

Research Article

Environmental Hazard of Polypropylene from Disposable Face Masks Linked to the COVID-19 Pandemic and Its Possible Mitigation Techniques through a Green Approach

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The COVID-19 outbreak again underlined plastic items' importance in our daily lives. The public has widely utilized disposable face masks constructed of polypropylene polymer materials as effective and inexpensive personal protective equipment (PPE) to inhibit virus transmission. The consequences of this have resulted in millions of tons of plastic garbage littering the environment due to inappropriate disposal and mismanagement. Surgical masks are among them, and this study aimed to assess the biodegrading efficiency of disposable face masks using *Pseudomonas aeruginosa* VJ 1. This work used a bacterial strain, *Pseudomonas aeruginosa* VJ 1, obtained from sewage water-contaminated surface soil in Tiruchirappalli, India, to investigate the biodegradation of polypropylene (PP) face masks. The mask pieces were incubated with *Pseudomonas aeruginosa* VJ 1 culture in three different solid and liquid media for 30 days at 37°C. Surface changes and variations in the intensity of functional groups and carbonyl index variations were confirmed using Field Emission Scanning Electron Microscopy (FE-SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis to ensure microbial degradation (up to 5.37% weight reduction of PP films within 30 days). These findings show that *Pseudomonas aeruginosa* VJ 1 could be a good choice for biodegrading PP masks without harming our health or the environment. There is a need for a novel solution for the degradation of PP. The methods and strain presented here reveal the potential biodegrading agents of PP masks.

1. Introduction

The rapid global spread of the SARS-CoV-2 pandemic has required essential attempts to reduce transmission, resulting in considerable and widespread socioeconomic damage [1]. According to World Health Organization, as of March 6, more than 445 million cases and 5.99 million deaths were reported.

The coronavirus pandemic has prompted the establishment of emergency solutions and progressive actions targeted at dealing with and defeating COVID-19 while reducing exposures and promoting a healthy lifestyle worldwide. Compulsory use of plastic-based PPE by healthcare staff and face masks for the general public leaving their homes for significant reasons is a preventive step meant to reduce the community spread of COVID-19 transmitted through droplets [2].

At the beginning of the COVID-19 pandemic, the use of medical masks as an infection control measure was wide-spread in East and South-East Asia, and it later gathered speed in the rest of the world in 2020 and 2021 [3]. Medical mask usage and production are currently at massive levels, and both are anticipated to rise further soon. For instance, China made roughly 450 million medical masks every day as of April 2020. In reality, China, the world's largest producer, was expected to produce 10 billion medical masks annually by 2020, up from 5 billion in 2019 [4]. In the current circumstances, three types of face masks can be used: (i) respirator mask (N95, FFP2), (ii) surgical or medical mask, and (iii) noncertified disposable mask (cloth mask) [5].

The top and bottom layers of disposable (one-time use) face masks were woven fabric, while the center layer was formed of melting polypropylene polymers. Each day, the authors' models are predicted to generate 6.88 billion (about 206,470 t) of material, subsequently disposed of or burnt [6]. Because of their lightweight, used face masks are easily carried into city streets, rivers, and oceans, where they are fragmented into microplastics (MPs) [7, 8]. Furthermore, single-purpose face masks are made of nonbiodegradable materials that take hundreds of years to degrade in the environment [8, 9]. Medical masks come in many styles and materials, including polyurethane, polyacrylonitrile, polyester, polyethylene terephthalate, and polypropylene. The last one, polypropylene, has been used for a very long time and is still by far the most popular material [10].

Poor waste management is the leading environmental risk linked to the growing use of disposable medical masks by the general public [7]. Thermochemical conversion of disposable medical masks into value-added goods has recently been proposed as a potential waste management strategy [11]. However, masks are frequently disposed of improperly in public areas and the environment at large [12]. This adds to the polluting of the world's oceans with plastics, which has harmful effects on the environment [13]. Additionally, as recently discovered, throwaway surgical masks could be a sizable new source of microplastics.

Accordingly, an immediate multidisciplinary approach is required to dispose of the waste generated by the pandemic. Biodegradation is a microorganism's best ability to persuade abiotic deterioration through physical, chemical, or enzymatic action [14].

Microorganisms were well suited for the biodegradation process because they possess enzymes and their small size, allowing them to contact the plastic surface [15]. Exoenzymes from bacteria can break down complex polymers into simpler ones that can pass through semipermeable outer membranes and be used as carbon and energy sources by the microbes [16]. Researchers have identified several bacterial and fungal taxa that may degrade MPs, including *Pseudomonas* sp. [17–20].

