Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan (2025) 14(2): 148-156

DEPIK
Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Journal homepage: www.jurnal.usk.ac.id/depik

Biodegradation activities of microplastic polymers by *Agelas conifera* spongesymbiotic bacteria

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ARTICLE INFO

Keywords:
Dumbo catfish
Leukocytes
Erythrocytes
Glucose
Musa Acuminata
Aeromonas hydrophila

DOI: 10.13170/depik.14.2.35939

ABSTRACT

Research on microplastic (MP) accumulation in marine organisms' tissue have been widely conducted, as well as research on microplastic biodegradation by microbial organisms from the environment. Agelas conifera sponge is proven to accumulate MP in its tissue as well as having bacteria as symbiotic organism. This research aims to screen and study biodegradation activities of A. conifera symbiotic bacteria on MP polymers, also to identify the species of those bacteria isolates. Bacterial Isolation of A. conifera sponge tissue was using Zobell 2216E agar media. Screening and biodegradation assay of the bacteria isolates was conducted on Marine Salt Media agar that was added with 0,5 g of MP polymers, using Kroc-Boral method. Diameter of clear (bacterial growth) zone surrounding Kroc-Boral indicated the biodegradation activity of the bacterial isolates. Statistical analysis on the activity was using Two Way ANOVA. Results showed that there were 9 bacterial isolates of 53 that could degrade several MP polymers, with different activities (ANOVA, p value <0.05). High and low-density polymers (Linear-Low Density PolyEthylene/ LLDPE, Polyethylene/PE, and Polyvinyl Chloride/ PVC) were found to be degraded by all 9 isolates. Meanwhile, Polyethylen Taraphtalene (PET) and Polystyrene (PS) only degraded by 8 and 3 isolates, respectively. There are only 5 isolates that have high biodegradation index (>1.5) on different polymers, which mostly on PET polymer. Isolate Ac8MP found to be the most bacteria that have degradation index on all polymers more than 1. This research proves that symbiotic bacteria could have a biodegradation mechanism on MP polymers that accumulates by sponge-host. However, further study needs to be done to understand the mechanisms and effectiveness of sponge-bacterial biodegradation activity.

Introduction

Research on the presence of microplastics in marine ecosystems and their impact on marine organisms have been widely conducted throughout the world (Corinaldesy *et al*., 2021; Thushari and Senevirathna, 2020; Kibria, 2023) including Indonesia (Samalwan *et al*., 2021; Rahim *et al*., 2021; Sulistiowati *et al*., 2021; Sari *et al*., 2023; Akbar *et al*., 2023). The research also has found that waters and sediments in coastal ecosystems on small islands or near land can contain various microplastic polymers, in terms of shape, size, color and type. Organisms in that mentioned environment will also accumulate MP that can be discovered on the waters and sediments. The biota, mainly biota that tend to get food by filtering waters like bivalves and sea sponges. Associative organisms on sponge tissue can come from the invertebrate group and from the bacterial group. The biomass of sponge symbiont bacteria is known to reach up to 40% of the sponge's own body biomass. The research found that there are differences in bacterial biomass in different types of sponges, based on body cell fractionation.

Sponge symbiont bacteria is known to have an ecological role on health and adaptation of sponges in their ecosystems. Sponge symbiont bacteria have been widely studied regarding their potential for the production of bioactive compounds from secondary

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p-ISSN 2089-7790; e-ISSN 2502-6194
Received 9 January 2024; Received in revised form 6 November 2024; Accepted 25 April 2025
Available online 14 June 2025

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 14, Number 2, Page 148-156

metabolism. Various studies on sponges obtained compounds that have biological activities including anti-cancer, anti-HIV, anti-bacterial, anti-fungal, antioxidant, and various potential bioactive compounds (Warsidah and Kartika 2013; Handayani *et al.*, 2015).

