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Exploration of the polypropylene degrading bacteria candidates from the passive zone of the Supit Urang landfill in Malang city by using the next generation sequencing (NGS) method

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Abstract

The buildup of plastic waste in the passive zone of the Supit Urang landfill located in Malang City has been ongoing since 2018. Currently, plastic waste in this area appears brittle and cracked, potentially providing a habitat for PP-degrading bacteria. This research aims to explore the potential of polypropylene-degrading bacteria using Next Generation Sequencing (NGS) techniques in the passive zone of the Supit Urang landfill, Malang City. Our study was conducted in four steps: 1) sampling and sample collection, 2) DNA sequencing, 3) bioinformatics analysis, and 4) bibliometric analysis for identification of polypropylene-degrading bacteria. Based on the results of full-length sequencing using Oxford Nanopore Technologies with whole amplicon sequencing techniques, a total of 2,496 sequences were read, and 1,713 sequences were identified as species in the passive zone of Supit Urang landfill. The most abundant bacterial phyla in this region were *Proteobacteria* (51%), *Firmicutes* (21%), *Acidobacteria* (7%), *Bacteroidetes* (6%), *Planctomycetes* (4%), *Actinobacteria* (3%), *Gemmatimonadetes* (2%), *Nitrospirae* (2%), and *Chloroflexi* (2%). These results indicate that *Proteobacteria* and *Firmicutes* are abundant in the passive zone of TPA Supit Urang and could potentially biodegrade microplastics such as polypropylene. The narrative review's research showed that numerous bacterial species, including *Bacillus thuringiensis*, *B. cereus*, and *Bacillus* sp., were identified by NGS analysis as possible polypropylene-degrading bacteria.

Keywords: Bacterial diversity, Next generation sequencing, Polypropylene degradation

Introduction

Reports of synthetic waste contamination began in the 1970s [1]. Since then, the number has continued to become a serious problem and global concern. This is caused by the natural biodegradation of plastic waste that takes a very long time, i.e., 50 to more 100 years [2]. One type of plastic waste that often becomes problematic is polypropylene (PP) waste. PP is an extremely versatile polymer that is preferred for its ease of processing, barrier properties, gloss, and dimensional stability, but as its use grows, so does the problem of managing its waste. PP is utilized in diverse commercial applications, including packaging, labelling, and fibre production for indoor and outdoor carpeting. More than 79.01 million tons of plastic waste is estimated to have reached our oceans. According to data Making Oceans Plastic Free (2017), the use of the PP in Indonesia reaches 1,278 million metric tons. Important to note, many types of PP plastic are scattered in the passive zone of the landfills [3; 4]. Synthetic polymers such as PP have the potential to become microplastics that are harmful to the environment and human health. Important proteins such as albumin, globulin and fibrinogen are altered by microplastics when they enter the body and become dysfunctional due to their interaction with blood particles [4]. Changes in chromosome structure caused by microplastics entering cells and tissues have been linked to cancer, obesity and infertility [5]. As a result, the presence of mountains of plastic waste will endanger the environment and human health [6].

In recent years, natural degradation has become critical as social pressures increase for safe plastic waste management. One alternative step is biodegradation by utilizing indigenous degrading bacteria. Several studies show that using indigenous bacteria can increase the degradation rate and support ecosystem recovery without causing adverse impacts and is environmentally friendly [7]. This leads to the need of an alternative solution in form of the development of biological degradation [8].

The soil microbial community may contain indigenous bacteria candidates that are more adaptive. Observation results found that waste in the zone that has been passive for a long time in Malang City, namely the Supit Urang Landfill, since 2018 contains a lot of plastic that has experienced degradation, became cracked and brittle. Since the condition and nutritional characteristics of each landfill soil are likely to be different, resulting in diversity, different isolates of PP degrading bacteria can therefore be obtained from different landfills. From contaminated sites, including landfills, bacterial species capable of biodegrading plastics have previously been isolated [9].

The exploration of PP-degrading bacteria isolates can be done using bacterial culture techniques or a genomic approach. The bacterial culture technique takes a relatively long period of time because of some stages as sampling, isolate culturing, screening and isolate identification are influenced by many parameters such as nutrition in growth medium and environmental conditions. Meanwhile, identifying the presence of PP-degrading bacteria with the fastest culture technique takes 40 days [4]. Thereby, isolate culturing is slightly abandoned. On the other hands, the genomic technique using Next Generation Sequencing (NGS) methods takes only a few days. NGS uses a parallel approach to sequencing, in which millions of DNA fragments can be sequenced at the same time in a single reaction, allowing for high throughput analysis [10]. NGS automation is very high because almost all steps in the sequencing process, including sample preparation, amplification, library preparation, sequencing, and data analysis, can be done simultaneously. After sequencing, the data is obtained and subjected to bioinformatics analysis to identify colonizing bacterial species.

This research aims to explore candidate polypropylene degrading bacteria isolates in the passive zone of Supit Urang Landfill, Malang City using NGS methods. It is hoped that the findings of this study could serve as evidence for the search of polypropylene degrading bacteria from landfills.

