

## Comparison of Poly Ethylene (PE) Degrading Bacteria of Plastic Biofilm in the North Coast of Semarang Based on in Vitro Tests and Metabarcoding

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### Abstract

Marine bacteria play an important role in maintaining marine ecosystems, including plastic biodegradation by producing bioactive compounds. This study examines the potential of polyethylene (PE) plastic-degrading bacteria found in plastic waste biofilms on the north coast of Semarang. The focus of this study was to identify bacteria that can utilize polyethylene glycol as a carbon source and to determine the diversity of PE plastic-degrading bacteria in various regions to provide insight into how the diversity of culturable plastic-degrading bacterial colonies from the north coast of Java Island compared with metabarcoding analysis. Bushnell Haas Agar selective media enriched with 2% polyethylene glycol as the main carbon source was used to isolate bacteria. Metabarcoding analysis was carried out by DNA extraction, library preparation, and next-generation sequencing using Illumina novaseq6000. DNA extracts were amplified in the V3-V4 hypervariable region. In vitro assays produced four morphologically distinct bacterial colonies from Semarang City. The results of the study obtained four isolates in vitro. Colony morphology observations showed two Gam-negative bacterial colonies and two Gram-positive bacterial colonies. Metabarcoding analysis successfully identified one hundred and four genera of plastic-degrading bacteria on the north coast of Semarang consists of *Pseudomonas* 18%, *Aquabacterium* 15%, *Hydrogenophaga* 9%, and *Rheinheimera* 8%. A comparison of PE-degrading bacteria from the north coast of Semarang based on in vitro testing and metabarcoding demonstrated a complementary alignment in identifying bacteria with potential for PE plastic degradation.

**Keywords :** PE, Pollution, Plastic-Degrading Bacteria, Semarang

### INTRODUCTION

Marine ecosystems are closely related to the terrestrial environment, so shifts in one system can have an impact on another. For decades, a variety of factors, including human activity, have put pressure on coastal ecosystems. These pressures include pollution and physical damage to the environment (Rahman *et al.*, 2023). These pressures include pollution and physical damage to the environment. The accumulation of garbage or waste is one of the serious threats caused by humans to marine and coastal systems. Marine plastic waste enters through land and sea sources, which cover two-thirds of Indonesia's total area. The problem of marine debris pollution has worsened year by year and has had a tremendous impact on marine ecosystems (Jing and Sutikno,

2020). Due to its strong and flexible nature, many industries use plastic (Ghayebzadeh *et al.*, 2020).

The commercial use of plastic in society has a direct impact on increasing plastic waste globally. Plastic pollution in Indonesia has reached 3.22 tons (Jambeck *et al.*, 2015). There is an increasing trend where in 2019 it has reached 9.52 tons of plastic. Indonesia is the second largest contributor of plastic waste in the world. Until 2020, Indonesia ranked second in the world in terms of plastic waste production in the marine environment, with an alarming amount of 187.2 million tons (Jing and Sutikno, 2020). Today, plastic pollution has become a serious problem in almost all parts of the ocean basin around the world, regardless of whether the region is developing or undeveloped (Thushari and Senevirathna, 2020).

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The coastal area of Semarang also experiences a similar thing, Tanjung Mas District is a very strategic coastal area on the north coast of Semarang consisting of ports (53%), ponds (6.5%), industries (4.2%) (Anisyah *et al.*, 2016). This condition has resulted in the growth of slums in Tanjung Mas which reached 37.63 hectares in the Tambak Lorok and Kebonharjo areas (Muvidayanti and Sriyono, 2019). The Tambak Lorok area is the center of a fishermen's settlement located in Semarang City. However, environmental conditions, pollution, the quality of human resources, and uneven poverty levels result in a low quality of life for the people in Tambak Lorok (Nugraha and Dwiyanto, 2023). The social activities of the local community and the disposal of household and factory waste in the Tanjung Mas area are the main factors in marine pollution. In addition, the geographical position of Tambak Lorok, which is the mouth of the Canal Flood River, results in the accumulation of garbage and plastic waste on the coast of Tambak Lorok (Geyer *et al.*, 2017).

