

## ISOLATION AND IDENTIFICATION OF DEGRADING BACTERIA MICROPLASTICS ON THE SURFACE WATER IN BAGANSIAPIAPI WATERS, RIAU PROVINCE

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### ABSTRACT

Microplastic pollution in the marine environment seriously threatens aquatic ecosystems, including in the coastal areas of Bagansiapiapi, Riau Province. This study aims to isolate and identify microplastic-degrading bacteria from the sea surface and test their degradation ability. Sampling was carried out at three different locations in the Bagansiapiapi waters, and then isolation, morphological identification, and biochemical tests were carried out at the Marine Microbiology Laboratory, Universitas Riau. Microplastic degradation tests using LDPE plastic were carried out for 30 days, and molecular identification was carried out using the 16S rRNA PCR method. The results showed significant differences in the number of bacteria between stations. Station I (port area) showed the highest bacterial density of  $31.8 \times 10^4$  CFU/ml. Six of the 14 bacterial isolates obtained showed the ability to degrade microplastics, with ISL 14 (*Bacillus* sp.) showing the highest degradation percentage of 30.38%. This finding shows the potential for utilizing local bacteria as bioremediation agents for microplastics in the marine environment effectively and environmentally friendly.

**Keywords:** *Bacillus* sp, Bacteria, Biodegradation, Bagansiapiapi, Microplastics, 16S rRNA

### 1. INTRODUCTION

The Bagansiapiapi waters, an industrial and densely populated area in Riau Province, are estimated to receive waste discharge from ports, settlements, shipping, agriculture, fisheries, and industry, contributing to increasing pollution levels in the area. Plastic waste is one of the most common pollutants. Plastic is one of the most widely used materials and is widely integrated into people's lifestyles. It also contributes significantly to almost all product and service industries<sup>1</sup>. One of the serious threats due to the presence of plastic waste is microplastics. Microplastics are plastic waste measuring 0.3 to 5 mm<sup>2</sup>.

Microplastics that pollute waters can be eaten by marine biota in the ecosystem, and the entry of microplastics into marine waters can endanger the survival of marine biota. In addition, microplastics can be

carriers of hazardous contaminants, both inorganic and organic<sup>3</sup>.

Efforts to reduce plastic waste by recycling the garbage are not optimal, so another alternative, biodegradation using microorganisms such as bacteria, is needed. Biodegradation is when microorganisms can degrade or break down natural polymers (such as lignin and cellulose) and synthetic polymers (such as polyethylene and polystyrene). During the polymer biodegradation process, two categories of enzyme activity are involved, namely extracellular and intracellular depolymerases<sup>4</sup>.

Many studies have been conducted on dealing with microplastic waste in waters physically and chemically, but very few studies have been conducted microbiologically to deal with water pollution. Handling bacteria is effective

because it does not cause side effects on the environment and does not produce toxins or blooms. Microorganisms that can degrade plastic are more than 90 genera, namely from the types of bacteria and fungi, including *Bacillus megaterium*, *Pseudomonas* sp, *Azotobacter*, *Ralstonia eutropha*, *Halomonas* sp, and others<sup>5</sup>.

Based on this description, the author is interested in researching the isolation and identification of microplastic-degrading bacteria from Bagansiapiapi waters to control problems related to microplastic degradation in waters.

## 2. RESEARCH METHOD

### Time and Place

This study consists of several stages, namely sampling in the field and sample analysis in the laboratory, which was carried out from June to December 2024. Sampling was conducted in Bagansiapiapi Waters, and sample analysis was performed at the Marine Microbiology Laboratory, Department of Marine Sciences, Faculty of Fisheries and Marine, Universitas Riau. Molecular testing and sequencing were carried out at PT. Genetika Science Indonesia, West Jakarta.

### Method

The sampling location was determined by purposive sampling. Bagansiapiapi waters will be used as the sampling location, determining the location of the sampling point through several considerations based on the source of pollution and the criteria for water conditions. The research location will be divided into three Station points that are considered to be able to represent the research area. Station 1 is around the port, Station 2 is in the fisheries area, and Station 3 is in the Rokan River estuary area.

