Potential Bacteria Isolated from Contaminated Sites as Bio-degraders of Various Types of Plastic

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***Abstract*—The growing demand for plastics and their improper disposal have resulted in a significant environmental pollution problem. While various management strategies have been employed to tackle this issue, the persistence of plastic pollutants in the environment is still a major concern. Therefore, exploring and developing sustainable and environmentally safe techniques, such as biodegradation using potential bacteria, can help mitigate plastic pollution and provide a viable solution. The purpose of this study was to isolate and identify potential bacteria for degrading plastics from six soil samples collected from five plastic-contaminated sites. The population of microorganisms in the soil ranged from 1.9×10^5 to 8.2×10^4 CFU/g. The screening of biodegradation abilities to degrade various types of plastics, including Polypropylene (PP), Polystyrene (PS), Polyethylene (PE), Polyethylene terephthalate (PET), and Polylactic acid (PLA) (Bioplastic), as measured by the diameters of the clear zones surrounding the colonies, revealed that out of 40 strains, only 8 strains could degrade various types of plastics. These bacteria were identified using 16S rRNA genes, which showed that NBI0106, NBI0108, NBI0109, and NBI0111 tend to be *Streptomyces ardesiacus* with similarity 99%, NBI0113 tend to be *Pseudomonas plecoglossicida* with similarity 99%, and NBI0305 tend to be *Streptomyces cellulosae* with similarity 100%. In addition, The *Streptomyces ardesiaca* strain NBI0111 demonstrated the highest degradation efficiency for PP plastic, with a clear zone diameter of 32.19 ± 0.34 mm. This study shows the importance of identifying bacteria in plastic-contaminated soils and landfills, which may lead to the discovery of more effective bacteria strain with the capacity to degrade various types of plastic in real environmental conditions.**

***Index Terms*—Biodegradation, plastic pollution, bacteria, microorganism, microplastics**

I. Introduction

The world has prioritized waste problems. The environmental effect of particular concern is the disposal of plastics that remain in the environment. This is anticipated to get worse each year because plastic plays an essential part in human activities. Plastic is used for various containers since it is durable, lightweight, and inexpensive. In 2019, the manufacturing of plastic increased to 368 million metric tons as a result of the growth in the widespread consumption of plastic. In comparison with 2010, the use has grown by 3.5% of the total amount of plastic [1]. The COVID-19 pandemic brought about an unprecedented shift in consumption patterns, particularly due to the lockdowns, which led to a surge in online shopping and home delivery services, resulting in an increase in the use of single-use plastic and food packaging. This has resulted in the generation of significant volumes of plastic waste [2, 3].

Plastics are one of the main contributors to environmental pollution since they remain in the environment long after they have been used. Disposal or single use in both terrestrial and marine ecosystems has led to the accumulation of these materials because of their low rates of biodegradation, which has made the environment unsightly and may have harmful effects on the health of humans, animals, and other organisms [4]. Additionally, when plastics accumulate for a long time, they can break down into microplastics through physical, chemical, and biological processes (biodegradation). Microplastic is becoming a major concern [5] because microplastics are plastic with a size of 1 μm to 5 mm. Therefore, microplastics can enter various parts of the food chain because they are so small [6]. Microplastics were found in human blood for the first time in 2022, indicating that they may be taken into the bloodstream and affect human health [7].

Currently, there are many ways to eliminate of used plastic, such as recycling, incineration, landfilling, chemical processes, and others [8]. Nevertheless, some research revealed that only roughly 9–12% of plastic waste was disposed of by being incinerated and recycled, while the remaining 79% of plastic waste was disposed of by being dumped in landfills and into the environment [9]. Some methods have disadvantages, for example, some types of plastic cannot be recycled; incinerating plastics produces several toxic chemicals, most of which are emitted into the atmosphere; landfills consume a lot of land that could be used for other purposes; and chemical processing produces some toxic substances in the environment [8]. In light of these considerations, it is essential that research should be conducted towards the development of new methods for the management of plastic waste.