Similar to the presence of symbiotic bacteria in different benthic organisms, symbiotic bacteria within sponges are recognized for generating compounds that are either identical to or resemble those produced by their host sponges (Radjasa *et al.*, 2011; Ismet *et al.*, 2021). This phenomenon is associated with the degree of adaptation exhibited by symbiotic bacteria to the specific environment of their host sponges. Additional research (Selvin *et al.*, 2009) suggests that symbiotic bacteria, characterized by their ability to accumulate and resist pollutants, can transfer pollutant-tolerant plasmids, such as those for heavy metals, to their host sponges. Various studies have delved into the capability of symbiotic bacteria to both adsorb and break down pollutant compounds. Sponges, along with their symbiotic bacteria, have been proven to adsorb heavy metals like arsenic (As) and mercury (Hg) (Marzuki and Angela, 2021). Furthermore, this investigation provides evidence that symbiotic bacteria within sponges possess the capacity to degrade polycyclic aromatic hydrocarbon (PAH) pollutants in aquatic environments (Marzuki *et al.*, 2020; Marzuki *et al.*, 2021).

Research on the ability of bacteria to biodegrade microplastics has begun in response to the increasing presence of microplastics in marine ecosystems. The extensive focus has been on the microbial degradation of various types of microplastics (PEMPs, PS-MPs, PP-MPs, PET-MPs, PVC-MPs, etc.). Microorganisms can utilize microplastics as an energy source for survival. Hence *et al.*, (2016) investigated the degradation efficiency of *Bacillus subtilis* using PE as the sole carbon source, revealing a 9.26% reduction in mass after 30 days. Microplastics, fragments of plastic that have degraded to microscopic sizes, less than 5 mm, can have negative impacts on various organisms, particularly in marine environments. Several studies indicate that bacteria present in marine water and sediments, such as *Bacillus* sp. BCBT21, *Bacillus amyloliquefaciens* BSM-1, *B. amyloliquefaciens* BSM2, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus cereus*, *Brevibacillus borstelensis*, *Bacillus vallismortis* bt-dsce 01, *Pseudomonas protegens* bt-dsce 02, *Stenotrophomonas* sp. bt-dsce03, and *Paenibacillus* sp. bt-dsce04, have the ability to degrade microplastic polymers (Auta *et al.*, 2017; Chandra *et al.*, 2020). However, research on the ability of symbiotic bacteria to degrade microplastics in marine biota is still limited. The potential of symbiotic bacteria to degrade microplastics has the potential to be a solution in reducing the impact of microplastics on the health of marine biota, especially those of economic importance. This opens up opportunities to utilize these bacteria not only as biodegraders but also as probiotics for marine organisms exposed to microplastics in their environment.

Materials and Methods Experimental design

This research was conducted under laboratory experiment using bacterial isolates from *Agelas conifera* seagrass-sponge. The bacteria isolates were obtained from seagrass-sponge tissue of Pramuka Island, Jakarta, in March 2021, based on the study of Sari *et al.*, (2023).

Bacteria isolates were obtained from the tissue of *A. conifera* seagrass-sponge using Zobell 2216E (Difco) media. As much as 5gr of sponge tissue were cut after being high-pressure sprayed with sterilized seawater, to cleanse the detrital particulates from the exodermis. Sponge's tissues were then grinded using sterilized mortar, and 1 gr of the ground tissue was inoculated into 9 ml of Zobell 2216E broth media (Restuati and Gultom, 2021). After serial dilution (from 10^{-6} up to 10^{-8}), 0.1 ml of bacterial dilution were inoculated into Zobell 2216E agar media, and incubated for 72 h at room temperature. This procedure was conducted in triplicates, from two individuals of sponges.

The growth bacterial colonies were observed and chosen based on the morphological and pigmentation of bacterial colonies. The bacterial colonies were then purified using the streak method on Zobell 2216E agar media. The same media also used to make bacterial stock using the slant agar method. Each Bacterial cell is also characterized using Gram Staining method.

Screening of microplastic polymer biodegradation

Each different isolated bacteria was tested on its biodegradation potential against Linear Low-Density Polyethylene (LLDPE) microplastic polymer. This test is provided as the screening test of microplastic biodegradation. The LLDPE pellet used in this procedure was an industrial microplastic pellet (LLDPE Titanvene™ LL0209AA, LOTTE), with density of 915-930 kg.m^{-3} (ISO 1183). The assay was using Clear Zone method with minimal nutrient media, Mineral Salt Medium (MSM) (Himedia) 0.1%

(w/v), which has been added with 0.5 g of LLDPE microplastic polymer. The MSM (ATCC constant) contains 2g $NaNO_3$, 0.5g $MgSO_4$, 0.01f $FeSo_4$, 0.14g KH_2PO_4, 0.02g yeast extract, 30g agar, with adjusted pH to 7.