### Procedures

#### Sampling

At each sampling point, microplastic samples in water were taken. Water samples were taken using a 10 L bucket and filtered using a 100 L plankton net. The filtered

water samples were put into a 1 L sample bottle and then stored in an ice box. Meanwhile, samples of microplastic-degrading bacteria were taken by taking seawater using a bucket and putting it into a 250 mL sample bottle. Then, the samples were put into an ice box.

### Water Quality Measurement

The water quality parameters measured are: 1) temperature Measurement on the water surface using a thermometer. 2) salinity: Measure salinity using a hand refractometer. 3) Degree of Acidity (pH), pH measurement is carried out using a pH meter. 4) Current Speed, Current speed is measured using a current drogue and an activated stopwatch. 5) Transparency is measured using a secchi disk. 6) Dissolved Oxygen (DO): The measurement of dissolved oxygen can be done using a DO meter.

### Microplastic Analysis

A water sample of 500 mL was filtered, and 200 mL of 30% NaCl was added, stirred, and left for one night. After that, 10 mL of 30% H<sub>2</sub>O<sub>2</sub> was added, stirred for 5 minutes, and left for 2 nights. The sample solution was filtered using the vacuum pump method. It dried the filter paper in a desiccator for 1 night and was analyzed using an Olympus CX 21 microscope and grouped into four types: film, fiber, fragment, and pellet<sup>6</sup>. The microplastic particles found were calculated using the microplastic abundance formula and analyzed at each location. The following is the Microplastic Abundance formula<sup>7</sup>:

$$C = \frac{n}{v}$$

Description:

- c = abundance of microplastics  
(Number of particles/m<sup>3</sup>)
- n = number of microplastic particles
- v = volume of filtered water (m<sup>3</sup>).

### Isolation and Purification of Bacteria

Seawater samples are homogenized using a vortex and then put into a

physiological solution (0.9% NaCl). Furthermore, dilution is carried out using the dilution series method, which takes 1 mL of the sample and puts it into the first test tube containing 9 mL of physiological solution to obtain a dilution of  $10^{-1}$  is obtained. Then, another 1 mL was taken from the dilution tube  $10^{-1}$  and put into the second test tube containing 9 mL of physiological solution to get a dilution of  $10^{-2}$  and so on until a dilution of  $10^{-1}$  [1].

Next, inoculation was carried out on the Zobell Marine Agar media by taking 0.1 ml of the sample and putting it into a petri dish containing solid Zobell Marine Agar media, then leveled using an L rod, and then incubated with the dish upside down for 24-48 hours at a temperature of  $37^{\circ}\text{C}$ . The bacteria are calculated for the number of bacterial cells from the calculation multiplied by the dilution factor expressed by the formula:

$$\text{number of bacteria } \left(\frac{\text{cfu}}{\text{mL}}\right) = \text{number of colonies} \times \frac{0,1}{\text{Dilution Factor}}$$

Bacterial purification was carried out using the streak plate method. Each petri dish from the results of bacterial cultivation was taken from several bacterial colonies that showed different morphologies. The bacterial colonies were streaked on the Zobell Marine Agar media, then incubated at room temperature for  $1 \times 24$  hours<sup>8</sup>.

### Morphological and Biochemical Tests of Bacteria

Colony morphology observation was done by measuring the colony's diameter, color, shape, size, edge, and elevation<sup>9</sup>. While the biochemical tests carried out consist of: Gram staining, catalase, oxidase, motility, indole, MR-VP, and  $\text{H}_2\text{S}$  test

### Microplastic Degradation Test

The biodegradation test of microplastic waste was carried out to see the percentage of reduction in LDPE plastic samples by degrading bacteria, which consisted of several stages, namely<sup>1</sup>: Samples of plastic waste measuring  $1 \times 1$  cm were weighed initially, washed with sterile distilled water, and sprayed with 70%

alcohol. Furthermore, the plastic sample was put into a glass sample bottle containing 50 ml of TSB media aseptically. Then, two loops of bacterial isolates were inoculated into the media, in a shaker at room temperature with 130 RPM aeration for one month.

