Indigenous plastic-degrading species of *Massilia* and *Brevundimonas* from the Wonorejo Mangrove plastic waste to support sustainable development goal 14: Life below water

Lisa Lisdiana[1]*, *Anggi M.* Arista[1], *Farah A.* Nafidiastri[1], *Guntur* Trimulyono[1], *Mahanani* T. Asri[1], and *Sanjar* Tillayev[2]

[1]Undergraduate Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Kampus Unesa 1, Jl. Ketintang Bld C14, Surabaya 60231, Indonesia
[2]Institute of Biochemistry, Department of Organic Synthesis and Bioorganic Chemistry, Samarkand State University, Uzbekistan

Abstract. Plastic pollution in coastal ecosystems poses a significant threat to aquatic life and biodiversity, directly undermining Sustainable Development Goal (SDG) 14: Life Below Water. This plastic pollution also occurs downstream of the Wonorejo Mangrove, whose function is expanded as a tourist destination. This study aims to identify indigenous bacterial species from the plastic waste plastisphere in the Wonorejo Mangrove, East Java, Indonesia, with the capability to degrade plastic. Plastic degradation potential was screened by assessing the formation of clear zones around colonies during growth. The selected colonies were then identified by using the 16S rRNA gene as a marker. The result showed that two isolates formed clear zones around the colonies, which were then identified as members of the genera *Massilia* and *Brevundimonas*. However, the ability of these two isolates to degrade plastic was assessed only qualitatively in this research. Further research is needed to quantitatively assess the plastic-degrading capabilities of these isolates. By exploring the potential of these indigenous bacteria to reduce plastic waste, this study supports United Nations global initiatives to achieve SDG 3 (Good Health and Well Being), SDG 12 (Responsible Consumption and Production), SDG 14 (Life Below Water), and SDG 15 (Life on Land).

1 Introduction

The use of plastic in daily life has both benefits and disadvantages. While plastic serves as a versatile material for numerous applications, excessive and improper use can significantly contribute to environmental pollution. In recent years, globally, plastic waste generation has reached approximately 6.3 billion tons. This number could double by 2030 if no intervention is taken [1]. This situation certainly opposes the objectives of United Nations Sustainable

---

* Corresponding author: lisalisdiana@unesa.ac.id

Development Goal No. 12 (Responsible Consumption and Production), which emphasizes minimizing waste and reducing pollution.

Extensive plastic consumption and slow degradation rates result in the accumulation of plastic waste in the environment [2], adversely affecting human and other organisms' health. Incomplete degradation of plastics produces microplastics (MPs), which are defined as plastic residues with diameters less than 5 mm [2, 3]. In addition to large plastic debris in aquatic and terrestrial environments, the pervasive contamination by MPs has emerged as a significant concern. As emerging organic pollutants, MPs exert substantial impacts on ecosystems. Their high molecular weight and exceptional durability enable MPs to persist in the environment for centuries [3]. Consequently, ingestion by organisms and subsequent transfer through the food chain pose considerable risks to human health and threaten life on land and water. Thus, plastics and MPs pollution may also hinder the attainment of Sustainable Development Goals (SDGs) No. 3 (Good Health and Well-Being), No. 14 (Life Below Water), and No. 15 (Life on Land) as well [4].

Previous studies identified microplastics in the forms of fragments, films, and granules, with concentrations of approximately 8×10^6 particles/km^2 in water and an average of 1.5×10^9 particles/km^2 in sediment within the Wonorejo Mangrove. The detected microplastics consist of polyethylene, polyvinyl alcohol, polypropylene, polyvinyl chloride, and polystyrene [5]. Immediate intervention is necessary to address this issue. In recent decades, an alternative strategy has been to use plastisphere bacteria. Plastisphere bacteria constitute a community of microorganisms associated with plastic debris [6]. Several plastisphere bacteria are capable of degrading plastic or microplastics (MPs), as demonstrated by their ability to bind to plastic surfaces and form biofilms. Notable species with plastic degradation capabilities include *Bacillus, Nocardioides, Solirubrobacter, Sphingomonas, Streptomyces, Rhodococcus, Corynebacterium, Pseudomonas, Micrococcus*, and *Arthrobacter* [1, 6, 7].