Biodegradation is an important factor that might be exploited to solve the problem of plastic accumulation. This process has no negative effects on the environment, hence it is considered ecologically friendly [10]. Biodegradation of plastic is the result of several microorganisms, such as bacteria and fungi, which break down plastic for use as their source of food [11]. Additionally, further research on the degradation of plastics by microorganisms has been

Manuscript received April 4, 2023; revised May 5, 2023; accepted June 3, 2023.

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doi: 10.18178/ijesd.2023.14.6.1454

extensively investigated for the isolation of microbes capable of degrading plastics from natural environments. For instance, *Bacillus* sp., *Pseudomonas aeruginosa*, *Ideonella sakaiensis*, and *Streptomyces* sp. are all capable of using plastics as a carbon source, and this process occurs under optimal conditions [11–13]. It was also demonstrated that these microorganisms could degrade plastics in three steps: first, by adhering to the surface of plastic particles, and then, by releasing extracellular enzymes to break down plastics [14]. Lastly, they break the chain of the polymer into a monomer that microorganisms could consume [15].

Several research findings have revealed that microorganism can degrade a wide variety of plastics, including high-density polyethylene (HDPE) and polyvinyl chloride (PVC), with PVC films losing up to 19% of their dry weight after bacterial treatment and the surface of HDPE colonized by dark brown fungus [16] –18]. *Pseudomonas* sp. have been studied for their ability to stimulate the breakdown of both natural and synthetic rubber (up to 18%) while also promoting the formation of characteristic biodegradation byproducts throughout the biodegradation process [18, 19].

Researchers are still attempting to identify new bacteria capable of degrading plastics to investigate the degradation of plastics. As prospective degraders of various types of plastic polymers, nonpathogenic bacteria that decompose organic matter in the soil are advantageous. The current study aims to determine the ability of bacteria isolated from contaminated plastic soils in several locations in Nonthaburi, Thailand, to degrade different types of plastic including Polypropylene (PP), Polystyrene (PS), Polyethylene (PE), Polyethylene terephthalate (PET), and Polylactic acid (PLA) (Bioplastic), then identify the potential bacteria that can degrade plastics based on their morphological and genetic characteristics. The findings of this study could provide valuable insights and information for researchers who are interested in studying the management of plastic waste. Specifically, the study could contribute to the existing knowledge about how microorganisms can be used to degrade plastic waste. This could aid in the development of new and more effective methods for the sustainable management of plastic waste.

II. Materials and Methods

A. Study Area

Soil samples were collected from the five locations of plastic-contaminated sites in Nonthaburi province, including plastic recycling plant 1, plastic recycling plant 2, plastic recycling plant 3, dumping site 1, and dumping site 2, in order to find microorganisms that can degrade plastic, as shown in Fig. 1. The five locations of the plastic contaminated sites are located at latitudes 13°52'14.9"N 100°23'52.8"E (plastic recycling plant 1), 13°52'42.1"N 100°17'58.8"E (plastic recycling plant 2), 13°55'19.1"N 100°17'51.7"E (plastic recycling plant 3), 13°52'49.9"N, 100°18'52.4"E (dumping site 1), and 13°49'11.5"N, 100°21'10.4"E (dumping site 2), respectively, where a lot of plastic waste comes from the recycling processing and waste disposal areas.