The Kroc-Boral method was chosen to inoculate 0.1ml of the bacterial isolates onto MSM media with LLDPE polymer, after being cultured in Zobell 2216E broth media for 48h at room temperature. Each isolate was tested triplicates to ensure replication. Negative control was using plate of MSM agar infused with LLDPE pellet, without any bacterial inoculation. The assay was observed after 2 weeks of incubation at 25°C-30°C/ room temperature (Soud, 2019).

Microplastic biodegradation assay on different polymers

Isolates that showed biodegradation activity then tested on the MSM with several different microplastic polymers (LLDPE Titanvene™ LL0209AA, LOTTE; polyethylene/PE cosmetics grade scrub powder TRK-70011-00666; Polyethyl-terephtalate/PET 39076100, Polystyrene/PS 1-2mm foam particlesJF21, Myer; and Polyvinyl chloride/PVC SIP655 Satomo). The objective of using this kind of polymers is to mimic the effects of microplastic pollutants in the environment. All the microplastic polymers that were used in this research were resin pellet polymers with industrial grade, that were widely retailed for commercial use.

The media of MSM 0.1% was added with 0.5g of each UV-sterilized microplastic polymers, then 0.1ml of bacterial isolates were inoculated into the KrocBoral wells within the MSM agar. The result was observed after 4 weeks of incubation, at 25°C-30°C/ room temperature, modified from Soud (2019).

Measurement of Biodegradation Activity

Biodegradation activity was observed by measuring the clear zone (or bacterial growth zone) surrounding the bacterial-inoculated wells in KrocBoral Method. The measurement was conducted through visual measurement. The diameters of the clear zone around the Kroc -Boral were measured and calculated to gain the degradation index. The degradation index of the microplastic polymer following Sumardi *et al.* (2018) based on enzymatic activity, with the formula of:

$$\pi = \frac{clear\ zone\ diameter - Kroc\ Boral\ diameter}{Kroc\ Boral\ diameter}$$

Data Analysis

Distribution of biodegradation activity on microplastic polymers was analyzed using ShapiroWilk formula. The significant test was using ANOVA analysis on normal distribution data and Kruskall-Wallis analysis on data that are not normally distributed. Correlation between bacterial isolates on its biodegradation activities against microplastic polymers was conducted using Correspondance Analysis. All data analysis were conducted using XLStat 2014 application.

Results Isolation of Agelas conifera sponge-symbiotic bacteria

Isolation of symbiotic bacteria was conducted on *Agelas conifera* sponge that was found in the seagrass ecosystem of Pramuka Island. This sponge has been found to accumulate microplastic polymers with different shapes, which are pellet, fragment, and fiber (Sari *et al.*, 2023). Results on the isolation procedure found that there are 53 different colonies of bacteria that had been isolated from this *Agelas conifera* sponge using Zobell 2216E agar medium. Observation on bacterial morphological features, as well as the cell's Gram staining is shown in Table 1.

Screening and assay of microplastic polymer biodegradation

This research is a pilot study on sponge symbiotic bacteria activities on pollutant biodegradation. Out of 53 isolates of sponge-bacteria, only 19 isolates have biodegradation activity on LLDPE microplastic polymers (Table 2). Those isolates are Ac1MP, Ac8MP, Ac10MP, Ac14MP, Ac18MP, Ac36MP, Ac37MP, Ac44MP, and Ac48MP. All 9 isolates showed biodegradation

Those 12 bacteria isolates, then tested on different polymers of microplastic, which are LLDPE, PET, PE, PVC and PS. The biodegradation activity that is represented as a clear (bacterial growth) zone could be seen in Figure 1. Meanwhile, Figure 2 showed the activity of microplastic biodegradation by all 12 isolates, in diameter of clear (bacterial growth) zone.

Table 1. Morphological characteristics and number of bacterial isolates from *Agelas conifera* sponges.