Experimental section

This research uses an explorative descriptive method to assess the biodiversity of bacterial communities in the passive zone of TPA Supit Urang Malang City, consisting of: 1) sampling site and sample colection, 2) DNA sequencing, 3) bioinformatics analysis, and 4) bibliometric analysis for identification of polypropylene degrading bacteria. The analysis of the passive zone samples begins with the isolation of genomic DNA from the samples from passive zone of TPA Supit Urang Landfill using Zymo Research's ZymoBIOMICS DNA MiniPrep Kit, followed by amplification of the isolated DNA with 16S 27F - 1429R primers using (NGS) using Oxford Nanopore Technologies technology, sequencing of the DNA amplification results using NGS, downstream analysis of the results using Pavian Krona software, instrumentation and library preparation using Oxford Nanopore Technology kits, and review of blibliometric analysis to identify native polypropylene-degrading bacterial candidates.

Sampling and Sample Collection

Soils were collected from the passive zone of the TPA Supit Urang in Mulyorejo Village, Sukun District, Malang City, East Java, with the coordinates of 7°59'20.04" latitude in the South and 112°38'17.52" longitude in the East. The sampling technique refers to Helen et al. (2017) [4] and Anah et al., (2020) [11]. Soil sediment samples were collected with a shovel from 30 to 60 cm above the soil surface at 5 different locations. Samples were taken at each end as much as 200 g to obtain a total piece of 1 kg sediment. Then, the samples from the five points were stirred evenly and sifted to get a homogeneous soil. Homogeneous samples were subjected to preliminary physico-chemical tests to determine the temperature, moisture, pH, organic carbon and total N content of the soil. The soil samples were then placed in dark container and containing ice gel to maintain a consistent temperature during transport.

DNA from the soil samples (Sample Code: CA20) were extracted using the ZymoBIOMICS DNA MiniPrep Kit from Zymo Research with minimum sample amount of 250 mg. The basic principle of using the ZymBIOMICS DNA MiniPrep Kit is to lyse and disrupt all microbes in samples using lysis buffer and beads. Then, the purification step is repeated several times to ensure better separation and more efficient protein precipitation, and the final step uses affinity chromatography to obtain pure DNA extracts. DNA concentration measurements were performed using a Qubit fluorometer. Qubit provides DNA concentration information using fluorogenic dyes that bind selectively to DNA or RNA. The dye emits a signal only when bound to the target. It can then be used to determine the purity of DNA using the NaNoDrop spectrophotometer, providing a direct measurement of the A260/280 purity ratio. Using the two together can help ensure that the DNA sample is of sufficient concentration and purity for sequencing applications.

DNA Sequencing

All obtained amplicons were sequenced using Full-Length next-generation sequencing (NGS) techniques. Full-Length Sequencing using Oxford Nanopore Technologies technology. Oxford Nanopore Technologies' technology is a nanopore-based DNA sequencing technology in which DNA or RNA molecules can be passed through nanopores from end to end, causing electrical changes that produce a complete sequence of the molecule.

Bioinformatics analysis

DNA amplification results were sequenced with the specialized software for Oxford Nanopore Technologies' technology, namely GRIDION instrument using MinKNOW software version 20.06.9. Base calling was carried out using Guppy software to visualize the data from genetic sequence data resulting in ±2400 sequences. Quality control was performed using nano plots and classified as bacteria and archaea indices downloaded from the website (https://ccb.jhu.edu/software/centrifuge). The analysis was carried out downstream using the software Pavian Krona Tools: to visualize microbial diversity data using krona visualization.

Bibliometric Analysis for Identification of Polypropylene-Degrading Bacteria

The data sources used were scientific articles in the 1960-2021 period, sourced from the Scholarly Database (https://scopus.com). Search for scientific articles was made by using Publish or Perish (*PoP*) Version 7 software with the keywords degradation of polypropylene. The software collects and processes data from sources such as Scopus to provide statistics about one's scholarly work, such as number of citations, h-index, g-index, and other indicators. Data obtained from Publish or Perish was filtered based on the suitability with research, then stored in CSV (Comma Separated Values) and RIS (Research Information System) files. The results obtained were analysed using VOSviewer version 1.6.20 software for bibliometric map analysis to determine the development of the topic of polypropylene-degrading bacteria. VOSviewer is software used to visualize and analyze bibliometric networks and overall concept maps emerging from the scientific literature.

Results and Discussion

Extracted DNA from the sample with the ZymBIOMICS DNA MiniPrep Kit were 50 μL with a concentration of 57.4 ng/μL, which means there is 2.86 μg of DNA. Amplification of the 16S rRNA gene by polymerase chain reaction (PCR) using the primers resulted in an amplicon size of around 1,500 bp (**Figure** 1) and 2,496 sequences. According to Sacchi et al. (2002), the PCR band sizes of about 1,500 bp 1,600 bp are those of the bacterial 16S rRNA sequences as they can distinguish between specific taxa or strains and are indispensable for describing new species [12].