Plastisphere bacteria possess the potential to degrade plastic or MPs. The source of these potential plastisphere bacteria could be plastic waste, such as found in the Wonorejo Mangroves. Accordingly, the present study investigates plastisphere bacteria from plastic waste in the Wonorejo Mangrove estuary and identifies bacterial isolates with potential for plastic waste degradation through preliminary screening.

2 Methods

This research consists of procedures as follows.

2.1 Sampling

Sampling was conducted using a randomized purposive sampling method in the Wonorejo Mangrove. Plastic waste was collected from a depth of approximately 5 cm from the surface of the plastic waste pile in the estuary area. The collected plastic samples were placed in sterile containers and transported to the laboratory for subsequent analysis.

2.2 Preliminary screening of plastic-degrading potential isolates

Plastic waste collected from the Wonorejo Mangrove was thoroughly washed and weighed to 1 gram. The sample was cut into small pieces and placed in 20 ml of MSM broth media containing 2.5 mg/ml PCL, followed by aerobic incubation at 150 rpm for 7 days, room temperature. This procedure was repeated twice. Upon completion of the incubation period, 1 ml of culture was subjected to serial dilution and pour plate with MSM Agar supplemented with PCL. Incubation was continued for 7 days at room temperature, after which colony growth and the formation of clear zones were observed. Colonies that formed clear zones, which indicated their potential as plastic-degrading bacteria [2], were subsequently purified and maintained as stock cultures on NA supplemented with PCL.

2.3 Total DNA isolation

For total DNA isolation, a loopful of the stock culture was inoculated into 3 mL of Luria Bertani broth (Merck), and the culture was incubated aerobically at 200 rpm overnight, room temperature. The total DNA of each isolate was isolated using the Bacteria DNA Preparation - Column Kit (Jena Biosciences cat# PP-214S). One milliliter of the bacterial culture was harvested by centrifuging at 10,000 xg for 1 minute. The supernatant was discarded, and the pellet was processed for DNA isolation according to the manufacturer's manual. The total DNA obtained was assessed for both quantity and quality prior to storage at -20 °C and further amplification procedure.

2.4 16S rRNA gene amplification and sequencing

A PCR protocol comprising 30 cycles was used to amplify the 16S rRNA gene. Each cycle included 30 seconds of denaturation at 96°C, 30 seconds of annealing at 55°C, and 1.5 minutes of extension at 72°C. An initial pre-denaturation step at 96°C for 2 minutes preceded the cycling. Following completion of the cycles, a final extension was conducted at 72°C for 4 minutes. The total PCR reaction volume was 25 µl, containing 1 µl of template DNA, 12.5 µl of 2x PCR master mix, 10.5 µl of nuclease-free water, and 0.5 µl of 10 µM of each universal primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GTTTACCTTGTTACGACTT-3'). Amplicons were analyzed on a 2% agarose gel containing 0.5 mg/ml ethidium bromide and a 1 kb DNA marker. Amplicons meeting quality standards were submitted to 1st Base Malaysia for sequencing analysis.

2.5 Identification of selected isolates

The identification process commenced with contig analysis, which entailed examining the forward and reverse sequences of the 16S rRNA gene using BioEdit. Pairwise alignment of these sequences was conducted to generate a consensus sequence. Subsequently, the 16S rRNA consensus sequences for each isolate were compared to the database (https://www.ncbi.nlm.nih.gov/) using the web-based BLAST2 program (version 2.13.0).

3 Results and discussion

Wonorejo Mangrove's expanded function as an ecotourism destination increases the potential for plastic waste accumulation. Additionally, its location within an estuary also contributes to the deposition of plastic waste due to tidal activity [8]. The pile of plastic waste in the Wonorejo Mangrove area is depicted in **Fig. 1**. Previous studies have indicated the potential of isolates from sediment and water samples in this area as plastic-degrading bacteria [9]. However, the similar potential of plastisphere bacteria associated with plastic waste in this area remains unknown. Recent studies found that bacteria isolated from the plastisphere may exhibit the potential to degrade plastics [2]. Therefore, plastic samples obtained from this pile were used as a source for isolating plastisphere bacteria to assess their potential.

