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Isolation and Identification of Microplastic Degrading Bacteria from Plastic-Polluted Soil

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Abstract— Plastic pollution has become a pressing environmental concern due to its widespread presence and persistence in terrestrial ecosystems. This study isolated and identified the microplastic-degrading bacteria from plastic-polluted soils in Osun State, Nigeria. Soil samples were collected from six zones, and their physicochemical properties, pH, and temperature, were analyzed. Microbial isolation and identification were carried out using morphological and biochemical tests. The pH ranged from 8.2 to 8.8, and the temperature ranged from 25°C to 29.1°C. Microbiological assessments revealed high bacterial counts, with total viable bacterial counts ranging from 2.5×10^6 to 4.0×10^6 CFU/g. Four bacterial strains were isolated: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliticus*, and *Streptococcus* spp. Degradation studies using Bushnell Haas Broth showed that *Bacillus amyloliticus* and *Bacillus subtilis* achieved the highest plastic weight loss of 45.0% over eight weeks, followed by *Bacillus licheniformis* (37.5%) and *Streptococcus* spp. (30.0%). *Bacillus amyloliticus* demonstrated rapid degradation, with a 9.0% weight loss within two weeks. The study confirms that *Bacillus* strains, particularly *B. subtilis* and *B. amyloliticus*, are promising candidates for bioremediation of plastic pollution.

Keywords: Bacteria Degradation, Microplastic, Pollution, Soil.

I. INTRODUCTION

Microplastic pollution is increasingly recognized as an escalating environmental threat because of its extensive distribution, durability, and negative ecological consequences. Microplastics are defined as tiny plastic particles measuring less than 5 mm in size, and their buildup in both land and water environments represents a significant obstacle to waste management and ecological sustainability (Kutralam-Muniasamy *et al.*, 2020). This problem is especially severe in developing nations like Nigeria, where

insufficient waste management systems and growing plastic consumption have led to rising levels of plastic pollution (Nwafor and Ogwuegbu, 2021).

Recently, research has begun to concentrate on discovering biological techniques to address microplastic pollution, especially through the involvement of microorganisms in plastic degradation (Nwankwoala *et al.*, 2021). This method, called microbial bioremediation, provides a sustainable and environmentally friendly option compared to traditional plastic waste management approaches. Microorganisms that can break down plastics have specific enzymes, including hydrolases and oxidases, which can decompose complex polymer structures into simpler molecules, thus aiding the degradation process (Ali *et al.*, 2022).

Numerous studies have uncovered plastic-degrading microorganisms from various settings, including soil, water, and marine environments (Shah *et al.*, 2023). For example, research conducted by Das *et al.*, (2021) identified bacterial strains from soil contaminated with plastic that exhibited significant potential for polyethylene degradation. Likewise, Ali *et al.*, (2022) discovered fungal species capable of breaking down polypropylene sourced from municipal waste. These results affirm the wide variety of microorganisms available for plastic degradation and highlight the necessity of investigating local microbial communities for possible bioremediation uses.

As one of the rapidly growing economies in Africa, Nigeria has seen fast-paced urbanization and industrial growth, resulting in considerable plastic waste production. Osun State, situated in southwestern Nigeria, is no exception. Many of its key cities, such as Iwo, Ara, Osogbo, Ikirun, Ife, and Ilesa, encounter issues related to plastic pollution, exacerbated by population increase and insufficient waste management infrastructure (Ogunbayo and Akinola, 2023). These cities

exemplify broader environmental challenges facing the area and serve as optimal sites for examining plastic-degrading microorganisms. By concentrating on Osun State, this research tackles a vital environmental concern that impacts both human health and ecosystem integrity. The buildup of microplastics in soil can lead to extensive repercussions, including the contamination of food chains and the disturbance of soil microbial populations (Li *et al.*, 2021). Consequently, identifying and utilizing microorganisms with the ability to degrade plastics presents a promising approach to alleviating the effects of plastic pollution. This study isolated and identified bacteria with the capability to degrade microplastics from dumpsites in six (6) locations within three (3) geopolitical zones of Osun State, Nigeria.