Several gut bacterial species were isolated from worms and validated for their potential to facilitate plastic breakdown directly. *Bacillus* sp. YP1 and *Enterobacter asburiae* YT1 are two bacterial strains obtained from wax worms that can depolymerize polyethylene (PE) in vitro [21]. *P. aeruginosa* isolated from the intestines of super worms can biodegrade three different types of plastics in unusual ways (PE, PP, and PPS (polyphenylene sulfide)). Biodegradation efficiency varies from one plastic to the next; the fastest biodegradation happens on PE [22]. *Pseudomonas fluorescens* and *P. aeruginosa* are highly able to degrade polyethylene [23]. This study aimed to assess *Pseudomonas* sp. biodegradation ability on a surgical face mask in an environmentally acceptable manner to safeguard our environment from pandemic-related garbage.

2. Materials and Methods

2.1. Chemicals and Reagents. The soil sample was taken from sewage water-contaminated surface soil, Geetha Nagar, Uyyakondan Thirumalai, Tiruchirappalli, Tamilnadu, South India. It is located at longitude of 78°40'22.01"E (78.672779) and latitude of 10°48'56.12"N (10.81559). The sample was packed with collection bags and then securely transported to our laboratory, where the plastic-degrading *Pseudomonas* sp. was isolated. Due to laboratory norms and regulations in this COVID-19 pandemic, the utilized face masks were not allowed to be employed in our laboratory test. The trials employed clean surgical face masks (ear loops were removed). The HiMedia laboratory in India provided the chemicals used in our research.

2.2. Isolation of Bacteria from a Soil Sample. Serially diluted soil samples were put onto a sterile nutrient agar (NA) medium that had previously been prepared and incubated at 37°C for 24–48 hours to isolate bacteria from the sample. Individual colonies were taken after incubation and used to make pure cultures. Gram staining was used to identify the pure colonies for the first time. The isolated bacterium was then kept at 4°C in our laboratory on nutrient agar for the biodegradation experiment in our current investigation.

2.3. Identification of Bacteria. Bergey's Manual of Systematic Bacteriology was used to identify the isolated culture based on morphological, staining reaction, culture, and other biochemical properties. Basic biochemical tests were used to determine the isolated bacterial strain. The biochemical assays were carried out on cultures cultivated on nutrient agar medium for 24 hours at 37°C. According to the supplier's procedure, an indole test was done to verify their use of tryptophan. According to [24], a catalase study was performed. Bacterial colonies were inoculated on Simmons Citrate Agar to study citrate metabolism (according to supplier protocol). The oxidase test determined which bacteria possessed the cytochrome oxidase enzyme. Sugar fermentation tests (glucose, lactose, and sucrose) were investigated (according to supplier protocol). SIM media was used to measure motility and H₂S generation (according to supplier protocol). The MR-VP test was used to determine whether or not the isolate was a facultative anaerobe based on their sugar fermentation patterns. The bacterium was also streaked over selective agar Cetrimide for confirmation. 16S rRNA sequencing was used to identify the isolated bacterial strain ISJ14. For molecular identification, using forward and reverse 16S rRNA primers together with DNTP, buffer, and Taq polymerase, PCR of the extracted genomic DNA was carried out. In order to amplify the 16SrRNA genes, a universal primer sequence was used: CGGTTACCTTGT-TACGACTT and AGAGTTTGATCMTGGCTCAG. 100 ng of template DNA is present in the mixture of PCR amplified product. The 16S rRNA sequence was submitted to the GenBank, and accession number was obtained [17].

2.4. Investigation of Biodegradation of Polypropylene Mask

2.4.1. Pretreatment of Polypropylene Mask Pieces. The masks were stripped of their metal strips and ear loops. The masks were then prepped using the procedure outlined by [17]. The masks were cut into 33 cm sections for this procedure and soaked for 30–60 minutes in a solution containing 7 ml Tween-80. Tween-80 is used as a wetting agent, and it will moisten the mask's surface and cause it to repel air, making the treatment more effective: 10 ml bleach for disinfection and 983 ml sterile water with constant stirring. The mask parts were then washed at room temperature with distilled water. The pieces were then sterilized with 70% ethanol for 30 minutes before drying at 45°C. The mask parts were weighed using a weighing balance after drying, and the initial weight of the pieces was recorded.

