IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 9, Issue 2 (Mar. – Apr. 2023), PP 01-06
www.iosrjournals.org

Isolation and Characterization of Polystyrene-Starch Polymer Degrading Bacteria Coating of Slow-Release Urea Fertilizer

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Abstract:
Research on the isolation and characterization of bacteria degrading polystyrene-starch polymer coatings of slow release urea fertilizer was carried out to see the efficiency of decomposition of slow release urea fertilizer coating polymers assisted by bacterial microorganisms. The research was conducted using a field survey method and followed by an experimental method. The stages of the research were carried out starting from taking soil samples in the plant root area and isolating bacteria using certain media to obtain bacteria that have the potential to degrade the coating polymer, then testing the weight reduction of the coating polymer film to test the potential ability of the bacteria. Bacteria capable of reducing the weight of the coating polymer film were examined by observing the surface characteristics of the coating polymer film to prove the interaction between the bacterial isolates and the PS-Starch coating polymer film which had been degraded and followed by biochemical testing. The results obtained in this study found the presence of bacteria that play a role in the process of degrading the coating polymer of slow release urea fertilizer isolated from bacteria taken from the soil around the roots of plants that have been treated with slow release urea fertilizer.
Key Words*: bacteria, bioblend polymer, characterization, degradation, slow-release.*

Date of Submission: 01-03-2023 Date of Acceptance: 11-03-2023

I. Introduction

Fertilizer plays a role in providing nutrients to plants to increase or maintain optimal crop yields. Thus increasing the efficiency of fertilizer use in fulfilling the intake of nutrients that are beneficial to plants and affecting crop yields which is very important for fertilizer producers and farmers [1]. In general, the use of fertilizer that is found is urea, because it has a high nitrogen content (45% -46%) [2]. High nitrogen content is very important for plants to stimulate growth, fertilize leaves and increase protein levels in plants[3]. However, the use of urea in high quantities is a problem for the environment. Approximately 20% -70% of the urea used pollutes the environment due to the leaching process, while only 30% -50% of the urea is absorbed by plants resulting in repeated use of fertilizers resulting in increased costs [1].

There are efforts made by researchers to reduce the loss of nitrogen and nutrient elements in conventional urea fertilizer by modifying the physical and chemical forms into slow release urea fertilizer or Slow Release Urea Fertilizer (SRUF) because modified urea can slow down the hydrolysis of nitrogen in the soil [4]. The manufacture of SRUF is an alternative in increasing efficiency and overcoming problems with the application of urea. The benefits of using SRUF are reducing fertilizer loss by rainwater and sustainable release, and saving fertilizer use which means saving production costs and minimizing environmental pollution [5].

Previous studies on the use of polystyrene as a coating to produce SRUF can be carried out by adding other biodegradable polymers so as to increase the efficiency of using N and increase the biodegradability of polystyrene as a coating material [6]. However, the problem is that polymeric materials that settle in the soil can inhibit soil permeability, thereby inhibiting the ability and aeration of the soil and causing contaminants to accumulate [7].

Several recent methods are potential biological systems to degrade polymers. In this case, bacterial organisms have been shown to be able to degrade polymers and convert them into environmentally friendly carbon compounds. Plastic biodegradation by bacteria is a bioprocess carried out by microbial enzymes such as lipase, pronase, proteinase K and hydrogenase [8]. Bacteria that play a role in the biodegradation process are thought to be able to destroy the urea coating wall periodically, so that the urea contained in the coating can come out after the biopolymer wall is destroyed by bacteria by biodegradation process.

The release mechanism of slow-release urea fertilizer depends on several factors that are the nature of the coating material, the type of slow-release fertilizer, agronomic conditions and many more. The release mechanism of slow release urea fertilizer coated with polymer occurs with the help of microorganisms which can damage the coating polymer resulting in internal pressure that disrupts the membrane resulting in the release of nutrients. Nitrogen will be released when the coating is damaged or due to diffusion through the pores in the coating layer. The rate of release is also affected by temperature, humidity and the thickness of the coating polymer layer [9]. In this study, the isolation of bacterial species that play a role in the biodegradation of urea coating was carried out and the biochemical characteristics of the bacterial isolates were studied.