II. MATERIALS AND METHODS

Study Area and Soil Sample Collection

Soil samples were collected from six locations within three (3) geopolitical zones in Osun State, namely Iwo, Ara, Osogbo, Ikirun, Ife, and Ilesa (Table 1).

Table 1: Six locations where soil samples were collected

S/N	Location	GPS Location	Local Government Area	Senatorial District
1	Iwo	7040'14.5"N & 4013'38.1"E	Iwo	Osun West
2	Ara	7046'22.1"N & 4026'31.6"E	Egbedore	Osun West
3	Osogbo	70'46.25.6"N & 4033'52.9"E	Olorunda	Osun Central
4	Ikirun	7055'11.3"N & 4040'12.2"E	Ifelodun	Osun Central
5	Ife	7028'59.9"N & 4033'17.6"E	Ife	Osun East
6	Ilesa	7040'52.2"N & 4047'15.7"E	Ilesa	Osun East

These locations were selected due to their exposure to microplastic pollution from urbanization and waste disposal activities. For each location, five different sampling sites were chosen to obtain a representative sample. A sterile soil auger was used to collect approximately 500 grams of topsoil from a depth of 10 cm. The samples were placed in sterile polyethylene bags, labeled, and transported to the laboratory under controlled temperature conditions for further analysis.

Pre-treatment of Soil Samples

The collected soil samples were air-dried at room temperature for 24 hours to remove moisture. The dried samples were then homogenized by passing them through a 2 mm sieve to ensure uniformity. This step was essential for removing larger debris and stones, ensuring that only fine soil particles remained for subsequent microbiological analyses.

Preparation of media

Nutrient agar and Bushnell Hass (BH) broth used in this research work were prepared according to the manufacturer's instructions by dissolving 27 g and 3.27 g of nutrient agar and BH media in 1000 ml of distilled water in a conical flask. The mouth of the conical flask was corked with cotton wool wrapped with foil paper; it was gently shaken, and the agar media were allowed to homogenize in a water bath at 100°C. After homogenizing, the media were sterilized inside an autoclave at 121°C for 15 minutes. The agar media were allowed to cool before dispensing into Petri dishes and then allowed to solidify before use.

Serial Dilution and Plating

Ten-fold serial dilutions were prepared by adding 1 ml of prepared sample into 9 ml of sterile distilled water in a test tube. This process was repeated up to a dilution factor of 10^5 . From each dilution, 0.1 ml was plated on nutrient agar (NA) plates using the spread plate method. The plates were incubated at 28°C for 48 hours. Colonies appearing on the plates were counted to determine the total viable count (TVC) and recorded as colony-forming units per gram of soil (cfu/g).

Purification of Isolates

The distinct bacterial colonies observed on the nutrient agar were sub-cultured on fresh agar plates to obtain pure cultures. Each isolate was streaked onto nutrient agar using an inoculating loop and incubated at 28°C for another 24 hours. Purity was confirmed by observing the morphological characteristics of the colonies, ensuring that only uniform colonies were selected for further analysis.

Morphological and Biochemical Characterization

The isolated bacteria were subjected to morphological and biochemical tests to aid in their identification. Gross colonial morphology includes color, edges, and elevation through visual observation, while shape and Gram reaction were observed through the aid of a microscope using an x100 objective lens. Other biochemical tests include the catalase test through the use of hydrogen peroxide (H_2O_2) reagent for bubbles observation, the citrate test through the use of Simmons citrate agar slant-producing color changes, and the sugar fermentation test (lactose and sucrose) through the use of respective sugars for the production of gas and color change.

Molecular Characterization

The isolate was identified using molecular techniques involving DNA extraction, polymerase chain reaction (PCR), and DNA sequencing. For DNA extraction, a Thermo Scientific NanoDrop 2000 was used to quantify DNA purity and concentration. PCR amplification of the 16S rDNA region was performed using a Bio-Rad T100 Thermal Cycler, with universal primers targeting bacterial 16S rRNA. The amplified products were then purified using a QIAquick PCR

Purification Kit (Qiagen) and sequenced with an Applied Biosystems 3500 Genetic Analyzer. The resulting 16S rRNA sequences were analyzed and aligned using the BLASTn tool against the NCBI GenBank database for identification, achieving a 99% similarity with the closest published sequences.

