Isolation of Bacteria and Analysis of Microplastic Abundance from Bagansiapiapi Waters in Riau Province, Indonesia.

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Abstract. Microplastic contamination in the marine environment has disturbed organisms such as plankton, benthos, nekton and fish. Plastic waste also has potency to cause economic losses in fishing, tourism and shipping industries. This study aims to isolate and identify bacteria and to analyse microplastic in Bagansiapiapi waters by a survey method. Sampling was carried out at three locations in the range of geographical coordinates of 2.110717° - 2.131641° Latitude, and 100.745152° - 100.795530° Longitude. The water samples were collected from the surface water, subsurface water (1-2 m depth), and surface sediment. Range of bacterial counts in the surface, sub-surface waters and sediment were $11.4 \times 10^4 – 31.8 \times 10^4$ cfu/ml; $0.2 \times 10^4 – 13.6 \times 10^4$ cfu/ml and $17.9 \times 10^4 – 33.1 \times 10^4$ cfu/mg, respectively. There were 37 bacterial isolates identified based on morphological, physical and biochemical characters. The ranges of microplastic abundance in surface and subsurface waters and in sediment were 73.333 ± 30.551 – 83.333 ± 15.275 particles/L, 50.000 ± 20.000 – 76.667 ± 20.817 particles/L and 50.000 ± 26.458 - 70.000 ± 17.321 particles/Kg, respectively. The types of microplastic found were fiber, filament, pellet and film.

1 Introduction

Increase in world population and life' activities had resulted in negative impact on environmental quality, especially in aquatic ecosystem. One of serious problems is pollution due to plastic wastes found in the ocean [1, 2]. The growth of plastic was along with the increase in urbanization, economic development, and population [3]. In 2022, the worldwide plastic production is calculated to be 400.3 million tons. The production is estimated to grow exponentially thereafter [4]. Indonesia ranks among the top largest contributors of plastic waste into the coastal environment, along with the United States and China [5, 6]. Plastic waste causes some problems, due to its low densities results in it is easily distributed, moreover, the degradation takes hundred years [7]. This is due to the chemical structure of plastic consisted of polymers of long chain hydrocarbon with high molecule weight.

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Degradation process of plastic into microplastic in seawaters is influenced by solar radiation, high temperature and current condition [8].

Microplastics are found in any compartment of environment, mainly in the oceans and remote islands. In general, microplastics are synthetic polymer compounds that form when large plastic materials are fragmented and micronized to a size ≤5 mm [9]. Due to its small size, microplastics can easily ingested and enter the digestive, blood circulation, endocrine and reproductive systems of exposed marine biota [10, 11]. Accumulation of microplastics in marine biota would result in negative impact to organism health because of the toxic additive and carcinogenic compound of the plastics [12]. The abundance of plastic waste nowadays has been reported to cause economic loss in fishery, tourism and navigation industries [13]. The most types of plastics found in the aquatic environments are polymers such as high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET) [14]. Microplastics can exist as fragments, films, fibres, and foam [9].

Some efforts have been done to solve problem of plastic waste in the environment, either with physical or chemical treatments. However, the efforts sometimes give rise to new problem in the environment. For example, combustion process is considered less effective and dangerous because the plastic can release toxic gases such as CO_2 and CO [15]. An alternative to overcome those problems is by the use microorganisms which have enzymes to degrade the plastic polymers.

Many researches have found the presence of plastic-degrading microorganisms in the world marine environment, mainly from bacteria, fungi and algae species. *Pseudomonas* spp. was among the different bacterial genera associated with plastic degradation, account for 21% [16]. In the marine environment, *Pseudomonas* spp. have been widely detected on plastic biofilms [17]. *Pseudomonas rhodesiae* isolated from Brazilian deep-sea sediments could form biofilms on high density polyethylene (HDPE) and cause structural changes in the plastic [18]. Other bacteria also have potential to degrade plastics. *Bacillus* spp. can effectively degrade different types of plastics, represented by *Bacillus cereus*, *Bacillus safensis*, and *Bacillus subtilis* [17]. For example, *B. cereus* isolated from mangroves in Peninsular Malaysia resulted in weight losses of 1.6%, 6.6%, and 7.4% for PE, PET, and PS, respectively, in 40 days [19]. *Pseudomonas, Streptomyces, Corynebacterium, Arthrobacter, Micrococcus, Rhodococcus, Polaromonas, Micrococcus, Subtercola, Agreia, Leifsonia, Cryobacterium*, and *Flavobacterium* are among microorganisms prominently found in cold environment [20].