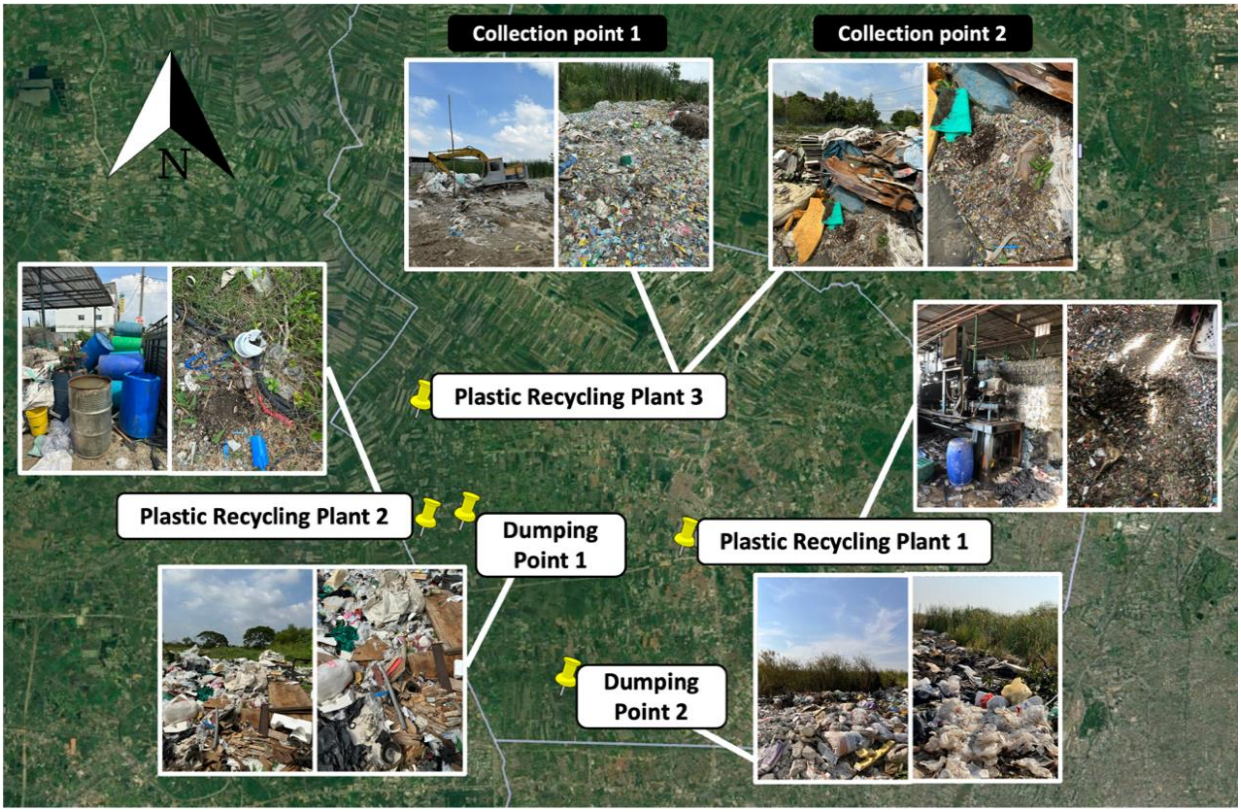

Fig. 1. A satellite image depicting the locations and sampling sites (marked in yellow).

B. Collection of Soil Samples

The soil samples were randomized by random collection at approximately 20 cm of depth. Approximately 10 samples were collected from each sampling location, and each soil sample was collected around 1 kg. The soils were also measured for temperature and pH. The soil samples were kept in sterile bottles and transported to the Environmental and

Water Resources Engineering laboratory of Mahidol University at 4 ℃ for the isolation of microorganisms [20].

C. Isolation of Microorganisms from the Soil Samples

Before microorganisms were isolated, ten-fold dilutions of soil samples were prepared. The 10 g soil sample was transferred to 90 mL of 0.1% Tween 80, shaken for good mixing, and then made into a 10^{-1} concentration. Aseptically, 1 mL of the above suspension was transferred to a tube containing 9 mL of 0.85% sodium chloride (NaCl) and shaken for good mixing. The serial ten-fold dilutions ended up with a 10^{-6} dilution. Approximately 0.1 mL serial dilutions of the 10^{-3}-10^{-6} concentrations were poured into sterile Petri dishes in two types of different medium, one of which contained Nutrient Agar (NA). The other contained Actinomycete Isolation Agar (AIA). This step was repeated in triplicate. The microorganism isolation plates were incubated at 35°C for 3-4 days. The number of microorganism colonies per gram of soil was determined using the plate count technique. The acquired colonies were repeatedly sub-cultured in their appropriate medium in order to produce pure cultures of the microorganisms. The acquired bacteria were labeled as "NBIxxyy," consisting of 3 elements. NBI refers to Nonthaburi province. The letters "xx" refer to the location point, where number 01 represents the plastic recycling plant 1; number 02, the plastic recycling plant 2; number 03, the plastic recycling plant 3; number 04, dumping sites 1, and number 05, dumping sites 2. The letters "yy" refer to the number of samples collected from each collection point.