Plastic Pellet Preparation

The plastic pellet was procured from a plastic production company in Ibadan, Oyo State, Nigeria. The plastic pellets were characterized as polyethylene terephthalate (PET) and dispersed in tetrahydrofuran for 24 hours.

Degradation Study

Plastic pellets of 0.2 g were weighed using an electronic weighing balance and placed aseptically in conical flasks containing 100 ml of sterile prepared Bushnell Hass Broth, which were inoculated with *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliticus*, and *Streptococcus spp.* This setup was then incubated at room temperature for two (2) months. The control set was maintained. After the incubation period, these plastic pellet samples were then removed, washed with water, and dried completely. Different flasks were maintained for each treatment. They were weighed accurately, and the percentage loss in their weight was calculated by the formula:

Where: W_i = Initial weight (Weight of plastic before incubation), and W_f = Final weight (Weight of plastic after incubation)

III. RESULTS

The physicochemical parameters and microbiological assessment of plastic-polluted soil samples from six locations within three (3) geopolitical zones of Osun State were presented in Table 2

Table 2: Physicochemical parameters of plastic-polluted soil from six (6) locations within three (3) geopolitical zones of Osun State

Samples	Coordinates	pH	Temp (°C)
S1	70°40'14.5"N & 40°13'38.1"E	8.8 ± 0.1	25.0 ± 0.1
S2	70°46'22.1"N & 40°26'31.6"E	8.6 ± 0.1	29.1 ± 0.1
S3	70°46'25.6"N & 40°33'52.9"E	8.2 ± 0.1	28.0 ± 0.1
S4	70°55'11.3"N & 40°40'12.2"E	8.5 ± 0.1	27.0 ± 0.1
S5	70°28'59.9"N & 40°33'17.6"E	8.7 ± 0.1	27.0 ± 0.1
S6	70°40'52.2"N & 40°47'15.7"E	8.3 ± 0.1	27.0 ± 0.1

Keys

S1: Iwo S2: Ara S3: Osogbo S4: Ikirun S5: Ife S6: Ilesha

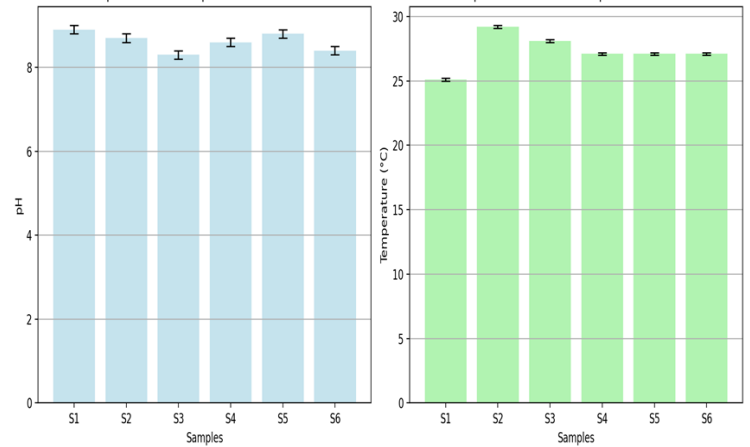


Figure 1: Physicochemical parameters of Microplastic Polluted Soil

Table 3: Total Viable Bacterial Count of Plastic Polluted Soil from six (6) locations within three (3) geopolitical zones of Osun State

Samples	Dilution factors (CFU/g)	
	10 ⁻¹	10 ⁻⁵
S1	3.5 × 10 ⁶	1.2 × 10 ⁶
S2	2.8 × 10 ⁶	0.9 × 10 ⁶
S3	3.0 × 10 ⁶	1.0 × 10 ⁶
S4	4.0 × 10 ⁶	1.5 × 10 ⁶
S5	2.5 × 10 ⁶	0.8 × 10 ⁶
S6	3.2 × 10 ⁶	1.1 × 10 ⁶