A total of 2,496 sequences were successfully identified. All sequences were then subjected to quality control using NanoPlot to ensure that the sequences obtained were Bacteria or Archaea, and classified using the source data downloaded from the website (https://ccb.jhu.edu/software/centrifuge). Based on this classification, 2,496 sequences were bacterial sequences [12]. A total of 2,496 bacterial sequences were identified from the full-length 16S rRNA sequence results as Kingdom (1), Phylum (25), Class (10), Order (10), Family (16), Genus (19), Phylum (659), Species (1,713), and Subspecies (43). In addition, the data obtained was further analyzed using Pavian Krona Tools software to visualize

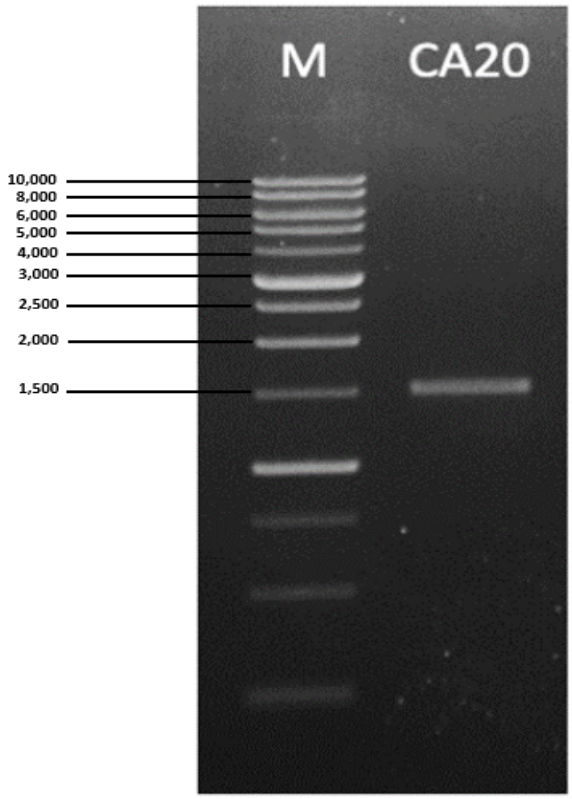

Figure 1. Electrophoregram of amplified bacterial DNAs from CA20 (passive zone sample of Supit Urang Landfill, Malang City).

microbial diversity data using Krona visualization.

Based on the results of the Krona graph, the diversity of bacterial phyla abundant in the passive zone of Supit Urang landfill are Proteobacteria (51%), Firmicutes (21%), Acidobacteria (7%), Bacteroidetes (6%), Planctomycetes (4%), Actinobacteria (3%), Gemmatimonadetes (2%), Nitrospirae (2%), and Chloroflexi (2%). Meanwhile, relative abundance less than 0.6% is categorized into other categories. Proteobacteria and Firmicutes exhibited the highest bacterial abundance among the analyzed samples. The overall structure of the present bacteria in the passive zone of Supit Urang Landfill, Malang, is visualized in a Krona Graph (Figure 2), showing a broader taxonomic hierarchy. The style of reading the Krona Graph from inside to outside showed the abundance of the bacterial domain consisting of 9 phyla (Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, Gemmatimonadetes, Nitrospirae, Chloroflexi) (Figure 3). Each consisted of classes, orders, families, genera, and species. The advantage of the Krona Graph visualization is that it has a more extensive or highly detailed taxonomic hierarchy [13].

Another similar study with the same conditions and research methods but different sampling sites [14] from Bestari Landfill, Probolinggo, resulted in different phyla identified (Table 1). This

