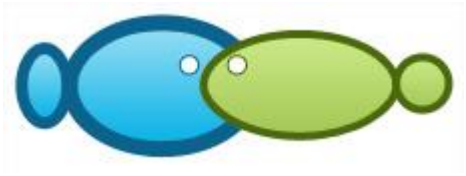

Low-density polyethylene biodegradation by the *Pantoea* sp. TR-C2 isolated from seabed sediment

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Abstract. Various problems caused by waste in the environment, especially the sea, require special handling because they impact the decline in water quality and ecosystem structure. Safe handling actions using biological agents are a promising solution. We tried to find potential bacterial isolates from seabed sediments and obtained TR-C2 isolates that can degrade low-density polyethylene (LDPE). We succeeded in identifying the isolates molecularly and found a phylogeny relationship with *Pantoea* sp. The ability to degrade LDPE by TR-C2 isolates was observed and analyzed for six weeks. The results of the observations with SEM showed that the LDPE film treated with bacterial culture experienced surface morphological damage and the presence of holes or cavities, indicating that degradation by bacteria had occurred. In addition, LDPE film underwent alterations in bond breakage, chemical transformation, and the creation and removal of new functional groups, as revealed by IR spectrum analysis. The occurrence of chemical bond modifications to form new compounds in the treatment with the TR-C2 strain indicates biodegradation by the bacterial strain. This study is quite promising for further analysis to be applied to plastic biodegradation agents from the sea.
Key Words: biodegradation, deep-sea bacteria, low-density polyethylene, *Pantoea* sp.

Introduction. Marine debris, including plastic, is a global problem. It continues to increase because unlimited production is not in line with monitoring and control, so plastic increases and harms the biodiversity, economic activity, human health, and ecological factors (Thushari & Senevirathna 2020). The sea becomes the last reservoir for waste and seabed sediments become a dumping ground for plastic waste (Woodall et al 2014). Plastics that turn into microplastics due to photo-degradation become abundant in the sea and, worse, have entered the food chain and can cause toxic effects on biota and humans (Thushari & Senevirathna 2020).

Various studies have been carried out to find solutions to the plastic problem. The persistence of plastics, environmental toxicity, and large-scale accumulation demand action to develop efficient and environmentally friendly methods for their degradation and explore the potential of microbial catabolism for plastic biodegradation (Montazer et al 2018; Danso et al 2019). The biodegradation process not only breaks down plastic into smaller fragments but is also a process of utilization and mineralization by bacteria (de Vogel et al 2024). The process of polyethylene (PE) biodegradation (high density, i.e., HDPE and low density or LDPE) by microorganisms involves the action of enzymes, including alkane hydroxylases, proteases, laccases, cutinases, lipases, peroxidases (Tokiwa et al 2009; Bhardwaj et al 2013; Wei & Zimmermann 2017; Ahmed et al 2018).

Plastic waste recovery, especially polyethylene in the sea, dramatically requires biodegradation. However, information on marine bacteria for polyethylene biodegradation is minimal (Lyu et al 2024). Some microbes found in marine environments can be used in biotechnology, particularly in the bioremediation of plastic waste (de Fretes & Sohilait 2022). Currently, it has been found that certain marine bacterial species are capable of breaking down PE, including *Alcanivorax*, *Acinetobacter*, *Exiguobacterium*, *Halomonas*, and *Ochrobactrum* (Delacuvellerie et al 2019; Gao & Sun 2021; Lyu et al 2024).

In this study, we searched bacterial isolates to degrade LDPE from seabed sediments in the Makassar Strait. We conducted molecular identification of bacterial isolates using the 16S rRNA gene. Furthermore, we measured the degradation ability of bacteria by observing changes in the fourier transform infrared spectroscopy (FTIR) analysis spectra and the morphology of the plastic surface after treatment with bacterial culture with surface morphology analysis (SEM). This study will be an initial study to further characterize potential bacterial isolates in marine ecosystems.