After one month of being placed in the shaker, the plastic waste sample was washed with sterile distilled water and sprayed with 70% alcohol. The plastic waste sample was air-dried (UV light on Laminar Air Flow) for 30 minutes, and the final weight was measured. The plastic pieces were weighed using an analytical balance. Determination of the percentage of degradation of plastic waste samples by bacteria was calculated using the formula:

$$\% \text{ degradation} = 1 - \frac{\text{final weight}}{\text{initial weight}} \times 100\%$$

Next, take the media that has been overgrown with colonies, dilute it with a physiological solution from  $10^{-1}$  to  $10^{-8}$ . Then, plant it on the Plate Count Agar media by taking 0.1 mL of the dilution and putting it into a petri dish containing the solid Plate Count Agar media, then level it using an L rod. Then incubate with the dish upside down for 24-48 hours at a temperature of  $37^{\circ}\text{C}$ . After 24-48 hours, count the bacteria, then calculate using the following formula:

$$\text{Number of bacteria } \left(\frac{\text{cfu}}{\text{ml}}\right) = \text{number of colonies} \times \frac{0,1}{\text{Dilution Factor}}$$

### 16S rRNA Molecular Testing and DNA Sequencing

Bacterial inoculum was inoculated on TSA media in a test tube with a swab and sent to PT Genetika Science Indonesia, West Jakarta, to undergo molecular testing using the PCR 16S rRNA method and sequencing at First Base. The sequencing data were converted into Paste using Bioedit software.

### Data Analysis

Observation data, in the form of water quality, bacterial morphology, bacterial count, and microplastic degradation tests, are presented in tables and figures for differences in the number of bacteria between research stations using the One-

Way ANOVA (Analysis of Variance) test. Data obtained from the sequence results were analyzed using the BLAST (Basic Local Alignment Search Tool) technique, namely matching the DNA sequence of the test bacteria with the DNA sequence of the bacteria on the website <https://www.ncbi.nlm.nih.gov/>, with the MEGA 6 and Bioedit applications

### 3. RESULT AND DISCUSSION

#### Water Quality

The water quality parameters measured in this study include temperature, salinity, pH, current speed, transparency, and dissolved oxygen. These parameters aim to determine the water conditions that can support the growth of bacteria for the bioremediation of microplastic pollutants.

**Table 1.** Water quality conditions in Bagansiapiapi

| Station                   | Water Quality Parameters |                |      |               |              |           |
|---------------------------|--------------------------|----------------|------|---------------|--------------|-----------|
|                           | Temperature (°C)         | Salinity (ppt) | pH   | Current (m/s) | Clarity (cm) | DO (mg/L) |
| I (Port)                  | 30.3                     | 4.7            | 5.58 | 0.5           | 0            | 2.0       |
| II (Fishing Area)         | 33.4                     | 10.7           | 5.34 | 0.5           | 10           | 2.5       |
| III (Rokan River Estuary) | 32.2                     | 15.0           | 5.83 | 0.4           | 23.8         | 2.2       |

Water quality parameters at three stations show an average temperature value ranging from 30.3-33.4°C (Table 1). Water temperature plays a role in controlling the condition of the aquatic ecosystem. An increase in temperature causes an increase in the decomposition of organic matter by microbes<sup>10</sup>. An average value of between 4.7 and 15 ppt was obtained in salinity measurements. The salinity of Bagansiapiapi waters is good enough to support microbial growth. Marine bacteria can grow in a salinity range of 20-60 ppt (Minister of State for the Environment No. 51 of 2004); the salinity value at the observation station is still within the seawater quality standards for marine biota.

In pH, an average value of between 5.34 and 5.83 was obtained. The pH condition of the waters is also one of the parameters of bacterial growth because it is related to enzyme activity that affects what bacteria need to catalyze reactions related to bacterial growth<sup>11</sup>. The average current velocity value obtained is 0.4-0.5 (m/s). These nutrients are helpful for the development of aquatic organisms such as plankton and bacteria. The use of current movement by biota is a means of propulsion, in addition to the role of the old current, which is to supply food, oxygen solubility,

and removal of CO<sub>2</sub> and waste products of marine biota.

The average water transparency level value varies between stations, ranging from 0 to 23.8 cm. Microorganisms that utilize oxygen provide oxygen in the process of decomposing organic matter. Light energy is used for photosynthesis (euphotic zone), so that the ability of light to penetrate a certain depth greatly determines the vertical distribution of aquatic organisms. DO find average results quite different at each station, ranging from 2 to 2.5 mg/L. DO is very important for bacterial growth because bacteria need oxygen for respiration and growth; high DO levels usually support the development of aerobic bacteria. In addition, the higher the DO concentration, the efficiency of organic matter removal by microorganisms also increases. This shows that sufficient DO supports the metabolic activity of bacteria that reduce organic matter.

