Degradation of polyethylene plastic by bacteria isolated from seawaters, in the Rokan River estuary in Riau Province, Indonesia

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Abstract. The presence of microplastics in the marine environment results in a negative impact on organism health and causes economic loss in the fisheries, tourism, and navigation industries. Microorganisms such as bacteria can degrade plastics and microplastics. The objective of this research was to examine the ability of 25 bacterial isolates from seawater and sediment in the estuary of the Rokan River in Bagansiapiapi of Riau Province to degrade PE plastic. The isolates, consisting of 14 isolates from surface waters (ISW) and 11 isolates from subsurface waters (ISS), were examined for their ability to degrade PE plastic. The ability to degrade the plastic was determined by measuring the decrease in microplastic in dry weight after a 30-day incubation period. Six isolates from ISW indicated a higher degradation rate (12.30±14.69-30.39±9.03%) than three isolates from ISS (9.23±3.59-11.63±6.48%). The ability to degrade plastic was in line with the growth of bacteria, as the higher total bacterial counts were shown by ISW (9.00-8.29 CFU mL^{-1}) than isolates from ISS (7.71-8.23 CFU mL^{-1}). Molecular identification based on 16S ribosomal RNA gene analysis, two isolates indicate a high degradation rate ISW14 and ISS 1, which have high similarities to *Bacillus* sp. strain PR79 and *Stutzerimonas stutzeri* strain SM12, respectively.

Key Words: PE plastic, degradation, bacteria, estuary, seawater.

Introduction. The presence of plastics in the marine environment has become one of the serious problems in the world. Plastics can be found in any compartment of the marine environment, in waters, sediments, and biota. Some factors may influence the degradation process of plastics into microplastics, such as solar radiation, high temperature, and current conditions (Wang et al 2019). Microplastics are man-made polymer substances that result from the breakdown of larger plastic items into particles measuring 5 mm or smaller (Lee et al 2023). Because of their tiny size, microplastics can be readily consumed and can infiltrate the digestive, circulatory, endocrine, and reproductive systems of marine organisms that come into contact with them (Avio et al 2019; Kabir et al 2020). The accumulation in marine biota would result in a negative impact on organism health because of the toxic additives and carcinogenic compounds of the plastics (Lahimer et al 2017). Microplastics can hinder cyanobacterial growth by inhibiting photosynthesis (Wu et al 2021; Lu et al 2022) and causing cell damage (Chen et al 2020). In addition, the abundance of plastic waste has been reported to cause economic loss in the fisheries, tourism, and navigation industries (Beaumont et al 2019).

Polymers such as high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyvinyl chloride, polystyrene (PS), polypropylene, and polyethylene terephthalate (PET) are among the most common types of plastics present in aquatic environments (Wu et al 2017). Microplastics can manifest in various forms, including fragments, films, fibers, and foam (Lee et al 2023). A previous study revealed that microplastic concentrations were higher in sediment (ranging from 193.33 to 746.67 particles kg^{-1}) compared to seawater (0.130 to 0.200 particles L^{-1}) in the Dumai waters of

Riau Province. The identified microplastic types in this study included fibers, fragments, and films (Yoswaty et al 2021). Due to their low density, microplastics are readily dispersed in the environment, and their degradation process can take hundreds of years (Haward, 2018). This is attributed to the chemical structure of plastics, which consists of long-chain hydrocarbon polymers with high molecular weights. The degradation of plastics into microplastics in marine environments is influenced by factors such as solar radiation, elevated temperatures, and current conditions (Wang et al 2019).

Microplastics in the environment can be easily colonized by microorganisms and form biofilm, which influences the behaviors and potential risks of microplastics (Wang et al 2021). Microplastics also behave as an artificial microbial reef, attracting a variety of bacterial microbiota communities known as the plastisphere (Vidal-Verdú et al 2022). The plastisphere plays a role as a new micro-ecosystem in pelagic waters, including primary producers, predators, symbionts, and decomposers (Amaral-Zettler et al 2020). The microbial communities in the plastisphere are composed of a diverse range of bacteria, fungi, viruses, archaea, algae, and protozoans (Wright et al 2020).