Material and Method

Collecting sample. The seabed sediment samples used in this study were taken by the TRIUMPH Eastern Indonesia Expedition in February 2021 from Makassar Strait with coordinates of 118°51.2155' East and -002°51.2155' South. The sediments were obtained using a box corer on the seabed at a depth of 605 m. The samples were placed in sterile falcons and stored at -20°C until they were taken to the laboratory.

Low-density polyethylene preparation. The production of LDPE powder was done according to the procedure by Sah et al (2010), which involved boiling LDPE beads (Goodfellow, UK) with xylene and then evaporating the solvent at room temperature (29°C) to turn them into powder. LDPE powder was utilized as a primary carbon source for biodegradation investigations after being air-dried and repeatedly cleaned with 70% ethanol.

Isolation of LDPE-degrading bacteria. One gram of the sample was added to 9 mL of mineral salt (MS) medium and vortexed until homogeneous. Then put in 2 cm^2 LDPE film (thickness 0.05 mm, Goodfellow, UK) and incubated at 35°C for 48 hours. After that, the LDPE film was inoculated into MS agar and incubated for seven days. Bacteria that grew on agar were purified to obtain pure isolates and stored as stocks in glycerol at -80°C. LDPE degrading bacteria was screened using MS medium with 1% LDPE powder as a carbon source. Bacterial isolates were streaked on the test medium and incubated for seven days at 35°C. Isolates that were able to grow and showed a clear zone around the colony were considered capable of degrading plastic.

Identification of LDPE-degrading bacteria. Marine bacterial DNA was isolated using the Presto™ Mini gDNA Bacteria Kit (Genaid). The strain TR-C2 was amplified using polymerase chain reaction (PCR) and identified as undertaken by de Fretes et al (2021). The universal 16S rRNA gene primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'TACGGYTACCTTGTTACGACTT-3') were used for PCR. The obtained sequences were compared with the sequences in NCBI GenBank using the Basic Local Aligment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The selected sequences were aligned with CLUSTAL X (Thompson et al 1997). Phylogenetic tree construction was using MEGA X with Neighbor-joining tree and bootstrap method with replications 1000.

LDPE biodegradation. The plastic degradation potential of the isolated bacterial isolates was assessed using the method developed by Dey et al (2020). In each sample, 10 sterile LDPE films of approximately 2 cm^2 were employed as the solitary carbon source, and 1 mL of freshly grown cells were added to 100 mL of MS broth. Their incubation was conducted aerobically at room temperature under a shaking condition. The biodegradation of the plastic films was assessed after 1, 3, and 6 weeks of the set-ups. The samples were incubated in conjunction with a control that lacked an inoculum.

Surface morphology analysis of LDPE films by scanning electron microscopy (SEM). LDPE films were also visualized using SEM to investigate the modifications in surface morphology that result from microbial action. The microbial cells and associated derbies were removed from the LDPE films by treating them with 2% SDS and drying them at 50°C. In the final step, they were gold-coated and examined under an SEM (SEM

JSM-6510LA, JEOL, Japan) with a Cu grid at 1000x and 3000x magnification (Das & Kumar 2015).

Fourier transform infrared spectroscopy (FTIR) of LDPE films. Chemical bond modifications of LDPE caused by isolated bacterial strains were evaluated using FTIR (Shimadzu, Japan). Microbiologically treated and untreated LDPE films were removed after 6 weeks and rinsed with 2% SDS and dried at 50°C. Finally, the control and treated LDPEs were examined using transmission mode on an infrared spectrophotometer (Kowalczyk et al 2016). All the spectra shown in the results were acquired within the 600-4000 cm^{-1} wavenumber range.

Results and Disccusion. We successfully isolated five isolates (TR-C1, TR-C2, TR-C3, TR-C4, TR-C5) in the experiment with LDPE film, but only TR-C2 was positive in the screening on the medium with the addition of 1% LDPE as a carbon source. Molecular identification using the 16S rRNA gene showed that the TR-C2 had a close relationship with strains from *Pantoea* (Figure 1).

