Investigation into the microbial degradation potential of *Bacillus cereus* and *Pseudomonas aeruginosa* on disposable surgical masks

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Abstract

An unexpected accumulation of polypropylene (PP) waste resulted from the extensive use of disposable surgical masks during the COVID-19 pandemic, underscoring the urgent need for sustainable degradation strategies. In the current study, soil samples collected from a municipal dumping site were screened for plastic-degrading microorganisms and identified using 16S rRNA sequencing. Two bacterial strains were obtained, belonging to the species *Bacillus cereus* and *Bacillus anthrasis*; *B. cereus* was selected for further investigation. In addition, a laboratory strain of *Pseudomonas aeruginosa* was included for comparative assessment. Standard PP mask pieces were incubated for 60 days in nutrient broth inoculated with B. cereus and P. aeruginosa. Post-incubation analysis demonstrated a substantial reduction in the dry weight of PP films, with *B. cereus* and *P. aeruginosa* achieving 43.3% and 33.3% weight loss, respectively. Scanning electron microscopy revealed distinct morphological deterioration on treated PP surfaces, including cracks and erosion features, indicative of microbial degradation. These findings highlight the importance of environmentally sourced bacteria for the effective biodegradation of PP-based surgical masks, offering a promising biological route for mitigating pandemic-related plastic pollution

Keywords: *Biodegradation, face mask, Bacillus cereus, Pseudomonas aeruginosa*

INTRODUCTION

One preventive measure to reduce the transmission of illness from person to person during the SARS-CoV-2 epidemic was the introduction of face masks (Worby and Chang 2020). In April 2020, the Centres for Disease Control and Prevention (CDC) and the World Health Organisation (WHO) advised the public to use masks to reduce community transmission. Surgical mask usage surged globally as a key public health measure, despite challenges in supply, compliance, and consistent guidance (Wong et al., 2020; (Dharmaraj et al., 2021). Face masks come in a wide variety of sizes and styles on the global market, and among them, the surgical face mask is mainly made up of polypropylene. The extensive usage of these masks has led to the reckless disposal of discarded masks in the environment and their mishandling, which has become a significant concern (Vijayalakshmi et al., 2022). The gradual decomposition of polypropylene and polyethene fibres from masks led to significant reservoirs of microplastic pollutants, which have both acute and chronic impacts on the physiology of aquatic organisms (Rebelein et al., 2021). Conventional waste management practices such as incineration and landfilling often result in secondary pollution, toxic emissions, or limited biodegradation, thereby underscoring the need for environmentally sustainable alternatives (Prata et al., 2020). Thus, the disposal of these masks has become an environmental hazard (Dissanayake et al.,2021)

Microorganisms capable of polymer breakdown have been studied and isolated from the natural environment. Various studies have documented specific bacterial and fungal species involved in the microplastic degradation process (Vague et al., 2019; Gupta and Devi, 2020; Roberts et al., 2020). Certain bacteria, like *P. aeruginosa*, show potential for partial PP degradation (Knicker and Molina, 2022). Studies have shown that bacteria belonging to the genera *Bacillus* and *Pseudomonas* are widely recognised for their biodegradative versatility and ability to colonise hydrophobic polymeric surfaces (Shah et al., 2008; Ojha et al., 2017). Despite increasing attention to microbe-mediated degradation of plastics, investigations focusing specifically on the biodegradation of PP-based surgical masks remain limited. Given the rapid accumulation of mask waste in landfills and dumping sites, identifying naturally occurring microbial strains capable of degrading PP components is of critical importance for sustainable post-pandemic waste management (Dubey & Thalla, 2025).

The present study explores the biodegradation potential of the *B. cereus* strain isolated from soil collected at a garbage dumping site, alongside a comparative strain of *P. aeruginosa*. By evaluating weight loss, surface morphological changes, and degradation patterns in PP mask

pieces over a 60-day incubation period. This work provides a new understanding of the microbial breakdown of polypropylene surgical masks, which will contribute to the development of eco-friendly plastic waste management methods.