The presence of plastic-degrading bacteria has also been reported from the Indonesian marine environment. Enterobacteriaceae, *Moraxella* spp. and *Psudomonas* spp. isolated from the Marina Beach Semarang, Central Java had high polyethylene degradation potential with the speed of degradation 0.0091%, 0.0066% and 0.0076% per week, respectively [15]. *Vibrio alginolyticus, Pseudoalteromonas caenipelagi, Microbulbifer pacificus, Pseudomonas marincola*, and *Bacillus subtilis* isolated from Muara Angke Jakarta Bay had capacity to degrade PE and PET microplastics in a liquid medium [21].

Research on the presence of plastic-degrading bacteria from Riau marine environment is still limited. *Bacillus* sp., a bacterial isolate from sediment of the Dumai marine area of Riau, is able to degrade Polyethylene Terephthalate (PET) with a 7% of degradation rate in 30 days [22]. Therefore, this research aimed to isolate and identify bacteria which may potential in degrading microplastic from another Riau marine area, that was in the Rokan River estuary of Bagansiapiapi city.

2 Materials and Methods

2.1 Sampling locations

The research was conducted in Bagansiapapi as the capital city of Rokan Hilir District in Riau Province, Indonesia. Surface and subsurface water and top sediment samples were collected from three stations. Station 1 was in the domestic harbour; station 2 was in the Rokan River estuary, and station 3 was in waters toward the open ocean. Geographycal position of the sampling areas is presented in Table 1.

Table 1. Geographycal position of sampling locations in the Bagansiapiapi waters

Sampling location	Latitude coordinates	Longitude coordinates
Station 1: Domestic harbor	2.110717° - 2.110728°	100.795512° - 100.795530°
Station 2: Rokan River estuary	2.123533° - 2.129586°	100.765514° - 100.745152°
Station 3: Waters toward the open ocean	2.131411° - 2.131641°	100.745152° - 100.745514°

2.2 Materials and equipment

Bacteria in each of water and sediment samples was isolated and grown in the Zobell Marine Agar (Himedia, India). The water samples were collected both from the surface and from subsurface in a depth of 50 cm. While, surface sediment samples (0 10 cm) were collected from the river estuary bench. Isolates with different morphological characters were reinoculated and purified in trypticase soy agar (TSA, Oxoid, UK) and nutrient agar (NA, Oxoid, UK) for identification. Other media and chemicals such as sulfide indole motility agar (SIM, Oxoid, UK), Methyl-Red Voges Proskaeur (MR-VP, Oxoid, UK), crystal violet, safranin, Kovac's reagent, alcohol, hydrogen peroxide (H_2O_2) 3% which were used in bacterial identification, and McFarland standard solution. trypric soy broth (TSB, Oxoid, UK) and polyethylene (PE) plastic were used in bacterial degradation test.

Environmental condition of sampling location was also observed and measured. Water temperature, salinity, pH and dissolved Oxygen (DO), water transparency and current speed were measured by using Celsius thermometer, hand refractometer, Water Checker Tester EZ9909, Secchi disk and current meter, respectively. Water sediment samples were collected using VanDorn water sampler and scoop sampler.

2.3 Bacterial isolation and identification

Bacteria from each of water and sediment samples was isolated and grown on the Zobell Marine Agar (Himedia) by using the spread plate technique in triplicate. Number of grown colonies was counted; different size and colour of the colonies were reinoculated and purified on the fresh agar medium. Each isolate was then identified based on morphological characteristics (i.e. colony's shape, size diameter, colour, elevation and edges) and biochemicals tests (i.e. Gram staining, productions of catalase, oxidase, indole, H_2S and methyl red). All of different isolates were refrigerated at ± 4 ^oC for the next uses.