D. Screening of Potential Microorganisms and Their Abilities for Plastic Degradation

This step was adapted from Nakei in 2022 [4]. Microbial acquired were tested on Bushnell-Haas agar (BH agar) [21] with various types of plastic powders to determine their ability to degrade different types of plastics, including PP, PS, PE, PET, and PLA (Bioplastic). The plastic powder was mashed plastic, sieved through a 0.6-mm sieve. After sieving, 1 g of plastic powder was added to 1,000 mL (0.1% w/v) of this medium and mixed for 1 hour at 120 rpm in a shaker [4]. pH was adjusted to 7.0 ± 0.2, and the medium was autoclaved for 15 minutes at 1.05 kg/cm^2 and 121 ℃. The colonies that could degrade plastics were identified by the clear zones surrounding them. The diameters of both the colonies and the clear zones were measured with a vernier caliper. This step was performed in triplicate.

E. Morphological Characteristics of the Potential Microorganisms

The potential bacteria were characterized morphologically based on the color of colonies, the shape of cells, and gram stain. The Gram staining of the bacteria was performed using the same technique as Alfred (2009) had described previously. The slides were then observed at 1,000X magnification under a light microscope [22].

F. Genotypic Characteristics of the Selected Microorganisms

The selected bacteria were transported to a sequencing company (Macrogen Co., Ltd., Seoul, South Korea) for DNA extraction and sequencing. In order to identify bacteria, PCR of 16S rRNA genes was performed using 27F and 1492R primers. Sequencing was performed using 785F and 907R primers, which are inter-primers. The BLAST program from the GenBank database of the National Center for Biotechnology Information (NCBI) was used to evaluate the 16S rRNA gene sequences of the bacteria. The Molecular Evolutionary Genetics Analysis version 11.0 (MEGA11) program was used in order to construct a neighbor-joining phylogenetic tree.

III. Results and Discussion

A. Microbial Population in Plastic Contamination Sites

The results demonstrated that the microbial populations in all soil samples range from 8.2×10^4 CFU/g to 1.9×10^5 CFU/g as shown in Table I. CFU is a unit of measurement used to quantify the number of viable bacteria or fungi in a sample. These results imply that the microbial populations in the studied soils are quite low, indicating that the soils may have a limited ability to sustain populations of microorganisms exceeding 10^5 CFU/g of soil [4].

TABLE I: Microbial Populations and Properties of the Soils

Soil	pH	Temperature (°C)	Microbial Population (CFU/g)
Plastic Recycling Plant 1	6.8	32	1.2×10^5
Plastic Recycling Plant 2	7.0	37	1.9×10^5
Plastic Recycling Plant 3			
Collection point 1	4.7	34	1.7×10^5
Collection point 2	6.2	38	9.0×10^4
Dumping Point 1	5.2	33	8.2×10^4
Dumping Point 2	6.3	34	1.0×10^5

Similar findings were reported by Akande and Adekayode (2019) in a study that compared microbial populations across different soil types and also found the microbial populations in soils are relatively low [23]. This result indicated the low microbial populations observed in these soils might be attributed to suboptimal soil conditions such as pH, nutrient availability, temperature, and moisture content [24].