2.4.2. Biodegradation Experiment. Pseudomonas aeruginosa VJ 1 was aseptically inoculated on nutrient agar (NA), Bushnell Haas agar medium (BHM), and Mineral Salt agar medium (MSM) plates using the carpet culture method. Pretreatment polypropylene mask pieces (about 0.1 g) were aseptically placed over the inoculated plates using sterile forceps and incubated for one month at 30°C and 37°C, respectively, following inoculation (30 days). The negative control agar plates, which contained the same number of mask pieces but were not inoculated with bacteria, were kept at the same temperature as the positive control agar plates. In addition, the pretreated mask pieces were incubated with *Pseudomonas aeruginosa* VJ 1 in Nutrient Broth (NB), BH (Bushnell Haas broth), and MS (Mineral Salt broth) as well

as liquid media containing the same quantity of mask pieces but no culture, for the same amount of time as the control.

2.4.3. Experimental Setup

T1A: 0.1 g of pretreated mask pieces + *Pseudomonas aeruginosa* VJ 1 (in NA plates)

T1B: 0.1 g of pretreated mask pieces + *Pseudomonas* aeruginosa VJ 1 (in NB)

C1A: 0.1 g of pretreated mask pieces in NA plates (without inoculums)

C1B: 0.1 g of pretreated mask pieces in NB (without inoculums)

T2A: 0.1 g of pretreated mask pieces + *Pseudomonas aeruginosa* VJ 1 in BHM plates

T2B: 0.1 g of pretreated mask pieces + *Pseudomonas* aeruginosa VJ 1 (in BH)

C2A: 0.1 g of pretreated mask pieces in BHM plates (without inoculums)

C2B: 0.1 g of pretreated mask pieces in BH broth (without inoculums)

T3A: 0.1 g of pretreated mask pieces + *Pseudomonas* aeruginosa VJ 1 in MSM plates

T3B: 0.1 g of pretreated mask pieces + *Pseudomonas* aeruginosa VJ 1 (in MS)

C3A: 0.1 g of pretreated mask pieces in MSM plates (without inoculums)

C3B: 0.1 g of pretreated mask pieces in MS (without inoculums)

2.4.4. Monitoring the Planktonic Growth of Pseudomonas aeruginosa VJ 1 Strain. The growth of Pseudomonas aeruginosa VJ 1 in liquid and solid media such as NB, BHM, and MSM was studied for 30 days (1 month), with a 15-day interval due to the presence of a PP film and the properties of the biofilm generated on the polymer surface. Before analyzing the biofilm, sterile forceps were used to remove polymer samples from the media, which were then carefully rinsed with sterilized distilled water to remove the loosely adhered bacteria. The biofilm was then cleaned off the surface of the polymer by using a gentle water bath sonication for 4 minutes at 1-minute intervals in 1 ml of 0.85 percent saline solution. The resulting saline solution was serially diluted up to 10⁻⁷, and aliquots were disseminated on nutrient agar, with the number of colonies calculated as CFU/µL [25].

2.4.5. Viability Testing of Surface-Attached Bacteria. The survivability of bacterial strains adhered to the surface of PP films was calculated [26]. PP films were removed from the media at 10-day intervals and rinsed with sterile distilled water before being subjected to mild bath sonication with a 0.85 percent saline solution. The resulting solution was serially diluted, plated on NA, and incubated for 48 hours at

35°C. CFU/ml was used to calculate the number of viable bacteria species.

2.4.6. Assessment of Cell Surface Hydrophobicity of Pseudomonas aeruginosa VJ 1. The BATH test was used to measure the hydrophobicity of the bacterial cell surface with minor adjustments [27], which was explained as follows: The bacteria were grown in NB media until they reached the mid-log stage of growth. It was then centrifuged and twice rinsed with phosphate urea buffer (PUM containing 17g K₂HPO₄, 7.26 g KH₂PO₄, 1.8 g urea, and 0.2 g MgSO₄.7H₂O per liter). Following washing, the cells were suspended in PUM buffer with an OD 400 of 1.0-1.2. To facilitate phase separation, aliquots of 1.2 ml of the above-obtained suspension were introduced to a series of test tubes containing escalating quantities of xylene (ranges from 0 to 0.2 ml) and shaken well for 10 minutes before being left to stand for 2 minutes. The lower aqueous phase's OD 400 nm was measured and recorded as OD 1. The percentage of xylenebound cells reported as the fraction of cells expelled from the aqueous phase calculated the cell surface hydrophobicity. The blank was a plain PUM buffer. Therefore, the percentage of adhering cells is represented using the following formula: cell surface hydrophobicity (%) = $((OD_0 - OD_{10})/OD_0) \times$ 100 (OD₀ is the initial OD of the aqueous phase).