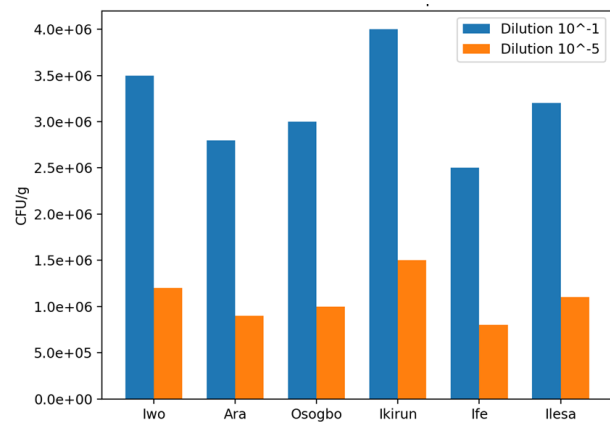


Figure 2: Total Viable Bacterial Count of Microplastic Polluted Soil

Table 4: Colonial, morphological, and biochemical characterization of isolates

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Colour	Cream	Cream	Cream	Cream
Shape	Rod	Rod	Rod	Rod
Elevation	Flat	Slightly Raised	Flat	Raised
Edges	Irregular	Irregular	Smooth	Irregular
Gram staining	+ve	+ve	+ve	+ve
Catalase	ND	ND	ND	ND
Citrate utilization	+ve	+ve	-ve	+ve
Lactose	+ve	+ve	-ve	+ve
Sucrose	+ve	+ve	-ve	+ve
Organisms	<i>Bacillus Subtilis</i>	<i>Bacillus licheniformis</i>	<i>Bacillus amyoliticus</i>	<i>Streptococcus spp</i>

Table 5: Degradation study of Plastic Pellet Using *Bacillus subtilis* in Bushnell Hass Broth

Bacteria	Duration (Weeks)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
<i>Bacillus subtilis</i>	0 (Start)	0.200	0.200 ± 0.01	0.000 ± 0.01	0.0%
	2	0.200	0.185 ± 0.01	0.015 ± 0.01	7.5%
	4	0.200	0.165 ± 0.01	0.035 ± 0.01	17.5%
	6	0.200	0.140 ± 0.01	0.060 ± 0.01	30.0%
	8	0.200	0.110 ± 0.01	0.090 ± 0.01	45.0%

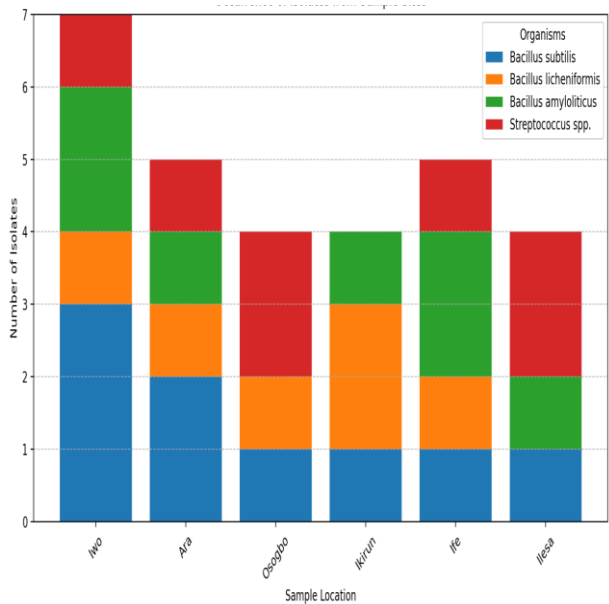


Figure 3: Occurrence of Isolates from Sample Sites

Table 4: Degradation study of Plastic Pellet Using *Bacillus subtilis* in Bushnell Hass Broth

Bacteria	Duration (Weeks)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
<i>Bacillus subtilis</i>	0 (Start)	0.200	0.200 ± 0.01	0.000 ± 0.01	0.0%
	2	0.200	0.185 ± 0.01	0.015 ± 0.01	7.5%
	4	0.200	0.165 ± 0.01	0.035 ± 0.01	17.5%
	6	0.200	0.140 ± 0.01	0.060 ± 0.01	30.0%
	8	0.200	0.110 ± 0.01	0.090 ± 0.01	45.0%