Fig. 1. Sampling location condition at Wonorejo Mangrove estuary

Based on the screening result, two isolates were expected to have the potential as plastic-degrading bacteria, as indicated by the formation of clear zones around their colonies. These isolates, which were coded PID4 and PID5, exhibit the characteristics listed in Table 1. Both isolates have a similar shape, elevation, margin, surface, and gram type, but differ in pigmentation. Isolate PID4 is white-pigmented, while isolate PID5 is yellow-pigmented. These two isolates were then processed for identification based on 16S rRNA gene similarity.

Table 1. Characteristics of potential isolates from plastic waste plastisphere

Isolate Code	Shape	Elevation	Margin	Surface	Pigmentation	Gram type
PID4	circular	flat	entire	smooth	white	negative
PID5	circular	flat	entire	smooth	yellow	negative

The identification of two potential isolates was performed by comparing the 16S rRNA gene sequences to the database (https://www.ncbi.nlm.nih.gov/) using BLAST analysis. The result indicated that isolates obtained from plastic waste plastispheres in the Wonorejo Mangrove exhibited the highest similarity to the genera *Massilia* and *Brevundimonas* (Table 2). Isolate PID4 demonstrated 94.85% similarity to *Massilia suwonensis* strain 5414S-25, while isolate PID5 exhibited 97–98% similarity to members of the genus *Brevundimonas*, with the highest similarity to *Brevundimonas aurantiaca* strain CB-R (98.42%). However, taxonomic boundaries for bacteria based on 16S rRNA gene sequence identities are set at 94.5% for genus and ≥ 98.7% for species [10]. According to these criteria, both isolates can be identified only at the genus level, with isolate PID4 as a member of *Massilia* and isolate PID5 as a member of *Brevundimonas*.

Table 2. Closest relatives of isolate PID4 and PID5 based on their 16S rRNA gene sequence similarity

Isolate Code	Closest strains	Sequence similarity*	Accession no. of the closest strains
PID4	*Massilia suwonensis* strain 5414S-25	94.85%	NR_116872.1
	Massilia haematophila strain CCUG 38318	94.63%	NR_042653.1

Isolate Code	**Closest strains**	**Sequence similarity***	**Accession no. of the closest strains**
	Massilia timonae strain UR/MT95	94.62%	NR_026014.1
	Massilia niastensis strain 5516S-1	94.55%	NR_044570.1
	Massilia chloroacetimidivorans strain TA-C7e	94.47%	NR_149216.1
	Massilia tieshanensis strain TS3	94.47%	NR_117795.1
PID5	*Brevundimonas aurantiaca* strain CB-R	98.42%	NR_028889.1
	Brevundimonas intermedia strain CIP 106444	97.77%	NR_116137.1
	Brevundimonas intermedia strain ATCC 15262	97.67%	NR_041966.1
	Brevundimonas vesicularis strain CIP 101035	97.62%	NR_116136.1
	Brevundimonas huaxiensis strain 090558	97.59%	NR_181184.1
	Brevundimonas vesicularis strain NBRC 12165	97.59%	NR_113586.1
	Brevundimonas vesicularis strain Busing	97.59%	NR_037104.1
	Brevundimonas nasdae strain W1-2B	97.52%	NR_028633.1

The genus *Massilia* comprises Gram-negative, rod-shaped, aerobic bacteria that are non-spore-forming, motile, and possess a relatively high DNA G + C content. Members of this genus have been identified in diverse ecosystems worldwide, including extreme environments. Currently, approximately 66 species have been reported. Certain *Massilia* species contribute to the detoxification of heavy metals and the degradation of organic pollutants, highlighting their potential for bioremediation [11]. Previous studies have also described this genus as a plastisphere bacterium capable of degrading plastic [6, 12]. However, some species within this genus have been reported as pathogenic bacteria [11]. Consequently, a comprehensive characterization is required prior to further application of these species.

