical recycling and incineration are considered not eco-friendly, because of the hazardous products (such as chemical waste or toxic gases) resulted during the process (Gajendiran et al., 2016; Hadad et al., 2005; Okan et al., 2019). As a consequence, the accumulation of plastic waste poses a global threat to natural ecosystems, human health, and sustainability (Prata et al., 2019; Silva et al., 2021).

The more eco-friendly way to reduce plastic accumulation is through biodegradation by microorganisms. Although biodegradation takes several decades depending on the types of polymer plastic (Gewert et al., 2015), it seems to be a

promising option, not only because it is pollutant-free, but also low-cost (Andrady, 2011; Shah et al., 2008; Webb et al., 2012; Zheng et al., 2005). Biodegradation, either through aerobic or anaerobic (Datta et al., 1998; Müller, 2005; Seymour, 1989), consists of four consecutive steps: (i) attachment of microorganisms to the plastic surface, (ii) secretion of extracellular enzymes to increase the hydrophobicity, (iii) degradation of high molecular weight polymer plastic to low molecular weight, (iv) absorption and hydrolysis of low-molecular weight molecules (Alshehrei, 2017). Numerous microorganisms isolated from plastic-contaminated soil, including yeast and bacteria (such as *Bacillus* sp., *Rhodococcus* sp. (Auta et al., 2018), *Pseudomonas aeruginosa* (Jeon and Kim, 2015), *Zalerionmaritimum* (Paço et al., 2017), and *Aspergillus clavatus* (Gajendiran et al., 2016), can efficiently hydrolyse plastic polymer and utilize them as their energy sources in cultivation media (in vitro condition) (26).

Furthermore, a previous study reported that 80% of untreated plastics have contaminated the ocean (Le Guern, 2018), with 77% predicted to be trapped in the coastal intertidal zone like mangrove forest, and the rest of them are floating in the coastal water (Onink et al., 2021). The presence of plastic waste in the mangrove forest or sediment disrupts oxygen penetration within the rhizosphere, creates an anoxic condition, and causes mangrove suffocation (Smith, 2012), leading to a pneumatophore deformation or low-growth (van Bijsterveldt et al., 2021). Despite that, we demonstrated herein that bacteria isolated from plastic-polluted mangrove sediment possess a potential plastic-degrading activity.

2 Materials and Methods

2.1 Plastics preparation

In this study, we used a commercial transparent plastic bag. Plastic was cut into pieces measuring 6×3 cm^2 and submerged in 70% ethanol for 30 minutes. Afterwards, the plastics were sterilized using UV light for 15 minutes. Subsequently, they were air-dried in an incubator at approximately 80°C and further dehydrated using a desiccator for 24 hours. The plastic weight was measured by analytical balance (Shimadzu).

2.2 Preparation of plastic-contaminated mangrove sediment

We obtained mangrove sediment from a plastic-contaminated area in the Wonorejo estuary, located on the east side of Surabaya. The sediment underwent a multilevel filtration process to remove gravel, macro-benthos, and other debris. After this cleaning process, the sediment was rinsed with marine water collected from the same area. For comparison, we also prepared sand following a similar method with the only difference being that the sand was washed using tap water.

2.3 Plastic degradation assay by soil burial method

After filtering the sediment as previously described, we evenly distributed it into a glass incubator until it reached a height of 2 cm. We then placed plastic bags onto the sediment surface. These plastic pieces were subsequently covered with an additional 2 cm layer of mangrove sediment and submerged in marine water to a depth of 1 cm. Four identical sets of plastics were prepared in separate incubators. We opened one incubator at each of the following time intervals: 4, 8, 12, and 16 weeks of incubation and observed the degradation activity. The plastics were collected and equally cut into two pieces. The first piece underwent bacterial cell density measurement, dry weight measurement, reducing sugar measurement, and FTIR observation. The second piece was subjected to observation for biofilm formation and Scanning Electron Microscope (SEM) observations. As a negative control for this experiment, we prepared four additional incubators containing a sandwich configuration of sand-plastic-sand, which were submerged in sterilized H_2O.

2.4 Plastic degradation assay by liquid immersion method

Commercially available transparent plastic bags and Low-density polyethylene (LDPE) (type GF57836537, from Aldrich in Huntingdon, England) were used in this experiment. The plastics were cut into 1×1 cm^2 and rinsed with sterilized ddH_2O. Afterward, they were submerged in 70% ethanol for 30 minutes and air dried in the incubator at approximately 60°C for 24 hours. The dried-plastic weight was measured by analytical balance (Shimadzu). Three pieces of each plastic were placed inside of 50 mL falcon containing 9 mL minimum salt medium (MSM) and 1 mL bacterial culture. The falcon was then incubated at 37°C with continuous rotary shaking (120 rpm). The experiments were repeated three times for each plastic.