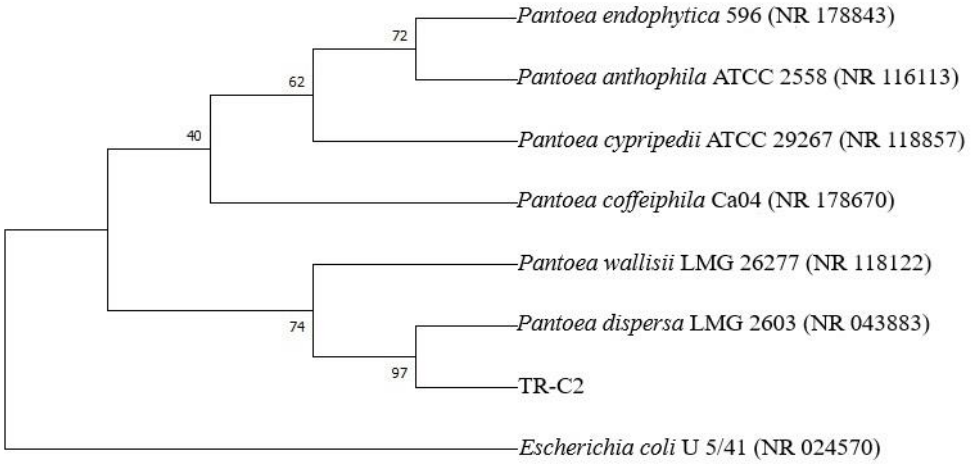

Figure 1. Phylogenetic tree of *Pantoea* species based on 16S rRNA gene sequences.

Research by Skariyachan et al (2016) showed that bacterial strains from the genus *Pantoea* can degrade LDPE films and pellets. *Pantoea ananatis* was isolated from marine snow and formed a quorum sensing in the degradation process of marine organic particles (Su et al 2019). This is likely influenced by the production of extracellular hydrolytic enzymes by the same species (Jatt et al 2015). Silvi et al (2013) successfully isolated a *Pantoea* species from seabed sediment capable of producing high exopolysaccharides (EPS) levels. These EPS have practical uses in environmental biotechnology, including soil and water bioremediation, decontamination, and detoxification (Moslemy et al 2004; Satpute et al 2010; Wood et al 2011).

TR-C2 was incubated aerobically in a carbon-free basal medium for six weeks, with LDPE films serving as the sole C source. Subsequently, the films were analyzed for changes in surface morphology and chemical modifications using SEM and FTIR. Analysis with SEM confirmed that the strains had significant modifications to the surface of LDPE films, which resulted in a weakening of their physical integrity (Li et al 2020).

After the LDPE film was treated with isolate TR-C2 in mineral salt medium, bacterial attachment and colonization were observed after one week, and cavities or holes continued to form on the film surface with increasing incubation time (Figure 2). However, the control sample showed no defects or bacterial biofilm (Figure 2a). Analysis of the films is conducted to detect any alterations in surface morphology, such as pinholes, cracks, and holes, following the introduction of microorganisms that degrade plastic. A significant factor contributing to biodegradation is the adhesion of bacterial film on the surface of polymers (Das & Kumar 2015). A probable explanation for pits and cavities is the uneven distribution of short branches or photodegradable products inside the polymer matrix. This suggests that the bacterial consortia may have more effective

contact with the plastic surface during the process of degradation (Manzur et al 2004). Alterations in the morphological structure suggest the breakdown of polymers into individual elementary units. Plastic product degradation is mainly caused by enzymatic processes involving bacteria (Bhatia et al 2014).