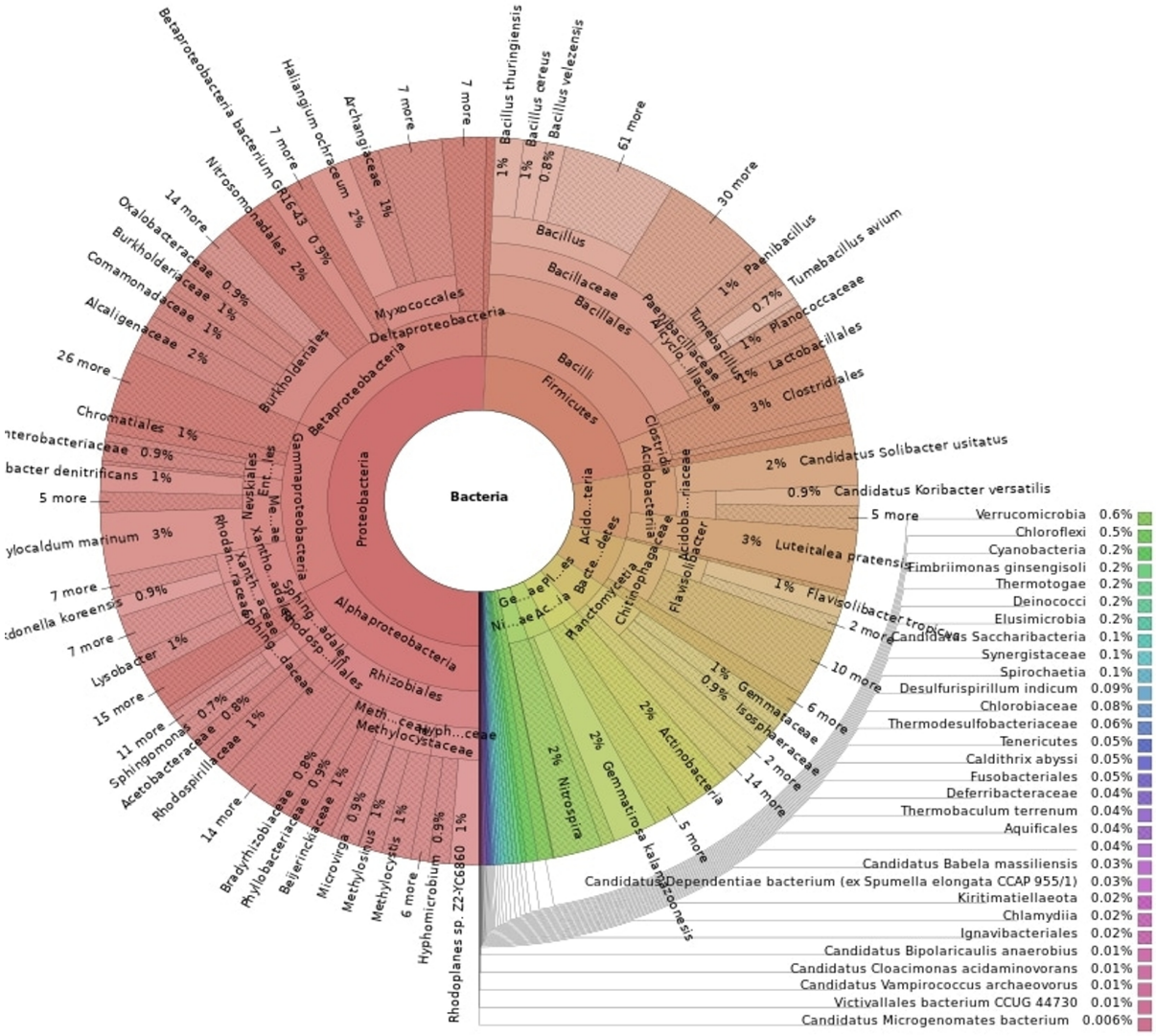

Figure 2. The Krona graph represents bacterial diversity distribution in the Passive Zone of Supit Urang Landfill Malang, East Java.

proves the initial assumption of this study that each landfill has its own characteristics resulting in different microbial diversity. The characteristic properties of each landfill mainly depend on factors of different environmental conditions, including pH, oxygen availability, temperature, humidity, and landfill age which will influence differences in microbial diversity [13]. Differences in environmental conditions at the Supit Urang Landfill and Bestari Landfill are shown in Table 2. The Bestari Landfill location is in the lowlands compared to the Supit Urang Landfill location, which is in the highlands. Thus, Bestari, Probolinggo Landfill is used as a comparison because it has different environmental conditions (physicochemical conditions, types of waste disposed, topographical differences) will affect the diversity of native bacteria that are adaptive to the environment.

Based on our preliminary investigations, the differences in the abundance of some phyla in the Supit Urang Landfill, Malang, and in the Bestari Landfill, Probolinggo, could be due to the differences in some of the environmental parameters in the different soil profiles. Based on research [15], several *Pseudomonas* strains have been reported to aid in PP degradation [3; 4]. In

Table 1. Comparison of the diversity of identified bacterial phyla with those in the reference [14].

Phylum	Supit Urang Landfill, Malang	Presence	Reference	Percentage (%)
Proteobacteria	identified	51	Identified	70
Firmicutes	identified	21	Identified	15
Acidobacteria	identified	7	not identified	
Bacteroidetes	identified	6	not identified	
Planctomycetes	identified	4	Identified	2
Actinobacteria	identified	3	not identified	
Gemmatimonadetes	identified	2	not identified	
Nitrospirae	identified	2	not identified	
Chloroflexi	identified	0.6	not identified	
Cyanobacteria	not identified		identified	0.3

Table 2. Comparison of identified environmental conditions with reference [14].

Parameter	Supit Urang Landfill, Malang	Bestari Landfill, Probolinggo
Temperature	33°C	36°C
Humidity	46%	52%
pH	7	7
Carbon Organic	5.71%	2.85%
Nitrogen Total	0.36%	0.15%

both aquatic and terrestrial environments, *Pseudomonas* has a pH range of 4.5 to 9.5 [16]. Unfortunately, in the abundance of microbial diversity of the NGS results did not show the presence of *Pseudomonas* (Figure 3). It is possible that the pH range of the Supit Urang Landfill habitat is within the growth range of *Pseudomonas*, but there are other nutrients that do not support *Pseudomonas* growth. Therefore, *Pseudomonas* has not been identified in the Supit Urang Landfill.

Most members of the phylum Firmicutes are Gram-positive bacteria with the ability to form endospores. They are primarily found in soil habitats [17]. The most frequently mentioned organisms are the genus *Bacillus* members [18]. Bacteria in this genus can produce oval or cylindrical endospores and can function as aerobes or facultative aerobes. Certain strains can generate extracellular hydrolytic enzymes that break down complex polymers, such as lipids, to be used as a carbon source and electron donor. Moreover, several of these bacteria synthesize antibiotics and insecticides [17]. Growth took place between pH values of 5.4 and 8.5, with the most favorable growth at 7.0 [19]. *Bacillus* is known to be able to degrade PP [20; 21; 22; 23].

Pseudomonas was suggested by 21.0% of the studies, *Bacillus* by 15%, and a combination of the two by 17% of the studies as the bacteria that effectively initiated the biodegradation of synthetic polymers. Thus, the environmental conditions of the waste piles are severe because of the existence of bio toxic compounds and various synthetic substrates [24].