2.5 Bacterial cell density measurement

The bacterial growth (OD_{600}) was regularly observed after 4, 8, 12, and 16 weeks of incubation. Plastics were homogenized in 10 mL of MSM medium by vigorous shaking for 5 minutes and followed by centrifugation at 5000 rpm for 15 minutes (Sekhar et al., 2016). While the cell suspension was subjected to OD_{600} detection and reducing sugar measurement, the plastics were subjected to dry weight measurement, and FTIR.

2.6 Plastic dry weight loss measurement

The treated plastics were rinsed using 70% ethanol for 30 minutes and air-dried in an incubator at approximately 80°C and further dehydrated using a desiccator for 24 hours. The

plastic weight was measured by analytical balance (Shimadzu). The percentage of plastic dry weight loss was used to evaluate the degradation activity. Below is the equation to calculate the percentage of dry weight loss (Phetwarotai et al., 2012):

Dry weight loss = (initial weight - final weight) / initial weight $\times$ 100%

2.7 Fourier transform infrared (FTIR) analysis

The dried plastics were cut into 1×1 cm^2 and continued to the FTIR analysis using Thermo Scientific Nicolet iS50 in the material characterization laboratory at the Material and Metallurgical Engineering, ITS.

2.8 Scanning Electron Microscope (SEM)

To detect morphological changes on the plastic surface during the degradation process, plastics underwent a stepwise washing process including distilled water, 2% sodium dodecyl sulfate, and distilled water again, to remove the remaining medium and the attached biofilm from the plastic surface. The plastics were then coated with gold using SC7620 sputter coated (EMITECH, Dubai, UAE) at 18 mA and 8 mbar before being visualized using SEM Evo Ma 10 (Carl Zeiss) (Sekhar et al., 2016).

2.9 Reducing sugar detection

Exopolysaccharide, one of the compounds in biofilm formation, was detected by the presence of reducing sugar. A 1.5 ml supernatant, from the previously mentioned method, was aliquoted and moved to a new Beaker glass containing 2 ml of 2N NaOH. The mixture was then incubated in a water bath at approximately 80°C for 15 minutes. 0.5 ml aliquots of the mixture were taken and mixed with 0.5 ml DNS (3,5-dinitro salicylic acid) solution. The exopolysaccharide concentration was detected using a spectrophotometer at 600 nm (Ochoa et al., 2013). The absorbance was then converted into units (mg/mL) by using a glucose standard curve.

2.10 Biofilm observation

The treated plastics were placed between two object glasses for a moment with some pressure. Afterward, the two glasses were gently separated from the plastics. The glass sides, which touch the plastic's surfaces containing biofilm, were placed upward and fixed using Bunsen for 2 minutes then stained by methylene blue for a minute. The stained glasses were then rinsed with H_2O and air-dried. The glass side containing biofilm was then covered by cover glass followed by the addition of oil immersion. Afterward, the biofilm formation was observed under a microscope with a magnification of 1000$\times$.

2.11 Genomic DNA isolation from a single bacterial colony

One gram of mangrove sediment was dissolved in sterilized ddH_2O. 1 ml of the suspension was aliquoted and poured onto the nutrient agar (NA) plate. The plate was then incubated at 37°C for 12 hours. The resultant colonies were inoculated onto a new NA plate by streaking method and incubated at 37°C for 24 hours. This step was repeated till a single pure colony was obtained. Afterward, 10 pure colonies were picked and cultured into a liquid medium. The culture was incubated at 37°C for 24 hours. The 10 bacterial cultures were then precipitated in different Eppendorf through centrifugation with 13000 rpm for a minute. The cell pellet was then proceeded to DNA isolation by following the Promega Kit Protocol. The DNA concentration was detected using Nanodrop at 260/280 nm and the remaining DNA was stored at -20°C.

2.12 Amplification of *16S rRNA* Gene and Sequencing

Genomic DNA isolated from 10 different colonies were tested for their *16S rRNA* gene via polymerase chain reaction (PCR) using a set of primers, which are 27F (5'AGAGTTTGATCCTGGCTCAG 3') and 1525R (5'AAGGAGGTGWTCCARCC 3'). The PCR mixture contained 1 μl template, 2.5 μl forward primer (100 pmol), 2.5 μl reverse primer (100 pmol), 1.5 μl dNTPs, 0.5 μl Q5 polymerase and 42 μl ddH_2O. The PCR products were resolved by 0.8% agarose gel, stained by Ethidium Bromide (EtBr), and observed under UV transilluminator. The desired amplicon was then purified by NucleoSpin® Gel and PCR Clean-up of Macherey-Nagel. The purified DNA was sent out for *16S rRNA* sequencing at Cosmo Genetech Co., Ltd, South Korea.