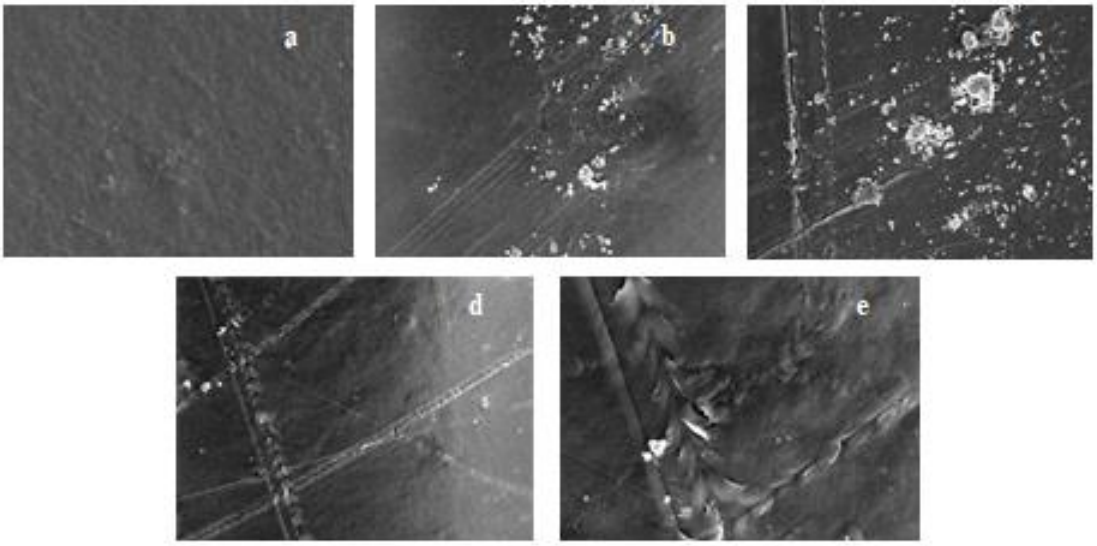

Figure 2. Surface morphology of LDPE with TR-C2 isolate treatment. Untreated controls a), 1 week (b), 3 weeks (c), 6 weeks (d and e). Images (a) to (d) used 1000x magnification while (e) used 3000x magnification.

FTIR was conducted to analyze LDPE films grown with TR-C2 culture broth at 1, 3, and 6 weeks. The resulting spectra are presented in Figure 3. Aliphatic CH exhibited prominent absorption bands at 2954 and 2839 cm^{-1} in the spectra of all samples. The spectra of these bacteria yielded the typical bands with modest intensities at 1615, 1550, and 1512 cm^{-1} observed in LDPE films. Broadband with low intensity at 3414 cm^{-1} was also observed in LDPE films produced following cultivation with this bacterial culture broth. Spectra of LDPE films exhibited strong bands at 1471 and 1463 cm^{-1} corresponding to CH_3 deformation, and 1369 and 1305 cm^{-1} corresponding to C-O-C stretching.