B. The Ability of Isolated Bacteria to Degrade Different Types of Plastics

The results indicated that only 8 out of 40 bacterial isolates from the 5 plastic-contaminated sites demonstrated the capability to degrade plastic as shown in Table II. This suggests that not all bacteria have the ability to degrade plastic and that specific bacteria are required to perform this function. 6 strains of the plastic-degrading bacteria were isolated at plastic recycling plant 1, while the remaining 2 strains were isolated at plastic recycling plant 3. Both locations had large amounts of plastic waste which had accumulated several years, indicating that long-term exposure to plastic waste may have contributed to the adaptation and evolution of bacteria strains with the ability to degrade plastic. These findings align with the hypothesis that exposure to plastic waste over an extended period may lead to the emergence of plastic-degrading bacteria.

TABLE II: SCREENING THE BIODEGRADATION ABILITIES OF ISOLATED MICROBIAL FROM 6 DIFFERENT SOILS IN CONTAMINATED SITES

Soil	Number of isolates tested	Number showing biodegradation ability
Plastic Recycling Plant 1	14	6
Plastic Recycling Plant 2	6	0
Plastic Recycling Plant 3		
Collection point 1	4	0
Collection point 2	7	2
Dumping Point 1	4	0
Dumping Point 2	7	0

Table III presents a comparison of the ability of microorganisms to degrade different types of plastic. This comparison assesses the efficiency of microbial degradation, which refers to the rate and extent at which microorganisms can break down the plastic material.

TABLE III: COMPARATIVE EFFICIENCY OF MICROBIAL DEGRADABILITY OF VARIOUS TYPES OF PLASTIC

Isolates	Diameters of clear zones (mm)				
	PS	PP	PET	PE	Bioplastic (PLA)
NBI0106	++	+++	++	++	++
NBI0108	++	+++	++	+++	++
NBI0109	+++	+++	++	++	+
NBI0111	++	++++	++	+++	++
NBI0112	+++	+++	++	++	++
NBI0113	+++	+++	+++	+++	-
NBI0305	+++	+++	+++	+++	++
NBI0309	+++	+++	+++	+++	++

(Diameters of clear scale: + 0-10 mm; ++ 11-20 mm; +++ 21-30 mm; ++++ 31-40 mm; – absent)

The biodegradability of various types of plastic, including PP, PS, PE, PET, and PLA (bioplastic) was conducted and demonstrated that different strains of bacteria exhibit varying degrees of degradation ability for each type of plastic. Notably, strain NBI0111 displayed a higher level of capability for degrading PP plastic than other strains, as shown in Table III. This could be attributed to it being a different or distinct species. It is possible that differences in the habitats from which different microbes are isolated contribute to disparities in the capacities of different bacteria to degrade plastics. Microorganisms evolve to adapt to their specific environments, and their metabolic capabilities and enzymatic pathways can vary depending on the specific conditions they experience. This means that microorganisms isolated from different environments may have different abilities to degrade plastics based on the plastic types, chemical compositions, and availability of nutrients and environmental factors [4].

The finding highlights the importance of recognizing that not all bacterial species found in nature have the same potential to degrade different types of plastic. For example, microorganisms that have been isolated from a landfill or a place with a lot of plastic waste may have evolved to make enzymes that can break down a wider range of plastics than microorganisms that have been isolated from the normal environment [25]. In addition, the environmental adaptability of different types of microorganisms can vary based on the properties of their enzymes, which may be involved in the process of mineralizing different types of plastic polymers, such as cutinase from *Fusarium solani*, which helps in degrading PBS plastic [26] laccase-like multicopper oxidases from *Aspergillus flavus*, which display on PE degradation [27], and PETases from *Ideonella sakaiensis*, which are involved in degrading PET [28]. In this step, it can be helpful to select the most effective bacterial strains for the biodegradation of specific types of plastic.

C. Morphological Characterization of Potential Plastic-Degrading Bacteria

Table IV shows the macromorphological and micromorphological characteristics of the bacteria that were isolated with the naked eye and a light microscope, respectively. The majority of the bacterial isolates exhibited large, desiccated colonies with aerial mycelia displaying a range of colors from white to gray and red to gray. The reverse side of the colonies exhibited an almost brownish color, resembling that of actinomycetes. Under a light microscope, it seems that NBI0113 is the only strain of the isolated bacteria that is gram-negative and is in the shape of a rod. The other strains are gram-positive and are made up of multicellular, filamentous bacteria that produce well-developed vegetative hyphae with branches, which is a common trait of actinomycetes.