Bacteria from marine environments exhibit significant potential for plastic degradation, primarily through species of bacteria, fungi, and algae. Several bacterial species, such as *Pseudomonas* spp., are commonly found on plastic biofilms, while *Bacillus* spp. have demonstrated notable efficiency in degrading various types of plastics, including species like *Bacillus cereus*, *Bacillus safensis*, and *Bacillus subtilis* (Lv et al 2024). Marine isolates identified as *Pseudomonas sp.* and *Lysinibacillus sp.* have shown a reduction in biofilm formation on weathered HDPE, particularly within the first 24 hours of incubation (Oliveira et al 2021).

The presence of plastic-degrading bacteria in the Indonesian marine environment has been extensively studied. For instance, three bacterial genera (*Moraxella* spp., *Enterobacteriaceae*, and *Pseudomonas* spp.) isolated from Marina Beach in Semarang, Central Java, exhibited significant polyethylene (PE) degradation potential (Rachmawati et al 2021). Additionally, *Pseudoaltoromonas caenipelagi*, isolated from mangrove sediment in Pasir Putih, Wonorejo, demonstrated the ability to degrade PE microplastics (Novitasari et al 2023). The sulfur-oxidizing bacterium *Sulfurovum sp.*, found in the coastal waters of Jakarta Bay, has been identified as a dominant biofilm-forming species on both PE and PET (Azizi et al 2023). Furthermore, eight bacterial species from the Musi River estuary have been shown to possess plastic-degrading capabilities, including *Staphylococcus hominis*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *A. baumannii*, *A. variabilis*, *Shewanella* sp., *Micrococcus luteus*, and *Bacillus amyloliquefaciens* (Meiyerani et al 2024).

The occurrence of plastic-degrading bacteria in the marine environment of Riau has been insufficiently studied. *Bacillus* sp. was a bacteria isolated from the Dumai seawaters, which indicated the highest plastic degradation (Pakpahan et al 2021). Recently, 25 bacterial isolates have been isolated from surface water (ISW) and subsurface waters (ISS) of the Rokan River estuary in Bagansiapiapi, Riau (Nursyirwani et al 2024). However, the potency of the degradation of PE plastic has not been examined yet. Therefore, the objective of this research was to examine the ability of all isolates from the Rokan River estuary to degrade PE plastic.

Material and Method

Source of bacterial isolates. Bacterial isolates used in this study were obtained from the collection of the Marine Microbiology Laboratory of the Faculty of Fisheries and Marine Science. The 25 isolates were isolated from seawater in the estuary of the Rokan River in Bagansiapiapi, Riau Province. Before being used, 14 surface waters (ISW) and 11 isolates from ISS were re-inoculated in fresh Zobell Marine Agar (Himedia, India), containing ingredients such as peptone 5 g L^{-1}, yeast extract 1 g L^{-1}, agar 15 g L^{-1}, sodium chloride 19.45 g L^{-1}, and ferric citrate 0.1 g L^{-1}. All inoculated Petri dishes were then incubated at 35^{o}C for 48 hours.

Preparation of plastic. PE plastic was used in this assessment. This type of plastic is commonly used for carrying bags, commonly used in Indonesia. The plastic was cut into pieces measuring 1 × 1 m^2. Before being used, the plastic pieces were sterilized in 70% ethanol, dried in a drying oven at 40°C for 24 hours, and weighed as the initial weight (Wi).