II. Materials and Methods

Materials

The materials needed in this study were soil samples from the agricultural land of the Buluah Sarumpun farmer group, Banuhampu District, Agam Regency. Nutrient Agar (NA) Medium, Bacto Agar Specific Medium with carbon sources : Polystyrene-Starch coating polymer Bioblend, Liquid Minerals, Polystyrene (Stryrofoam), Starch (*Manihot esculenta*).

Soil Sampling

Soil samples were taken using purposive sampling technique, in which taking soil samples from the soil on the roots of the bean plants after applying slow-release urea fertilizer. Sampling was carried out approximately one month after harvest.

Soil Bacterial Isolation

Dissolve 1.7g of technical NaCl in 200mL of aquadest as a physiological solution and sterilize at 121^{o}C with 15 lbs pressure for 15 minutes. Furthermore, the soil sample was weighed as much as 1g then diluted into 10mL of physiological solution in Erlenmeyer, then vortexed until homogeneous. Dilutions were made up to 10^{-4}. Then from the dilution series, 1 mL was pipetted into a petri dish and poured into a specific polymer medium using the pour plate technique, incubated at room temperature for 72 hours. The growing bacterial isolates were purified by the quadrant method. Single colonies that grow inoculated on the slanting culture were used as stock cultures.

Coating Polymer Film Degradation Test by Soil Bacterial Isolates

Bacterial isolates were selected of their degradation ability on polymer coating polymer films. Bacterial isolates were allowed to stand for 72 hours. Furthermore, the selection of the ability of bacteria using agar mineral medium was carried out. Coating polymer film was weighed initially and aseptically inserted and incubated at room temperature for 4 weeks with observations made every 1 week. Weigh the final weight of the coating polymer film. Bacterial isolates that had the greatest final weight loss activity of the coating polymer film are bacteria that had the potential to degrade the coating polymer.

Calculation of Weight Loss Percentage of Polymer Coating Film

The percentage reduction in the weight of the polymer coating film can be calculated using the following formula [10]:

$$\% \text{ Reduction of polymer coating film: } = \frac{R_1 - R_{2X}}{R_1} 100\%$$

Note: R1 = Initial Weight of the polymer film layer
R2 = Final weight of the polymer film layer

Morphological Characterization Using Scanning Electron Microscopy (SEM)

Coating polymer film samples that had been degraded by bacterial isolates were observed using Scanning Electron Microscopy (SEM). Comparison of the surface morphology of the coating polymer film sample before degradation was carried out with the polymer film surface after being degraded by bacteria.

Characterization of bacterial isolates

In this test, bacteria with the greatest potential were observed macroscopically by growing isolates on NA media using a streakplate. After that, look at the morphology including elevation, margin, shape and pigment. Furthermore, microscopic observations were carried out including cell shape, gram staining and spores on the bacterial preparations. To see the characteristics of bacterial isolates, biochemical tests were carried out.

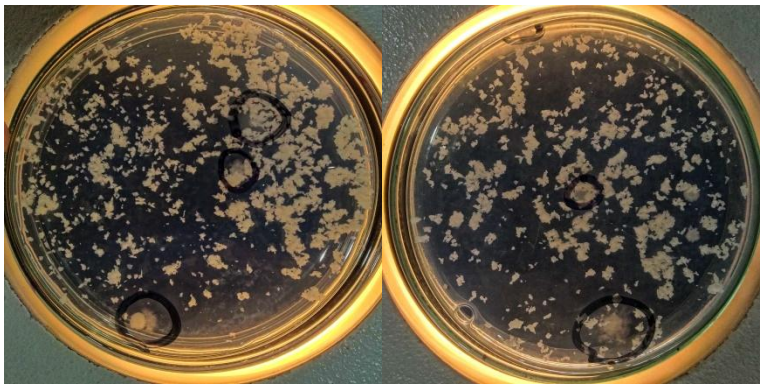
III. Results

Soil Bacterial Isolation

The total soil bacteria isolated from soil that had been treated with polymer-coated slow-release urea fertilizer can be seen in (Table 1).