2.13 Enzymatic activity assay

2.13.1 Lipase activity

The bacterial culture was inoculated onto plate medium, which contained 10 g/L peptone, 5 g/L NaCl, 0.1 g/L $CaCl_2 \cdot 5H_2O$, 70 g/L $MgSO_4 \cdot 7H_2O$, 70 g/L KH_2PO_4, 10 g/L Tween 80 and 15 g/L agar, and incubated at 37°C for 48 hours. The lipolytic activity was indicated by the formation of a precipitation zone around the bacterial colony (Kumar et al., 2012).

2.13.2 Alkane hydroxylase activity

Basal salt medium (BSM) plate (3.815 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.825 g/L $(NH_4)_2HPO_4$, 1.2625 g/L KNO_3, 0.2 g/L Na_2SO_4, 0.02 g/L $CaCl_2$, 0.002 g/L $FeCl_3$, and 0.02 g/L $MgCl_2$) was used in this assay. The plate was coated with 500 mg/mL sterile alkane-hexane solutions. The bacterial culture was streaked onto the pre-coated BSM plate and

incubated at 37°C for 48 hours. The resultant colonies on the plate indicated that the bacteria can degrade n-hexadecane. Note that the n-hexane (solvent used for alkane dilution) was neither toxic to the strain nor was it utilized by the strain (Liu et al., 2014).

2.13.3 Ligninolytic enzymes activity

A 10 μl of 24-hour bacteria culture was spread onto an LB plate containing methylene blue (with a concentration of 0.25 g/L) and incubated at 30°C for 72 hours (Bandounas et al., 2011). Growing colonies surrounded by a clear zone were indicated as positive results of ligninolytic activity.

3 Results

3.1 A consortium of indigenous bacteria successfully degrades commercial plastic bag

To investigate whether indigenous bacteria from plastic-contaminated mangrove sediment can degrade plastic, we buried a commercially available plastic bag in the polluted mangrove sediment for 16 weeks. Our results showed that indigenous bacteria formed a biofilm on the plastic surface, with bacterial density reaching an OD_{600} of up to 1.5 (Figure 1A). Along with the bacterial growth, the exopolysaccharide content on the plastic surface increased 3-fold over time, reaching a final exopolysaccharide content of 1.5 mg/ml (Figure 1B). This may indicate that bacteria already secreted exopolysaccharide to form biofilm on the plastic surface before degrading it. In contrast, the control experiment (with plastic buried in a sandy beach) demonstrated that bacterial growth was 2-fold lower, with only minimal exopolysaccharide detected.

Further observation of biofilm formation, representing the bacterial density, was shown in Figure 1C. The biofilm formation was improved over time from 4 weeks to 16 weeks. Over the 16-week incubation period, the dry weight of the plastic was decreased by up to 12% (Figure 2B), suggesting that the plastics underwent biodegradation by indigenous bacteria. Moreover, the conformation of plastic polymer, as demonstrated by FTIR transmittance (within the wavelength range of 1200 - 850 cm^{-1} and 700 - 400 cm^{-1}), was also reduced over time (Figure 2B), laying further emphasis that the chemical properties of degraded has been changed.

3.2 Bacillus majorly dominates the indigenous consortium

To identify the composition of the indigenous consortium, we characterized ten pure colonies from polluted mangrove sediment using several biochemical tests following Bergey's manual of determinative bacteriology. As shown in Table 1, nine out of ten were identified as Bacillus albeit with different

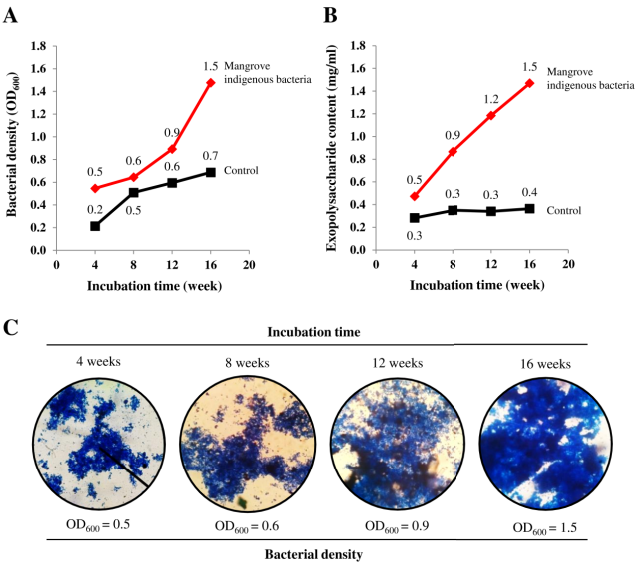

Figure 1. The growth of indigenous bacteria on plastic surface. (A) Bacterial growth and (B) exopolysaccharide production on plastic surfaces incubated with and without indigenous bacteria from plastic-contaminated mangrove sediment are colored in red and black, respectively. (C) Biofilm formation, which corresponds to bacterial growth, on the plastic surface after 4, 8, 12, and 16 weeks of incubations.