2.4 Analysis of microsplastic

Analysis of microplastic in water samples was conducted based on procedure of [23]. The water was filtered using a sieve (300 μm) to separate the meso trash with micro. The filtrate

was filtered through a whattman filter paper size 45 μm [24]. Then, the filtrate was visually observed under the microscope, and microplastics found were counted based on the type found. The abundance of microplastic was calculated based on the number of particles found in the filtered water [25]:

$$C = N/V$$

Where, C is abundance (particle/L); n is number of particles; and V is the volume of filtered water (L).

Analysis of microplastic in sediment samples were performed after drying 50 g sediment in an oven at 105 °C for 24 hours and grounded using mortar [26]. The fine sediment was put into a glass beaker and was mixed with NaCL solution for for 24 hours. Then, the sample was observed under a microscope and recorded every type of microplastic found. Based on the initial weight of the sample used as much as 50 g, the result of each sample analysis was converted into 1 kg by transferring it to 20 [27] with particle/Kg parameter units.

2.5 Data analysis

Data of total bacterial counts, morphological and biochemical characteristics from each water and sediment samples were presented in tables. Morphology of microplastic types observed was presented in figures. The data were then analysed descriptively and referred to related references.

3 Results and Discussions

3.1 Water quality conditions of sampling area

Rokan River is one of important rivers in Riau Province, which has 350 km length flows on the edge of Bagansiapiapi, the capital city Rokan Hilir district, ends into the Malacca Straits [28], The river and its estuary are important as transportation route and as source of livelihood of people, mainly for the local fishermen. The results of observation on the water quality of the Rokan River estuary in this research is presented in Table 2.

Table 2. Average values of physical and chemical parameter in the Bagansiapiapi waters

No	Water quality parameter	Station 1		Station 2		Station 3	
		Surface	Sub-surface	Surface	Sub-surface	Surface	Sub-surface
1.	Temperature (°C)	30.3	31.0	33.4	33.9	32.2	31.3
2.	Salinity (ppt)	4.7	5.0	10.7	10.3	15.0	15.0
3.	pH	5.6	5.6	5.3	5.1	5.8	5.7
4.	DO (mg/L)	2.0	2.4	2.5	2.7	2.2	2.4
5.	Transparency (cm)	0.0	0.0	0.0	10	0.0	23.8
6.	Current speed (m/s)	n.d	0.8	n.d	0.5	n.d	0.4

Note: n.d, note detected

Data in Table 2 indicate there is no much difference of some water quality parameters in each sampling location. Water temperature, salinity and pH are in normal value for seawaters in estuary. The pH values are relatively low, as the characteristics of pH in estuaries are influenced by a variety of factors (e.g. urban and agriculture) and processes, including the pH in river inflow. The pH in river inflow is influenced by catchment vegetation through the

amount of dissolved organic carbon in runoff; catchment vegetation is dominated by peat and humus, such substances convert to humic acid in solution, resulting in a decrease in water pH [29]. The DO and water transparency are also low. This is might be due to high suspended particle content in the waters as the Rokan River estuary experiences shallowing and sedimentation as a result of erosion up stream of the river [30].

3.2 Total bacterial counts

Total number of bacteria in water and sediment samples from each of sampling stations was counted as it is presented in Table 3. The bacterial counts range from 0.2×10^4 cfu/ml - 33.1×10^4 cfu/mg. Based on the sampling stations, the highest bacterial count in Station 1 was found in the surface waters and the lowest was in subsurface water; while, in Station 2, the highest count was found in sediment and the lowest was in subsurface waters; and in Station 3 the highest count was found in sediment and the lowest was in sediment subsurface waters.

Table 3. Average bacterial counts in the Bagansiapiapi waters

No.	Sampling site	Bacterial counts in water and sediment samples		
		Surface (cfu/ml)	Subsurface (cfu/ml)	Sediment (cfu/mg)
1	Station 1	31.8×10^4	13.6×10^4	25.0×10^4
2	Station 2	13.0×10^4	0.6×10^4	17.9×10^4
3	Station 3	11.4×10^4	0.2×10^4	33.1×10^4

In general, number of bacteria as shown in Table 3 is high in sediment. The high bacterial count in sediment could due to bacteria tend to accumulate and attach in sediment or to the particulate materials [31]. Estuary sediments contain organic and an-organic materials which are transported from river water mixes with seawater. Sediment bacterial communities act as primary regulators of biogeochemical processes and nutrient cycling at initial marsh development [32].