#### Microplastic Analysis

In this study, the types of microplastics found in the surface seawater of Bagansiapiapi waters were only microplastics of the fragment, fiber, and pellet types. Table 2 shows that the average abundance of microplastics ranges from 0.06

to 0.08 (particles/m<sup>3</sup>). The highest abundance of microplastics was found at Station I around the port area with an average of 0.08 (particles/m<sup>3</sup>), and the lowest was found at Station II around the fisheries area with an average of 0.06 (particles/m<sup>3</sup>). The abundance of microplastics can be seen in Table 2.

**Table 2.** Abundance of microplastics

| Station | Microplastic abundance<br>(Particle/m <sup>3</sup> ) |
|---------|--|
| I       | 0.08   |
| II      | 0.06   |
| III     | 0.07   |

Fiber-type microplastics come from the degradation of textiles, ropes, and fishing equipment, including nets and plastic bags<sup>12</sup>. Fibers have a thread-like shape with the same thickness throughout their length<sup>11</sup>. Fragment-type microplastics have an

irregular shape. Fragments come from the degradation of thick and stiff macroplastics due to exposure to ultraviolet light, river currents, plastic oxidative materials, and others<sup>13</sup>. Films are microplastics resulting from the degradation of plastic bags and food wrappers that are flexible, have a sheet shape, and are transparent<sup>8</sup>.

The color of microplastics can provide information about the source of waste pollution or their condition. The length of time they are exposed to sunlight and the oxidation process that results in color changes cause differences in the color of the microplastics<sup>14</sup>.

### Isolation of Bacteria

Based on the repeated culture and purification results, 14 bacterial isolates were obtained based on colony morphology identification. For morphological observations, see Table 3.

**Table 3.** Morphology of bacterial isolates

| No | Isolate | Diameter (cm) | Color       | Colony Shape | Edge    | Surface |
|----|---------|---------------|-------------|--------------|---------|---------|
| 1  | ISL 1   | 0.1           | milky white | round        | smooth  | hilly   |
| 2  | ISL 2   | 0.3           | dark yellow | round        | smooth  | raised  |
| 3  | ISL 3   | 0.2           | yellow      | irregularly  | grooved | convex  |
| 4  | ISL 4   | 0.8           | white       | round        | wavy    | convex  |
| 5  | ISL 5   | 0.7           | Kuning      | round        | smooth  | raised  |
| 6  | ISL 6   | 0.9           | dark yellow | round        | smooth  | raised  |
| 7  | ISL 7   | 0.5           | yellow      | round        | smooth  | raised  |
| 8  | ISL 8   | 0.1           | yellow      | round        | smooth  | raised  |
| 9  | ISL 9   | 0.4           | pale yellow | round        | smooth  | raised  |
| 10 | ISL 10  | 0.6           | pale yellow | round        | smooth  | hilly   |
| 11 | ISL 11  | 0.7           | yellow      | round        | smooth  | raised  |
| 12 | ISL 12  | 1.2           | milky white | concentric   | wavy    | convex  |
| 13 | ISL 13  | 1.1           | white       | concentric   | grooved | convex  |
| 14 | ISL 14  | 1.9           | white       | concentric   | wavy    | convex  |

Observations of the morphology of bacterial colonies showed quite striking differences between the 14 bacterial isolates. The bacterial isolates had a diameter ranging from 0.1 to 1.9 cm. Based on the results of observations, it can be seen that three bacterial isolates were white, two isolates were milky white, two isolates were pale yellow, five isolates were yellow, and two isolates were dark yellow. The shape of the

colonies obtained was known to be that 10 bacterial isolates were round, three bacterial isolates were concentric, and one bacterial isolate was irregularly shaped.

In observations of the colony edges, nine bacterial isolates had smooth edges, three bacterial isolates had wavy edges, and two others had grooved edges. Meanwhile, in observations of the surface of the bacterial colony, seven bacterial isolates had a raised



surface, five bacterial isolates had a convex surface, and two bacterial isolates had a hilly surface. Several groups of bacteria showed different colony characteristics, both in shape and elevation of the colony edge. Cell size, shape, and arrangement are gross morphologies of bacterial cells<sup>15</sup>.