MATERIALS AND METHODS

Sample collection

The soil samples were collected from a garbage dumping site in Chennai, Tamil Nadu, India. The samples were collected in sterile collection boxes and transported to the laboratory. Because of the laboratory regulations, the experiments were carried out in sterile surgical face masks, instead of used face masks.

Isolation of bacteria from soil samples

Samples were serially diluted and inoculated into nutrient agar (NA) medium, then incubated at 37°C for 24 to 48 hours. Isolated individual colonies led to the creation of pure civilisations. Gram staining was done for the initial identification of the isolated bacteria. The pure cultures were preserved at 4°C on nutrient agar until further tests.

Identification of Bacteria

The bacterial isolates were inoculated in nutrient broth and incubated for 24 hours and used for genomic DNA isolation and purification. The isolated DNA was amplified by polymerase chain reaction (PCR) using universal primer 27F (5′ GAGAGTTTGATCCTGGCTCAG-3′) and 1495R (5′ CTACGGCTACCTTGTTACGA-3′) (Weisburg et al., 1991). 16S rRNA sequencing was carried out to identify the selected pure cultures. For molecular identification, using the forward primer CGGTTACCTTGTTACGACTT and reverse primer GAGTTTGATCMTGGCTCAG. The sequences were analysed through NCBI BLAST (https://www.ncbi.nlm.nih.gov/), and the sequences were submitted to the GenBank database, which is accessible at NCBI.

Biodegradation Experiment

The metal nose strips and ear loops were removed, and the remaining mask material was cut into (1cm ×1 cm) fragments. These pieces were soaked in sterile water for 30-60 minutes with continuous stirring, after which they were surface sterilised with 70% ethanol for 30 minutes. The sterilised fragments were dried at 45°C and subsequently weighed to record the initial mass.

The bacterial cultures – *B. cereus* and *P. aeruginosa* were inoculated in nutrient broth, and the pre-treated mask pieces were inoculated into the tubes and incubated at room temperature for 60 days. Mask pieces were also kept in autoclaved water as a control. All the procedures were carried out in sterile conditions.

Dry Weight determination of recovered mask pieces

After a two-month incubation period, the mask fragments were retrieved from the culture tubes. To remove the bacterial biomass adhering to their surfaces, the pieces were washed with 2% (v/v) sodium dodecyl sulfate for 2 hours, followed by thorough rinsing with distilled water (Hadad et al 2005). The cleaned fragments were then air-dried and weighed, and the percentage weight loss was determined using the following formula

$$Percentage\ of\ weight\ loss = [(initial\ weight - Final\ weight) \div Initial\ weight] \times 100$$

Field Emission- Scanning Electron Microscopy (FE- SEM)

To assess surface modifications in the treated mask fragments, samples were first washed with 2% SDS and warm water for 10–20 minutes to ensure complete removal of adherent bacterial biomass. Following cleansing, the fragments were fixed in 4% glutaraldehyde at 4 °C for 2 hours and subsequently dehydrated through a graded ethanol series, beginning with 50% ethanol for 30 minutes. The samples were then immersed in 70% ethanol and incubated overnight at room temperature. After drying, the mask fragments were mounted, sputter-coated with gold for 40 s, and examined using FE-SEM

RESULTS

Identification of Bacteria

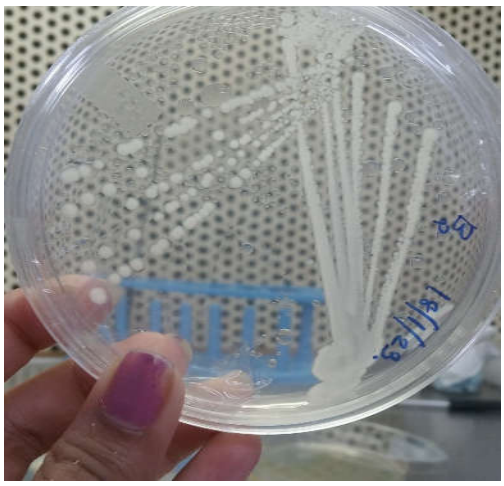

Fig.1 Pure cultures of isolated bacteria

Gram Staining

Based on the preliminary identification of bacteria by the Gram staining protocol, *B. cereus* was Gram-positive, rod-shaped, *B. anthracis* was Gram-positive rod, and *P. aeruginosa* was Gram-negative, rod. From this, only *B. cereus* and *P. aeruginosa* were taken for the biodegradation study.