Bacterial Characteristics		Sponge 1	Sponge 2	Total
		27	26	53
Gram	Gram Cocci	23	26	49
Staining	Negative Bacilli	4	0	4
	Gram Cocci	0	0	0
	Positive Bacilli	0	0	0
Edges	Smooth	25	26	51
	Surge	2	0	2
Shape	Round	20	26	46
	Centroid	7	0	7
Elevation	convex	20	18	38
	Flat	7	5	12
	Raised	0	3	3
Form	Dot	14	10	24
	Small	9	10	19
	Moderate	4	6	10
Appearance	Shiny	27	26	53
Optical Property	Opaque	27	26	53
Texture	Silken	10	7	17
	Slimy	11	11	22
	Buttery	6	8	14
Pigment-ation	White	20	24	44
	Yellow	6	2	8
	Red	1	0	1

Note: Activity within a range of 4,00 – 11,5 mm of clear (bacterial growth) zone. The lowest activity shown by Ac48MP with biodegradation index of 0,57, whilst the highest shown by Ac18MP, with biodegradation index of 1,62. The screening procedure was conducted in triplicates. Even though it showed a ranging deviation standard of the treatment replicates, statistical analysis of ANOVA showed that there were no significant differences in each sample's replication (p value>0,005).

All 9 isolates showed a different range of biodegradation activities that could be considered low to high activities. Figure 1 showed that at least two isolates could use all microplastic polymers as its sole carbon source. Those two isolates are Ac8MP with biodegradation zone of 7,7±2,1 mm up to 10,66±0,4 mm, and Ac10 MP with the range of biodegradation zone of 4,13±0,4 mm - 10,03±2,3 mm. The other 7 isolates have varied activities in using different polymers of microplastic (Figure 2).

Table 2. Biodegradation activity of 9 bacteria isolates on LLDPE polymer, from 53 bacterial that successfully isolated from *Agelas conifera* sponge, with ANOVA significant value of p> 0.05 (0,524).

No	Code of Isolate	Diameter of Clear Zone (±deviation standard)	Biodegradation Index
1	Ac1MP	9,3±3,05	1,33
2	Ac8MP	6,3±2,08	0,90
3	Ac10MP	11,3±3,51	1,61
4	Ac14MP	9,5±3,53	1,36
5	Ac18MP	11,3±3,05	1,62
6	Ac36MP	7,0±0,0	1,00
7	Ac37MP	5,7±0,58	0,81
8	Ac44MP	5,0±2,0	0,71
9	Ac48MP	4,0±3,46	0,57

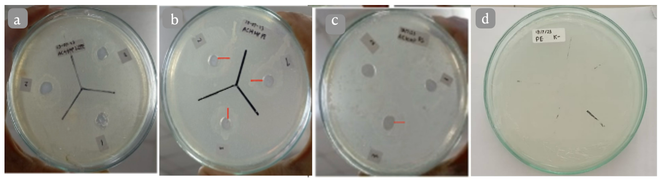

Figure 1. Biodegradation activity of bacterial isolates on different microplastic polymers shown by the clear (bacterial growth) zone (red line) surrounding the Kroc-Boral wells: a. Ac44MP on LLDPE, b. Ac14MP on PE, and c. Ac1MP on PE, d. Negative control test on PE.

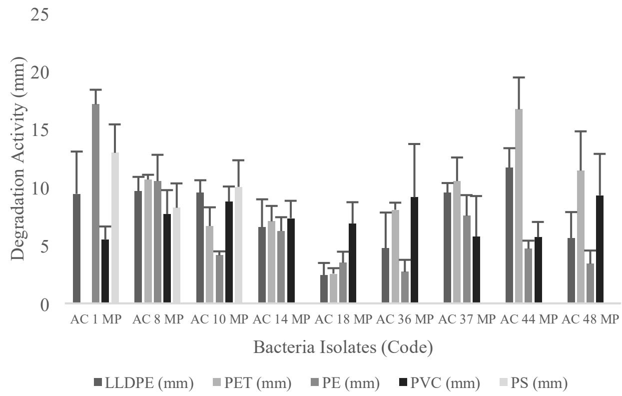

Figure 2. Biodegradation activities of spongebacteria isolates on different polymers of microplastic (with sig of Levene statistic for homogeinity (p= 0.524), and sig. value of ANOVA p=0.388). Spike indicated a deviation standard of replication.