Table 1.Total of Soil Bacteria Colonies treated with Polystyrene-Starch Coating Polymer

No	Isolates code	pH	Incubation temperature
1.	PSPT 1	7,3	28°C
2.	PSPT 2	7,3	28°C
3.	PSPT 3	7,3	28°C
4.	PSPT 4	7,3	28°C
5.	PSPT 5	7,3	28°C



A B

Fig. 1. Dilution of 10^{-4} soil bacteria colonies on specific polystyrene-starch media (A) found 3 bacterial colonies (B) found 2 bacterial colonies

Selectivity was carried out by adding PS-Starch polymer coating powder to the media with the aim of obtaining bacterial isolates that utilize PS-Starch polymer coatings as the main carbon source. Modification of this specific media was carried out with the aim of obtaining tolerant conditions, so that only bacteria that have the ability to degrade PS-Starch coating polymers can grow in these conditions.

Qubra and Febria (2017) that bacterial colonies that successfully grow in extreme environmental conditions indicate that these bacterial colonies are able to survive and adapt to that environment by having certain mechanisms [11]. This opinion is also supported by Febria *et al*., (2016) and Gilan *et al*., (2004), that microbial groups found in polluted locations are able to adapt naturally to that environment and even these microbial groups can use the components contained in the waste as a source of nutrition and indicates its ability to carry out metabolic processes [12,13].

Testing the Ability of Bacterial Isolates to Degrade Polystyrene-Starch Coating Polymers

The results of testing the ability to degrade the PS-Starch coating polymer of slow-release urea fertilizer (Figure 2), showed a decrease in the weight of the PS-Starch coating polymer of slow-release urea fertilizer.

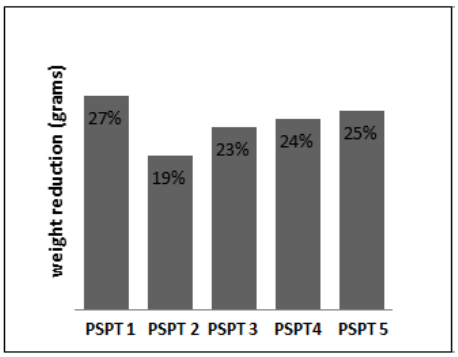


Fig. 2. Average Weight Loss of PS-Starch Coating Polymer for 4 Weeks

From the data obtained, the decrease in coating polymer by bacterial isolates was suspected because the bacterial isolates produced extracellular and intracellular depolymerase enzymes. The main function of

extracellular enzymes is to change the surrounding nutrients to enter the cell as energy for cell growth. The depolymerase enzyme in the depolymerization process carried out by bacteria is able to decompose polymers into monomers or monomer mixtures on PS-Starch coatings or plastic films. According to Usha (2011), the breakdown of polymers is assisted by extracellular and intracellular depolymerase enzymes which facilitate the transportation of compounds that are broken down into carbon and a source of energy for these microorganisms. Microorganisms will absorb the plastic and metabolism occurs. The products produced in aerobic metabolism are carbon dioxide and water while the products produced in anaerobic metabolism are methane, carbon dioxide and water [14].

Characterization of Urea Coating Polymer Film Decomposed by Potential Bacteria by Scanning Electron Microscopy (SEM)

Scaning Electron Microscopy (SEM) characterization of all PS-Starch polymer coating film samples can be seen in (Figure 3).