### Number of Bacteria

The bacteria isolated from the Bagansiapiapi, Riau Province's water samples were counted, and their colonies are presented in Table 4.

**Table 4.** Number of bacteria found on the surface of the waters.

| Station | Number of Bacteria (CFU/mL) |
|---------|-----------------------------|
| I       | $31.8 \times 10^4$          |
| II      | $13.0 \times 10^4$          |
| III     | $11.4 \times 10^4$          |

Description: CFU = Colony Forming Unit

The number of bacterial cells in this study tended to show very different results between stations. Most bacterial cells were seen at Station I in the port area, while the lowest bacterial cells were found at Station III around the mouth of the Rokan River. The waters of Bagansiapiapi can be

represented by the difference in the number of bacteria in an area, which is caused by several factors, namely environmental conditions such as salinity, pH, depth, current speed, and organic matter<sup>16</sup>. The quality parameters of the Bagansiapiapi waters measured were good enough to support bacterial growth in these waters.

Meanwhile, an ANOVA-One Way test was carried out to compare the average number of bacteria between the three stations in this study, followed by a Normality Test. Based on the Normality Test. The significant number of each station is more than 0.05 ( $> 0.05$ ), indicating that the data is usually distributed, and further testing can be carried out as an ANOVA-One Way test. The results of the ANOVA-One Way test showed a significant figure of 0.003 or less than 0.05 ( $0.003 < 0.05$ ), which means that the average number of bacteria at the three stations was significantly "different".

### Biochemical Tests

The biochemical test results of each bacterial isolate from Bagansiapiapi waters obtained 14 biochemically tested isolates (Table 5). There were quite striking differences between each isolate. For more details, see Table 5.

**Table 5.** Biochemical tests of each bacterial isolate

| Isolate | Biochemical Tests |          |         |          |        |            |                       |
|---------|-------------------|----------|---------|----------|--------|------------|-----------------------|
|         | Gram Staining     | Catalase | Oxidase | Motility | Indole | Methyl Red | H <sub>2</sub> S Test |
| ISL 1   | +                 | (coccus) | +       | +        | -      | +          | -                     |
| ISL 2   | +                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 3   | +                 | (bacil)  | +       | +        | -      | -          | +                     |
| ISL 4   | -                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 5   | -                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 6   | +                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 7   | -                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 8   | -                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 9   | -                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 10  | +                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 11  | +                 | (coccus) | +       | +        | -      | -          | +                     |
| ISL 12  | +                 | (bacil)  | -       | +        | -      | -          | +                     |
| ISL 13  | +                 | (bacil)  | -       | +        | -      | -          | +                     |
| ISL 14  | +                 | (bacil)  | +       | +        | -      | -          | +                     |

Description: +: Test is positive; -: Test is negative

There were nine isolates of Gram-positive bacteria and 5 Gram-negative bacteria. Catalase was 12 positive and two negatives. Based on Gram staining, the shape of the bacteria (*Bacillus* and *Coccus*) can be seen. For the oxidase test, 14 isolates were positive, and motility was negative. The indole test had one positive isolate and 13 negatives; the Methyl Red test had one negative isolate and 13 positives, while the sulfide test showed two positive isolates and 12 negative.

Biochemical reactions that occur in bacterial cells are influenced by enzymes, where enzymes play a role in accelerating chemical reactions and showing a change after the action occurs<sup>17</sup>. Gram-negative bacteria have thin peptidoglycan (10-15 nm) and high lipid content (11-22%). Lipids are soluble in acetone and alcohol, causing the crystal violet dye in Gram-negative bacteria to not be retained, and then the bacterial cells bind the red color of safranin so that the bacterial cells appear red during observation. Gram-positive bacteria have thick peptidoglycan (15-30 mm) and low lipid content (1-4%). This thin peptidoglycan causes the crystal violet dye to be retained because peptidoglycan is insoluble in acetone and alcohol<sup>18</sup>.

### Microplastic Degradation Test by Bacteria

The degradation test determines whether the bacteria found can degrade microplastic waste. The ability of bacteria to degrade microplastics can be seen by looking at the formation of a biofilm layer on the surface of the plastic and the reduction in plastic mass. Table 6 shows the results of the degradation test of LDPE microplastic waste after being shaken for 30 days by bacteria.