Figure 2 also showed that there were at least 5 isolates that could use at least one polymer of microplastic with a high to moderate activity. Those isolates are: Ac1MP, Ac18MP, Ac36MP, Ac44MP, and Ac48MP. It also can be seen that all isolates could use PVC, which is a high density polymer, as its carbon source, and at the same time, could also use the low density polymers (LLDPE and PE). Meanwhile, PET polymer only could be degraded by 11 bacterial isolates, except Ac1MP. The least degraded polymer is PS that has a low-density polymers bond. Figure 2 showed that only 3 bacterial isolates could degrade or use this PS polymer as its carbon source.

Those bacterial isolates were: Ac1MP, Ac8MP and Ac10MP, with high biodegradation index of 1.85, 1.18 and 1.43, respectively (Table 3).

Table 3. Biodegradation index of microplastic polymers from the activity of bacterial isolates (with sig of Levene statistic for homogeneity (p= 0.523), and sig. value of ANOVA (p<0,005).

Code of Bacterial Isolates	Biodegradation Index of Microplastic Polymers				
	LLDPE	PET	PE*	PVC	PS*
AC 1 MP	1,35	0	2,45	0,78	1,85
AC 8 MP	1,38	1,52	1,5	1,1	1,18
AC 10 MP	1,36	0,95	0,59	1,25	1,43
AC 14 MP	0,94	1,01	0,89	1,05	0
AC 18 MP	0,35	0,37	0,5	0,98	0
AC 36 MP	0,68	1,15	0,38	1,3	0
AC 37 MP	1,36	1,5	1,08	0,82	0
AC 44 MP	1,67	2,38	0,67	0,81	0
AC 48 MP	0,8	1,63	0,48	1,33	0
Negative Control**	0	0	0	0	0

*Not normally distributed based on Shapiro-Wilk significant analysis (p value <0.005)
**Negative Control were using MSM media + microplastic polymer

Table 4. Distribution analysis on degradation activity based on types of the polymers

	Types of Polymer	Shapiro-Wilks		
		Statistic	df	Sig.
Clear Zone	LLDPE	0,916	9	0,363
	PET	0,971	9	0,906
	PE	0,81	9	0,026
	PVC	0,903	9	0,269
	PS	0,687	9	0,001

Correlation between the degradation of bacterial microplastic activity and the polymer

The activity value (mm) based on the diameter of the growth zone was then analyzed using Correspondence Analysis (CA) to determine the correlation between the degradation of bacterial microplastic activity and the type of polymer used. Figure 3 shows a graph of the results of this analysis.

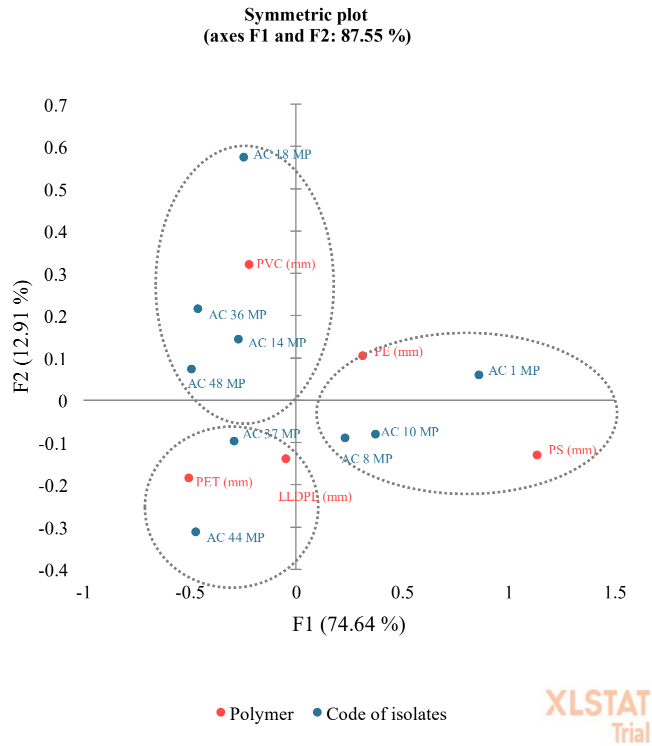

Figure 3. Correlation of microplastic degradation activity by selected bacterial isolates with the type of microplastic polymer using CA analysis.