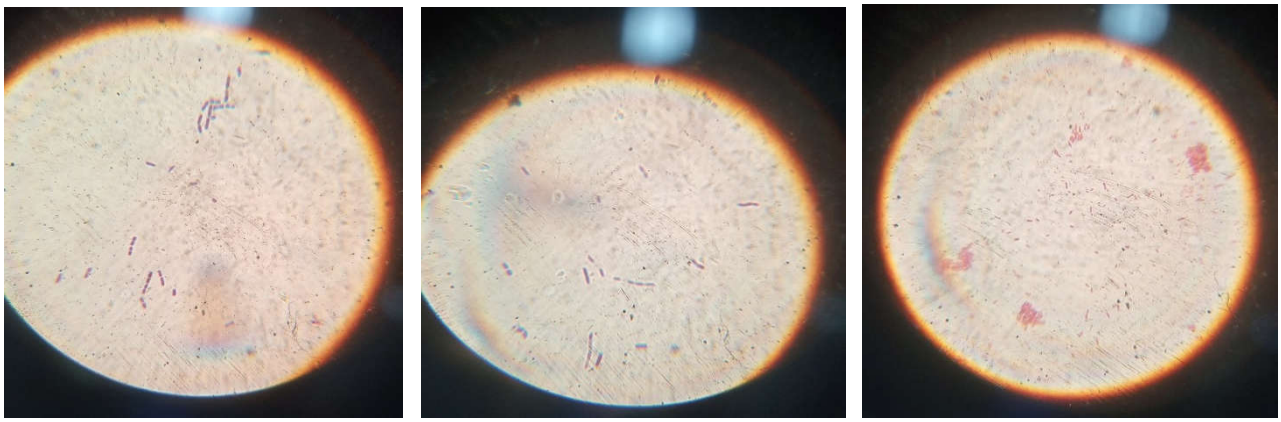

Fig. 2 Gram staining images of *B. cereus, B. anthracis, P. aeruginosa*

16S rRNA sequencing

Based on the 16S rRNA gene sequencing, the isolates exhibited high similarity to Bacillus cereus and Bacillus anthracis. The sequence of B. cereus has been deposited in GenBank (NCBI) and the accession number of it is OQ633421

Dry weight determination of recovered mask pieces

$$\text{Biodegradation (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Table 1 Dry weight determination of recovered mask pieces

Source	Initial Weight (g)	Final Weight (g)	Weight Reduction (%)
P. aerugenosa	0.09	0.06	33.3