2.4.7. Determination of Dry Weight of the Recovered Mask Pieces. After a month of incubation, the remaining mask parts were collected from the culture plates. The bacterial biomass that had adhered to the polypropylene mask surface was rinsed for 2 hours with a sodium dodecyl sulfate (SDS) solution (2 percent v/v). After that, the pieces were cleaned with distilled water to remove any contaminants from the surface and dried at 45° C overnight. Then, the degraded mask pieces were weighed using a weighing balance, and the percentage of biodegradation (weight loss) was estimated using the following formula:

biodegradation (%) =
$$\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100.$$
 (1)

2.4.8. Surface Analysis of Mask Pieces

(1) Scanning Electron Microscopy. The mask pieces were taken from the media and exposed to FE-SEM to observe biofilm growth and surface degradation after being infected with *Pseudomonas aeruginosa* VJ 1 for 30 days. The bacterial morphology of a biofilm on the surface of a PP mask was examined. Before the observation, the treated mask pieces were rinsed for 2 minutes in a 0.01 M phosphate buffer solution to eliminate any excess media that had adhered to the bacterial colonies. The treated mask pieces were washed with 2 percent SDS and warm water for 10–20 minutes to aid in the complete removal of bacterial biomass and to observe surface modification. After the process, the mask parts were fixed in 4% glutaraldehyde at 4°C for 2 hours and dehydrated

in 50% ethanol for 30 minutes. The recovered mask parts were treated in 70% ethanol at room temperature overnight. The pieces were dried, mounted, and sputter-coated with gold for 40 seconds before scanning with an FE-SEM.

2.4.9. Fourier Transform Infrared Spectroscopy (FTIR Analysis). PP mask films were washed with 2 percent SDS and warm distilled water for 10 to 20 minutes to remove bacterial biomass. The PP films were then fixed in 4% glutaraldehyde at 4°C for 2 hours after being dehydrated with 50% ethanol for 30 minutes. The PP films were treated with 70% ethanol at room temperature overnight before being dried. Small pieces of dried mask pieces were analyzed using FTIR spectra in the 4000-400 cm⁻¹ which were employed at a 1 cm^{-1} resolution to investigate the structural and functional group modifications. The following formula was used to calculate the relative absorbance intensities of the keto carbonyl bond, ester carbonyl bond, terminal double bond (vinyl), and internal double bond with methyl bond: keto carbonyl bond index (KCBI) = I1715/I1465, ester carbonyl bond index (ECBI) = I1740/I1465, vinyl bond index (VBI) = I1650/I1465, and the internal double bond index (IDBI) = I908/I1465. The carbonyl index was used to determine the degree of biodegradation because its value depends on the degree of degradation. The percentage of crystallinity of the polypropylene mask film was determined using the previously described method by Zerbi et al. (1989).

3. Results

3.1. Isolation and Identification of Pseudomonas aeruginosa VJ 1. Typical bacterial isolate colonies were subcultured on nutrient agar and incubated for 24 hours at 37°C. Pseudomonas aeruginosa VJ 1 was recognized as a Gram-negative rod based on a presumptive identification of bacteria using Gram's staining procedure. The isolated bacteria were identified as a Pseudomonas aeruginosa VJ 1 strain based on various biochemical characteristics (Figure 1(a)). A Pseudomonas aeruginosa VJ 1 specific substrate for isolation was further validated by streaking the strain onto cetrimide agar. After incubation, the isolates had a green-pigmented, round, and opaque colony shape.

Further, the isolate was identified through 16S rRNA sequence analysis. The 16S rRNA was compared with the other sequences in GenBank (NCBI). The results indicated that the isolate was identified as *Pseudomonas aeruginosa* VJ 1. Also, the nucleotide sequence of the isolates was deposited in NCBI and obtained the accession number (ON626420).