Meanwhile, the genus *Brevundimonas* is characterized as Gram-negative, rod-shaped, and aerobic. Members of this genus are widely distributed in various environments, including mangrove ecosystems [13], and are frequently classified as opportunistic bacteria. Previous studies have identified *Brevundimonas* species as plastic-degrading bacteria commonly found on the surface of plastic waste. Additionally, *Brevundimonas* spp. have demonstrated potential as bioremediators of hydrocarbons [13, 14]. However, similar to the genus *Massilia*, certain *Brevundimonas* species exhibit pathogenicity. Thus, comprehensive safety assessments prior to field application are needed to ensure its safety.

In this study, two isolates with potential plastic-degrading capabilities were identified from plastic waste plastispheres in the Wonorejo Mangrove. These isolates belong to the genera *Massilia* and *Brevundimonas*, both of which are recognized as potential bioremediation agents and as pathogens. Thus, further investigation is required to assess both the bioremediation potential and associated risks of these isolates.

4 Conclusion

Two isolates of plastisphere bacteria from Wonorejo Mangrove plastic waste, presumed to possess plastic-degrading potential, are identified as members of the genera *Massilia* and *Brevundimonas*. Both genera have previously been reported to be found in the plastisphere, and several studies have demonstrated their potential as agents for degrading organic pollutants. However, further analysis is required to assess the potential and safety of these indigenous bacteria for field applications. Comprehensive characterization of these bacteria may contribute to achieve the Sustainable Development Goals (SDGs) in the future.

Acknowledgment

The authors thank to LPDP EQUITY for supporting this publication. This publication is funded by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology, and managed under the EQUITY Program (Contract No. 4312/B3/DT.03.08/2025).

References

1. S. Dey, A.K. Rout, B.K. Behera, K. Ghosh, Environ. Microbiome. **17**, 32 (2022)
2. S.P. Singh, P. Sharma, A. Bano, A.K. Nadda, S. Varjani, Green Anal. Chem. **3**, 100030 (2022)
3. D. Zhou, J. Chen, J. Wu, J. Yang, H. Wang, Sustain. Mater. Technol. **28**, e00251 (2021)
4. W. Xuyang, K. Khalil, T. Zhuo, C. Keyan, M.Y.M. Nazir, W.S. Aqma, N.Q. Hong, Sci. Total Environ. **959**, 178105 (2025)
5. A.L. Nafisyah, H.S. Iswandi, A.D. Karisma, D.D. Nindarwi, S.M. Praveena, F. Schneider, IOP Conf. Ser.: Earth Environ. Sci. **1273**, 012059 (2023)
6. G.P.F. Macan, M. Anguita-Maeso, C. Olivares-García, Q.N.P. Le, C. Halsall, B.B. Landa, Appl. Soil Ecol. **206,** 105900 (2025)
7. N. Tiwari, M. Bansal, D. Santhiya, J.G. Sharma, Arch. Microbiol. **204,** 216 (2022)
8. S.B. Kurniawan, M. F. Imron, Environ. Technol. Innov. **15,** 100420 (2019)
9. L. Lisdiana, G. Trimulyono, A. Amaliah, Rifda, F.A. Nafidiastri, E3S Web of Conf. **513**, 03010 (2024)
10. P. Yarza, P. Yilmaz, E. Pruesse, F.O. Glöckner, W. Ludwig, K.H. Schleifer, W.B. Whitman, J. Euzéby, R. Amann, R. Rosselló-Móra, Nat. Rev. Microbiol. **12**, 9 (2014)
11. K. Amirhosseini, M. Alizadeh, H. Azarbad. Microb, Biotechnol. **18**, e70156 (2025)
12. T. Ran, H. Liao, Y. Zhao, J. Li, Ecotoxicol. Environ. Saf. **270,** 115946 (2024)
13. P. Singh, A. Nagarajan, K.O. Chua, A.S.Y. Ting, Bioremediation J. **28**, 4 (2023)
14. J. Koh, S. Bairoliya, M. Salta, Z.T. Cho, J. Fong, M.L. Neo, S. Cragg, B. Cao, Environ. Int. **179**, 108153 (2023)