B. cereus	0.09	0.051	43.3
Control	0.09	0.09	0

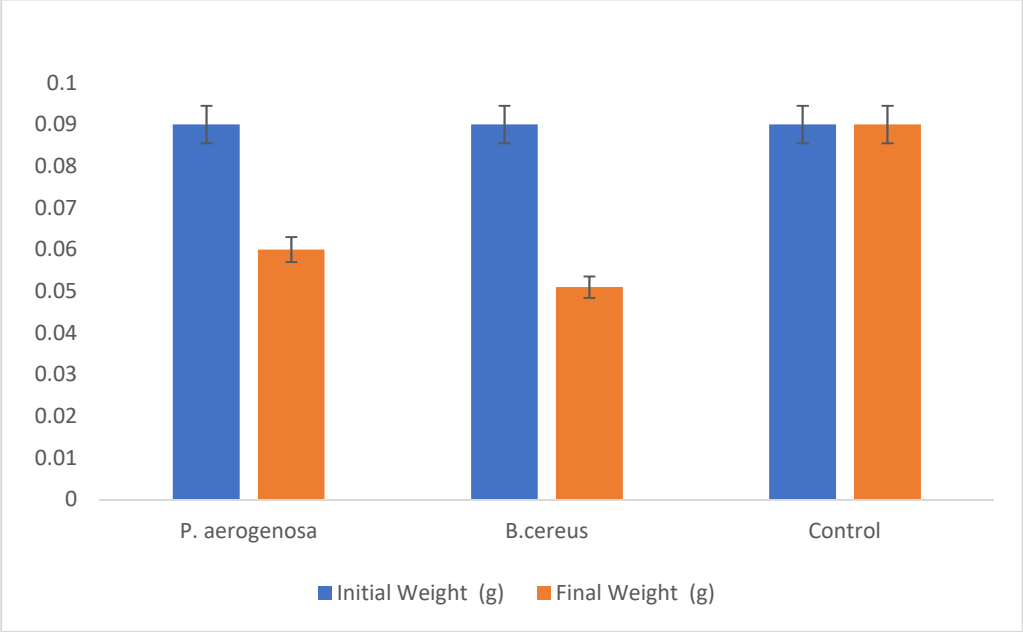

Fig. 3 Bar diagram of weight reduction compared with control

Surface Analysis using FE- SEM

The surface morphology of mask pieces incubated in the microbial cultures was analysed through FE-SEM

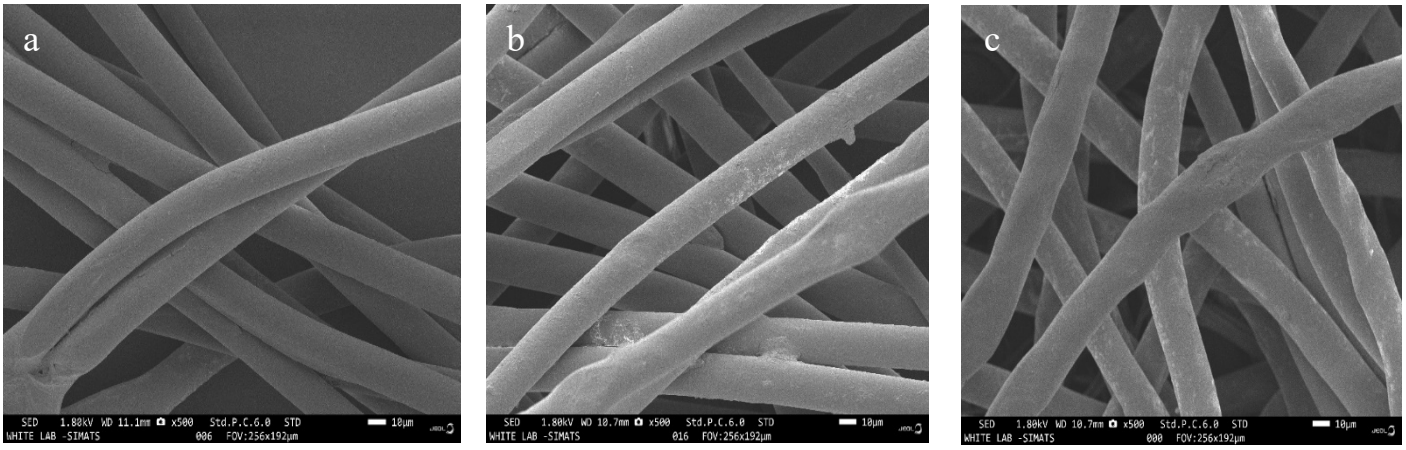

Fig. 4 FE- SEM images at 10 µm magnification (a) Control, (b) *B. cereus*, (c) *P. aeruginosa*