Degradation assessment. Plastic degradation test following the procedure of Azizi et al (2024). For the test, two loops of each bacterial culture were inoculated into a 150 mL bottle containing 100 mL of sterile tryptone soya broth (TSB, Oxoid, UK) medium. Three pieces of the PE plastic were put into each bacterial culture. The cultures were then incubated with agitation at 130 rpm at 30°C. After a 30-day incubation period, the plastic was taken out to measure the dry weight. The plastics were cleaned with 70% ethanol before being dried and weighed (Wf). The ability of each bacterial isolate to degrade the plastic was determined by measuring the decrease in plastic weight using an analytical balance. To calculate the percentage decrease of plastic by bacterial culture, the formula of Auta et al (2017) is used:

$$\text{Percentage of degradation (\%)} = \frac{wi - wf}{wi} \times 100$$

wi = Dry weight before degradation (g)
wf = Dry weight after degradation (g)

Determination of bacterial growth. Development of bacterial count in each treatment was determined by using the standard plate count method after 48 hours of incubation. Growth medium used was tryptone soy agar (TSA, Oxoid, UK), which contains ingredients such as tryptone 15 g L^{-1}, soya peptone 5 g L^{-1}, Sodium chloride 5 g L^{-1}, and agar 15 g L^{-1}. A volume of 0.1 ml from each bacterial culture was taken and diluted in a 1% NaCl solution (10^{-2} - 10^{-6}). Afterward, 0.1 ml of each dilution was spread onto the TSA medium in triplicate. All inoculated media were incubated for 48h at 37°C.

DNA extraction and characterization of 16S rRNA bacteria. Three isolates of bacteria, each from ISW14, ISS1, and from sediment (ISD5), were subjected to the amplification process by PCR techniques (Senbadejo, 2017). Quick-DNA Magbead Plus Kit (Zymo Research, D4082) (B/7.2.1/IKP/009) was used to extract the bacterial DNA. The DNA was amplified by using MyTaq HS Red Mix, 2X Kit (Bioline, BIO-25048) (B/7.2.1/IKP/002). Electrophoresis was performed to visualize the amplified products of Gen 16S (B/7.2.1/IKP/005). The Sanger DNA Sequencing method, using Capillary Electrophoresis, was employed to sequence the PCR products in both directions (forward and reverse). The products of Sanger Sequencing were analyzed through the Bioinformatic system (B/7.2.1/IKP/006). Similarity of species level if the "percentage identity" is more than 97.5%, and at genus level if the "percentage identity" is above 95% (Stackebrandt & Goebel 1994).

Phylogenetic tree construction. The bacterial species samples were aligned with bacterial sequences downloaded from the GenBank database using ClustalW software. The phylogenetic tree was constructed using the Neighbor-Joining (Unrooted Tree) by the NCBI Blast Tree Method.

Results and Discussion

Degradation of PE plastic. The degradation of plastics by bacterial isolates varies and depends on the species. The ability of bacterial isolates to degrade plastics can be determined from the percentage decrease in dry weight before and after the degradation test. Data on plastic weight before and after degradation test by bacterial isolates from the ISW and ISS were presented in Tables 1 and 2. In general, the ability of bacteria from ISW to degrade PE plastic is higher than bacteria from subsurface waters.

Table 1

Degradation rate of PE plastic by bacterial isolates from surface waters

Isolate	*Average (%)*
ISW 1	0
ISW2	27.14±4.95
ISW3	12.30±14.69
ISW4	0
ISW5	0
ISW6	16.43±4.69
ISW7	0
ISW8	0
ISW9	0
ISW10	12.50±12.50
ISW11	0
ISW12	0
ISW13	16.25±2.73
ISW14	30.39±9.03

ISW1- ISW14 = code of bacterial isolates from surface seawaters.

Table 2

Degradation rate of PE plastic by bacterial isolates from subsurface waters

Isolate	*Average (%)*
ISS1	9.45±4.65
ISS2	0
ISS3	11.63±6.48
ISS4	0
ISS5	0
ISS6	0
ISS7	0
ISS8	0
ISS9	9.23±3.59
ISS10	0
ISS11	0

ISS1 - ISS11 = code of bacterial isolates from subsurface seawaters.