Based on Table 2, the temperature in the passive zone is 33°C; mesophilic bacteria grow well at that temperature. The optimal temperature for plastic-degrading bacteria to grow optimally is 20°C-40°C [25]. Humidity in the sampling zone is 46% (moderate), which is not suitable for bacterial growth [26]. The pH at the side locations ranged from 6.97.2, indicating that the sampling location was neutral. Furthermore, in the analysis, the organic C content was found to be 5.71%, which was classified as very high (> 5%), and the organic N content was 0.36% (classified as moderate). Soil total nitrogen is used as an essential index of soil fertility. Based on the physicochemical characteristics of the soil, dealing with *Proteobacteria* or *Firmicutes* bacteria requires selective media to inhibit the growth of other unwanted species.

Identity of Polypropylene-Degrading Bacteria Based on Bibliometric Analysis

The data source used was scientific articles based on the Scopus.com database, with the keyword "degradation of polypropylene" in the keywords. Article searches are based on the Scopus database, because Scopus is one of the databases whose scope of reputable international journals can be recognized or well received by all researchers around the world [27]. As shown in Table 3, 200 articles were published by scopus.com, filtered according to research on polypropylene degradation from 1960-2021 in the scopus.com database, which has increased significantly yearly. The bibliometric analysis was performed at the end of 2022, but the articles that could be filtered by PoP only reached 2021. This is because Scopus-indexed research on bacterial degradation lasts until 2021.

Based on the graphical data of the number of publications from 1960-2021, it can be seen that the number of publications on polypropylene degradation has an unstable trend from 2001-2022. The beginning of the research topic on polypropylene degradation started in 1960 with one study and was significant until 1992. However, since 2001, research on polypropylene degradation has experienced ups and downs in the number of publications. Based on the graph of the number of publications (**Figure** 4), there were 12 publications in 2006 and 2018. In addition, only 1 publication was recorded in 2022, and even no publication records were found in 2023.

In terms of document type (**Figure** 5), most publications were research articles (166; 83%), followed by reviews (29; 14.5%), conferences (3; 1.5%), and short surveys (2; 1%). Information from the Scopus database showed that most of the publications were all open access.

Table 3. Analysis of PoP Metrics of Relevant Articles

Results	Metrics
Publication years	1960-2021
Citation years	62
Papers	200
Citations	58412
Cites/year	942.13
Cites/paper	292.06
Authors/paper	1.00
h-index	156
g-index	200
hI, norm	156
hI, annual	2.42
hA-index	38

The terms in the title, abstract, index keywords, and year of publication were identified for each publication. A circle represented each time. The size of the process reflected the number of publications. Meanwhile, the distance between the two terms offered an approximate indication of the relatedness of the terms. On the other hand, colors represent groups of words that are strongly related to each other [28]. The development map of the publication of polypropylene-degrading bacteria showed that there were 4 clusters (**Figure** 6 & Table 4). Keyword analysis provides a comprehensive overview of research trajectories and research topics (Donthu et al., 2021). Based on the keyword analysis (Table 4), cluster 1 has the highest number of selected keywords, which means that it contains keywords that are frequently used in the research context, which is "ability". In terms of main keywords, "ability" has the highest occurrence, followed

