

Isolation and Identification of Bacterial Isolate Associated with the Plastisphere from Plastic Waste at the Sumompo Landfill, Manado, Indonesia

Erminda Halawa*, Feky Recky Mantiri, Trina Ekawati Tallei

Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Kampus Bahu Street, Manado 95115, North Sulawesi, Indonesia, Telp./Fax. (0431) 864386/85371.

*email correspondence: ermindahalawa@gmail.com

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Abstract. Plastic waste accumulation has become a major environmental concern, particularly in landfill ecosystems where plastic surfaces serve as habitats for microbial communities known as the plastisphere. This study aimed to isolate and identify bacterial isolate associated with the plastisphere associated with plastic waste collected from the Sumompo Landfill, Manado City, Indonesia. Plastic bag (SPK) and plastic bottle (SPB) samples were processed using serial dilution and spread plate techniques on Nutrient Agar (NA), followed by bacterial purification and molecular identification based on 16S rRNA gene analysis. A pure bacterial isolate was successfully obtained only from the plastic bag sample, whereas the plastic bottle sample failed to yield a representative isolate due to contamination. PCR amplification produced a 16S rRNA gene fragment of approximately 1400–1500 bp. After sequence trimming, a 989 bp sequence showed 99.72% similarity to *Bacillus cereus* strain ATCC 14579 using BLAST analysis. Phylogenetic analysis with the Neighbor-Joining method confirmed its placement within the *Bacillus cereus* clade with 100% bootstrap support. These findings identify *Bacillus cereus* as a member of the plastisphere bacterium in the Sumompo Landfill and provide baseline data for future investigations of landfill plastisphere microbial diversity.

Key words: plastisphere; plastic waste; landfill; *Bacillus cereus*; 16S rRNA

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INTRODUCTION

Plastic pollution has become one of the most pressing environmental challenges worldwide due to the extensive production and consumption of synthetic polymers. The durability of plastic materials, resulting from their resistance to natural degradation processes, has led to the continuous accumulation of plastic waste in terrestrial and aquatic ecosystems. This accumulation poses significant ecological risks, including habitat alteration, biodiversity loss, disruption of ecosystem functions, and potential impacts on human health (Geyer et al., 2017). Indonesia is among the countries facing serious challenges in plastic waste management, where increasing urbanization, population growth, and consumption patterns contribute substantially to the generation of plastic waste that is predominantly disposed of in landfills (Jambeck et al., 2015).

One of the major landfill sites in North Sulawesi is the Sumompo Landfill, Manado City, which has operated since 1972 and receives approximately 350–400 tons of municipal solid waste per day (Polii et al., 2021). The long-term accumulation of plastic waste under tropical climatic conditions, characterized by high temperature, humidity, and rainfall, provides favorable conditions for microbial colonization and biofilm development on plastic surfaces. These plastic-associated microbial communities, collectively known as the plastisphere, consist of bacteria, fungi, algae, and other microorganisms that colonize plastic surfaces and form complex biofilm communities under environmental conditions (Zettler et al., 2013; Amaral-Zettler et al., 2020).



Previous studies have demonstrated that plastsphere microbial communities differ considerably among ecosystems because they are influenced by environmental conditions, polymer type, waste age, and local microbial diversity (Oberbeckmann et al., 2018). Most plastsphere research has been conducted in marine environments, freshwater ecosystems, or controlled laboratory experiments, whereas comparatively fewer studies have investigated plastsphere communities inhabiting tropical landfill ecosystems (Amaral-Zettler et al., 2020). Furthermore, information regarding bacterial isolate associated with the plastsphere associated with plastic waste in Indonesian landfills remains very limited, particularly in North Sulawesi and the Sumompo Landfill. Considering that microbial isolate characteristics is strongly influenced by local environmental characteristics, data obtained from other geographical regions cannot be directly extrapolated to tropical landfill ecosystems in Indonesia. Therefore, studies focusing on indigenous bacterial isolate associated with the plastsphere from this region are needed to improve our understanding of landfill microbial diversity and provide baseline information for future ecological and microbiological investigations.