The largest plastic pollutants that are dominant are found in Indonesian waters, including the north coast of Semarang, namely PE. Due to its inherent non-biodegradability, PE must be incinerated, landfilled, or recycled using thermochemical and mechanical methods. It will release a surplus of toxic gases into the atmosphere as airborne pollutants which threaten all life, such as bisphenols, phthalates, mercury, and polychlorinated biphenyls. Derivatives of PE consist of High Density PE (HDPE) and Low Density PE (LDPE) which reach 46% of total plastic waste in Indonesian waters (Kyaw *et al.*, 2012; Hariadi *et al.*, 2021). Although the threshold for microplastic pollution in the environment has not been determined, the increase in plastic and microplastic pollution in Semarang waters directly impacts the health of the aquatic ecosystem. Microplastic contamination in marine organisms has been found in coastal fisheries commodities in Semarang City, such as mackerel, shellfish, squid, and shrimp, as well as other fish. Various marine products obtained from the Tambak Lorok Fish Auction Site were found to contain microplastic contamination in the intestines of mackerel and cod fish as many as 15 - 111 microplastic particles (Senduk *et al.*, 2021), in tentacle and squid intestine samples were found as many as 212 - 343 microplastic particles (Makrma *et al.*, 2022).

This condition is dangerous for the quality of life and health of the people on the north coast of Semarang. The solutions taken by the government to overcome microplastic contamination tend to be preventive, so they have not been able to overcome the problem of abundant contamination in the environment. In addition, the uncontrolled amount of various sources of microplastic contamination (household products, comics, and so on that contain microplastics) has resulted in ineffective solutions to the plastic waste problem to date (Yulina *et al.*, 2021). Since 2019, the Semarang City government has launched the development of the Kampung Bahari Tambak Lorok through the *Urban Community Based Tourism* (UCBT) concept approach, as a long-term solution to the community's environmental and economic problems, but the technical feasibility that has not been met and the lack of community participation have made this solution run quite slowly and has not been realized (Rais and Hariyanto, 2021).

The microbial community began to develop on the plastic surfaces one week after deployment in the sea. Biofilm formation by bacteria on plastic surfaces allows them to form colonies and proliferate, thus benefiting biodegradation (Meera *et al.*, 2022; Peng *et al.*, 2023; Zhao *et al.*, 2024; Gulizia *et al.*, 2025). Microbes produce extracellular enzymes and free radicals to degrade plastic polymers (Liu *et al.* 2023; Gambarini *et al.*, 2024). Dense biofilms can form on the surfaces of plastic due to this biomineralization. Less than one percent of bacteria can be isolated using traditional culturing techniques, leaving the vast majority of these microorganisms inaccessible. The emergence of metabarcoding techniques has provided the tools to determine the full extent of the uncultured microbial diversity. Metabarcoding has provided a quickly, comprehensively, and efficiently methods to determine the diversity of microbes. However, metabarcoding information for microbes in marine environments that form biofilm on PE material surfaces in Indonesia is still very limited.

The isolation and identification of bacteria capable of facilitating PE biodegradation through biofilm formation are crucial—whether conducted in vitro or via metabarcoding—to ensure accurate and representative results Anggoro *et al.*, 2024, Wahyuningsih *et al.*, 2025; Kusumaningrum *et al.*, 2026). The development of the potential of plastic biodegradable bacteria is the main solution to help address plastic waste. Microorganisms including bacteria, fungi, and algae have absorption and

metabolic abilities that can adapt to substrates in nature. Bacteria are capable of producing certain enzymes that allow them to utilize persistent plastics as a source of carbon (Gao and Sun, 2021). The condition of plastic pollution and the stability of the increasing number of microplastics in marine waters make marine bacteria have great potential in the development of plastic biodegrading agents. This study aims to compare the potential of plastic-degrading bacteria—obtained from biofilms on plastic surfaces in the waters north of Semarang—using both in vitro methods and metabarcoding, in the Tambak Lorok area of Tanjung Mas Urban Village, Semarang.

## MATERIALS AND METHODS

### Sampling

Bacterial samples were taken from PE plastic waste biofilm on the north coast of Semarang at three sampling points. Environmental factors such as water temperature, pH are measured to see the environmental conditions at the time of sampling. Plastic samples are stored in sample bottles in submerged conditions, environmental conditions during the transfer of samples to the laboratory are maintained. Samples for metabarcoding are stored in sterile vials containing protective RNA Shield from RNA degradation. Samples are labeled, the place and date of sampling.

### DNA Extraction

DNA extraction is carried out using Zymo Fecal/Soil Kits according to the manufacturer's protocol. The stage begins with lysing the sample using the bead-beating method and then adding a DNA binding buffer. Next, the DNA is purified using a Zymo-Spin column and washed to remove DNA impurities. The DNA is eluted in a buffer and then visualized with electrophoresis. The results of the extraction are stored in a refrigerator at a temperature of -20°C for further use.

