Wonorejo mangrove indigenous bacteria: An insight into their potential as plastic-degrading agents

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Abstract. The use of bioplastic, such as polycaprolactone, to substitute conventional plastic remains a problem to solve. The bioplastic degradation time is still relatively low when compared to the rate of plastic consumption by the public. Therefore, exploration of indigenous bacteria with plastic-degrading potential is needed. This study aims to reveal the potential of indigenous bacteria isolated from Wonorejo Mangrove as plastic-degrading bacteria based on their growth in selective media and biofilm formation. Bacterial isolates obtained from water bodies and sediments of Wonorejo's mangrove were inoculated on minimum salt media with the addition of 0.25% polycaprolactone as the sole carbon source and then incubated for four weeks to determine the bacterial growth based on its total number. The total number of bacteria was calculated by the direct counting method using a hemocytometer. The results indicated a slight decrease in the number of cells for each isolate. Isolate T1A.1 obtained from mangrove water samples encountered a decrease in the total number of bacteria by 2 times the initial number. Meanwhile, isolate T2.1, which was isolated from mangrove sediments, was decreased by 1.4 times from the initial number. However, the enumeration did not cover the cells that formed the biofilm, which was observed in this study. Based on the ability of the isolates to live in the minimum media and the biofilm formation indicated their potential as plastic-degrading agents, specifically for polycaprolactone. Identification and further studies of both isolates are needed to get a better insight into their potential as polycaprolactone-degrading agents.

1 Introduction

The use of synthetic polymers (plastics) for various needs such as bottles, packaging, household appliances, medical equipment, and so on is unavoidable. The continuous consumption of these synthetic polymers causes the accumulation of plastic waste in soil and water bodies. Biodegradable polymers are a potential environmentally friendly alternative because it is easy to degrade through hydrolytic or enzymatic breakdown [1]. Until now, many biodegradable polymers have been developed, one of which is polycaprolactone (PCL).

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PCL is widely used in various fields of life as a substitute for conventional synthetic polymers, including for packaging, the medical world, tissue engineering, and trash bags [2 - 5]. The increase in the use of PCL is due to its superior properties, such as biodegradability, high strength, and biocompatibility [2, 4].

The increasing use of PCL as a substitute for conventional plastics has attracted the attention of researchers to explore microorganisms capable of degrading it. Studies were carried out to isolate, characterize, and even commercialize the PCL-biodegradation agents [2, 6, 7]. PCL can be naturally degraded by microorganisms with varying degradation times [2, 8, 9]. PCL biodegradation can occur due to the activity of extracellular PCL depolymerase enzymes, such as esterase, lipase, and cutinase [8]. Several microorganisms have been known to have the ability to degrade PCL, such as *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Paecilomyces lilacinus*, *Penicillium* spp., *Rhizopus* spp., *Acinetobacter* spp., *Alcaligenes faecalis*, *Brevundimonas* sp., *Clostridium* sp., *Lactobacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. which are found in many soil and aquatic ecosystems [8]. These potential isolates can be obtained from areas polluted by plastic waste [10].

The Wonorejo Mangrove was originally a conservation area created to prevent abrasion in the eastern region of Surabaya City [11]. However, in its development, the Surabaya City government expanded the function of this location as a natural and educational tourism area [11]. The expansion of this function indirectly has an impact on the volume of waste, one of which is plastic waste that has been buried near the estuaries of the Wonorejo Mangrove area. Hence, this area may be occupied by potential indigenous plastic-degrading bacteria.

Two bacterial isolates were obtained from sediment and water bodies in the estuaries of the Wonorejo Mangrove area, namely isolate T2.1 and isolate T1A.1. However, further study on these isolates has not been done. Therefore, a preliminary study to get an insight into their potential as PCL-biodegradation agents is needed. In this study, these isolates were tested on their ability to live in minimum growth media with PCL as the sole carbon source to disclose their potential.

2 Methods

2.1 Preparation of the isolates

Bacterial isolates were prepared before proceeding to further analysis. A loop of the stock culture of each isolate was inoculated into 5 ml of sterile Nutrient Broth media independently. Then, the culture was incubated at room temperature for 24 hours. Bacterial growth was observed based on the turbidity of the culture media.