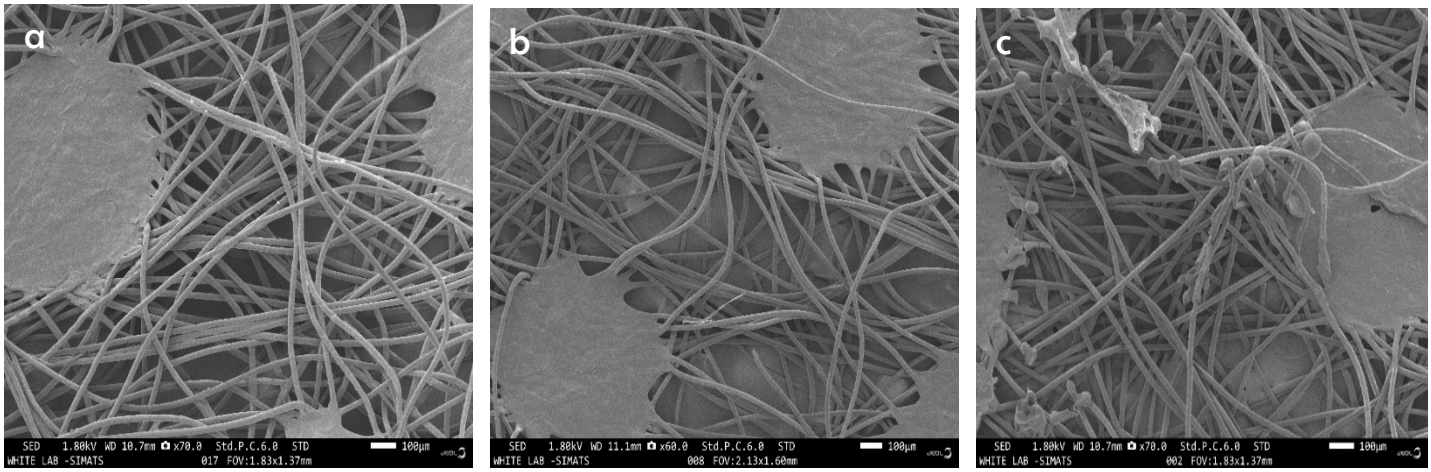

Fig. 3 FE- SEM images at 100 µm magnification (a) Control, (b) *B. cereus*, (c) *P. aeruginosa*

DISCUSSION

Bacteria isolated from municipal waste-dumping sites are often enriched for their ability to colonise and metabolise recalcitrant substrates due to prolonged exposure to mixed waste, which creates selective pressure favouring microbes capable of degrading complex polymers. Such environments harbour diverse microbial consortia that produce enzymes facilitating the breakdown of recalcitrant polymers, including plastics and organic waste fractions, especially under anaerobic digestion conditions (Blair et al., 2021). Studies have isolated novel bacterial strains like *Stenotrophomonas* sp. and *Achromobacter* sp. from waste dumpsites that show significant biodegradation of low-density polyethylene (LDPE), evidenced by biofilm formation, surface damage, and chemical modifications of the polymer (Dey et al., 2020). *B. cereus* utilised in our study is isolated from soil samples from waste dumping sites for this reason. Our study also reports that *B. cereus* and *P. aeruginosa* strains are capable of biodegradation of surgical face masks made up of hardy PP-like substances. Prior research by Kathiresan (2003) indicated the active involvement of soil bacteria in the biodegradation of plastics and polyethene bags (Kathiresan, 2003). Priyanka and Archana performed a comparative study on the biodegradation of polythene and plastic, utilising five different soil samples sourced from various locations. Various species of bacteria and fungi, including *B. subtilis, Aspergillus. niger, Penicillium, Pseudomonas* have demonstrated efficient degradation of polythene and plastics (Priyanka & Archana, 2011)