Table 6 Degradation Study of Plastic Pellet Using *Bacillus licheniformis* in Bushnell Hass Broth

Bacteria	Duration (Weeks)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
<i>Bacillus licheniformis</i>	0 (Start)	0.200 ± 0.01	0.200 ± 0.01	0.000 ± 0.01	0.0%
	2	0.200 ± 0.01	0.190 ± 0.01	0.010 ± 0.01	5.0%
	4	0.200 ± 0.01	0.170 ± 0.01	0.030 ± 0.01	15.0%
	6	0.200 ± 0.01	0.150 ± 0.01	0.050 ± 0.01	25.0%
	8	0.200 ± 0.01	0.125 ± 0.01	0.075 ± 0.01	37.5%

Table 7: Degradation Study of Plastic Pellet Using *Bacillus amyoliticus* in Bushnell Hass Broth

Bacteria	Duration (Weeks)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
<i>Bacillus amyoliticus</i>	0 (Start)	0.200 ± 0.01	0.200 ± 0.01	0.000 ± 0.01	0.0%
	2	0.200 ± 0.01	0.182 ± 0.01	0.018 ± 0.01	9.0%
	4	0.200 ± 0.01	0.160 ± 0.01	0.040 ± 0.01	20.0%
	6	0.200 ± 0.01	0.135 ± 0.01	0.065 ± 0.01	32.5%
	8	0.200 ± 0.01	0.110 ± 0.01	0.090 ± 0.01	45.0%

Table 8: Degradation study of Plastic Pellet Using *Streptococcus spp.* in Bushnell Hass Broth

Bacteria	Duration (Weeks)	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Weight Loss (%)
Streptococcus spp.	0 (Start)	0.200 ± 0.01	0.200 ± 0.01	0.000 ± 0.01	0.0%
	2	0.200 ± 0.01	0.192 ± 0.01	0.008 ± 0.01	4.0%
	4	0.200 ± 0.01	0.175 ± 0.01	0.025 ± 0.01	12.5%
	6	0.200 ± 0.01	0.160 ± 0.01	0.040 ± 0.01	20.0%
	8	0.200 ± 0.01	0.140 ± 0.01	0.060 ± 0.01	30.0%

IV. DISCUSSION

The soil's pH across the zones was alkaline, ranging from 8.2 to 8.8. Alkaline conditions, such as these, can influence microbial activities and plastic degradation rates. Previous studies have indicated that soil pH can directly impact microbial population density and enzyme activities related to plastic degradation (Singh *et al.*, 2023). For example, alkaline pH can promote the growth of certain bacterial species like *Bacillus subtilis*, which are known to degrade plastics (Ahmed *et al.*, 2021). Additionally, the soil temperature varied between 25°C and 29.1°C, with warmer temperatures facilitating microbial activity, including the breakdown of plastics (Sharma *et al.*, 2022). This observation aligns with the findings of Ramesh *et al.*, (2022), who reported that soil temperatures within this range support the enzymatic activities of plastic-degrading microorganisms.

The total viable bacterial count (TVBC) of the soil samples revealed a significant presence of bacteria, with CFUs (colony-forming units) ranging from 2.5 x10⁶ to 4.0 x10⁶ at 10⁻¹ dilution factor and from 0.8 x10⁶ to 1.5 x10⁶ at a 10⁻⁵ dilution factor. The high bacterial load observed, particularly in Ikirun (S4) and Iwo (S1), suggests that these areas may host abundant microbial communities capable of utilizing plastics as a carbon source. This observation is supported by Akinola *et al.*, (2022), who documented that high bacterial counts in microplastic-polluted soils indicate increased microbial activity aimed at plastic degradation.