The identification of plastsphere-associated bacteria requires an integrative approach combining phenotypic and molecular analyses. Morphological and biochemical characterization provides preliminary information on bacterial characteristics, whereas sequencing of the 16S rRNA gene remains one of the most reliable molecular markers for bacterial identification and phylogenetic analysis (Janda & Abbott, 2007; Yarza et al., 2014). Accurate identification is essential for documenting bacterial diversity and understanding the taxonomic composition of plastsphere microbial communities.

Therefore, this study aimed to isolate and identify bacterial isolate associated with the plastsphere associated with plastic waste collected from the Sumompo Landfill, Manado City, Indonesia, using morphological, biochemical, and molecular (16S rRNA gene) analyses (Aslinda & Ahyar, 2016). This study provides baseline information on the diversity of bacterial isolate associated with the plastsphere inhabiting a tropical landfill ecosystem in Indonesia and contributes to the growing body of knowledge on plastsphere microbiology in Southeast Asia.

METHODS

Study Area and Sampling

The study was conducted at the Sumompo Landfill (Tempat Pembuangan Akhir/TPA), located in Bailang Village, Manado City, North Sulawesi, Indonesia (1°30'02"N, 124°50'17"E). Sampling was carried out from August to October 2025 in the daily dumping zone. Two types of plastic waste were collected: plastic bags (Low-Density Polyethylene/LDPE, coded as SPK) and plastic bottles (Polyethylene Terephthalate/PET, coded as SPB). The selected plastic samples exhibited physical characteristics indicating long-term environmental exposure, such as a blackish discoloration, foul odor, dull surface, and brittle texture, which suggest the potential formation of a plastsphere-associated bacterium.

Materials and Equipment

The equipment used in this study included an autoclave, laminar air flow (LAF) cabinet, incubator, light microscope, analytical balance, micropipettes, vortex mixer, petri dishes, Erlenmeyer flasks, test tubes, eppendorf tubes, inoculation loops, spreader glass, object glasses, and standard laboratory glassware. The materials included Nutrient Agar (NA), Nutrient Broth (NB), phosphate buffer, sterile distilled water, 70% alcohol, crystal violet, safranin, Lugol's iodine, Simmons citrate medium, and various biochemical test media. Commercial DNA extraction kits and PCR reagents were also utilized for molecular analysis.

Sample Preparation and Bacterial Isolation

Plastic samples were cut into approximately 2×2 cm pieces and cleaned of adhering soil. The surface of the plastics was scraped using a sterile cutter, and the scrapings were transferred into a tube containing 1 mL of phosphate buffer. The suspension was homogenized by vortexing for 1 minute and subsequently serially diluted from 10^{-1} to 10^{-5} using sterile distilled water following standard microbiological procedures (Atlas & Bartha, 2010; Cappuccino & Welsh, 2017).

For bacterial isolation, 100 μ L of each diluted suspension was inoculated onto Nutrient Agar (NA) plates using the spread plate method. The plates were incubated at 34°C for 24 to 48 hours. Distinct colonies were selected based on their morphology and purified through repeated subculturing using the streak plate method on fresh NA media to obtain pure isolates (Cappuccino & Welsh, 2017). The pure isolates were stored in 20% glycerol at -20°C for further analysis. All glassware was sterilized in an autoclave at 121°C for 15–30 minutes, while heat-sensitive equipment was sterilized using 70% alcohol.

Morphological and Biochemical Characterization

Pure isolates were characterized based on their macroscopic and microscopic properties. Macroscopic observation included the assessment of colony shape, margin, elevation, and color. Microscopic observation was performed using Gram staining to determine cell morphology and Gram reaction, followed by examination under a light microscope at 100 $\times$ magnification. Gram staining was performed to determine bacterial cell morphology and Gram reaction following standard microbiological procedures (Tortora et al., 2022; Cappuccino & Welsh, 2017).