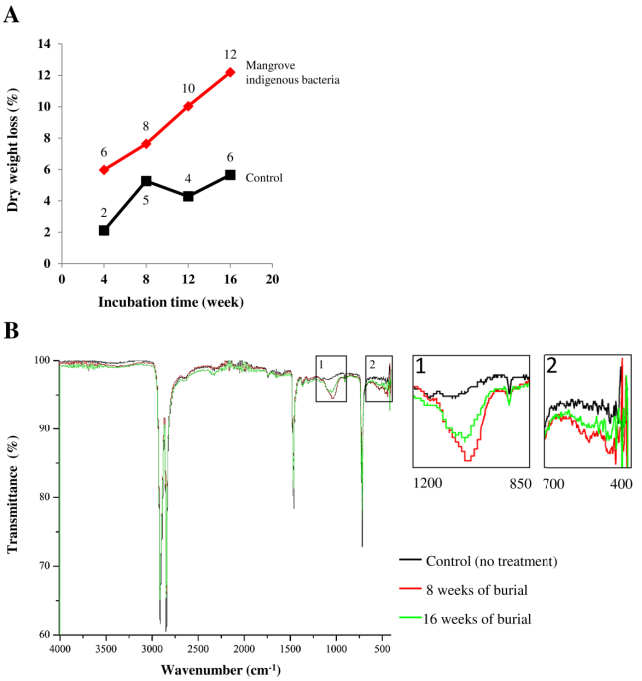

Figure 2. Commercial plastic bag degradation by indigenous bacteria isolated from plastic-contaminated mangrove sediment. (A) Dry weight loss percentage of plastic incubated with and without indigenous bacteria from plastic-contaminated mangrove sediment are colored in red and black, respectively. (B) FTIR analysis of plastic before, after 8 weeks, and after 16 weeks of incubation with bacteria from plastic-contaminated mangrove sediment colored in black, red, and green, respectively.

Table 1. Biochemical characteristics of bacteria isolated from contaminated mangrove sediment

Characteristics/ Bacterial test	Bacterial isolate									
	BIO-A	BIO-B	BIO-C	BIO-D	BIO-E	BIO-F	BIO-G	SOI-A	SOI-B	SOI-C
Gram staining	+	+	+	+	+	+	-	+	+	+
Endospore	+	+	+	+	+	+	-	+	+	+
Oxygen demand*	FA	OA	OA	OA	OA	OA	FA	M	FA	OA
Oxidase	-	+	+	-	+	-	+	-	-	+
Glucose fermentation	+	-	-	-	+	-	-	+	+	-
Catalase	+	-	-	+	+	-	+	+	+	+
Motility	+	+	+	+	+	-	+	-	+	+
Genus (Bergey's)	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Pseudomonas	Bacillus	Bacillus	Bacillus

Note: *FA : facultative aerobes, OA : obligate aerobes, M : microaerophile.

Table 2. BLAST sequence results of 16S rRNA gene of isolated bacteria

Bacterial isolate	Bacterial species from the BLAST search	Similarity (%)	No Accession
BIO-A	*Bacillus cereu* s ATCC 14579	99	NR_074540.1
BIO-B	*Brevibacillus formosus* strain NBRC 15716	99	NR_113801.1
BIO-C	*Bacillus vietnamensis* strain 15-1	99	NR_024808.1
BIO-D	*Bacillus drentensis* strain LMG 21831	99	NR_118438.1
BIO-E	*Bacillus stratophericus* strain 41K2a	100	NR_042336.1
BIO-F	*Bacillus enclensis* strain SGD-1123	100	NR_133700.1
BIO-G	*Stenotrophomonas maltophilia* strain IAM 12423	99	NR_118008.1
SOI-A	*Bacillus pseudomicoides* strain NBRC 101232	99	NR_113991.1
SOI-B	*Bacillus cereu* s ATCC 14579	100	NR_074540.1
SOI-C	*Lysinibacillus pakistanensis* strain NCCP-54	99	NR_113166.1

characteristics. For example, BIO-A and SOI-B had identical characters, so did BIO-B and BIO-C, whereas the rest of them were different. Only one colony, named BIO-G, was identified as Pseudomonas (Table 1).

