Screening of Gram-Positive Bacteria from Poultry Farms in Tarlac, Philippines for Potential Presence of Low-Density Polyethylene (LDPE) Degrading Actinomycetia spp. And Bacilli spp. Population

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Introduction

Plastic pollution remains a major environmental concern, largely due to the widespread use of low-density polyethylene (LDPE) in consumer goods, which accumulates in ecosystems (Abd, M. E., et al., 2024). Researchers are now exploring eco-friendly solutions through microorganisms such as *Actinomycetia* spp. and *Bacilli* spp., gram-positive bacteria known for producing plastic-degrading enzymes and commonly found in soil (He, Y., et al., 2024). This study investigates poultry farms in Tarlac, Philippines, as potential sources of such bacteria, aiming to identify strains capable of degrading LDPE for future waste management practices (Salam, N., et al., 2020; Saini, A., et al., 2015).

Objectives

1. To **isolate and identify a population of *Actinomycetia* spp. and *Bacilli* spp.** from soil samples through macroscopic, microscopic, and molecular identification.
2. To assess the biodegradation activity of the isolated *Actinomycetia* spp. and *Bacilli* spp. on LDPE by:
   - a. Clear Zone Formation
   - b. Optical Microscopy
   - c. Weight Loss Analysis
3. To **compare the plastic degradation capabilities** of *Actinomycetia* spp. and *Bacilli* spp. on LDPE

Methodology

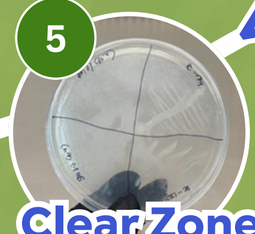
1. **Soil Sample Collection**

2. **Serial Dilution**

3. **Cultivation**
   - Nutrient Agar
   - Starch Casein Agar for ***Actinomycetia*** spp.

4. **Gram Stain**

5. **Clear Zone Formation Assay**



6. **Weight Loss Analysis**

Isolates were sent to **Macrogen Inc.** for PCR Amplification and 16s rRNA sequencing.

Results

Identification of Isolates

After Gram staining, 10 Gram-positive isolates were sent to **Macrogen Inc.** via Kinovett Scientific Solutions for 16S rRNA sequencing. 4 out of 10 isolates were positively confirmed under *Actinomycetia* spp. and *Bacilli* spp.

NCBI Scientific Name	Accession number (GenBank)	Percent Identity and Family
Arthrobacter pascens strain DSM 20545	NR_026191.1	99.03% Micrococcaceae
Kocuria rhizophila strain TA68	NR_026452.1	99.79% Micrococcaceae
Brevibacillus brevis strain DSM 30	NR_112204.1	99.25% Paenibacillaceae
Bacillus cereus strain CCM 2010	NR_115714.1	99.73% Bacillaceae

Table 1. *Molecular Identification of Isolates*

Clear Zone Formation Assay

All four isolates showed growth after 7 days of incubation at 37 °C in LDPE-supplemented Mineral Salt Medium (MSM) agar, and showed a zone of clearance, which is described as a visibly transparent, bacteria free area on the solid medium around each microbial colony (Gohel et al., 2014). This is indicative of the bacteria's ability to utilize the LDPE as its sole carbon source.

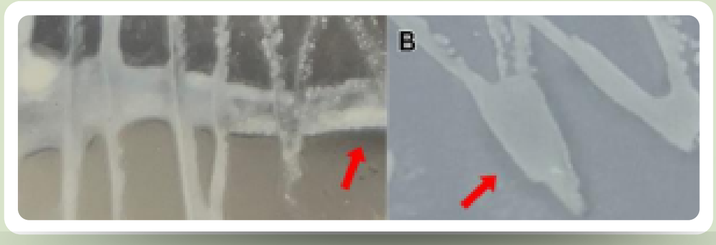


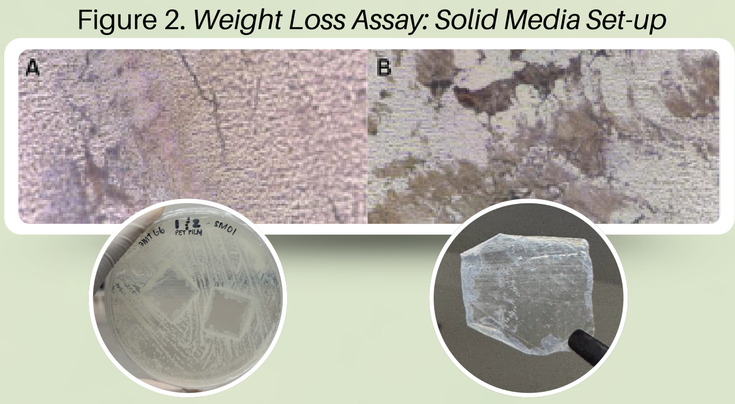
Figure 1. *Clear Zone Formation Assay*

Weight Loss Analysis

Solid Media Set-up

In the **solid media** set-up, homogenous growth of the 4 isolates were observed. Furthermore, microscopic analysis of the LDPE films showed notable growth when incubated with ***Kocuria rhizophila***, showing values as high as 0.13%. These explain the evidence of possible degradation such as bacterial growth and structural changes such as cracks and spots.

Figure 2. *Weight Loss Assay: Solid Media Set-up*



Liquid Media Set-up

In the **liquid media set-up,** minimal weight loss percentages are observed, with ***Kocuria rhizophila*** also showing the highest value of 0.16%.

Table 2. *Weight Loss Analysis of Isolates*

Set Up	p-value	t-value	Decision
Plate	0.340	1.075	Accept Ho (Not Significant)
Tube	0.380	1.116	Accept Ho (Not Significant)

Note: Significant at p-value < 0.05

Statistically, the weight loss analysis of the LDPE films before and after incubation in both set-ups showed n**o statistically significant difference**. This can also be observed in the T-test for comparison of the plastic degrading capabilities of isolates from *Actinomycetia* spp. and *Bacilli* spp.

Discussion

The researchers were able to isolate bacteria within the targeted populations, identified via sequencing. The isolates were proved to be capable of plastic degradation, evident in the results of the assays, which indicate their ability to break down and utilize LDPE as a source of carbon by enzymatic activity. However, none of the isolates were able to show significant deterioration of LDPE.

Conclusion

In this study, the bacteria isolated from the collection sites were not able to prove the long-term viability of bacterial degradation, but have shown their capability to aid in the degradation of plastic. Moreover, there is no significant difference in both of the population's capability in plastic degradation.

Recommendations

For future researchers, we recommend the following:

1. **Increase the time allotted for incubation**, approximately up to 60 days for plastic degradation.
2. **Increase the scope** of the analyzed bacterial species to increase the sample size.
3. **View the films under Scanning Electron Microscopy** (**SEM**) to observe better evidence of degradation activity.

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REFERENCES