TABLE IV: MORPHOLOGY OF PLASTIC-DEGRADING MICROORGANISMS

Isolates	Macro morphology (Colony appearance)	Micromorphology (Shape of the cell)	Gram stain
NBI0106	Greyish large, and dry colony	Filamentous	Gram +
NBI0108	White to dark large, and dry colony	Filamentous	Gram +
NBI0109	White to dark large, and dry colony	Filamentous	Gram +
NBI0111	White large, and dry colony	Filamentous	Gram +
NBI0112	White to red large, and dry colony	Filamentous	Gram +
NBI0113	White surrounded by brown shadow large, and dry colony	Rods	Gram -
NBI0305	Whitish large, and dry colony	Filamentous	Gram +
NBI0309	Whitish large, and dry colony	Filamentous	Gram +

These findings are similar to those of Ng *et al*. (2013) and Nakei *et al*. (2022), who proved that *Streptomyces* sp. may create a non-fragmenting substrate mycelium that can contain spores and, in the majority of genera, a well-developed aerial mycelium with long or short spore chains [4, 29].

D. Molecular Identification of Selected Plastic-Degrading Bacteria

The study used 16S rRNA gene analysis to identify the bacteria with the potential to degrade plastics. The results of the analysis revealed that strains NBI0106, NBI0108, NBI0109, and NBI0111 tend to be *Streptomyces ardesiacus*, with a 99% similarity in their 16S rRNA gene sequence. Strain NBI0113 was identified as *Pseudomonas plecoglossicida*, with a 99% similarity in the 16S rRNA gene sequence. Strain NBI0305 was identified as *Streptomyces cellulosae* with a 100% similarity in the 16S rRNA gene sequence as shown in Table V.

TABLE V: IDENTIFICATION OF PLASTIC-DEGRADING MICROORGANISMS

Strain	Identification	Similarity of current isolate to GenBank		Sources
		Accession numbers	% Identity	
NBI0106	*Streptomyces ardesiacus*	NR_043486.1	99	Plastic Recycling Plant 1
NBI0108	*Streptomyces ardesiacus*	NR_043486.1	99	Plastic Recycling Plant 1
NBI0109	*Streptomyces ardesiacus*	NR_043486.1	99	Plastic Recycling Plant 1
NBI0111	*Streptomyces ardesiacus*	NR_043486.1	99	Plastic Recycling Plant 1
NBI0113	*Pseudomonas plecoglossicida*	NR_114226.1	99	Plastic Recycling Plant 1
NBI0305	*Streptomyces cellulosae*	NR_043815.1	100	Plastic Recycling Plant 3

The contigs of the 16S rRNA gene were assembled using the BioEdit Sequence Alignment Editor (version 7.0) software. The bacterial strains were compared against their respective type strains in the GenBank database of the National Center for Biotechnology Information (NCBI). To obtain the aligned nucleotides of the potential strains and type strains, cluster W in the BioEdit software was employed. Gaps and unknown nucleotides were subsequently expunged. The distance matrices for the aligned sequences were computed using the two-parameter method of Kimura (1980) [30]. The constructed phylogenetic tree of the bacteria strains that showed plastic degradation ability is depicted in Fig. 2.

Nakei *et al*. (2022) similarly demonstrated that *Aspergillus terreus* F4 degraded the ground plastic bottle with a clear zone diameter of 60.10 ± 0.02 mm, while *Aspergillus terreus* F5 degraded the gunny plastic bag with a clear zone diameter of 33.3 ±0.04 mm. *Aspergillus terreus* F8 was isolated from a different site, and it degraded the ground plastic bottle with a clear zone diameter of 59.1 ± 0.02 mm [4]. There is a possibility that these strains are not identical. It is plausible that a specific microorganism, such as bacteria, could possess other strains that exhibit a higher capacity to break down a particular form of plastic, whereas some strains may not possess such a capability [4].