To confirm the bacterial species, we conducted 16S rRNA gene sequencing and nucleotide BLAST. The results showed that two out of nine bacillus colonies, named BIO-B and SOI-C, showed 99% similarity to *Brevibacillus formosus* and *Lysinibacillus pakistanensis*, respectively (Table 2). Phylogenetic analysis indeed showed that BIO-B and SOI-C clustered with other bacteria from *Lysinibacillus* and *Brevibacillus* genera, respectively, belonging to gram positive bacteria (Figure 3). As for BIO-G, which was previously identified as Pseudomonas, it shared 99% similarity with *Stenotrophomonas maltophilia* (Table 2). However, phylogenetic analysis showed that it clustered together with *Stenotrophomonas* and *Pseudomonas* genera. Besides, BIO-G belonged to the gram negative bacteria (Figure 3).

3.3 BIO-G displays the highest plastic-degrading activity toward LDPE

Table 3. Enzymatic activities of isolated bacteria

Bacterial isolate	Lipase	Alkane hydroxylase	Ligninolytic
BIO-A	+	+	++
BIO-B	+++	+	+++
BIO-C	+	+	-
BIO-D	+	+	-
BIO-E	+	+	+
BIO-F	+++	+	-
BIO-G	+	+	+
SOI-A	+++	+	++
SOI-B	+	+	+++
SOI-C	++	+	+

Previous studies reported that the presence of several enzymatic activities including lipase (Premraj & Doble, 2005), alkane hydroxylase (Yoon et al., 2012), and ligninolytic enzyme (Bhardwaj et al., 2013) involved in plastic degradation.