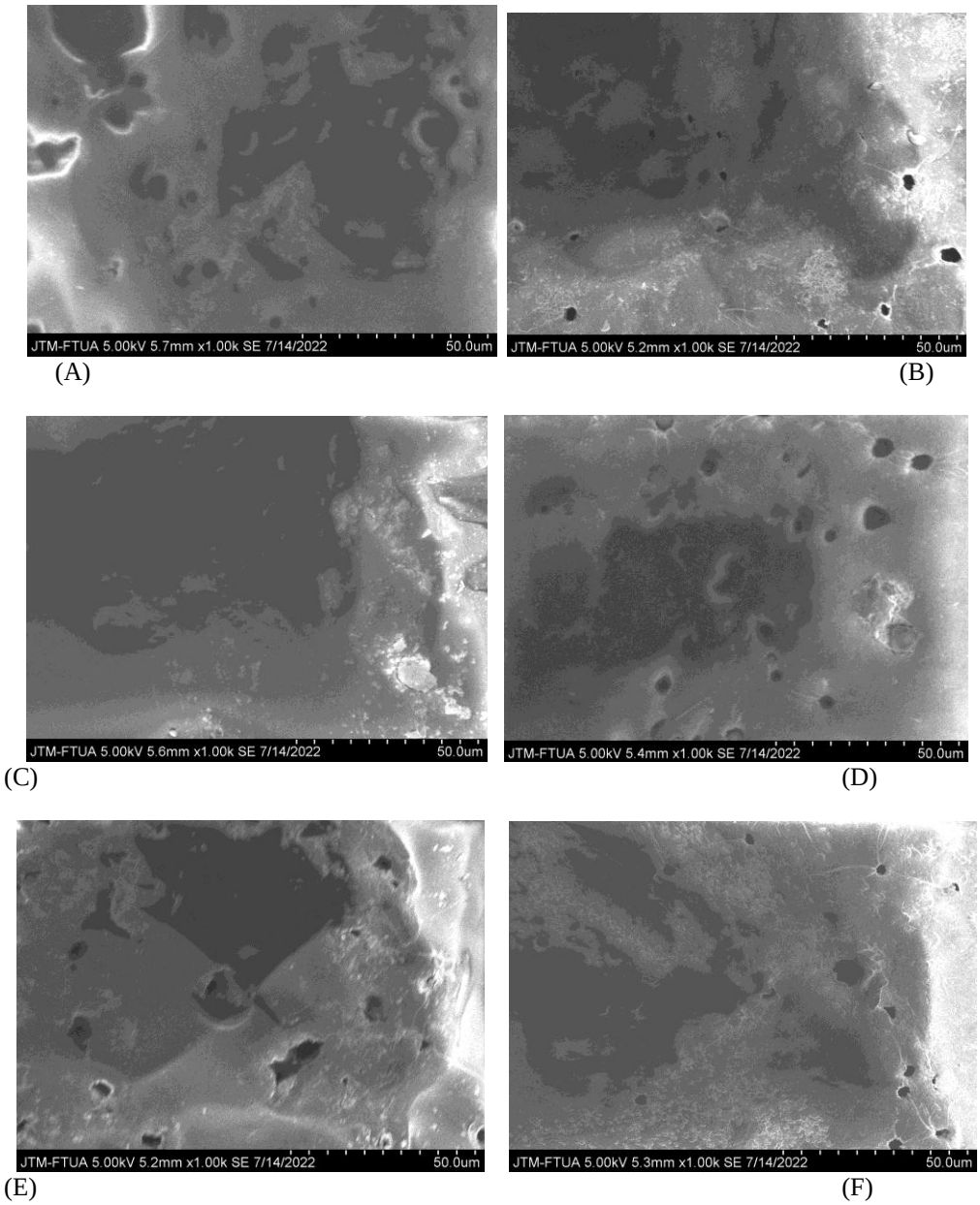


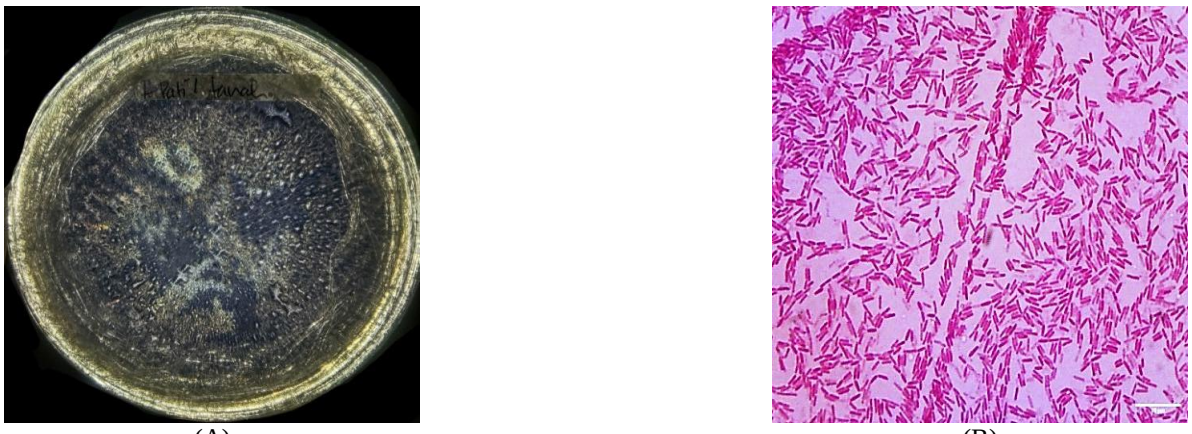
Fig. 3. Characterization of coating polymer films: (A) PS-Starch control, (B) PS-Starch 1 (C) PS-Starch 2, (D) PS-Starch 3, (E) PS-Starch 4, (F) PS-Starch 5.

Macroscopic, Microscopic Observation and Biochemical Testing of Polystyrene-Starch Coating Polystyrene Polymer Degrading Bacteria Isolates

Potential bacterial isolates obtained in the biodegradation process were observed macroscopically, microscopically and in biochemical tests (table 2).

Table 2.Macroscopic, microscopic and biochemical tests of isolates on bacterial isolates that have the potential to degrade PS-starch coating polymers

No	Treatment	Isolate PSPT1
1.	Form	Filament
2.	Margins	Lobate
3.	Elevation	Flat
4.	Pigment	White
5.	Gram stain	Positive
6.	Cell shape	Baccil
7.	Spores	-
8.	Blood agar	+
9.	Aerobic/ anaerobic	Aerobic
10.	Tsia	Yellow/Yellow
11.	Gas	-
12.	Catalase	-
13.	Oxidase	-
14.	Mortality	+
15.	Indole	-
16.	Urea	-
17.	Citrate	-
18.	Lactose	+
19.	Glucose	+
20.	Sucrose	-
21.	Mannitol	-
22.	Mr	+
23.	Vp	-
24.	Of	-
25.	Kcn	
26.	Lysine	
27.	Ornithin	
28.	Phenylalanine	
29.	Nitrate	+
30.	Gelatin	-



(A) (B)

Fig. 4. Morphology and gram staining of PSPT 1 bacterial isolates that have the potential to degrade PS-Starch coating polymers

Biochemical test is a way to identify bacteria by knowing their physiological or biochemical properties. The purpose of the biochemical test is to reduce the error rate in the identification process because some bacteria have almost the same properties whereby macroscopic observations or gram staining are carried out, the results are less