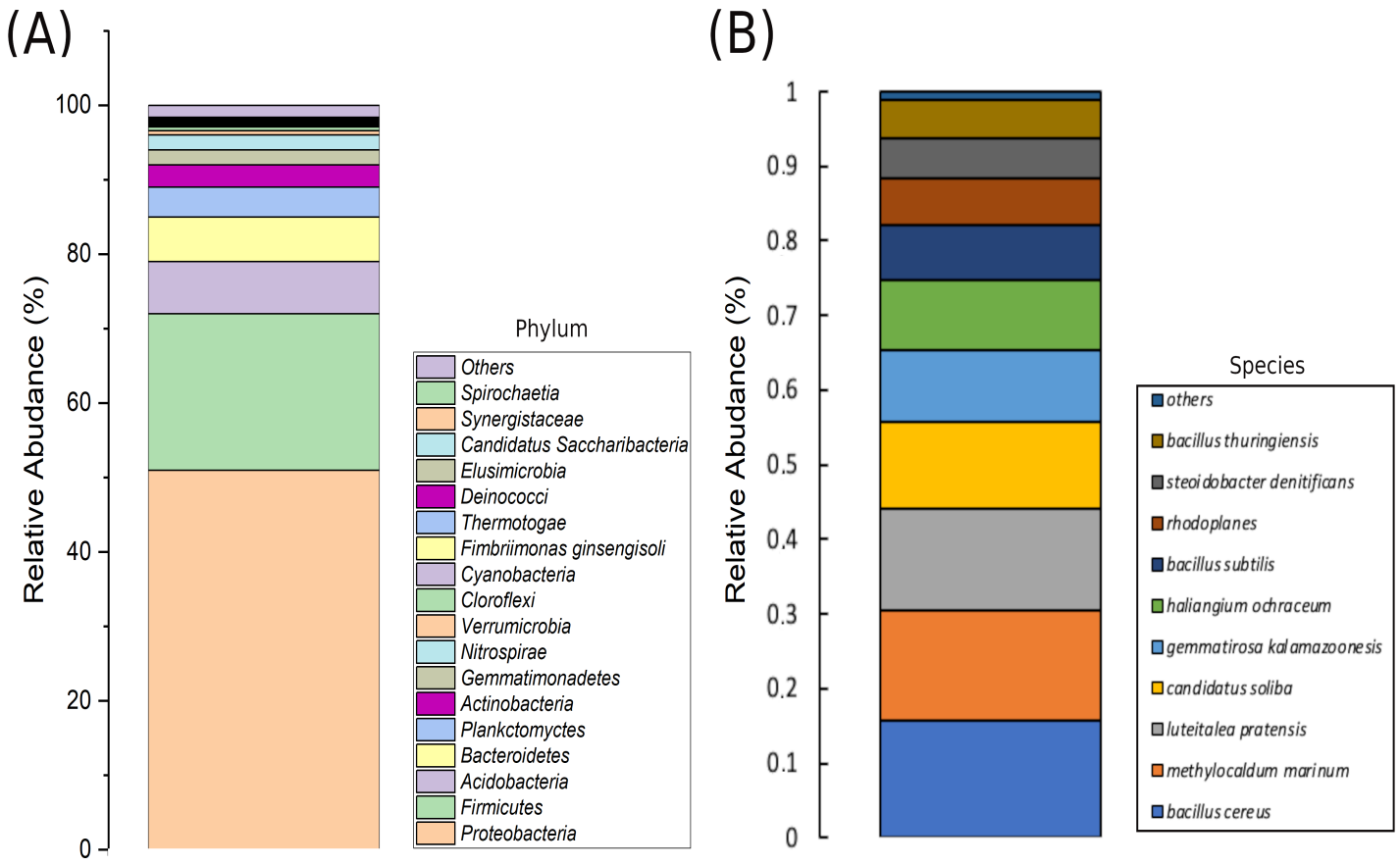

Figure 3. Relative abundance (%) at the phylum (A) and species (B) levels obtained from the Passive Zone of the Supit Urang Landfill Malang City, East Java, Indonesia.

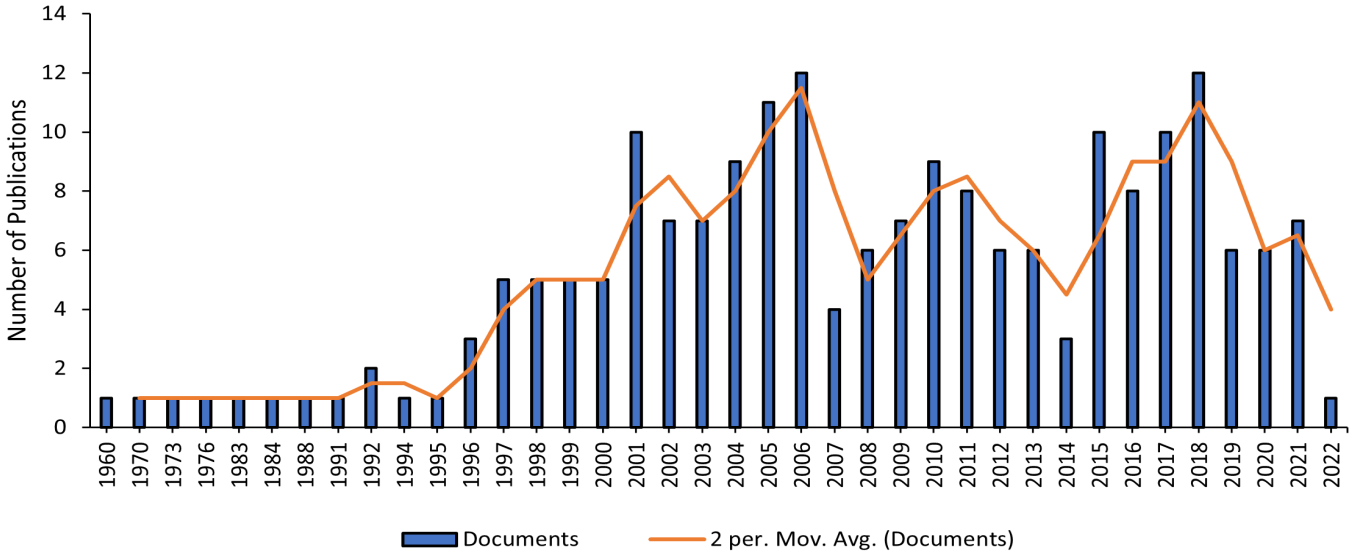

Figure 4. Global publication trend by document types.