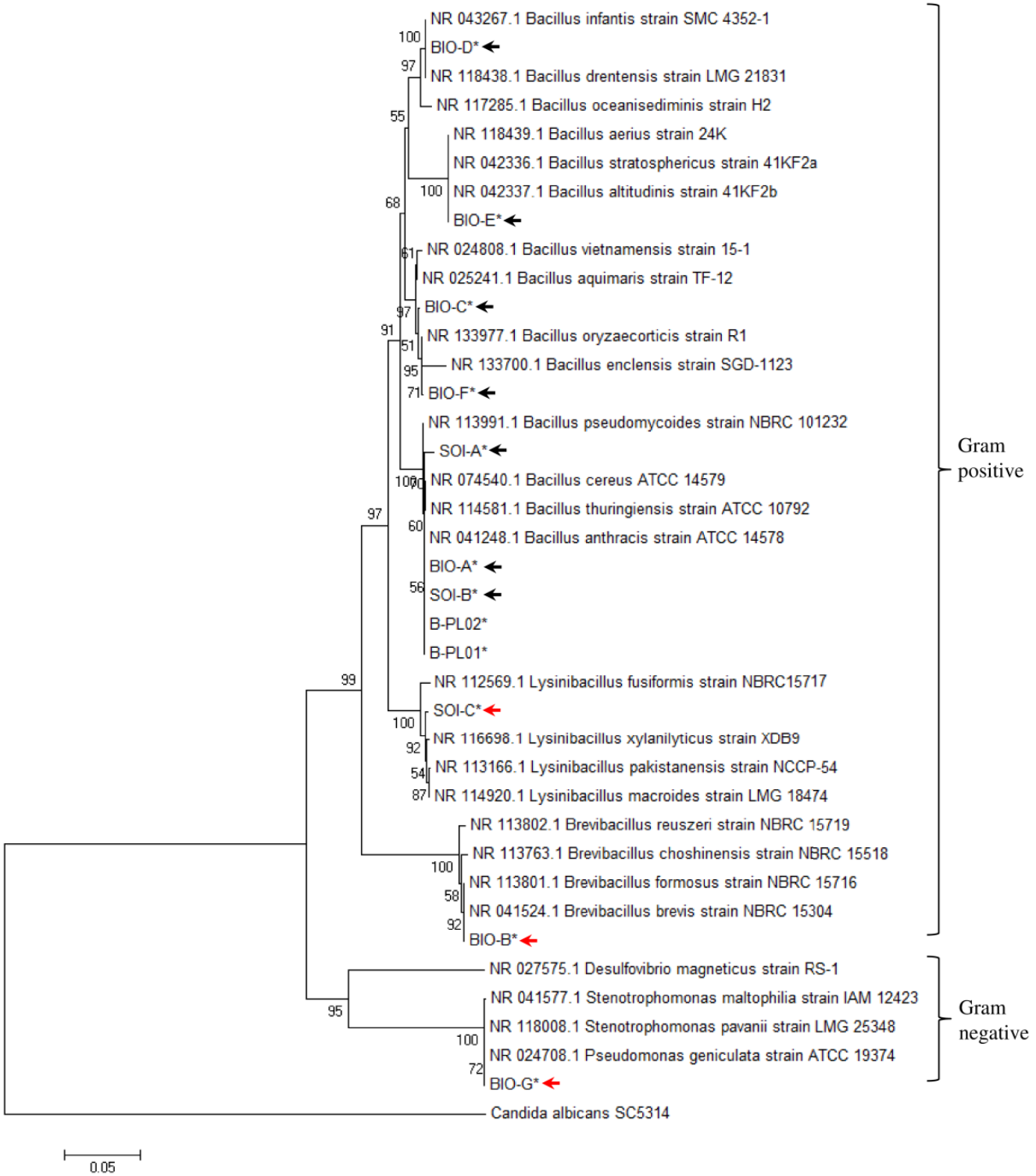

Figure 3. Phylogenetic tree analysis of ten characterized indigenous bacteria. The initial tree was obtained automatically by applying the neighbor-joining model of Kimura's two-parameter algorithm in MEGA 6 with 1,000 replicates of bootstrap analysis. The evolutionary relationship between one and another is represented by scale bar.

Therefore, to examine the plastic-degrading activity of ten isolated and characterized bacteria, we tested their enzymatic activities. As shown in Table 3, all of them exhibited lipase and alkane hydroxylase activities, albeit with varying efficiency in lipase activity. For example, BIO-B, BIO-F, and SOI-A exhibited strong lipase activity. Moreover, seven of them displayed moderate to strong ligninolytic activity, whereas the remaining three showed no such activity.

Two candidates from gram-positive bacteria (BIO-B and SOI-C) were selected for a further plastic degradation test. Although BIO-B and SOI-A exhibited stronger enzyme activity than others. BIO-B and SOI-C were chosen because they belong to *Lysinibacillus* and *Brevibacillus* genera, respectively, which are less studied for their plastic degradation activity, compared to other characterized bacteria belonging to Bacillus genus. In addition to gram-positive bacteria, the only gram-negative characterized bacterium (BIO-G) was also selected for the subsequent assays (Figure 3).

Unlike previous tests using endogenous bacteria, the three characterized bacteria are considered as single isolates, relying on their individual ability to degrade plastic. Therefore, to observe which bacteria exhibit efficient plastic-degrading activity, they were subjected to only 4 weeks of incubation. After 4 weeks of incubation, BIO-B, BIO-G, and SOI-C were observed to plastic degradation from the percentage of plastic dry weight loss by up to 2%, 5%, and 1.5%, respectively. FTIR analysis further revealed chemical changes in the plastic polymer treated by BIO-G within the wavelength range of 1300 - 1000 cm^{-1}. In contrast, plastic treated by BIO-B and SOI-C remained unchanged (Figure 4).

To further examine the BIO-G, two different types of plastic were used including LDPE and commercial plastic bags. As shown in Figure 5, BIO-G was able to degrade LDPE 4-fold better than commercial plastic bags within 4 weeks (with the percentage of plastic dry weight loss up to 8.9% and 2.2% for BIO-G and control, respectively). FTIR analysis indicated that the polymer conformation of LDPE and commercial plastic bag were changed (within the wavelength range of 1300 - 750 cm^{-1} and 700 - 400 cm^{-1}) (Figure 5).

SEM further indicated that the surface of LDPE and commercial plastic bag were changed after 2 and 4 weeks of incubation (Figure 6). These data suggested that indigenous bacteria BIO-G degraded plastic by breaking down the polymer compound of plastic leading to a morphological disruption. SEM analysis further indicated that there are erosions, cavities, and pores formed on a plastic surface.

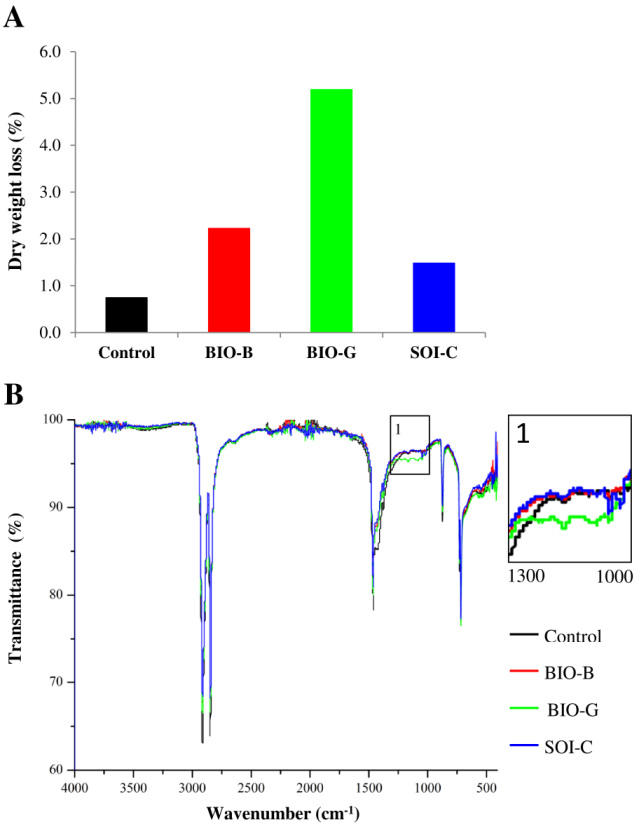

Figure 4. Degradation activity of BIO-B, BIO-G, and SOI-C. (A) Dry weight loss percentage and (B) FTIR analysis of commercial plastic incubated with three chosen-characterized bacteria.