3.3 Characteristics of bacterial isolates

As many as 37 bacterial isolates have been isolated and identified from the three sampling stations in the estuarine area of Rokan River based on morphological and biochemical characteristics. From the surface water, 14 isolates (ISW) were identified as shown in Table 4 and 5; while in the subsurface waters 11 isolates (ISS) have characteristics as shown in Table 6 and 7; and the characteristics of 12 isolates (ISD) from sediment as presented in Table 8 and 9. All of bacterial isolates shows differences in morphological characters based on size, colour, shape, edge and surface of the grown colonies.

Table 4. Morphological characters of bacterial colonies isolated from surface water

No.	Isolate code	Diameter (cm)	Colony colour	Shape of colony	Edge of colony	Surface of colony
1	ISW 1	0.1	White yellowish	Punctiform	Convex	Entire
2	ISW 2	0.3	Deep yellow	Round	Convex	Entire
3	ISW 3	0.2	Yellow	Irregular and spreading	Umbonate	Irregular
4	ISW 4	0.8	White	Irregular and spreading	Raised	Entire
5	ISW 5	0.7	Yellow	Round	Convex	Entire
6	ISW 6	0.9	Deep yellow	Round	Convex	Entire
7	ISW 7	0.5	Yellow	Round	Convex	Entire

No.	Isolate code	Diameter (cm)	Colony colour	Shape of colony	Edge of colony	Surface of colony
8	ISW 8	0.1	Yellow	Round	Convex	Entire
9	ISW 9	0.4	Pale yellow	Round	Convex	Entire
10	ISW 10	0.6	Pale yellow	Punctiform	Convex	entire
11	ISW 11	0.7	Yellow	Round	Convex	Entire
12	ISW 12	1.2	Cream	Irregular and spreading	Raised	Wavy
13	ISW 13	1.1	White	Irregular and spreading	Umbonate	Wavy
14	ISW 14	1.9	White	Irregular	Raised	Wavy

Table 5. Biochemical characters of bacterial isolates from surface water

Isolate code	Biochemical characters						
	Gram staining	Catala-se	Oxida-se	Motility	Indole product.	Use of methyl red	H_2S gas product.
ISW 1	+ (coccus)	+	+	-	+	-	-
ISW 2	+ (coccus)	+	+	-	-	+	+
ISW 3	+ (bacil)	+	+	-	-	+	-
ISW 4	- (coccus)	+	+	-	-	+	+
ISW 5	- (coccus)	+	+	-	-	+	-
ISW 6	+ (coccus)	+	+	-	-	+	-
ISW 7	- (coccus)	+	+	-	-	+	-
ISW 8	- (coccus)	+	+	-	-	+	-
ISW 9	- (coccus)	+	+	-	-	+	-
ISW 10	+ (coccus)	+	+	-	-	+	-
ISW 11	+ (coccus)	+	+	-	-	+	-
ISW 12	+ (bacil)	-	+	-	-	+	-
ISW 13	+ (bacil)	-	+	-	-	+	-
ISW 14	+ (coccus)	-	+	-	-	+	-

Data in Table 4 and 5 indicates slightly difference between all colonies obtained. Diameter of bacterial colonies from the surface water varies from 0.1 – 1.9 cm; almost all of the colonies have round shape and yellow in colour. Nine of 14 isolates are of Gram-positive bacteria, 11 isolates produced catalase enzyme, but all isolates were non-motile and produced oxidase enzyme; only one isolate produced indole and two isolates produced H_2S, and 13 isolates used methyl red as the carbon source.

In the meant time, bacteria isolated from the subsurface water also indicated variation in morphological and biochemical characters. Colony diameter of 11 isolates varies from 0.1 – 0.8 cm. The colony shapes were round (7), fillamentous (3) and punctiform (1) which were dominantly white to yellow in colour.