CA analysis showed that there was a correlation between the type of polymer and the degradation activity of bacterial isolates, amounting to 87.55%. Isolates AC1MP, AC8MP, and AC10MP are related to PE and PS on the F1 axis. Isolates AC37MP and AC44MP were closely related to PET and LLDPE while isolates AC18MP, AC36MP, AC14MP, and AC48MP likely had a correlation with PVC. Generally, isolates of AC1MP, AC8MP, and AC10MP are related to the PE and PS polymer due to its moderate to high degradation index to the polymer. Furthermore, only these 3 isolates that showed degradation activity against PS polymer. Meanwhile, AC37MP and AC44MP showed a high index to LLDPE and PET, as well as AC18MP, AC36MP, AC14MP, and AC48MP showed moderate to high degradation index to PVC, amongst other polymers.

Discussion Isolation of Agelas conifera sponge-symbiotic bacteria

Results showed that the number of bacteria isolates with different morphologies of colony from each sponge individual have quite similar numbers of culturable bacteria (27 and 26, respectively). This result is higher than result from Kelly *et al.* (2005) that found only 4-5 isolates from the surface of *A. conifera* sponge. However, culturable symbiotic bacteria still relatively lower than the unculturable species, due to difficulties in mimicking the environmental and nutritional condition in the laboratory (Kiran *et al.*, 2018).

Most sponge-bacteria that have been successfully isolated have the colonies with morphological characteristics of smooth edges, round shape, convex elevation, dot formation, shiny, opaque and slimy appearances, with white pigmentation. Meanwhile, the cell characteristics were mostly cocci with Gram negative cell walls. These characteristics are commonly found in the pelagic bacteria of the seawater (Ginting *et al.*, 2019). However, these characteristics alone are very lacking to determine the identification of the bacteria. Therefore, other characteristics, such as physiological characteristics and /or molecular identification need to be conducted to determine the identity of the bacteria.

Screening and assay of microplastic polymer biodegradation

Concordance to the microplastic biodegradation activity, index of microplastic biodegradation showed that all tested bacterial isolates had varied activities between 0 up to 2.4. This index indicated that each isolate has different capabilities on degrading microplastic polymers. The biodegradation activity index was used based on several previous research that found that plastic degradation, either in the form of macro and micro, is basically came from enzymatic production of the degrading-bacteria (Oliviera *et al.*, 2020; Yang *et al.*, 2021; Cai *et al.*, 2023). Results showed that the isolate of Ac8MP is found could have biodegradation capabilities to all microplastic polymers, with moderate to high degradation index of 1.1-1.5. Whilst the most degraded polymers with high degradation index was found to be PVC, with degradation index of 0.78-2.44.

Data of biodegradation activities showed that there was a variance of distribution in the test. Shapiro-Wilk test showed that biodegradation of PE, and PS was not normally distributed (p value< 0,05), meanwhile LLDPE, PET and PVC polymers were normally distributed (Table 4). Moreover, significant differences between and within variables of treatment (microplastic polymers) using statistical analysis of Two Way ANOVA showed that each bacterial isolate is significantly different in its ability to degrade different polymers of microplastic, with p value of < 0,005 (1,2 E-07)). All data considered as homogeneity (without any outliers), since the homogeneity test using Levene Test showed that data is equally distributed, with p value of 0.388 (>0.005). Thus, even if the deviation standard of replication varied in each sample's treatment, it is still considered insignificant (Mishra *et al.*, 2019).

Microplastic could be reduced by physical, chemical and biological methods. There are several common mechanisms of microplastic reduction, such as thermal and chemical degradation, photodegradation, oxidative degradation and biodegradation (Haider *et al.*, 2019). Biodegradation could be defined as the process of biodeterioration, bio fragmentation and assimilation in synthetic polymers, yet also could be considered as the weathering process of polymers (Oberbeckmann and Labrenz, 2020). Furthermore, Wright *et al.* (2020), add that in the end of the biodegradation process, polymers, particularly microplastic, should be mineralized, and therefore, eventual removal in the natural environment. However, the mineralisation process of MP in the environment is still unclear (Cai *et al.*, 2023; Ruthi *et al.*, 2023).