In our study, *B. cereus* was identified as Gram-positive rod, and *P. aeruginosa* was identified as Gram-negative rod. Molecular identification was further done through 16S rRNA

sequencing. In this study, the mask pieces were incubated for 60 days in the bacterial cultures in liquid media. After 60 days of incubation, the mask's components had a mild yellow fading from their original colour. Final weight loss for the mask pieces is provided in Table 1 and Figure 3. The *B. cereus* and *P. aeruginosa* strains' growth kinetics in media showed that it has colonised the surface of the PP mask pieces. Weight reduction was observed following the utilisation of pp mask film as a nutrient source. This study demonstrates the high efficacy of the *B. cereus* and *P. aeruginosa* strains by showing a weight reduction of 33.3% and a weight loss of 43.3% respectively, in the case of PP films placed in NB broth after two months (60 days). Similar findings were reported by several other researchers on the LDPE surface (Shah et al, 2008). In our study, surface morphology changes on the PP mask pieces after exposure were investigated with the help of FE SEM and PP mask films treated with *B. cereus* and *P. aeruginosa* strains showed signs of surface deterioration after 60 days of incubation. On the other hand, the control film (untreated) keeps hold of a smooth surface under the same incubation condition. In a previous study, surface morphology changes in LDPE films treated with *Pseudomonas* spp. after extended incubation periods (40, 80, and 120 days) show significant biodegradation effects, including biofilm formation, surface fissures, and roughening. For example, *P. aeruginosa* strains isolated from waste sites have demonstrated the ability to degrade LDPE by up to 9-26% weight loss over 30 to 100 days, confirmed by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) showing increased carbonyl groups and surface cracks (Gupta & Devi, 2020; Shilpa, 2024). Novel bacterial consortia including *P. aeruginosa* combined with *Enterobacter* species achieved even higher degradation rates, with weight reductions exceeding 60% after 160 days, accompanied by clear structural changes on LDPE surfaces due to biofilm activity (Skariyachan et al., 2021). A study also reported that the *P. aeruginosa* ISJ14 used to treat LDPE film showed maximum deterioration after 60 days of treatment when observed under the FE-SEM (Gupta and Devi, 2020). In a cross-reference to the earlier research studies on LDPE biodegradation, many authors have reported similar morphological changes on LDPE degradation by Aspergillus spp. (Nasrabadi et al., 2023).

In the current study, the bacterial isolates demonstrated clear potential to initiate and accelerate the biodegradation of PP, as evidenced by weight reduction and SEM analysis. While the results provide compelling evidence for biodegradation, it is important to recognise that the rates observed in controlled laboratory settings may not directly correlate to environmental conditions. The findings in this study establish that the bacterial isolates can initiate multi-step biodegradation of PP and provide a mechanistic basis for further optimisation. Their

performance under controlled conditions, combined with their native adaptation to plastic-contaminated environments, positions them as promising candidates for future development of microbial or enzyme-based strategies for managing PP mask waste.

CONCLUSION

The global increase in surgical face mask usage, driven by their vital role in medical and diagnostic settings, has intensified concerns regarding the environmental burden of polypropylene-based waste. As polypropylene remains one of the most widely manufactured and persistently non-degradable plastics, its accumulation denotes a major ecological challenge. In this study, *B. cereus* and *P. aeruginosa* demonstrated notable biodegradation capabilities, reducing the weight of polypropylene films by 43.3% and 33.3 % respectively, within 60 days of incubation. These findings highlight the substantial potential of soil-derived microorganisms in facilitating the breakdown of polypropylene components of surgical masks. This work offers a promising framework for developing microbially driven bioremediation strategies that can be utilised for environmental applications. Future studies focusing on metabolic pathway elucidation, enzyme characterisation, and optimisation of degradation conditions will be crucial for enhancing efficiency and enabling real-world implementation. Overall, this research contributes valuable insight towards sustainable management of mask-derived plastic waste and advances the pursuit of eco-friendly solutions for polymer degradation.

Author contributions: Anu Thomas designed and performed the experiments, analysed the data, and wrote the manuscript. T.G. Nithya supervised the work and critically revised the manuscript. All authors have read and approved the final manuscript

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Competing interest: The author has no financial or nonfinancial interests related to this work.

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