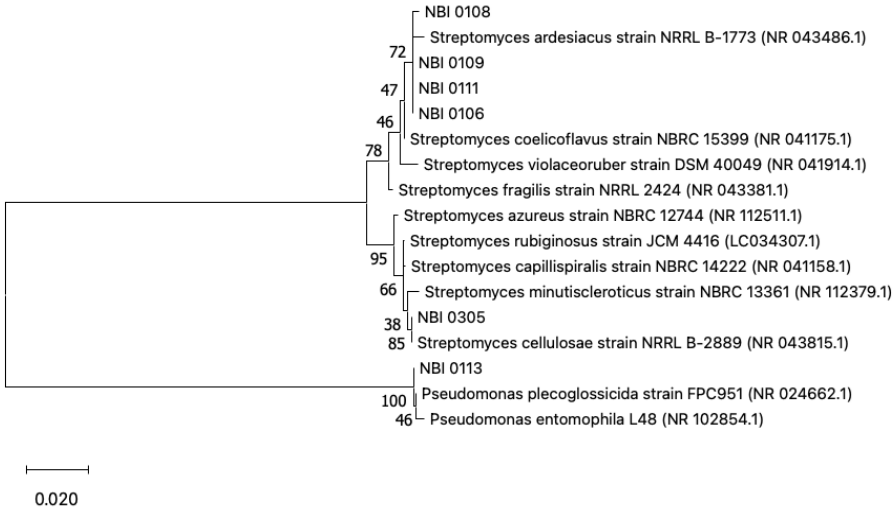

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences using maximum likelihood method for six isolates of bacteria that showed plastics degradation ability and their closely related strains.

It is therefore important to note that the same species may not have the same ability to degrade plastics. It is also possible that different strains of the same organism could exhibit varying abilities to degrade a specific type of plastic. It cannot be assumed therefore that any isolates of the same genus or species will have the same capability to degrade a particular plastic.

As the study only focuses on identifying the species, and not the subspecies, it is important to note that differences in subspecies may still exist, especially since the samples were collected from various sites. Consequently, even bacteria belonging to the same genus or species may exhibit dissimilar external features, including variations in enzyme production or substrate degradation processes [31]. For example, identification of *Lactobacillus delbrueckii* obtained from diverse sources revealed that different subspecies were present in each source, exhibiting distinct characteristics and activities of the bacteria [32]. That is why this study is necessary to test the plastic degradation of all isolated bacteria before applying the bacteria for use on a larger scale or in real environmental conditions.

E. Comparative Plastic Degradability of Three Bacteria Isolated with Different Types of Plastic

The comparative efficiency of three different species of bacteria in degrading a number of different types of plastic, including PP, PE, PS, PET, and PLA (bioplastic), is shown in Fig 3. The ability of the bacterial isolates to degrade plastic varies, as evidenced by the variation in the diameters of the clear zones on the BH agar plates, which were supplemented with 1 g of different types of plastic powder. The results show that different species of bacteria showed differences in growth during the 14-day period of plastic degradation. *Pseudomonas plecoglossicida* strain NBI0113 has the

greatest ability to degrade PS plastic, as determined by the diameter of the clear zone, which measures 26.83 ± 0.57 mm. *Streptomyces cellulosae* strain NBI0305 has the greatest ability to degrade PE plastic, as measured by the clear zone diameter of 25.10 ± 0.42 mm. Interestingly, as measured by the diameter of the clear zone, *Streptomyces ardesiacus* strain NBI0111 has the greatest ability to degrade PP plastic up to 32.19 ± 0.34 mm, evaluated by the clear zone diameter. The results of the screening were consistent with Sriyapai *et al*. (2018) and Yottakot *et al*. (2019), which showed that *Streptomyces* sp. isolated was capable of degrading PLA (bioplastic) [33, 34].