Biochemical tests were conducted to determine the metabolic and enzymatic profiles of the isolates. The tests included citrate utilization, carbohydrate fermentation, sugar utilization, and catalase activity. The isolates were inoculated into specific media and incubated at 34°C for 24–48 hours. Changes in media color or gas production were recorded as indicators of positive enzymatic activity reactions (Tortora et al., 2022; Cappuccino & Welsh, 2017).

Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted from the pure bacterial isolates. The quality and quantity of the extracted DNA were evaluated using a spectrophotometer and agarose gel electrophoresis. The 16S rRNA gene was amplified via Polymerase Chain Reaction (PCR) using universal bacterial primers (27F and 1492R) (Weisburg et al., 1991). The PCR products were visualized on a 0.8% agarose gel and subsequently sent for Sanger sequencing. The obtained nucleotide sequences were trimmed, assembled, and edited using Geneious Prime version 10.1.3 (Kearse et al., 2012).

The sequences were compared against the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) to determine the species identity based on sequence similarity (Altschul et al., 1990). For phylogenetic analysis, reference sequences with the highest percent identity from the BLAST results were downloaded in FASTA format. Multiple sequence alignment was performed using the ClustalW algorithm in BioEdit version 7.2 (Hall, 1999). A phylogenetic tree was constructed using MEGA X (Kumar et al., 2018). software with the Neighbor-Joining (NJ) method based (Saitou & Nei, 1987). on the Kimura 2-parameter model (Kimura, 1980). The statistical support of the tree topology was assessed using bootstrap analysis with 1,000 replicates.

RESULTS AND DISCUSSION

Isolation and Purification of Bacterial Isolate Associated with The Plasticsphere

Plastic waste samples collected from Sumompo Landfill displayed visible signs of prolonged environmental exposure, including dark coloration, surface deterioration, and brittle texture (Rillig *et al.*, 2024). These characteristics suggest long-term colonization by environmental microorganisms and biofilm formation (Ainiyah & Shovitri, 2014). Following serial dilution and cultivation on Nutrient Agar, bacterial growth was observed in both SPK and SPB samples. However, only the SPK sample produced well-separated colonies suitable for purification (Putri, S. (2021). The SPB sample exhibited excessive microbial growth and colony overlaps at all dilution levels, preventing the isolation of individual colonies (Sari et al., 2018; Atlas 2010).

The SPK sample yielded a single representative isolate at dilution 10^{-1} (Figure 1). Colony counts ranged from 10 colonies in the first replicate to three colonies in the third replicate, while higher dilutions showed no detectable growth (Putri, 2021). In contrast, SPB plates were categorized as too numerous to count (TNTC) and unsuitable for further purification. The inability to obtain pure cultures from SPB may be attributed to the presence of highly diverse microbial communities and intense interspecific competition. Furthermore, partial melting of agar media during incubation negatively affected colony separation. Similar difficulties have been reported in environmental samples with high microbial density and heterogeneous community structures (Sari et al., 2018; Atlas 2010).



Figure 1. Pure isolate SPK 10^{-1} obtained through repeated purification on nutrient agar medium.

Morphological Characterization

Macroscopic examination revealed that the SPK isolate formed irregular colonies with undulate margins and raised elevation. Colonies exhibited yellowish-white pigmentation, suggesting adaptation to stressful environmental conditions. Microscopic analysis following Gram staining demonstrated that the isolate consisted of rod-shaped cells and stained purple, indicating a Gram-positive bacterium. The thick peptidoglycan layer characteristic of Gram-positive bacteria enables retention of the crystal violet-iodine complex during decolorization. The observed morphological characteristics correspond closely with members of the genus *Bacillus*, which are commonly isolated from soil and waste-associated environments. Species belonging to this genus are commonly isolated from soil and waste-associated environments because of their physiological adaptability and ability to survive under diverse environmental conditions (Naufal et al., 2021).