accurate[15]. Biochemical test refers to the concept that each microorganism produces different metabolites. These metabolites will later be detected using chemical reagents to produce a color change. Based on the color change, it will be known whether the bacteria produces metabolites (positive) or do not produce metabolites (negative) [16].

IV. Conclusion

The results obtained in this study were that bacteria that play a role in the biodegradation process of slow release urea fertilizer coating polymers were successfully isolated as many as 5 bacterial isolates grown on specific media. Of the 5 bacterial isolates, there was one bacterial isolate that had the most potential to degrade the polystyrene-starch coating polymer, namely PSPT 1bacterial isolate with an average reduction in the weight of the coating polymer film by 27% for 4 weeks and on the surface morphology of the coating polymer films PS-starch 1 there were also holes or pores indicating an interaction between the bacteria and the PS-starch coating polymer.

Acknowledgements

The Author would like to gratitude Prof. Akmal Djamaan, PhD and Dr. Fuji Astuti Febria who were author's supervisors and guided and supported me by their wisdom throghout my research.The Author would like to thank to all supporters who supported me to study at Universitas Andalas and friends from the research laboratory.

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Tawaffani Qubra, et. al. "Isolation and Characterization of Polystyrene-Starch Polymer Degrading Bacteria Coating of Slow-Release Urea Fertilizer."*IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, *9*(2), (2023): pp. 01-06.