Data in Table 1 indicate that only six of 14 bacterial isolates from the ISW2, ISW3, ISW6, ISW10, ISW13, and ISW14, were able to degrade PE plastic. The degradation rate ranges from 12.30±14.69-30.39±9.03%. The highest degradation ability was indicated by isolate ISW14. While three isolates (ISS1, ISS3, and ISS9) of 11 isolates from the subsurface seawater (Table 2) indicate the degradation ability ranges from 9.23±3.59 to 11.63±6.48%. The highest degradation rate was indicated by isolate ISS3.

The data indicate that each bacterial isolate can use PE plastic as a carbon source for growth. Microorganisms are the most promising source for microplastics degradation due to their ability to produce enzymes that can break down the complex polymer structures of plastics and use them as a carbon source for their growth and metabolism (Jain et al 2023). Among the microorganisms, bacteria and fungi are known to produce alkane hydroxylase (AHs), which is a key enzyme involved in the aerobic degradation of PE in the beta oxidation pathway (Efendi 2016). Similarly, Yoon et al (2012) reported that alkane hydroxylase, which was produced by *Pseudomonas* sp. E4 played an important role in the degradation of PE through the oxidation of the PE main chain. Laccase, an enzyme produced by *Aspergillus flavus*, degraded PE significantly (Zhang et al 2020). The enzymes divide polymers into short chains or smaller molecules, which can pass through membranes using the mechanism known as depolymerization. In turn, these short molecules are subjected to the mineralization of end products, such as CO_2, H_2O, or CH_4, through the TCA cycle and utilized as sources of carbon and energy (Gu 2003; Zhai et al 2023).

This finding indicates that the ability of bacteria isolates from surface and ISS in the PE degradation was higher than that reported by some previous studies. A range of 0.42-17.27% after 30-day incubation was demonstrated by bacteria isolates from the Dumai seawaters in Riau (Mardalisa et al 2021), while Azizi et al (2024) found lower values of PE-degradation percentage (0.3 to 4.5%) throughout 14-day tests. The difference in plastic-degradation rates is due to the difference in the ability of each bacterial species. In addition, environmental parameters could influence the degradation process. Environmental parameters such as moisture/water content, pH value, temperature, availability of oxygen and/or nutrients, oxidative and photo-oxidative environment, etc., affect polymer degradation (Viel et al 2023).

The ability of bacterial isolates to degrade plastic is due to their ability to colonize the plastic surface. Some other studies reported that adhering bacteria on the plastic surface is the initial step of microbial colonization, and the premise of enzymatic degradation (Cai et al 2023). The second step is hydrolysis, which comprises the combination of enzyme hydrolase and polymer matrix, and then catalytic hydrolysis and cracking (Tokiwa et al 2009). PE degradation involves biocatalysts hydroxylase, laccase, peroxidase, and reductase (Amobonye et al 2021).

Growth of bacteria. The number of bacteria after a 30-day incubation period also indicates an increase during the plastic degradation test, particularly for bacteria that demonstrate high degradation ability. The average count of each bacterial isolate is presented in Figures 1 and 2. The highest log bacterial count numbers of bacterial isolates from surface and subsurface were isolates ISW14 (Log_{10} 8.29) and ISS3 (Log_{10} 8.23), respectively.