Table 1. Macroscopic and Microscopic Characteristics of The Plastisphere Bacterial Isolate

Isolate Code	Form/Shape	Macroscopic		Microscopic		
		Margin	Elevation	Color	Cell Shape	Gram Group
SPK	Irregular	Undulate	Raised	Yellowish-white	<i>Bacillus</i>	Positive

Biochemical Characterization

The SPK isolate exhibited positive reactions in all biochemical assays performed. Positive citrate utilization indicated the ability to metabolize citrate as a sole carbon source. Positive fermentation and sugar utilization tests demonstrated the capacity to metabolize carbohydrates and produce acidic end products. Catalase activity was also detected, indicating the presence of catalase enzymes capable of detoxifying hydrogen peroxide into water and oxygen. This enzymatic capability provides protection against oxidative stress and contributes to bacterial survival in fluctuating landfill environments (Sonkar & Singh, 2020; Tortora et al., 2022).

Table 2. Biochemical Characterization of Bacterial Isolate Associated with The Plastisphere

Isolate Code		Biochemical			
		Citrate utilization	Fermentation	Sugar utilization	Catalase
SPK 10 ⁻¹	+	+	+	+	+

Molecular Identification Based on 16S rRNA Gene Analysis

PCR amplification of the 16S rRNA gene successfully produced a DNA fragment approximately 1,400–1,500 bp in length, corresponding to the expected amplicon size generated by primers 27F and 1492R (Figure 2). Following sequence editing and trimming, a high-quality sequence of 989 bp was obtained. BLAST analysis revealed that the isolate shared 99.72% sequence identity with *B. cereus* strain ATCC 14579 (Figure 3).

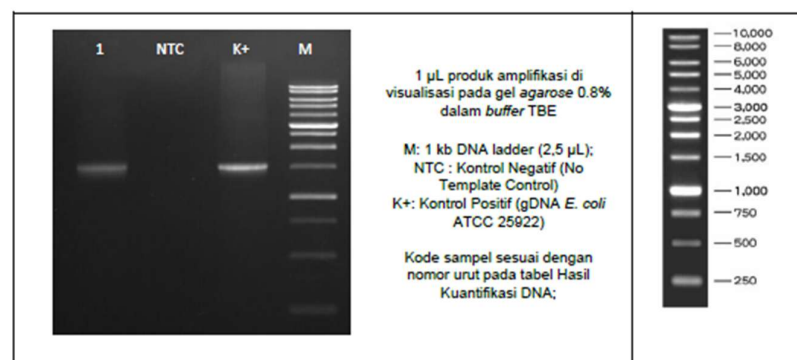


Figure 2. Agarose gel electrophoresis (0.8%) of PCR-amplified 16S rRNA gene fragments from bacterial isolate associated with the plastisphere isolates showing DNA bands of approximately 1500 bp.

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBankGraphicsDistance tree of resultsMSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per ident	Acc Len	Accession
☒ Bacillus cereus strain T3M4 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1341	MG737476.1
☒ Bacillus sp. (in: Firmicutes) strain 5MUR 41 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: Firmicutes)	1807	1807	100%	0.0	99.60%	1209	MK482166.1
☒ Bacillus cereus strain T3M13 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1476	MW550371.1
☒ Bacillus cereus strain OKF61 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1449	KC569074.1
☒ Bacillus cereus strain K1M33 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1464	MW550324.1
☒ Bacillus cereus strain T3M12 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1447	MW550254.1
☒ Bacillus cereus strain 13.1 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1154	GQ756385.1
☒ Bacillus albus strain JB48-AB5 16S ribosomal RNA gene, partial sequence	Bacillus albus	1807	1807	100%	0.0	99.60%	1312	MT378505.1
☒ Bacillus anthracis strain C10 16S ribosomal RNA gene, partial sequence	Bacillus anthracis	1807	1807	100%	0.0	99.60%	1446	KX833689.1
☒ Bacillus paretanthracis strain YRT2 16S ribosomal RNA gene, partial sequence	Bacillus paretanthracis	1807	1807	100%	0.0	99.60%	1451	MW455818.1
☒ Bacillus sp. (in: Firmicutes) strain bas201 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: Firmicutes)	1807	1807	100%	0.0	99.60%	1229	FQ721012.1
☒ Bacillus sp. UR2 16S ribosomal RNA gene, partial sequence	Bacillus sp. UR2	1807	1807	100%	0.0	99.60%	1344	JN20269.1
☒ Bacillus cereus strain st2 16S ribosomal RNA gene, partial sequence	Bacillus cereus	1807	1807	100%	0.0	99.60%	1489	MF102134.1