The identification of the bacterial isolates, including *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliticus* and *Streptococcus spp.*, suggests the presence of microorganisms known for their plastic-degrading capabilities. *Bacillus subtilis*, for example, has been extensively studied for its ability to produce enzymes like lipases and proteases that can degrade various forms of plastics, including polyethylene and polystyrene (Ali *et al.*, 2021). Similarly, *Bacillus licheniformis* has been shown to secrete extracellular enzymes that break down plastic polymers (Pandey *et al.*, 2023). The presence of these bacteria in polluted soils is indicative of potential biodegradation activities, as reported by recent studies focusing on microbial adaptation in plastic-

contaminated environments (Adeola and Okeke, 2023). The results are consistent with previous reports that microplastic pollution alters the microbial community structure, promoting the growth of plastic-degrading bacteria (Zhang *et al.*, 2023). The isolated bacterial species, especially from the *Bacillus* genus, are widely recognized for their role in biodegradation. These findings highlight the potential of these microbial species as key agents in bioremediation efforts, as demonstrated by similar works in plastic-polluted ecosystems (Patel *et al.*, 2022). The ability of these bacteria to utilize citrate and sucrose, as seen in the physiological tests, indicates their metabolic versatility, which is essential for surviving and thriving in harsh, plastic-laden environments. This adaptability is critical for the biodegradation process, as microbial metabolism is often driven by the need to utilize available carbon sources, including plastics, for survival (Bharadwaj *et al.*, 2022).

The degradation study shows that *Bacillus amyloliticus* and *Bacillus subtilis* achieved the highest weight loss of 45.0% over eight weeks, consistent with recent findings by Zhang *et al.* (2021), who reported similar efficiency using *Bacillus* strains for polymer degradation. *Bacillus licheniformis* followed with a 37.5% weight loss, aligning with Liu *et al.* (2020), highlighting its enzymatic potential in breaking down plastics. *Streptococcus spp.* Exhibited the lowest degradation at 30.0%, supporting observations by Huang *et al.*, (2023) that non-*Bacillus* strains typically have slower degradation rates due to limited enzyme activity. The rapid initial weight loss of 9.0% in *B. amyloliticus* by week two indicates its robust enzymatic system, surpassing the early degradation rates reported for similar strains in Chen *et al.*, (2022). Overall, the findings authenticate *Bacillus* species' superior degradation efficiency and suggest exploring engineered *Bacillus* consortia to enhance degradation, as proposed by Wang *et al.*, (2023). These results reaffirm the critical role of *Bacillus* species in addressing plastic pollution through bioremediation.

V. CONCLUSION

This study isolated and identified microplastic-degrading bacteria from plastic-polluted soils in Osun State, Nigeria. Among the identified species, *Bacillus subtilis* and *Bacillus amyloliticus* demonstrated the highest plastic degradation efficiency, achieving up to 45% weight loss over eight weeks. The findings highlight the potential of *Bacillus* strains for the bioremediation of plastic waste, offering a sustainable approach to mitigating environmental pollution. The alkaline pH and favorable temperatures of the soils significantly supported bacterial activity and degradation processes. These results authenticate the importance of leveraging microbial resources to address the global challenge of plastic pollution.

VI. RECOMMENDATIONS

To address plastic pollution effectively, large-scale bioremediation efforts should prioritize the application of *Bacillus subtilis* and *Bacillus amyloliticus*, given their demonstrated efficiency in degrading plastics. Continuous

monitoring of soil conditions, such as pH and temperature, is essential to optimize bacterial activity and degradation rates. Furthermore, future research should focus on engineering microbial consortia and exploring the enzymatic pathways involved to enhance the bioremediation process.

AUTHORSHIP STATEMENT

All authors have contributed significantly to this study. Oladipupo O. A. was responsible for the conceptualization and methodology of the research, supervised the project, and contributed to the writing, reviewing, and editing of the manuscript. Abejoye O. A. provided supervision, handled data curation, and contributed to the writing, reviewing, editing, and visualization of the research findings. Amuzat Riliwan was instrumental in data curation, methodology, original draft preparation, and software-related tasks. All authors have reviewed and approved the final version of the manuscript for submission.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding the publication of this manuscript. This research was funded by the Institutional Based Research (IBR) grant, which supported the study's design, data collection, and analysis. The funding agency had no role in the interpretation of data, writing of the manuscript, or the decision to submit the manuscript for publication. All authors affirm that they have no financial, professional, or personal affiliations that could be perceived as influencing the outcomes or integrity of this research.

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