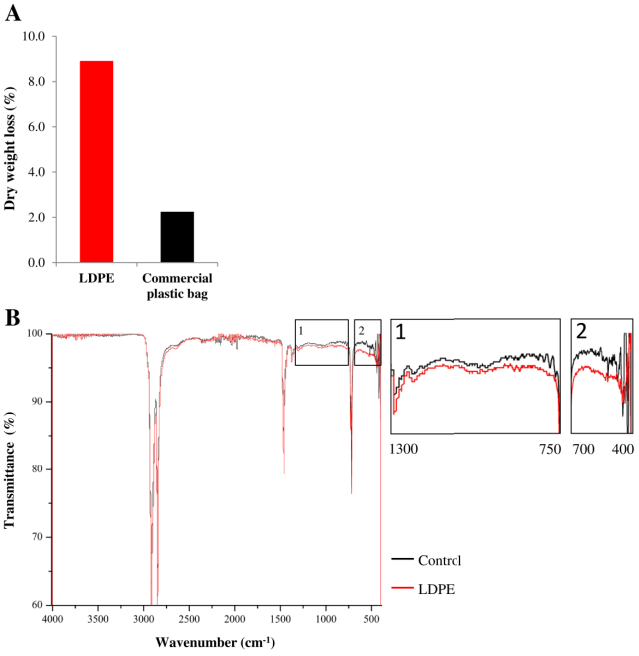

Figure 5. Degradation activity of BIO-G toward LDPE and commercial plastic bags. (A) Dry weight loss percentage and (B) FTIR analysis of LDPE and commercial plastic colored in red and black, respectively.

4 Discussions and Conclusion

4.1 Indigenous bacteria evolve a coping mechanism in plastic-polluted habitats

It is well known that microorganisms are versatile and prevalent, existing everywhere from habitual to extreme environmental conditions, such as high/low temperature (Pérez et

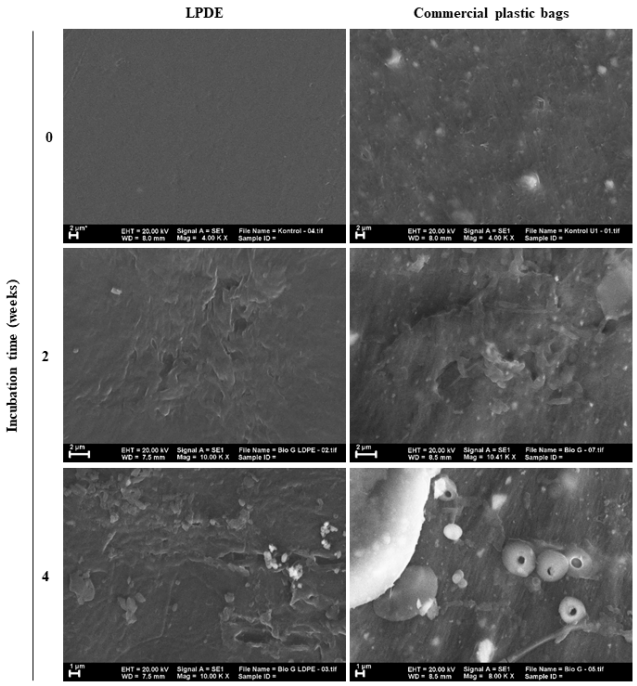

Figure 6. Morphology of plastic surface. SEM results depict the morphological changes occurring on the plastic surface during the biodegradation process.

al., 2006), acidic/basic pH levels, high/low pressure, and high/low salinity (Borroni and Benussi, 2019). Microorganisms can adjust their metabolic pathways under extreme conditions and endure them by forming colonies (Tan et al., 2022). Not only do they adapt to tough environments, but microorganisms also often require these conditions to maintain their survival. This suggests that they have evolved adaptive metabolic pathways different from the norm (Rampelotto, 2013).