Table 6. Morphological characters of bacterial colonies isolated from subsurface water

No.	Isolate code	Diameter (cm)	Colony colour	Shape of colony	Edge of colony	Surface of colony
1	ISS 1	0.2	White yellowish	Fillamentous	Flat	Curled
2	ISS 2	0.1	Yellow	Round	Convex	Entire
3	ISS 3	0.8	Yellow	Round	Convex	Entire

No.	Isolate code	Diameter (cm)	Colony colour	Shape of colony	Edge of colony	Surface of colony
4	ISS 4	0.2	White	Round	umbonate	Entire
5	ISS 5	0.2	White	Round	Convex	Entire
6	ISS 6	0.6	Deep yellow	Round	Convex	Entire
7	ISS 7	0.5	White	Fillamentous	Flat	Curled
8	ISS 8	0.6	White yellowish	Fillamentous	Flat	Curled
9	ISS 9	0.8	Yellow	Round	convex	Entire
10	ISS 10	0.3	White yellowish	Round	Convex	Entire
11	ISS 11	0.1	White	Punctiform	convex	Entire

Table 7. Biochemical characters of bacterial isolates from subsurface water

Isolate code	Biochemical characters						
	Gram staining	Catala-se	Oxida-se	Motility	Indole product.	Use of methyl red	H_2S gas product.
ISS 1	- (coccus)	-	+	-	-	+	-
ISS 2	+ (coccus)	-	+	-	-	+	-
ISS 3	+ (coccus)	+	+	-	+	+	-
ISS 4	+ (coccus)	+	+	-	-	-	-
ISS 5	+ (coccus)	+	+	-	-	+	-
ISS 6	+ (coccus)	+	+	-	-	-	-
ISS 7	+ (bacil)	+	+	-	-	+	-
ISS 8	+ (bacil)	+	+	-	-	+	-
ISS 9	+ (coccus)	+	+	-	-	+	-
ISS 10	+ (bacil)	+	+	-	-	+	-
ISS 11	+ (bacil)	+	+	-	-	+	-

Data in Table 7 indicates that 10 bacterial isolates from subsurface water were of Gram positive bacteria which had dominantly coccus cells. Nine isolates could produce catalase enzyme, but all produced oxidase enzyme and were non-motile cells. Only one isolate produced indole and none of them produced H_2S gas. Nine isolates were able to use methyl red as the carbon source.

Sediment bacteria indicated few differences in morphological characters. The diameter of colony of 12 isolates varied from 0.1 – 1.2 cm. All colonies were round in shape, and were dominantly white in colour. Eleven bacterial isolates from the sediment samples were of Gram-positive bacteria, and cells were dominantly coccus. All isolates were non-motile and able to produce catalase and oxidase enzymes. Six and 10 of the 12 isolates produced indole and H_2S gases, respectively. Eleven isolates used methyl red as the carbon source.

Table 8. Morphological characters of bacterial colonies isolated from the sediments

No.	Isolate code	Diameter (cm)	Colony colour	Edge of colony	Elevation of colony	Shape of colony
1	ISD 1	0.3	White	Entire	Flat	Round
2	ISD 2	0.2	Yellow	Entire	Flat	Round
3	ISD 3	0.8	White	Entire	Flat	Round
4	ISD 4	0.9	White	Entire	Flat	Round
5	ISD 5	0.7	White	Wavy	Flat	Round
6	ISD 6	1.2	White	Entire	Convex	Round
7	ISD 7	1.1	White	Entire	Flat	Round

No.	Isolate code	Diameter (cm)	Colony colour	Edge of colony	Elevation of colony	Shape of colony
8	ISD 8	0.8	White	Entire	Flat	Round
9	ISD 9	0.5	Yellow	Entire	Convex	Round
10	ISD 10	0.1	White	Entire	Convex	Round
11	ISD 11	0.4	White	Entire	Flat	Round
12	ISD 12	0.6	White	Entire	Flat	Round

In general, 30 of 37 bacterial isolates found from both water and sediment in the Rokan River and its estuary were of Gram-positive bacteria, and dominantly were coccus. Many researches reported that some Gram-positive bacteria have an ability to degrade plastics and microplastics, such as *Kocuria palustris, Bacillus pumilus, B. subtilis* and *Staphylococcus epidermidis* [33, 34]. Genera of *Bacillus* and *Staphylococcus* are Gram positive which have bacil and coccus cells, respectively. Although there were only seven isolates which are of negative bacteria, they are also potential in plastic and microplastic degradation. Current study found seven isolates are of Gram-negative cocci bacteria. *Acinetobacter* and *Moraxella* are among Gram negative cocci. Antarctic bacterium *Moraxella* sp. produced polyesterase which was able to degrade highly crystalline synthetic polymers [35]. *Acinetobacter venetianus* F1, a deepsea bacterium can degrade 12.2% of polyethylene after 56 days [36].