2.2 Viability test and biofilm formation

The isolate cultures were taken as much as 1 ml and then inoculated into 20 ml of Minimum Salts Medium (MSM) with the addition of 0.25% of PCL as the sole carbon source. Afterward, the test cultures were incubated for 30 days at 150 rpm. Observation of the biofilm formation was performed daily. These experiments were independently carried out two times for each isolate.

2.3 Enumeration of the cell

Enumeration was done before the incubation which counted as the initial number of the cell and after the incubation which counted as the final number. The final number of the cells was calculated as the average of two replications. Enumeration was conducted by direct counting method using a hemocytometer.

3 Results and Discussion

In this study, isolate T2.1 and isolate T1A.1 obtained from the sediment and water bodies of the plastic-polluted area in the estuary of Wonorejo Mangrove were tested for their viability while cultured in minimum media containing PCL as the sole carbon source. Each isolate was first grown in nutrient broth media to ensure the viability of the isolate which was previously stored as stock culture at 5°C. Nutrient Broth is a liquid medium widely used for the cultivation of fastidious and non-fastidious microorganisms with non-specific nutritional requirements. This medium consists of peptone, beef extract (or yeast extract), and sodium chloride. Peptone and beef or yeast extract provide nitrogen compounds, amino acids, vitamin B complex, and other nutrients essential for growth.

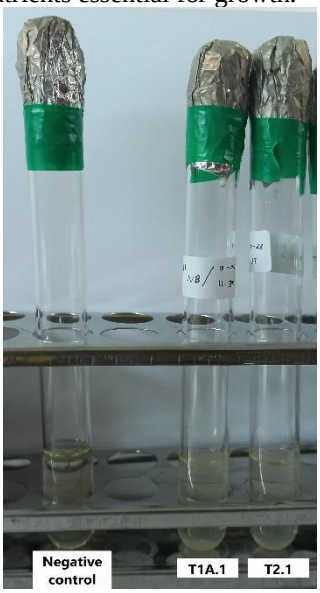

Fig. 1. Culture of tested isolates in nutrient broth media

Figure 1 showed that both isolates grew well on nutrient broth media which was characterized by media turbidity compared to negative controls. The negative control used in this study was nutrient broth media without any bacterial inoculation. Based on this result, the bacterial cultures can be used for further analysis.

Each of the culture's cells was enumerated for its number by using the direct counting method. The data were recorded as the initial number of the cell. Enumeration was also carried out after 4 weeks of aerobic incubation to get the final number of the cells of each isolate.

Table 1. The initial and final number of the cells of isolate T2.1 and T1A.1.

Isolate	**Cell number (cell/mL)**[†]	
	Initial	Final
T2.1	1.48×10^9	1.04×10^9
T1.A1	2.00×10^9	9.94×10^8

The results of the enumeration carried out on isolate T2.1 showed that there was a slight decrease in the number of cells which was initially as much as 1.48×10^9 to 1.04×10^9 or a decrease in the number of living cells by 1.4 times. Similar results were also obtained in the isolate T1.A1 culture which decreased the number of cells by 2 times, from the initial number of 2.00×10^9 to 9.94×10^8 (Table 1).

The decrease in the number of cells may be caused by a coping mechanism of both isolates. In extreme environmental conditions with a sole carbon source, the survival of microorganisms depends on their ability to utilize that carbon source. This circumstance may slow down the growth rate. However, if an isolate can survive in such conditions for more than 14 days, then the isolate may have the ability to degrade the compound which is the sole carbon source. Haedar et al. [12] stated accordingly, that bacteria which able to grow in a media with a specific plastic as the sole carbon can be assumed as bacteria with the potential to degrade plastic.

In this study, biofilm formation was also observed. Bacterial biofilms are clusters of bacteria attached to a surface and/or to each other and embedded in a self-produced matrix [13]. Biofilm formation is one of the bacterial coping mechanisms to survive in an extreme environment as well as the degradation mechanism of a specific material [14]. Biofilm formation was observed in both culture media (Fig. 2). The formation of biofilm may interfere with the enumeration of the total bacteria number. The biofilm formed in this study was intact and difficult to dissolve by vigorous vortex. So, the cells that formed the biofilm cannot be enumerated and cause a bias in the counted cell number. Hence, a slight decrease in the final number of each isolate may be caused by not all the cells in the culture can be counted.