### Library DNA Preparation

DNA Library was created using the Illumina platform following the manufacturer's protocol. The extracted DNA was amplified using a special primer for the V3-V4 region of the 16S rRNA gene. PCR products are then purified and then indexed and sequenced by sequence fragments using the Illumina platform.

### Data analysis

The DNA sequence data was analyzed using the QIIME2 or Galaxy program software, which is by filtering the raw data to remove low-quality reads and then grouped into operational taxonomic units (OTUs) based on sequence similarity. A series of OTU taxonomy programs are carried out using reference databases to calculate alpha and beta diversity. The data is displayed in the form of bar plots, heat maps, and PCoA plots. Prediction of metabolic pathways and gene functions from DNA metabarcoding data using 16S rRNA amplicon sequencing conducted on predictive metagenomics pipelines.

### Bacterial Growth Media

*Bushnell Haas Agar* (BHA) selective media with the addition of polyethylene glycol (PEG) 4000 as the main carbon source. *Bushnell Haas Agar* contains magnesium sulfate 0.2 g/L, calcium chloride 0.02 g/L, monopotassium phosphate 1 g/L, dipotassium phosphate 1 g/L, ammonium nitrate 1 g/L, agar 20 g/L, ferric chloride 0.05 g/L, which is suitable as a growth medium for saline bacteria with an average pH of  $7.0 \pm 0.2$ .

### Bacterial Isolation

Bacterial biofilm samples from plastic surfaces were dissolved in 0.9 mL of sterile aqueducts which were then homogenized with vortexes. Bacterial samples were then grown on *Bushnell Haas Agar* (BHA) Media using the *spread plate method*. The bacterial isolates were then incubated at room temperature for 7 days to obtain bacterial isolates that were able to survive in minimal nutritional conditions with an abundant source of polyethylene carbon.

### Identification of Bacterial Morphology

Identification of bacterial morphology was carried out by Gram Staining Test and microscopic observation with 1000x magnification. Furthermore, observations were made of colony color, colony shape, colony texture, colony surface, colony edge, and elevation.

## RESULTS AND DISCUSSION

The results of in vitro tests of bacteria from the north coast of Semarang obtained four bacterial isolates from the north coast of Semarang that have the potential to degrade PE. The isolates obtained have different morphological characteristics of the colony as shown in Table 1. The four bacterial

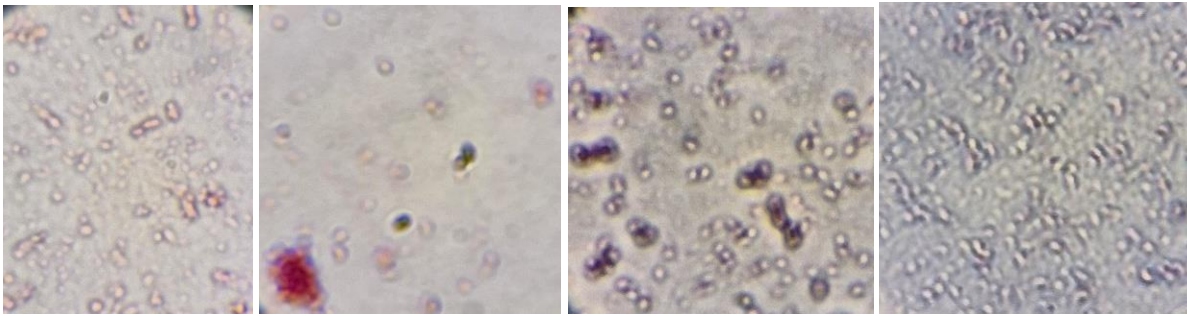
isolates were cultured in Bushnell Haas Agar (BHA) media with 2% PEG (*polyethylene glycol*). Bushnell Haas Agar (BHA) media is a bacterial growth medium without carbon source content that is ideal for growing bacteria potential for the degradation of special substrates including polyethylene (Viel *et al.*, 2023). The use of PEG as the only carbon source supports the degradation of plastic polymers as the primary substrate (Habib *et al.*, 2020). Bacteria that are able to grow on the selective medium of Zobel Marine and Bushnell Haas enriched by a special *substrate of polyethylene glycol* show the potential for *polyethylene* (PE) biodegradation (Mohanrasu *et al.*, 2018; Ozyurek, 2024). Plastic waste in the ocean hosts a unique community of bacteria known as a *plastisphere* that is different from the surrounding bacterial community. Comparative morphological analysis showed similarity in the shape of plastic degrading bacterial colonies in the marine waters of Semarang City with other areas. Groups of bacteria commonly found in marine plastics that have been successfully grown in the laboratory by other researchers include Rhodobacteraceae, Flavobacteriaceae, Erythrobacteraceae, and Cyanobacteria (Roager and Sonnenschein, 2019; Azizi *et al.*, 2023). The family Erythrobacteraceae have pleomorphic and Gram-negative stem-shaped or cocoid cells. The Rhodobacteraceae family consists of bacterial cells