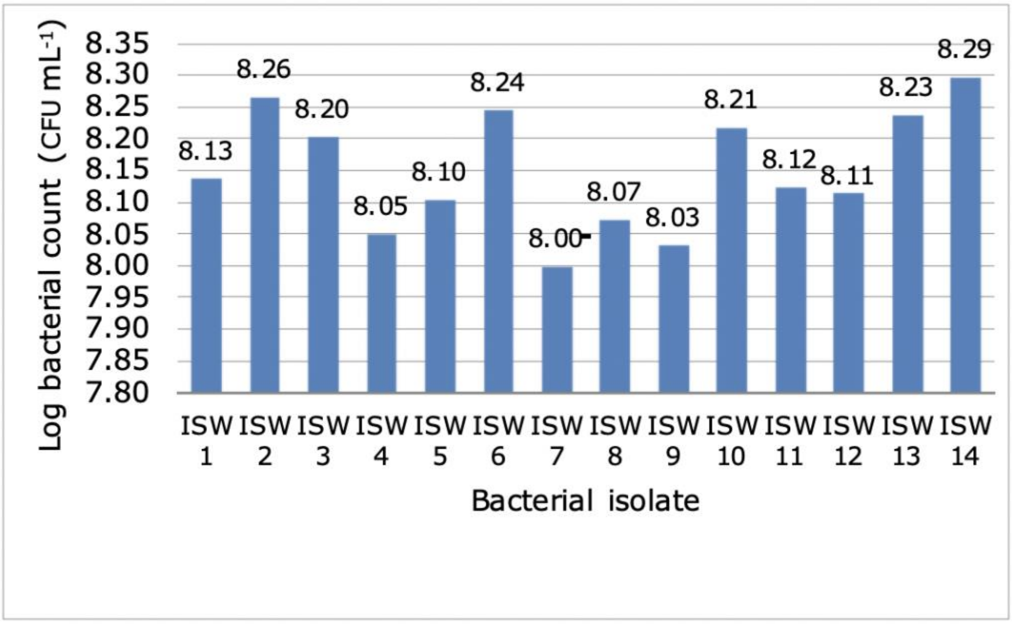

Figure 1. Log number of bacteria from surface waters after a 30-day incubation period.

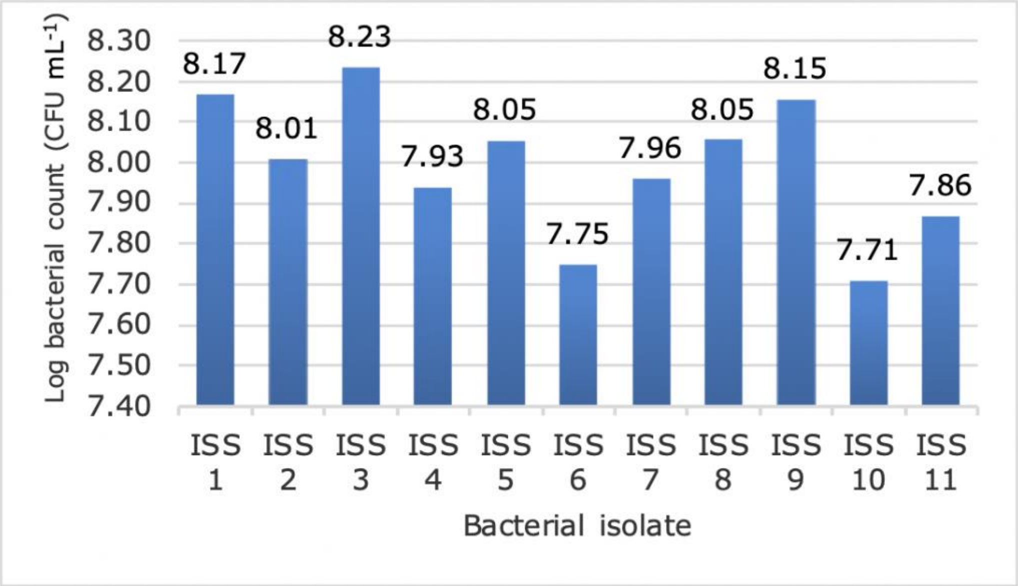

Figure 2. Log number of bacteria from subsurface waters after a 30-day incubation period.