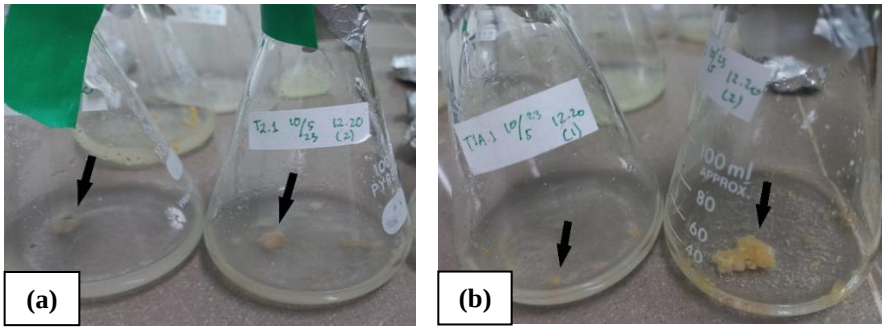

Fig. 2. Biofilm formation in MSM+0.25% PCL media (a) Isolate T2.1 isolated from mangrove sediment (b) Isolate T1A.1 isolated from water bodies of mangrove

Both of the isolates formed biofilm about two weeks after the inoculation (Fig 2). In accordance, a previous study also found that biofilm formation started from two weeks of incubation and is related to the degradation process of plastic waste [15].

These results disclosed the ability of bacterial isolates of Wonorejo Mangrove to survive in an environment with PCL as the sole carbon source by forming the biofilm. Re-enumeration of the cell numbers during the viability test should be done by using other methods, such as spectrophotometry, to confirm the growth of each isolate in the test media. Further studies are also needed to get a better insight into the mechanism of each isolate in PCL-degradation.

4 Conclusion

Based on the survival of the isolates during long-term incubation in the minimum media with PCL as the sole carbon source the biofilm formation may indicate their potential as plastic-degrading agents, specifically for polycaprolactone. However, further studies are needed to get a better insight into their potential as polycaprolactone-degrading agents.

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References

1. A. Alhanish, G. A. M. Ali, Biodegradable polymers. In: Ali, G.A.M., Makhlouf, A.S.H. (eds) Handbook of biodegradable materials (Springer Nature, Switzerland AG, 2022)
2. L. Li, X. Lin, J. Bao, H. Xia, F. Li, Frontiers in Bioengineering and Biotechnology **10** (2022)
3. P. Canale, Polycaprolactone and its use as a biodegradable trash bag (Honors Thesis, Salem State University, 2021)
4. J. S. Lyu, J. S. Lee, J. J. Han, Scientific Reports **9** (2019)
5. J. Abdelfatah, R. Paz, M. E. Alemán-Domínguez, M. Monzón, R. Donate, G. Winter, Materials **14**, 9 (2021)
6. N. Atanasova, T. Paunova-Krasteva, S. Stoitsova, N. Radchenkova, I. Boyadzhieva, K. Petrov, M. Kambourova, Biomolecules **11**, 10 (2021)
7. A. Nawaz, F. Hasan, A. A. Shah, FEMS Microbiology Letters **362**, 1 (2014)
8. J. Budkum, S. N. R. Thammasittirong, A. Thammasittirong, Folia Microbiologica **67** (2022)
9. M. Suzuki, Y. Tachibana, K. Kasuya, Polymer Journal **53** (2021)
10. S. Ali, A. Rehman, S. Z. Hussain, D. A. Bukhari, Saudi Journal of Biological Sciences **30** (2023)
11. S. Murtini, Kuspriyanto, A. Kurniawati, In Journal of Physics: Conf. Series. **953,** 1 (2018)
12. H. Haedar, T. Clara, Fahrudin, A. Abdullah, S. T. Fausiah, M. T. Rapak, Journal of Physics: Conference Series **1341** (2019)
13. L. Quintieri, S. Mani, G. Lentini, G. Maisetta, Frontier in Microbiology **13** (2022)
14. Y. N. Han, M. Wei, F. Han, C. Fang, D. Wang, Y. J. Zhong, C. L. Guo, X. Y.Shi, Z. K. Xie, F. M. Li, Microorganism **8**, 1979 (2020)
15. T. Morohashi, T. Oi, H. Aiso, T. Suzuki, T. Okura, S. Sato, Microbes Environ. **33**, 3 (2018)