that have short to oval stem cell forms and are Gram-negative. The Flavobacteriaceae family has most of the cells in the form of short rods and some in the form of filament rods where the results of Gram painting are negative. Cyanobacteria are a group of bacteria that have a single cell in the shape of a spheris, ovoid, or cylindrical and Gram-negative (Roager and Sonnenschein, 2019).

Based on this study (Table 1), the bacterial isolates obtained have several similarities that indicate the presence of degrading bacteria with the ability to colonize plastics in the North Coastal Waters of Java Island. Marine bacterial species that have the potential to degrade various types of plastics come from both Gram-positive and Gram-negative bacteria consisting of the genus *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Bacillus*, and *Pseudomonas* spp. *Ideonella*, *Klebsiella*, *Streptomyces*, *Mycobacterium*, *Flavobacterium*, *Rhodococcus*, *Escherichia*, *Azotobacter*. and *Kocuria* (Naveed and Mehmood, 2022; Lv *et al.*, 2024). This is in accordance with the results of the study where the bacteria grown consist of Gram-positive and Gram-negative bacteria. The microscopic characteristics of the four isolates generally show the shape of the cell in the form of bacilli. The basic morphological traits of microbial isolates generally align with the dominant bacterial groups detected in the metabarcoding data.

**Table 1.** Results of morphological and staining observations of Gram isolates of PE degrading bacteria

| Species | Colony Shape | Edges Colony | Colony surface | Color Colony | Gram Staining | Cell Shape |
|---------|--------------|--------------|----------------|--------------|---------------|------------|
| S1      | irregular    | undulate     | circular       | cream        | (-)           | bacilli    |
| S2      | circular     | entire       | circular       | white        | (-)           | bacilli    |
| S3      | circular     | lobate       | convex         | white        | (+)           | bacilli    |
| S4      | circular     | entire       | convex         | white        | (+)           | bacilli    |



**Figure 1.** Bacterial isolates from plastic pollutants

### Metabarcoding test results

The metabarcoding data show the best results at the genus level, but some data on bacterial species were also obtained. The results of the metabarcoding test obtained about 1650 genera from places containing a lot of plastic waste. The results of the metabarcoding test obtained and compared with the results of the *in vitro* test are shown in Table 2. The genera that are aligned with *in vitro* tests are 104 genera, most of which are dominated by Gram-negative bacteria and only a few are Gram-positive, namely *Paenibacillus*, *Flectobacillus*, *Limosilactobacillus*, *Roseibacillus*, *Deinococcus*, *Macellibacteroides*, *Ilumatobacter*, *Gordonia* and *Kocuria*. The results of the metabarcoding test obtained more and varied groups of bacteria. By comparing the usual group data obtained by the researchers using the *in vitro* test and metabarcoding test in Table 2., it can be seen that 95 genera of Gram-negative bacteria are found in plastic-contaminated waters. This is because most Gram-negative bacteria are species that have the ability to degrade plastics effectively and efficiently. The phylum of these bacteria were Proteobacteria, Actinobacteriota, Bacteroidota, Firmicutes, Campylobacterota, Deinococcota, and Nitrospirota.

The data in Figure 3 using metabarcoding analysis shows the ten highest genera in the sample with a percentage, nine of which are Gram-negative bacteria. In this case, *Pseudomonas* spp. has the highest ability in biodegradation of natural and synthetic polyethylene plastics through the formation of viscous and flocculent biofilms on the surface of plastics over a considerable period of time (Orr *et al.*, 2004; Nanda *et al.*, 2010; Tribedi *et al.*, 2012; Usha *et al.*, 2011; Thakur *et al.*, 2022).

Plastic biodegradation occurs through the enzymatic action of converting plastics into metabolic products such as methane, carbon dioxide, water, etc. The degradation of plastics that occurs depends on the availability of nutrients, surface area, temperature, molecular weight, salinity, hydrophilicity or hydrophobicity, crystallinity, functional groups, chemical structure, etc. (Mohan and Srivastava, 2010).