Previous studies have reported that microorganisms from heavy-metal-contaminated areas contain metal-resistant genes, which express numerous proteins to hydrolyze and utilize heavy metal to survive (Barman et al., 2020). Moreover, studies have revealed that gram-negative and gram-positive bacteria exhibit distinct mechanisms to cope with high organic solvent contamination. While gram-negative bacteria tend to change their morphology to reduce organic solvent accumulation (Heipieper et al., 2003; Shi and Xia, 2003; Vangnai et al., 2002), gram-positive bacteria possess organic solvent-emulsifying or deactivating enzymes (Sarwan et al., 2020; Sardessai and Bhosle, 2003).

As previously mentioned, plastics have become a major threat to the environment (Prata et al., 2019). To thrive in such conditions, bacteria from sewage wastewater, for example, hydrolyze polymer plastic, reducing plastic dry weight by up to 25% (Ali et al., 2023). Our result also indicated that bacteria from plastic-contaminated mangrove sediment exhibit potential plastic-degrading activity. The activity was observed from the plastic dry weight loss, changes in polymer formation (Figure 2), and the activity of plastic-degrading enzymes (Table 3), all of which enhance the solubility of polymer plastic for easy degradation (Alshehrei, 2017).

The activity of plastic-degrading enzymes (such as cutinase, lipases, protease, esterases, laccase, peroxidases, and pro-oxidant ions) has gained increased attention due to their eco-friendly role in plastic degradation. Furthermore, numerous studies have demonstrated that indigenous bacteria isolated from seawater, waste treatment areas, biofilm on marine plastic, and landfills possessed plastic-degrading enzymes (Bhardwaj et al., 2013; Pinnell and Turner, 2019; Popovic et al., 2017; Tchigvintsev et al., 2015). The indigenous bacteria from various contaminated habitats may have evolved the capability to degrade and utilize plastic as their carbon source.

4.2 Biodegradation disrupts both the chemical and morphological conformation of plastic

One of the suitable methods to analyze polymer plastic is the Fourier Transform Infrared (FTIR) Spectroscopy (Castaldi et al., 2005). It traces chemical changes, confirms the presence of plastic-degrading bacteria, and observes degradation activity (Masó et al., 2016). The presence of C-H bonds and the appearance of new bands such as C=O, C-O, O-H, O-C=O, and C=C indicate that the polymer plastic undergoes hydrolysis (Barbeş et al., 2014; da Luz et al., 2013; Hou et al., 2019). All treated plastic presented C-H stretching peak at around 2800-3000 cm^{-1} (Nishikida and Coates, 2003), CH_2 bending and ricking peaks at around 1400-1500 cm^{-1} (Coates, 2000), CH_3 and CH_2 rocking are at around 800-900 cm^{-1}, and 700-750 cm^{-1} (Mecozzi et al., 2016), respectively. These four peaks can be found in almost any plastic polymer (Veerasingam et al., 2020). Interestingly, some peaks were only detected in treated plastic, which lays further emphasis that plastic conformation has changed due to microbial activity during certain incubation time (Figures 2, 4, and 5). These peaks include C-O stretching peak at 1000-1300 cm^{-1}, as well as peaks at around 500-700 cm^{-1} denoting C-H bending, CH_2 rocking, C=O bending, C-Cl stretching, aromatic C-H out-of-plane bending, aromatic ring out-of-plane bending, N-H bending, CF_2 bending, and C-C-F bending (Jung et al., 2018). However, the transmittance attenuation percentage observed from the plastics treated with indigenous bacteria was higher than the one treated with BIO-G (Figures 2 and 5). One possibility is that indigenous bacteria consist of many potential plastic-degrading bacteria and BIO-G is one of them. Therefore, it is not surprising that the degradation activity of BIO-G is lower than indigenous bacteria.

Notably, despite producing lower enzymatic activity than BIO-B and SOI-C, BIO-G exhibited higher plastic degradation activity (Figure 4). Since BIO-G forms a thicker biofilm than that of Bacillus (Shovitri et al., 2017), it reduces plastic hydrophobicity, allowing plastic-degrading enzymes

to deeply penetrate and break down the plastic compounds. Moreover, other studies reported similar phenomena, demonstrating that Pseudomonas (such as BIO-G) is more efficient in plastic degradation than Bacillus (such as BIO-B and SOI-C) (Mahdy and Lahmood et al., 2021).

To further examine the degradation activity of BIO-G, SEM analysis revealed that the morphology of the plastic surface has changed after a certain period of incubation. The formation of bacterial colonies and cavities on the treated plastic surface (Figure 6) indicates that the bacteria attach to and break polymer plastic. A previous study reported that during the degradation process, the plastic surface becomes uneven and filled with cracks and grooves (Auta et al., 2017). The cavities and holes become bigger through time indicating the presence of bacterial activity (Sarwan et al., 2020). Taking all the data together, our study suggests that indigenous bacteria isolated from plastic-polluted mangrove sediment in Wonorejo possess a potential plastic degrading activity.

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Conflict of Interest

The authors declare no competing interests.

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