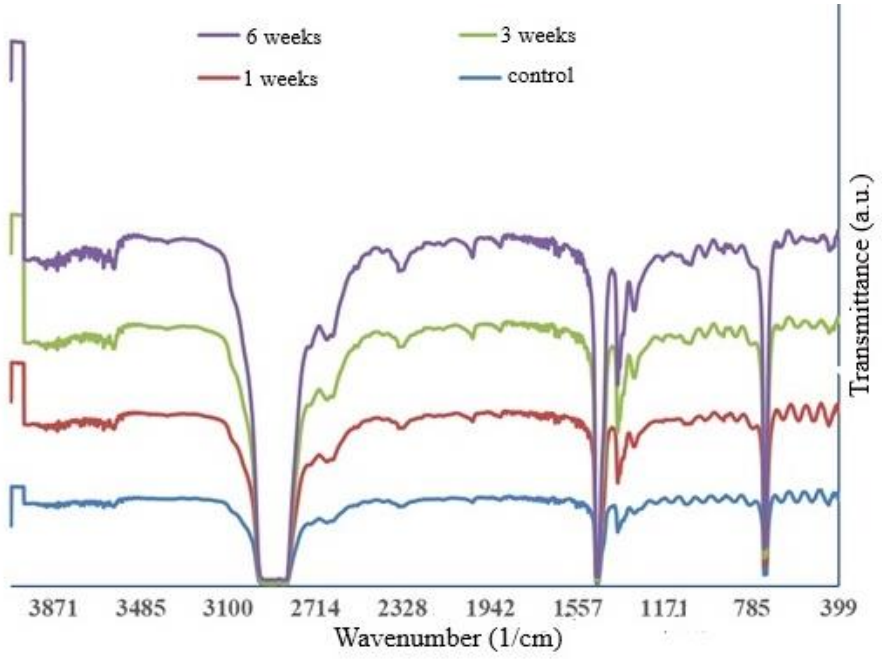

Figure 3. Comparative FTIR spectra of LDPE films cultured with TR-C2 isolate.

Furthermore, all LDPE films exhibited absorbance within the wavelength range of 719 to 729 cm^{-1}, indicating the stretching of -C=C- bonds and the existence of alkene groups. The primary observed structures were alkoxy groups (C-O) peaking at 1053 cm^{-1}, nitro groups (N-O) ranging from 1500 to 1600 cm^{-1}, acyl groups (C-O) peaking at 1305 cm^{-1}, and carbonyl groups (C=O peak 1840 cm^{-1}). Additional changes detected were chain scission, H_2C=C- (906 cm^{-1}), and N-O stretching (peak at 1377 cm^{-1}). The bacterial enzymes could readily employ these groups as the function site. Furthermore, bacteria would find the double bond of carbon more readily available than the C-C bond. The process of enzymatic degradation of LDPE was elucidated by the analysis of chemical bond formation and modification accomplished by FTIR spectroscopy (Dey et al 2020).

The degradation ability of LDPE by *Pantoea* sp. TR-C2 has been successfully analyzed by SEM and FTIR. The breakdown of LDPE films by bacterial strains was confirmed by SEM analysis, which revealed substantial morphological damages such as holes, fissures, and roughening on the surface of LDPE sheets caused by biofilm formation. The presence of proteinaceous materials, polysaccharides, and metabolites produced by bacteria, which are the significant components of biofilm, as well as alcohols, phenols, alkanes, amines, and alkenes, was determined by FT-IR analysis of degraded LDPE films. These compounds were produced after six weeks, suggesting that strain TR-C2 has successfully undergone degradation. The substantial rise in the index observed in the treated samples signifies a highly encouraging outcome of biodegradation. Bacteria utilize LDPE as a carbon source and facilitate essential modifications by integrating functional groups, transforming it into more basic compounds such as alcohols, ketones, and fatty acids (Peixoto et al 2017). Identifying prospective bacteria as plastic bioremediation agents, in conjunction with appropriate testing, will be expected to impact the resolution of the marine ecosystem pollution issue positively.

Conclusions. This study demonstrated the biodegradation potential of TR-C2 strain from seabed sediment with LDPE as the only carbon source during the isolation and enrichment procedure. Molecular identification of the bacterial isolate revealed a kinship tie with members of the genus *Pantoea* sp. Electron microscopy (SEM) examination verified that the TR-C2 strain effectively altered the surface structure of LDPE film cells. The present work also outlined the potential processes by which these strains may biodegrade LDPE. According to the FTIR analysis, the degradation of LDPE into smaller molecules resulted from a sequence of chemical changes. The bacteria subsequently utilized these molecules for their metabolism. Particularly in marine ecosystems, this will facilitate the development of stable microbes capable of degrading LDPE. Additional research on the metabolic pathways, enzymatic processes, and metabolites will provide a comprehensive understanding of the precise mechanism by which these bacterial strains degrade LDPE. This understanding will enable us to build an in situ technique for the biological degradation of LDPE.

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Conflict of interest. The authors declare that there is no conflict of interest.

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