The characterization study in the genus *Aquabacterium* discovered by Kalmbach *et al.* (1999) showed the morphology of rod-shaped cells with Gram-negative staining results and were motile. Although it tends to form cell aggregates, this bacteria actually tends to be a single cell and form a creamy-white flattened colony with smooth

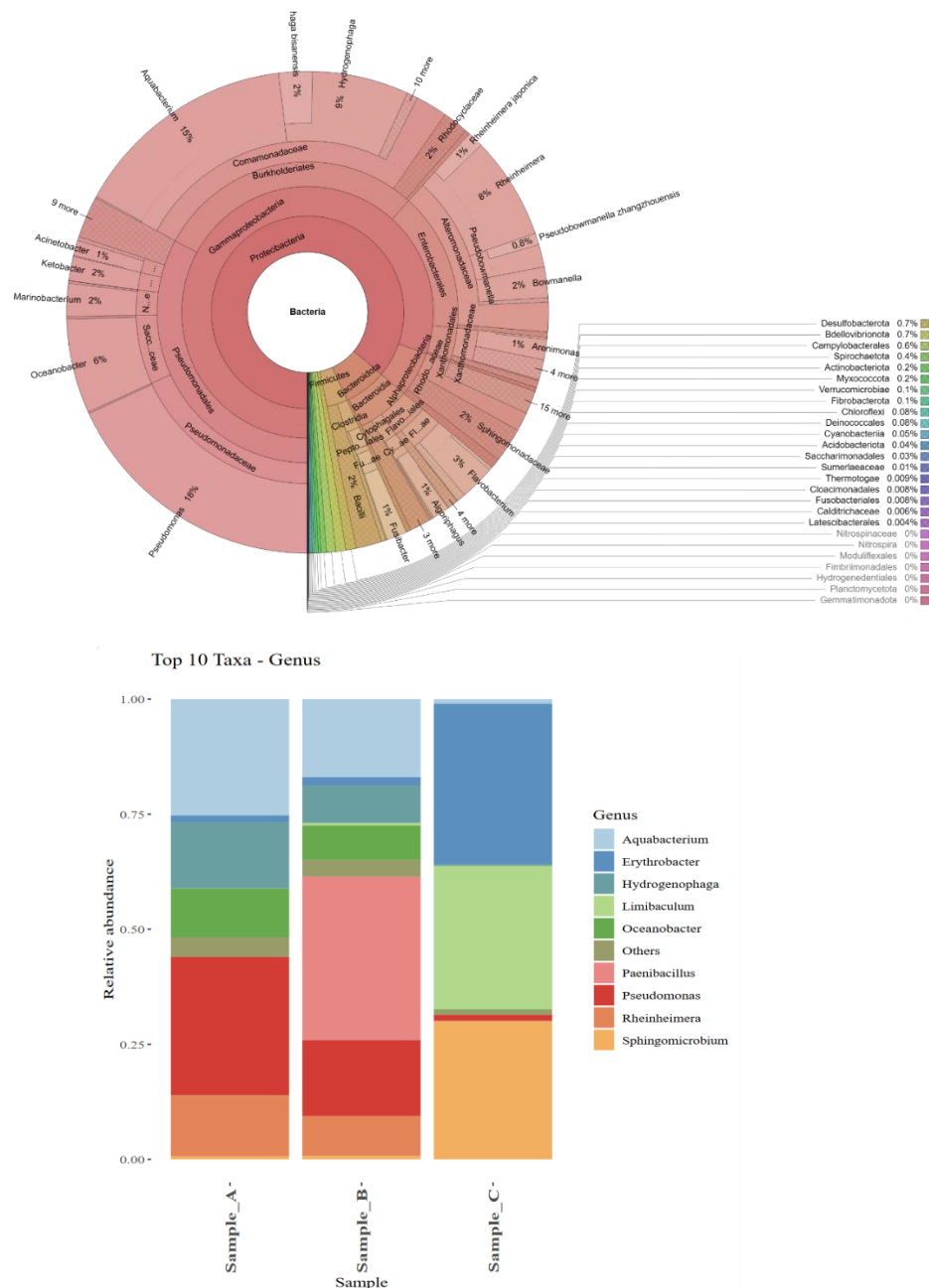
edges. *Aquabacterium* is a bacterium found in biofilms in the drinking water distribution system. Phylogenetic relationship analysis based on 16S rDNA sequences shows that they are members of *Proteobacteria* (Kalmbach *et al.*, 1997). *Aquabacterium* is also a bacterium that is active in degrading polypolymer ( $\epsilon$ -caprolactone) bioplastics (PCL) which are semicrystalline aliphatic polyesters (Yoon *et al.*, 2023). The other genus is also Gram-negative which has almost the same pattern as *Aquabacterium*. Based on several research results in plastic degradation, especially PE, it begins with the formation of biofilms on the surface of PE by the genus *Kocuria* which is carried out extracellular and intracellularly. Extracellular is carried out through the oxidation of C-C and C-H bonds with the help of enzymes that produce  $O_2$  &  $H_2O_2$ . In *Bacillus* the enzymes are alkane monooxygenase; lipase; cytochrome P450. In *Rhodobacter*, the enzymes that play a role are multicopper oxidase and catalase peroxidase. With the help of peroxidase and oxidase enzymes will both become alcohols and aldehydes. The enzymes esterase and lipase will then hydrolyze into fatty acids. Intracellularly, bacteria will use fatty acids into energy and amino acids. *Rhodococcus* will be used for amino acid oxidation & beta bond oxidation, phenylacetate metabolism, Krebs Cycle, glycolysis and amino acid synthesis (García-Depraet *et al.*, 2023; Tao *et al.*, 2023; Alaa *et al.*, 2024; Kumar *et al.*, 2024; Kumar *et al.*, 2025; Auta *et al.*, 2025; Ojo *et al.*, 2025).