Most of the previous research works on experimental works on microplastic biodegradation using microbial biofilm found in the "plastisphere" (Wright *et al.*, 2020). Most findings stated that the bacterial biodegradation in marine environments mostly comes from the pelagic, which is recruited into the biofilm on the plastisphere. The oligotrophic condition in the environment makes it possible for bacteria to use the plastic polymer as a source of carbon and nitrogen to gain their energy. Thus, the bacteria could form a complex community in the plastisphere (Cai *et al.*, 2023; Oberbeckmann and Labrenz, 2020; Ruthi *et al.*, 2023; Wright *et al.*, 2020). *A.conifera* sponge is benthic organism that could be found in the oligotrophic seagrass ecosystem with high abundance, and associated with mixed vegetation of *Thallasia hemprichii* and *Cymodocea rotundata* seagrass, with sparse vegetation structure (Ismet *et al.*, 2016; 2019). This sponge is also found to accumulate microplastic in its tissue with higher abundance compared to the sediment and column water (Sari *et al.*, 2023). Hence, it is almost predictable that the symbiotic bacteria of *A. conifera* sponge could also play a role in microplastic biodegradation.

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Sponge symbiotic bacteria is widely studied for its potential to produce secondary metabolites and extracellular enzymes. Amongst previous research, many have found that sponge symbiotic bacteria could help in polyaromatic hydrocarbons (Marzuki *et al.*, 2021; Marzuki *et al.,* 2023). However, research on microplastic biodegradation by sponge bacteria is still rare. This study highlighted the potential of sponge symbiotic bacteria as microplastic biodegradation agent, that could be useful in marine aquatic management and conservation. Most research on microplastic biodegradation focuses on the plastisphere bacteria community, for higher potential of application in the environment (Ruthi *et al.,* 2023). Several research works on how to implement the biodegradation mechanism in the environment, such as using synthetic sponges to absorb or coagulate the microplastic (Khan *et al.*, 2023; Sun *et al.,* 2021). This research proves that sponge, which accumulated microplastic, could also harbour symbiotic bacteria that is potential to be used to maintain, and rehabilitate marine ecosystems. These findings could be an alternative to have more environmentally friendly and cost effective rehabilitation on microplastic pollutants and at the same time increasing organisms biodiversity in the ecosystem. The reason sponge could become more efficient in degrading microplastic as well as enhancing biodiversity is because sponge could also play as marine biogenic habitat for mesofauna and benthic larvae (Ismet *et al.,* 2019), and its high capabilities in filtering surrounding water in the environment (Ledda *et al.*, 2014). Moreover, the symbiotic bacteria with capabilities of microplastic biodegradation, could also utilized as probiotic to enhance organisms resilience in polluted environment, likewise in current aquaculture application (Pringgienis *et al.*, 2020). Nevertheless, further research need be conducted to ensuring the improvement of host and environmental health, as well as large scale implementation (Felbeck and Distel, 1992; Mujeeb *et al.,* 2022).

Biodegradation of microplastic polymers depends on the molecule structure and energy that could produce from the polymer oxidation, thus related to the polymer's hydrolysable. Non hydrolysable plastic, such as polyethylene (PE) and polypropylene (PP), is more difficult to be assimilated into cells, for it needs to be cleavage into smaller molecules by redox reaction. On the contrary, hydrolysable polymers such as polyethylene terephthalate (PET) and polyamide (PA) is more easily degraded by enzymatic process or via hydrolysis, for its molecular similarity or analogous to natural substrate such as lignin and cellulose (Oberbeckmann and Labrenz, 2020). Interestingly, this study found that PE is one of the polymers that could be degraded by all tested bacteria, with varied degradation index (low-high). This result suggests that probably sponge symbiotic bacteria could have different mechanisms of enzyme production that could degrade polyethylene.