Figure 3. BLAST Result

Phylogenetic Analysis

Phylogenetic reconstruction using the Neighbor-Joining method demonstrated that the SPK isolate clustered tightly with *B. cereus* reference strains obtained from GenBank (Figure 4). The isolate grouped within the *B. cereus* clade and was supported by a bootstrap value of 100%, indicating a highly reliable phylogenetic relationship. The phylogenetic evidence strongly corroborated the molecular identification obtained through BLAST analysis and confirmed the taxonomic placement of the isolate.

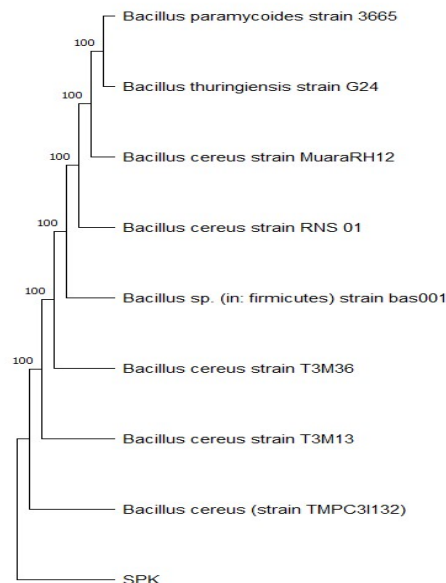


Figure 4. Phylogenetic Tree of SPK Isolate Constructed Using the Neighbor-Joining Method

Correlation Between Phenotypic and Genotypic Characteristics

The identification of the SPK isolate as *B. cereus* was supported by both phenotypic and molecular evidence. Morphological observations revealed a Gram-positive rod-shaped

bacterium, while biochemical tests demonstrated positive citrate utilization, fermentation, sugar metabolism, and catalase activity. These characteristics are widely recognized as typical traits of *B. cereus*. The consistency between phenotypic and genotypic analyses significantly increases the reliability of bacterial identification and minimizes the risk of misclassification.

The present study represents a preliminary investigation of a plastsphere-associated bacterium isolated from plastic waste collected at Sumompo Landfill. Since only one representative bacterial isolate was successfully purified and identified, the findings should be interpreted as baseline information rather than a comprehensive description of Plastsphere bacterial diversity (Amaral-Zettler et al., 2020; Wright et al., 2021). Further studies involving larger sample sizes, multiple plastic types, repeated sampling, and culture-independent approaches are needed to provide a more comprehensive understanding of plastsphere-associated bacteria inhabiting tropical landfill ecosystems.

CONCLUSION

This study successfully isolated and identified a plastsphere-associated bacterium from plastic bag (LDPE) waste collected at the Sumompo Landfill, Manado, Indonesia, using morphological, biochemical, and 16S rRNA gene analyses. The isolate was identified as *B. cereus*, providing baseline information on Plastsphere-associated bacteria inhabiting a tropical landfill ecosystem in Indonesia. Since only one representative isolate was successfully obtained, the present study should be regarded as a preliminary investigation. Further studies involving larger sample sizes, different plastic types, and additional molecular or culture-independent approaches are needed to achieve a more comprehensive understanding of plastsphere-associated bacterial diversity in tropical landfill environments.

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