Metabarcoding analysis also discovered biochemical pathways used by microorganisms in plastic biodegradation, both directly and indirectly. These include the branched-chain amino acid synthesis pathway, which aids the metabolism of microbes that consume the smaller molecules resulting from the degradation of polymers like polyethylene (PE). Another pathway is the  $\beta$ -ketoadipate pathway, which processes and mineralizes aromatic compounds in plastic biodegradation. This pathway breaks down aromatic monomers into common metabolic intermediates that need into the central cellular metabolism. Metabolic pathways involving Coenzyme A (CoA) was allowing the byproducts break down and assimilation of plastic degradation in microorganisms.

CoA is a crucial molecule that allow bacteria to use plastic as a carbon source. The tricarballic acid cycle pathway is aided by the reductive acetyl

**Table 2.** Bacterial isolates from metabarcoding tests compared to *in vitro* test results

| Class               | Ordo                 | Genus                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Species                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|---------------------|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gammaproteobacteria | Pseudomonadales      | <i>Acinetobacter</i> , <i>Alkanindiges</i> , <i>Enhydrobacter</i> , <i>Hahella</i> , <i>Marinobacter</i> , <i>Permianibacter</i> , <i>Thalassolity</i> , <i>Vibrio</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <i>Acinetobacter marinus</i> , <i>Acinetobacter parvus</i> , <i>E. Aerosaccus</i> , <i>H. chejuensis</i> , <i>M. hydrocarbonoclasticus</i> , <i>M. lutaoensis</i> , <i>M. Taiwanensis</i> , <i>T. marinus</i> , <i>V. proteolyticus</i>                                                                                                                                                                                                                                                  |
|                     | Enterobacterales     | <i>Alishewanella</i> , <i>Alteromonas</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <i>Alishewanella jeotgali</i> , <i>Alteromonas halophila</i>                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                     | Burkholderiales      | <i>Aquaspirillum</i> , <i>Azonexus</i> , <i>Dechloromona</i> , <i>Thauera</i> , <i>Sphaerotilus</i> , <i>Vogesella</i> , <i>Methylophilus</i> , <i>Rivicola</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <i>Aquaspirillum serpens</i> , <i>Azonexus fungiphilus</i> , <i>Azonexus hydrophilus</i> , <i>D. agitata</i> , <i>M. rhizosphaerae</i> , <i>R. Pingtungensis</i> , <i>S. natans</i> , <i>T. aminoaromatica</i> , <i>T. phenylacetica</i> , <i>V. perlucida</i> , <i>I. andamanensis</i> , <i>Pseudoalteromonas fenneropenaei</i> , <i>Pseudobowmanella zhangzhouensis</i> , <i>S. fodinae</i>                                                                                            |
|                     | Enterobacterales     | <i>Idiomarina</i> , <i>Pseudoalteromonas</i> , <i>Pseudobowmanella</i> , <i>Shewanella</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <i>Aquimonas voraii</i> , <i>Arenimonas malthae</i> , <i>L. sediminicola</i> , <i>L. caeni</i> , <i>L. xinjiangensis</i> , <i>P. mexicana</i> , <i>S. lenta</i> , <i>A. parvum</i> , <i>E. citreus</i> , <i>G. lanyuensis</i> , <i>H. bisanensis</i> , <i>L. beolgyonensis</i> , <i>Paracoccus homiensis</i> , <i>Rhodobacter blasticus</i> , <i>T. pacifica</i> , <i>Rheinheimera japonica</i> , <i>S. lutaoense</i> , <i>S. flavum</i> , <i>S. marinum</i> , <i>Thioclava pacifica</i> |
| Alphaproteobacteria | Rhodobacterales      | <i>Aquabacterium</i> , <i>Actibacterium</i> , <i>Aestuariicoccus</i> , <i>Defluviimonas</i> , <i>Erythrobacter</i> , <i>Gemmobacter</i> , <i>Hydrogenophaga</i> , <i>Leisingera</i> , <i>Limibaculum</i> , <i>Litorisediminicola</i> , <i>Lutimaribacter</i> , <i>Maribius</i> , <i>Maritimibacter</i> , <i>Marivita</i> , <i>Oceanicella</i> , <i>Oceanobacter</i> , <i>Paracoccus</i> , <i>Pseudomonas</i> , <i>Pseudooceanicola</i> , <i>Rheinheimera</i> , <i>Rhodobacter</i> , <i>Roseibacterium</i> , <i>Ruegeria</i> , <i>Rubribacterium</i> , <i>Seohaecicola</i> , <i>Silicimonas</i> , <i>Spingomicrobium</i> , <i>Tropicibacter</i> , <i>Tropicymonas</i> , <i>Thioclava</i> , <i>Tabrizicola</i> | <i>A. oceanensis</i> , <i>N. arvoryzae</i> , <i>N. kunmingense</i> , <i>N. yanoikuyae</i>                                                                                                                                                                                                                                                                                                                                                                                                |