Another interesting finding is differences of biodegradation activities of 9 isolates on LLDPE polymer between the screening to the biodegradation assay. The differences in biodegradation activity could be result from complexity production of degradation enzyme to hydrolysed the same molecules, due to bioavailability of the polymers and differences caused by nutrient acclimatization, as well as environment condition, such as salinity, temperature and cultured condition (Oberbeckmann and Labrenz, 2020; Ozdemir *et al.,* 2022; Wright *et al.,* 2020; Ruthi *et al.,* 2023). However, there are still many questions on interaction between the factors complexity on biodegradation of microplastic by symbiotic bacteria. Nevertheless, further studies are still needed to prove the hypothesis, as well as the emerging questions about the mechanism of microplastic degradation.

Correlation between the degradation of bacterial microplastic activity and the polymer

Differences on degradation activity of all bacterial isolates are corelated with the mechanisms of each bacterial isolate to cleavage carbon polymers of the microplastic. LLDPE, as well as PE (with CH_2 bonds), which has low density of polymer, has short branches (10-30 CH_3/1000C), which could be obtained through the introduction of one or more co-monomers to ethylene molecules, which resulting to low crystallinity (40-60%) and lower melting temperature (~125□C) (Ojeda *et al.* 2011). This polymer is commonly used for many commercial and industrial purposes for its shorter degradation time than other long chain polymers. Previous research showed that poly-efins plastic such as PE and LLDPE could be degraded naturally (abiotic mechanism) in shorter degradation time, in months to one year, if the film were added with pro-oxidative additives (Ojeda *et al.,* 2011). Mechanism of the degradation is by oxygenic process that could cleavage the hydrocarbon chain of the polymers. This study found all bacterial isolates could degraded these two polymers, with higher degradation index onto LLDPE than PE, in general. This suggesting that the all the bacteria isolates could use its oxidative metabolisms in the polymers degradation mechanism.

Interestingly, high density polymers PVC, could also be degraded by all isolates. PVC as one of the most plastic polymers, usually degraded using pyrolysis mechanism in the waste treatment. However, the pyrolysis combustion (degradation) could emit toxic precursors such as polychlorinated dibenzodioxins (PCDD), dibenzofurans (PCDF) and polychloro-biphenyls (PCB) upon combustion afterwards (Huang *et al.,* 2018). This could lead another environmental problem for the chlorinated substances in the end of degradation. Thus, biotic degradation mechanisms, likewise this study could be a great alternative for PVC degradation.

Degradation activity against different polymers suggest that could be different mechanism of bacterial enzymatic metabolism. Syranidou *et al.* (2019) found that there were adhesive cells carrying xenobiotic and hydrocarbon degradation genes that act in the microplastic degradation mechanisms in the bacterial consortia on PE and PS. Moreover, polymers degradation by microorganism were favoured by consuming the carbonyl groups that leads to unsaturated chains, breaking the plastic chains. This is the mechanisms that could probably happened this study. However, further research is still needed to confirm this hypothesis. Moreover, identification of bacterial species also needed to understand and give more insightful view of the bacterial metabolisms that could lead to the microplastic polymers degradation mechanism.

Overall, this study showed that microorganism could potentially degrading microplastic polymers using single or many bacteria cultures. Nevertheless, optimalization procedures to leverage the bacterial activities under certain condition still be needed, to implement the potential of bacterial in the industrial scales or waste treatment facilities.

Conclusion

Nine of 53 bacteria isolates were screened and tested to degrade microplastic polymers. All isolates found could degrade LLDPE, PE and PVC, whilst PET and PS only by 8 and 3 isolates, respectively. High activities of microplastic biodegradation (index >1.5) showed by 5 bacterial isolates on different polymers. The most potential isolates are Ac8MP that could degrade all polymers with degradation index more than 1. Further study on enzyme characterization as well as characterization on polymer mineralization could be the next step to understand the mechanism of microplastic biodegradation, as well as determining the residual of toxic compounds after biodegradation mechanism.

Acknowledgements

This research was supported by Directorate of Higher Education Research and Technology with grant number: 11428/IT3/PT.01.03/P/B/2023.

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How to cite this paper:
Ismet, M. S., E. S. Srimariana, N. A. D. Cahyati, N. P. Anggraini, L. M. Simbolon, N. Cakasana, A. E. M. Sari, M. Rafi. 2025. Biodegradation activities of microplastic polymers by Agelas conifera sponge-symbiotic bacteria. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 14(2): 148–156.