Six isolates were found to be able to reduce the weight of microplastic waste. The six isolates are: ISL 2, ISL 3, ISL 6, ISL 10, ISL 13, and ISL 14. Among the six isolates, ISL 14 and ISL 2 are the most able to degrade microplastics because they can reduce the weight of plastic waste by around 30.38% and 27.14% after one month of

incubation. The degradation test results of ISL 14 and ISL 2 on the surface of the plastic sample revealed a biofilm layer. In ISL 3, ISL 6, ISL 10, and ISL 13, they could only degrade microplastic waste by <20%. In ISL 1, ISL 4, ISL 5, ISL 7, ISL 8, ISL 9, ISL 11, and ISL 12, after the degradation test was carried out, no reduction in weight was found in microplastic waste.

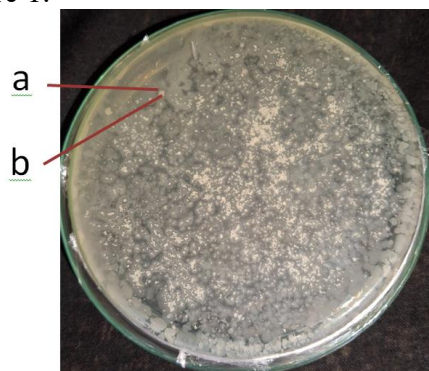
**Table 6.** Degradation of microplastic waste by bacteria

| Isolate | Average degradation (%) |
|---------|-------------------------|
| ISL 1   | -                       |
| ISL 2   | 27,14                   |
| ISL 3   | 12,30                   |
| ISL 4   | -                       |
| ISL 5   | -                       |
| ISL 6   | 16,43                   |
| ISL 7   | -                       |
| ISL 8   | -                       |
| ISL 9   | -                       |
| ISL 10  | 12,50                   |
| ISL 11  | -                       |
| ISL 12  | -                       |
| ISL 13  | 16,25                   |
| ISL 14  | 30,38                   |

Based on Table 6, through the biodegradation process, organic materials can be degraded aerobically and anaerobically. Some microorganisms, such as bacteria, can degrade synthetic plastics naturally (biodegradation). Generally, breaking the polymer chain into monomers requires several different organisms. For example, a bacterium can break down polymers into monomers, while other bacteria can use monomers and excrete simpler compounds. Other bacteria can even use the excreted compounds<sup>19</sup>.

Bacteria that degrade microplastics can produce enzymes that degrade plastic, namely serine hydrolase, esterase, and lipase. Plastic waste degradation by bacteria uses plastic as a carbon source for bacterial metabolism. These bacteria actively attach and form biofilms on the surface of plastic waste during the degradation process<sup>3</sup>. Bacteria that can degrade plastic are then

spread on ZMA media, and PE powder is sprinkled on it. It is found that bacteria grow around the PE powder, as can be seen in Figure 1.



**Figure 1.** Media with PE Powder: a. bacteria that grow around the PE powder. b. PE powder.

### BLAST Analysis of Bacterial Isolate DNA Sequencing Results

The isolate that underwent the sequencing process was ISL 14, which had the highest degradation percentage, around

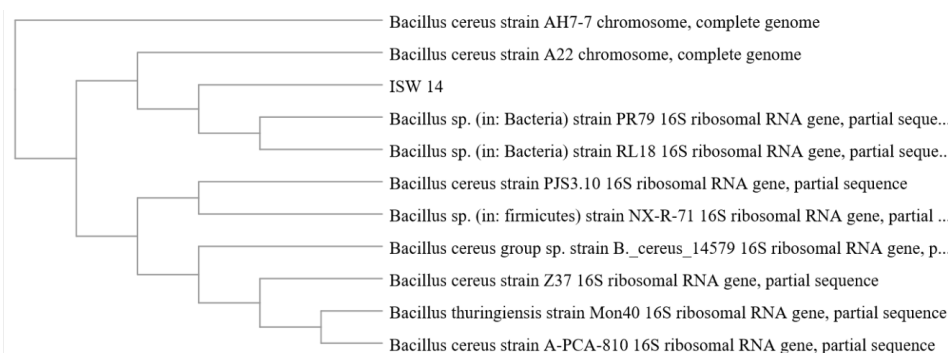
30.38%. Using Bioedit software, the sequencing data were converted into Pasta. The Pasta data were then analyzed using Genbank, accessed at <https://www.ncbi.nlm.nih.gov>.