Table 9. Biochemical characters of bacterial isolates from sediment

Isolate code	Biochemical characters						
	Gram staining	Catala-se	Oxida-se	Motility	Indole product.	Use of methyl red	H_2S product.
ISD 1	+ (coccus)	+	+	-	+	+	+
ISD 2	+ (coccus)	+	+	-	+	+	+
ISD 3	- (coccus)	+	+	-	-	+	+
ISD 4	+ (coccus)	+	+	-	-	+	+
ISD 5	+ (coccus)	+	+	-	+	+	+
ISD 6	+ (coccus)	+	+	-	-	+	+
ISD 7	+ (bacil)	+	+	-	+	+	-
ISD 8	+ (bacil)	+	+	-	+	-	+
ISD 9	+ (coccus)	+	+	-	+	+	+
ISD 10	+ (bacil)	+	+	-	-	+	+
ISD 11	+ (coccus)	+	+	-	-	+	+
ISD 12	+ (coccus)	-	+	-	-	+	-

3.4 Microplastic abundances

Analysis on the microplastic abundances in samples from each of sampling stations indicates a variation based on the sample types (Table 10). The highest abundance of microplastic was found in surface water of Station 1, followed by those in subsurface water and in sediment. While, in Station 2, the highest microplastic abundance was found in subsurface water, followed by those in surface water and in sediment. Then, in Station 3, the highest value was found in surface water, and same abundance was found in subsurface water and sediment.

Table 10. Average of microplastic abundances from the Bagansiapiapi waters

No.	Sampling site	Content of microplastic in water and sediment		
		Surface water (particle/L)	Subsurface water (particle/L)	Sediment (particle/Kg)
1	Station 1	83.333 ± 15.275	76.667 ± 20.817	70.000 ± 17.321
2	Station 2	63.333 ± 11.547	76.667 ± 15.275	60.000 ± 10.000

No.	Sampling site	Content of microplastic in water and sediment		
		Surface water (particle/L)	Subsurface water (particle/L)	Sediment (particle/Kg)
3	Station 3	73.333 ± 30.551	50.000 ± 20.000	50.000 ± 26.458

The higher abundance of microplastic in water could be due to sampling of water and sediment was conducted during the high tide, which indicated high current speed carrying solid and suspended particles from marine area and the Rokan River estuary toward the river. Moreover, the estuary is relatively shallow, so that during the high tide microplastic-containing material at the bottom could be lifted up. This finding is different from previous study which found the abundance of microplastic in the Dumai waters of Riau which was higher in sediment (193.33 - 746.67 particles/Kg) than in water (0.130-0.200 particles/L) [37]. At the Jakarta Bay in Indonesia, the abundance of microplastics in the sediment (166.8 particles/Kg, 95%CI: 148.0-185.0) was significantly higher ($p < 0.05$) than in surface water (70.9 particles/L, 95%CI: 55.6-86.2) [38]. Current study identified fibre, filament, pellet and film as the type of microplastic found in both seawater and sediment. These type microplastics had also been reported by the previous studies.

4 Conclusion

Bacteria and microplastic had been analysed from waters and sediments of the Rokan River estuary. Thirty of 37 bacterial isolates found are of Gram-positive cocci and bacil bacteria, and seven isolates are of Gram-negative cocci. Microplastic abundances in water was higher than in sediment. The bacterial isolates found could be potential in the degradation microplastics. Therefore, further study needs to be conduction to examine the ability of those bacteria in degrading microplastic.

Acknowledgements

This research was funded by DRTPM (the Directorate of Research, Technology and Community Service) of the Ministry of Education, Research and Technology of the Republic of Indonesia within the framework of project No. 083/E5/PG.02.00.PL/2024 under the Fundamental Research scheme.

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