In general, the growth of all bacterial isolates during the degradation test increases. Figure 1 indicates that the log count numbers of bacteria from the ISW are slightly higher than those from the ISS (Figure 2). The high bacterial count correlates with the ability of the bacteria to degrade PE plastic, which is also higher in bacterial isolates from the surface waters. This could be due to the bacteria colonizing plastic initially before the plastic floats in seawaters and sinks in the sediment, as it occurs in natural conditions. It has been reported that microbial community members initially present on floating plastics are quickly replaced by microorganisms acquired from deeper water layers, thus suggesting a limited efficiency of sinking plastic particles to vertically transport microorganisms (Vaksmaa et al 2022).

Species identification based on 16S ribosomal RNA gene analysis. Two isolates of bacteria, each from the surface (isolate ISW14) and subsurface waters (isolate ISS1), were selected for the molecular identification. Results of the 16S rRNA gene and bioinformatic analysis were presented in Table 4. Identification of kinship for the isolates and the reference species was as presented in Figures 3 and 4.

Table 4

BLAST analysis of isolates ISW14, ISS1 and ISD5

Isolate	*Species*	*Strain*	*Accession code*	*% Query coverage*	*% Similarity*
ISW14	*Bacillus cereus*	PJS3.10	MT299636.1	100	99.86
ISS1	*Pseudomonas stutzeri*	W41	KT380584.1	100	99.93

Identification based on 16S rRNA gene analysis showed that isolate ISW14 was highly similar to *Bacillus cereus* strain PJS3.10 (99.86%), with the accession no. MT299636.1, query coverage 100% and an e-value of 0.0. Data in the National Center for Biotechnology Information (NCBI) indicates that the bacteria are isolated from the seawater, shellfish, and sediment from the north coast of Java. Meanwhile, isolate ISS1 was highly similar to *Pseudomonas stutzeri* strain W41 (99.93%), which was isolated from the overlaying water in Liangshui River of Beijing with the accession no. KT380584.1, query coverage 100%, and an e-value of 0.0.

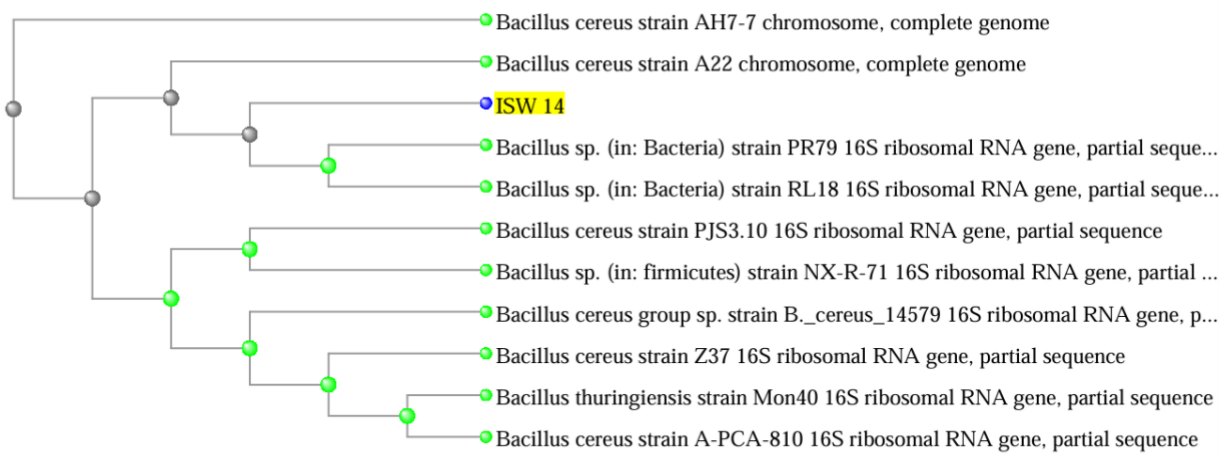

Figure 3. The phylogenetic relationship of isolate ISW14 with other related species was constructed by using Neighbor-Joining by the NCBI Blast Tree Method.