Determination of species diversity and genetic variation of bacteria can be done using 16S rRNA sequence analysis. The 16S rRNA sequence was used to analyze bacterial DNA because the 16S rRNA sequence is a target for monitoring genetic changes in organisms. The bacterial sequence database available in Genbank is 16S rRNA, so research is focused on this gene to facilitate data processing<sup>20</sup>.

The BLAST results of ISL 14 have a high percentage of homology with *Bacillus* sp. Strain PR79 has similarities to *Bacillus cereus* strain ZR452 with 99.86% homology. (Figure 2). To find out the closest kinship, BLAST data were converted into a phylogenetic tree using MEGA 11 software, as seen in Figure 2.

| Description  | Scientific Name                               | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession                  |
|--|---|-----------|-------------|-------------|---------|------------|----------|----------------------------|
| <input checked="" type="checkbox"/> <a href="#">Bacillus sp. (in: Bacteria) strain PR79 16S ribosomal RNA gene, partial sequence</a> | <a href="#">Bacillus sp. (in: firmicutes)</a> | 2590      | 2590        | 99%         | 0.0     | 99.93%     | 1425     | <a href="#">MN232168.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus sp. (in: Bacteria) strain RL18 16S ribosomal RNA gene, partial sequence</a> | <a href="#">Bacillus sp. (in: firmicutes)</a> | 2590      | 2590        | 99%         | 0.0     | 99.93%     | 1427     | <a href="#">MN234061.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain ZR452 16S ribosomal RNA gene, partial sequence</a>            | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1460     | <a href="#">OR294189.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain VLS-S-II (20) 16S ribosomal RNA gene, partial sequence</a>    | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1482     | <a href="#">MH114037.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain KSW-31 16S ribosomal RNA gene, partial sequence</a>           | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1438     | <a href="#">OR514176.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain PT318 16S ribosomal RNA gene, partial sequence</a>            | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1437     | <a href="#">OL824924.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain BXC13 16S ribosomal RNA gene, partial sequence</a>            | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1461     | <a href="#">MN227491.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain EA-CB1047 chromosome</a>                                      | <a href="#">Bacillus cereus</a>               | 2586      | 36154       | 100%        | 0.0     | 99.86%     | 5379554  | <a href="#">CP110129.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain PLB4 16S ribosomal RNA gene, partial sequence</a>             | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1484     | <a href="#">KY910157.1</a> |
| <input checked="" type="checkbox"/> <a href="#">Bacillus cereus strain LMU-81 16S ribosomal RNA gene, partial sequence</a>           | <a href="#">Bacillus cereus</a>               | 2586      | 2586        | 100%        | 0.0     | 99.86%     | 1450     | <a href="#">PP947801.1</a> |

**Figure 2.** BLAST analysis of ISL 14 bacteria



**Figure 3.** Phylogenetic tree of ISL 14 bacteria constructed using the neighbor joining method.

Based on the results of BLAST analysis of DNA sequencing using Genbank, ISL 14 is a *Bacillus* genus bacterium with a

homology percentage of 99.93%. Several studies with *Bacillus* sp. isolates showed the degradation of plastic shopping bags (black,



white, transparent) with a degradation percentage of around 8%, 5%, and 7% during 3 months of incubation<sup>21</sup>. Another study reported that the degradation of plastic bags by *Bacillus* PL01 reached 2-5% after 16 weeks of incubation<sup>22</sup>.

#### 4. CONCLUSION

Plastic-degrading bacteria that can be isolated are 14 isolates. The bacterial isolates with the highest degrading ability are ISL 14

and ISL 2, with a percentage of plastic weight loss of 30.38% and 27.14%. In ISL 3, ISL 6, ISL 10, and ISL 13, they could only degrade microplastic waste by <20. ISL 14 is a *Bacillus* sp. strain PR79 with 99.93% homology. This finding shows the potential for utilizing local bacteria as bioremediation agents for microplastics in the marine environment effectively and in an environmentally friendly way.

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