|                     | Sphingomonadales     | <i>Altererythrobacter</i> , <i>Novosphingobium</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <i>O. stylophorae</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Actinobacteria      | Caulobacterales      | <i>Oceanicaulis</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <i>C. indicus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                     | Micrococcal          | <i>Calidifontibacter</i> , <i>Kocuria</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <i>G. bronchialis</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Acidimicrobiia      | Corynebacterales     | <i>Gordonia</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | <i>I. fluminis</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                     | Microtrichales       | <i>Ilumatobacter</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <i>Chryseobacterium hominis</i> , <i>Cloacibacterium normanense</i> , <i>F. cheonhonense</i> , <i>F. indicum</i> , <i>H. neustonica</i> , <i>L. thermophila</i> , <i>M. aquimarina</i> , <i>P. luteus</i>                                                                                                                                                                                                                                                                                |
| Bacteroidia         | Flavobacteriales     | <i>Chryseobacterium</i> , <i>Cloacibacterium</i> , <i>Flavobacterium</i> , <i>Hanstruepera</i> , <i>Lutaonella</i> , <i>Muricauda</i> , <i>Phaeocystidibacter</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <i>A. zhangzhouensis</i> , <i>D. beijingensis</i> , <i>Fluviimonas pallidilutea</i> , <i>Fluviimonas roseus</i> , <i>Fulvivirga kasyanovii</i> , <i>Flectobacillus roseus</i> , <i>H. roseola</i>                                                                                                                                                                                                                                                                                        |
|                     | Cytophagales         | <i>Algoriphagus</i> , <i>Dyadobacter</i> , <i>Flectobacillus</i> , <i>Fluviimonas</i> , <i>Fulvivirga</i> , <i>Hugenholtzia</i> , <i>Flectobacillus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <i>Lacibacter cauensis</i> , <i>Lewinella nigricans</i>                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Bacilli             | Chitinophagales      | <i>Lacibacter</i> , <i>Lewinella</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <i>M. fermentans</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                     | Bacteroidales        | <i>Macellibacteroides</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <i>S. mizutaii</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|                     | Sphingobacteriales   | <i>Sphingobacterium</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                     | Paenibacillales      | <i>Paenibacillus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Clostridia          | Lactobacillales      | <i>Limosilactobacillus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                     | Peptostreptococcales | <i>Acidaminobacter</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <i>A. hydrogenoformans</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Campylobacteria     | Campylobacterales    | <i>Arcobacter</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <i>A. cryaerophilus</i> , <i>A. butzleri</i>                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|                     | Deinococcal          | <i>Deinococcus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <i>D. ficus</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Nitrospiria         | Nitrospirals         | <i>Nitrospira</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <i>N. bacterium</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |