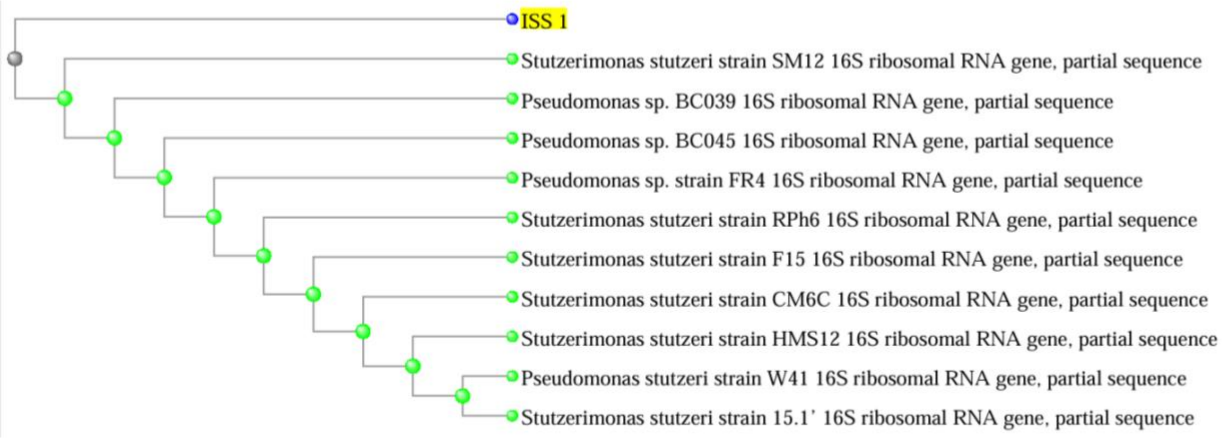

Figure 4. The phylogenetic relationship of isolate ISS1 with other related species was constructed by using Neighbor-Joining by the NCBI blast tree method.

Some studies have shown that *Bacillus* genera can degrade various types of plastics, for example, *B. amyloliquefaciens, B. mycoides, B. cereus, B. thuringiensis,* and *B. pumilus* (Sharma and Sharma 2004), *Bacillus* ISJ51 (Gupta & Devi 2019), *Bacillus* sp. strains BP4 and BP6 (Fibriarti et al 2021). From the marine environment, some species and strains of the *Bacillus* genera have also been reported to have potential in degrading plastics. *B. cereus* (OR268710) isolated from a plastic-polluted tropical coastal environment in Tamil Nadu, South India, was able to degrade LDPE and PS (Jebashalomi et al 2024). *Bacillus* sp. IBP-2, *B. paramycoides* IBP-3, and *B. cereus* IBP-4, isolated from the plastisphere of marine ecosystems, are also able to use LDPE as the sole carbon source (Febria et al 2024).

Pseudomonas genera are generally found in aquatic ecosystems, both in water and sediment. *Pseudomonas stutzeri* was found to be a common, abundant aerobic denitrifying bacterium in the phases of overlying water, biofilm, and sediment (Lv et al 2017). *Stutzerimonas stutzeri*, the current name of *Pseudomonas stutzeri* (Gomila et al 2022), isolated from mangrove sediment, also showed an ability to produce clear zones during the degradation of polyethylene glycol (PEG) and biofilm formation (Afianti et al 2024). *P. stutzeri* was predominantly of communities as the strongest polyester degrader, which revealed two putative polyesterases and one putative MHETase (Howard et al 2022).

Conclusions. Bacterial isolates from the Rokan River estuary indicate an ability to degrade PE plastic; those are six isolates from ISW and three isolates from subsurface waters. Among them, isolates ISW14 and ISS1 showed the most potential in PE degradation. Based on 16S rRNA gene analysis, one isolate from the ISW14 and one isolate from ISS were identified as *Bacillus cereus* and *Stutzerimonas stutzeri*, respectively.

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

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