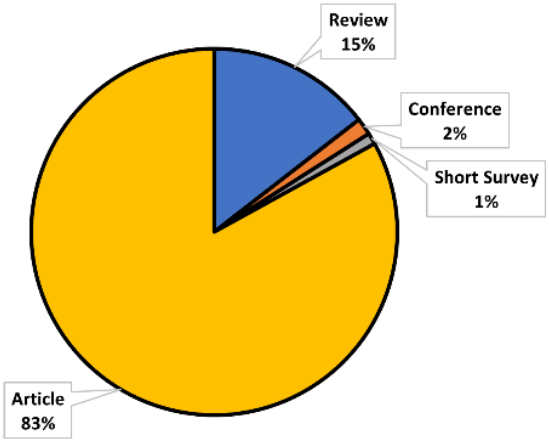

Figure 5. Publication types used for bibliometrics of 200 published articles.

by "biodegradability". This refers to the ability of a substance to be decomposed or broken down by microorganisms into simpler and less harmful components in the environment. The process of biodegradability is part of the natural cycle of plastic degradation, in which microorganisms such as bacteria and fungi break down complex compounds into simpler forms.

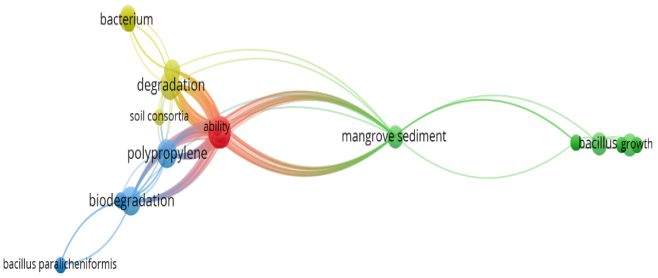

Figure 6. Knowledge map based on index keywords from Elsevier Scopus from 1960-2021.

The resulting clusters of keywords provide an overview of the research themes. For example, cluster 1 describes "ability" or "biodegradability", while the term in cluster 2 is the bacterial species *Bacillus*, which is most often found in research as a potential bacterium that can degrade plastics commonly isolated from sediments. Clusters 3 and 4, on the other hand, show interest in exploring bacterial species and sample locations to obtain more candidate bacterial species with biodegradation and bioremediation capabilities.

Table 5 shows that only some studies of PP biodegradation by bacteria have been reported. *Bacillus thuringiensis* has been reported to degrade PP by 12% for 40 days [3]. The study was conducted using PP pre-treatment, which involved irradiation or thermal treatment [29; 30] and was shown to reduce the hydrophobicity of the polymer. *B. flexus* has also been shown to degrade PP by UV treatment [23]. *Bacillus* sp. can degrade polypropylene plastic during the 40-day incubation period [31]. *B. cereus* showed 12% in degrading polypropylene for 40 days [4]. The *B. flexus* + *B. subtilis* consortium for one year showed a degradation of 1.45% [32]. This confirmed that the bacterial diversity, namely *B. thuringiensis*, *B. cereus*, and *Bacillus* sp., identified from the passive zone of the Supit Urang Landfill, could potentially degrade polypropylene.

Table 4. Keyword clusters in degradation of polypropylene research

Clusters	No. of keywords	Selected keywords	Color
1	37	Ability, *B. cereus*, bacteria isolate, bacteria isolated, bacterial strain, biodegradability, commercial application, day, environment, environmental pollution, extent, important role, incubation, isolate, mangrove ecosystems, medium, microorganism, microplastic material, mineral salt medium, polymer reduction, polypropylene degradation, polypropylene granule, polypropylene polymer, positive potential, the present study, rate, recalcitrance, reduction, remediation, *Sglobispora*, sole carbon source, *Sporasarcina globispora*, term, versatile polymer, vitro, weight loss	Red
2	13	*Bacillus*, *Bacillus* biofilm, biodeterioration, degradation potential, growth, growth kinetic, mangrove sediment, polypropylene microplastic, pretreated polypropylene, *Pseudomonas*, *Rhodococcus*, synergistic growth	Green
3	11	*Bacillus paralicheniformis*, biodegradation, low-density polyethylene, *Lysinibacillus fusiformi*, Madurai, microbe, municipal solid waste, polypropylene, polypropylene film, soil, Vaigai river	Yellow
4	11	aquatic ecosystem, *Bacillus* strain, bacterium, compost, degradation, mangrove ecosystem, microplastic degradation, peninsular Malaysia, polypropylene l lactated, screening, soil consortia	Blue