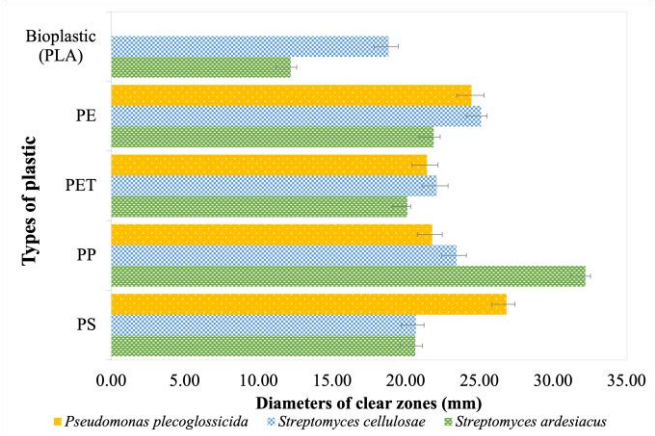

Fig. 3. Comparative efficiency of three bacteria with the plastic degradability of various types of plastic on day 14th and error bars represent the mean ± SD of three independent biological replicates.

The study found that PLA (bioplastic) is less biodegradable than synthetic plastics. The reason for this is attributed to the fact that the bacteria used in the study were isolated from sources of synthetic plastic waste, which made them more adaptable to breaking down synthetic plastics. The results of the study suggested that the adaptability of bacteria to different types of plastics plays a significant role in their ability to biodegrade these materials. The fact that the bacteria used in the study were isolated from sources of synthetic plastic waste implies that they may have evolved to be better suited to breaking down synthetic plastics than bioplastics [4, 27]. Other studies show that bacterial strains isolated from contaminated sites had higher yields, which could be attributed to their adaptation to xenobiotic compounds in their native environment [17]. Although PLA is a biodegradable polymer, it is only broken down under certain specific composting conditions, such as a lot of oxygen, high humidity (>60% moisture), high temperatures (58–80 °C), and the presence of microorganisms (thermophilic bacteria) [35].

IV. Conclusions

The increased demand for single-use plastics and their improper disposal leading to plastic pollution in various ecosystems have become a significant environmental concern. This study highlights the potential of using beneficial soil microorganisms for plastic degradation, which could provide a sustainable and eco-friendly solution to the problem of plastic waste. Microorganisms in this study were capable of degrading various types of plastic within 14 days, as evidenced by large clear zones of up to 32.19 ± 0.34 mm by *Streptomyces ardesiacus* strain NBI0111 degrading PP plastic, 25.13 ± 0.01 mm by *Streptomyces cellulosae* strain NBI0305 degrading PE plastic, and 26.83 ± 0.53 mm by *Pseudomonas plecoglossicida* strain NBI 0305 degrading PS plastic. Therefore, this study discovered that the plastic-contaminated sites have a variety of bacteria with the ability to degrade various types of plastic, such as *Streptomyces* sp. and *Pseudomonas* sp. The identification of microorganisms capable of degrading different types of plastics offers promising potential for the development of environmentally sustainable methods for plastic waste management. The high efficiency of some of the identified strains suggests that they could be further explored for industrial or environmental applications, such as in the development of bioreactors or landfill bioremediation. In addition, expanded exploration and bioprospecting efforts in areas contaminated with plastic and landfills offer the potential for the discovery of more effective microbial species with the ability to degrade plastics. Overall, this study highlights the potential of microbial plastic degradation as a viable solution for mitigating the negative impacts of plastic pollution on the environment.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

K.W.: conceptualization, methodology, analysis, visualization, writing original draft and editing; P.P.: conceptualization and writing review, N.B.: conceptualization and supervision; N.S: conceptualization and supervision; S.K.B.: conceptualization, supervision, resources, validation, writing review and editing. All authors had approved the final version.

Acknowledgment

This study was supported by the Mahidol University's Strategic Research Fund (MU-SRF-RS-16B/66), Thailand. The authors are grateful to the On-Site Laboratory Initiative of the Graduate School of Global Environmental Studies at Kyoto University for additional funding.

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