**Figure 3.** Genus that dominates plastic-contaminated waters

coenzyme A pathway (Sabri *et al.*, 2025). Another pathway discovered from metabarcoding analysis is the complete aromatic compound degradation pathway, a series of enzymatic reactions to break down and metabolize plastics containing aromatic rings. This pathway is crucial for biodegradation of polystyrene (PS), that are otherwise highly resistant to degradation. Fatty acid  $\beta$ -oxidation is a key metabolic pathway to break down certain types of plastics and related molecules for energy that is particularly relevant for the biodegradation of polymers like PE and polyhydroxyalkanoates

(PHAs). In plastic biodegradation, the gluconeogenesis pathway enables microbes to make glucose and other sugars from non-sugar carbon molecules from breaking down plastic polymers. This is a survival mechanism when glucose is not directly available that allows the microorganisms to build essential cellular components (Sutkar *et al.*, 2025). The glycol-glyoxdeg pathway is the aerobic degradation of polyethylene glycol (PEG), which is a component in some types of plastic, a specific route used to break down PEG, a substance that increase plastics

biodegradability. Glycolysis pathway will breaks down plastics, most commonly polyethylene terephthalate (PET), into their constituent monomers using ethylene glycol (Babaei *et al.*, 2024). During plastic biodegradation, glycolysis, the tricarboxylic acid (TCA) cycle, and the glyoxylate bypass are essential metabolic pathways to break down and assimilate plastic-derived molecules into cellular energy and biomass. These central metabolic pathways convert the resulting smaller fragments into useful metabolites. The glyoxylate bypass is a metabolic pathway used by microorganisms to assimilate two-carbon compounds into precursors for new biomass. Another pathway that plays a role in plastic biodegradation is the heme-biosynthesis-II pathway (Gates and Crook, 2024). The aceticlastic methanogenesis pathway, is the final stage of anaerobic biodegradation where certain plastics are converted into methane with acetate serving as the key precursor. The succinyl-CoA pathway to completely mineralize certain types of plastics, primarily biodegradable butylene succinate (PBS) (Kim *et al.*, 2022). The tricarboxylic acid cycle and glyoxylate bypass are central metabolic pathways in microorganisms plastic biodegradation to metabolize the breakdown specifically acetyl-CoA, and integrate them into their central metabolism for energy production and biomass synthesis (Tiso *et al.*, 2022; Tao *et al.*, 2023). The teichoic acid biosynthesis pathway plays indirect role in plastic biodegradation to create the cell wall components that enable Gram-positive bacteria to form biofilms, which initiate the degradation process (Lv *et al.*, 2024).

Major metabolic pathways found in marine bacteria from the research based on metabarcoding data, have an important role in the biodegradation of PE consist of branched-chain amino acid synthesis pathway,  $\beta$ -ketoadipate pathway, acetyl coenzyme A pathway, aromatic compound degradation pathway, the glyoxylate bypass, heme-biosynthesis-II pathway, aceticlastic methanogenesis pathway, succinyl-CoA pathway, glyoxylate bypass and teichoic acid biosynthesis pathway. Some studies showed that marine microbes are also capable of producing a wide variety of metabolites in adaptive response to extreme marine conditions, including biosurfactants, siderophores, antifouling agents, biofouling agents, and bioremediation (de Carvalho and Fernandes, 2010). The ability of bacteria to form biofilm complexes plays an important role in

the potential of bacteria to degrade various substrates including plastic degradation (Mangwani *et al.*, 2019; Omarova *et al.*, 2019). These biofilm communities are able to form complex structures with EPS (extracellular polymeric substances) that can increase the ability of bacteria to adhere to a surface and improve the degradation efficiency of the substance (Oliveira *et al.*, 2021). The results of this study have thus shown the potential for further development of isolates in plastic degradation.

## CONCLUSION

The results of the comparative study showed that metabarcoding analysis had provided more complete data on PE microplastic degrading bacteria by obtaining one hundred and four bacterial genera compared to in vitro tests that obtained four isolates. Metabarcoding analysis also revealed ten metabolic pathways that play a role in plastic biodegradation consist of branched-chain amino acid synthesis pathway,  $\beta$ -ketoadipate pathway, acetyl coenzyme A pathway, aromatic compound degradation pathway, the glyoxylate bypass, heme-biosynthesis-II pathway, aceticlastic methanogenesis pathway, succinyl-CoA pathway, glyoxylate bypass and teichoic acid biosynthesis pathway. Both microbiological and molecular tests are dominated by Gram-negative bacteria. However, both tests complement each other in showing potential isolates for PE plastic degradation.

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