Table 5. List of bibliography studies on the topic Degradation of Polypropylene

No	Publisher	Title	Bacteria	Comment	References
1	Elsevier	Degradation of unpretreated and thermally pretreated polypropylene by soil consortia	*Bacillus flexus*	Able to degrade polypropylene film (PP-TT), which has not been processed for 12 months, by 10.7%	[22]
2	Elsevier	Growth of *Pseudomonas* and *Bacillus* biofilms on the pretreated polypropylene surface	*Bacillus* and *Pseudomonas*	*Pseudomonas azotoformans*, *Pseudomonas stutzeri*, *Bacillus subtilis* and *Bacillus flexus* separately for 12 months. *P. azotoformans* and *B. subtilis* are relatively hydrophobic, produce biosurfactants, and form biofilms on polymers with higher carbohydrates and proteins than the other two organisms.	[23]
3	Taylor and Francis	Synergistic growth of *Bacillus* and *Pseudomonas* and its degradation potential on pretreated polypropylene	Consortium of *B. flexus* + *P. azotoformans*, Consortium *B. flexus* + *B. subtilis*	Consortium *B. flexus* + *P. azotoformans* for one year showed maximum degradation (22.7%). Consortium *B. flexus* + *B. subtilis* showed a 1.45% degradation for one year.	[32]
4	Elsevier	Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation	*B. gottheilii*	*B. gottheilii* was able to degrade PP for 40 days by 3.6%	[20]
5	Biochemistry and Bioinformatics	Screening for Polypropylene Degradation Potential of Bacteria Isolated from Mangrove Ecosystems in Peninsular Malaysia	*Bacillus cereus*, *Sporosarcina globispora*	*Bacillus cereus* showed 12%, and *Sporosarcina globispora* showed an 11% decrease in body weight in 40 days.	[4]
6	Taylor and Francis	Degradation of polypropylenepoly-*L*-lactide blend by bacteria isolated from compost	*Bacillus licheniformis* (isolate P6), *Bacillus thuringiensis* (isolate P8)	Isolate P8 was 12%, and P6 was 10%, capable of degrading PP for 40 days.	[3]
7	Elsevier	Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment	*Rhodococcus*, *Bacillus* sp.	Able to degrade PP by 6.4% *Rhodococcus* sp. strain 36 and lessen PP by 4.0% by *Bacillus* sp. strain 27 after 40 days of incubation.	[21]
8	Elsevier	Complete genome sequence of marine *Bacillus* sp. Y-01, isolated from the plastics contamination in the Yellow Sea	*Bacillus* sp.	*Bacillus* sp. isolated from plastic contamination in the Yellow Sea	[28]
9	Elsevier	Microplastic degradation by bacteria in aquatic ecosystem	*Bacillus* sp. BCBT21, *Bacillus amyloliquefaciens* BSM-1, *B. amyloliquefaciens* BSM-2, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus cereus*, *Brevibacillus borstelensis*, *Bacillus vallismortis* bt-dsce 01, *P. protegens* bt-dsce 02, *Stenotrophomonas* sp. bt-dsce03, and *Paenibacillus* sp.bt-dsce04	Extracellular hydrolytic enzymes such as CMCase, lipase, xylanase, keratinase, chitinase, and protease secreted by these bacteria play a charismatic role in plastic degradation. Polyurethanes depolymerize urethane and ester bonds due to the hydrolytic properties of urease, esterase, and protease enzymes. Papain and urease can degrade medical polyester due to their proteolytic properties.	[33]
10	PubMed	Microbial and Enzymatic Degradation of Synthetic Plastics	-	Outlining the progress made in the microbial degradation of synthetic plastics and an overview of the enzymes involved in biodegradation.	[34]
11	Elsevier	Plastic biodegradation: Frontline microbes and their enzymes	-	Literature study on plastic degradation by bacteria.	[35]
12	Springer	Biodegradation of low-density polyethylene and polypropylene by microbes isolated from Vaigai River, Madurai, India	*Bacillus paramycoides* (BP) and *Bacillus cereus* (BC)	The highest degradation of PP and PE was observed in BP (78.99 ± 0.005%) and BC (63.08 ± 0.009%) in the single approach, whereas in the combined system, BC & BP recorded the highest degradation in both PP (78.62 ± 2.16%) and PE (72.50 ± 20.53%).	[36]
13	Elsevier	Biodegradation of polypropylene films by *Bacillus paralicheniformis* and *Lysinibacillus fusiformis* isolated from municipality solid waste contaminated soil	*Bacillus paralicheniformis* and *Lysinibacillus fusiformis*	Growth of *Bacillus paralicheniformis* and *Lysinibacillus fusiformis* has shown OD values at 600nm after a 4-week degradation period increased from 0.131 to 0.334 and 0.148 to 0.213, respectively.	[37]
14	Elsevier	Microplastics spatiotemporal distribution and plastic-degrading bacteria identification in the sanitary and non-sanitary municipal solid waste landfills	-	The microplastic (MP) surface gradually fades, becomes rough, and even produces cracks and holes with landfill depth and age. Small-size MPs (<100 m) were the most abundant, and their numbers increased significantly from 28.14% to 49.13% in SL and from 24.54% to 59.51% in NSL, while prominent-size MPs decreased significantly.	[38]

Conclusion

NGS employing 16S rRNA analysis at the Supit Urang Landfill showed 1,713 bacterial species from 2,496 sequences. Bacterial species with high abundance, namely *Bacillus thuringiensis* (15%), *B. cereus* (13%), *B. velezensis* (10%), and *Bacillus* sp. (7%) were identified. Based on the bibliometric analysis results, *B. thuringiensis*, *B. cereus*, and *Bacillus* sp. showed potential in the degradation of polypropylene. These indigenous PP-degrading bacteria are expected to be cultured and used to develop PP biological degradation using suitable selective media. The limitation of the study is that it only provides the profile of candidate bacteria that can degrade plastics. To improve the results of this research in the future, confirmation can be carried out on a wet lab scale by performing proteomic analysis aimed at obtaining information on cellular protein expression, so that it can find specific proteins that can only be found in plastic degrading bacterial candidates, and not in bacteria that do not degrade plastic. From the specific proteins obtained, it can then proceed to bioinformatics analysis through molecular docking to determine the specific binding to the active site of the target protein. Bioinformatics analysis can specifically predict the target protein that causes the microbe to have the ability to degrade xenobiotic plastics, and it can be combined with molecular docking to see its ability to different types of xenobiotic plastics.

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