3.2. Cell Surface Hydrophobicity of Bacteria. Bacteria's capacity to exploit any substrate is determined by their development and adherence to that substrate. Several physical factors, including the pressures that help bacteria adhere to solid substrates, the substrate's qualities, and the bacteria's nature, influence bacteria's ability to stick to either hydrophilic or hydrophobic surfaces. The hydrophobicity of midlog phase *Pseudomonas aeruginosa* VJ 1 cells at 0.2 mL



FIGURE 1: (a) Pure culture of *Pseudomonas* sp. strain. (b) Pretreated PP mask pieces. (c) PP mask placed on the culture-inoculated nutrient agar plate. (d) PP mask pieces on nutrient agar after 30 days.



FIGURE 2: Cell surface hydrophobicity of Pseudomonas sp.

xylene revealed a considerable rise in hydrophobicity in this study (30.39 percent) (Figure 2).

3.3. Growth of Pseudomonas aeruginosa VJ 1 and Surface-Attached Cells on PP Films. After 15 and 30 days of incubation, the bacterial cell development was characterized by a rapid increase in planktonic cells and the surface-attached bacterial mass. In all of the liquid media employed, *Pseudomonas aeruginosa* VJ 1 reached a consistent growth rate of about 10⁷ CFU/ml after 15–20 days of incubation (Table 1). Biofilm production patterns were similar to planktonic cell growth in all three mediums (Table 2). According to the findings, *Pseudomonas aeruginosa* VJ 1 cells showed more significant colonization, biofilm formation, and fractional biodegradation of PP film in all three conditions. These findings indicate that *Pseudomonas aeruginosa* VJ 1 cell is a

TABLE 1: Viability of Pseudomonas sp. on different day intervals.

S. no.	Days	NB (CFU/ μ L)	BHM (CFU/ μ L)	MSM (CFU/µL)
1	0	1.07×10^{5}	0.8×10^5	0.92×10^{5}
2	10	2.01×10^{6}	1.9×10^{6}	1.92×10^{6}
3	20	2.02×10^{7}	1.95×10^{7}	1.99×10^{7}
4	30	1.97×10^{7}	1.98×10^{7}	1.98×10^{7}

TABLE 2: Monitoring the planktonic growth of Pseudomonas sp.

Media	Day 0 (CFU/µL)	15th day (CFU/ μ L)	30th day (CFU/ μ L)
NB	0.9×10^{5}	2.0×10^{5}	2.08×10^{5}
BHM	1.1×10^{7}	1.9×10^{7}	2.07×10^7
MSM	1.2×10^{7}	2.0×10^{7}	2.12×10^{7}

high affinity for the PP film. Still, they also suggest that *Pseudomonas aeruginosa* VJ 1 cultures can form biofilms through hydrophobic contacts even when carbon is limited.

On the other hand, this situation is not necessarily known to all biofilm-forming bacterial species. PP sheets served as a substrate for attachment and biofilm production and a carbon source for our study's *Pseudomonas aeruginosa* VJ 1. The incubation of *Pseudomonas aeruginosa* VJ 1 with PP films for an extended period resulted in forming a solid biofilm on the PP surface, leading to the fractional depletion of this polymer. Similarly, using low molecular mass elements in the polymer may aid in creating and maintaining active biofilm throughout the 30-day incubation period.

3.4. Dry Weight Determination of Recovered Polypropylene Mask Pieces. After a month (30 days) of incubation, the remaining polypropylene mask strips were retrieved from the media. The adhering media and bacterial biomass were rinsed and left to air dry after cleaning with suitable solutions. Table 3 shows the final weight loss for *Pseudomonas*

TABLE 3: Weight reduction percentage of PP mask pieces after 30day treatment with *Pseudomonas* sp. on different media sources.

Madia sources]	Incubation period	1
Media sources	10 days (%)	20 days (%)	30 days (%)
Nutrient agar	1.88	2.3	3.28
Nutrient broth	2.88	4.13	5.37
BHM agar	1.3	1.95	2.20
BHM broth	1.87	2	2.45
MSM agar	1.2	1.76	1.84
MSM broth	1.63	1.90	2.1

aeruginosa VJ 1 in various culture media (NA, NB, BHM agar, BHM broth, MSM agar, and MSM broth). The *Pseu-domonas aeruginosa* VJ 1 strain's growth kinetics in media revealed colonization on the Surface of PP mask pieces, resulting in a weight reduction due to using PP mask film as a nutrition source.

After one month, our study found a 5.37 and 3.28 percent weight decrease in the case of PP films placed in NB and NA, 2.20 and 2.45 percent weight reduction in BHM and BH, and 1.84 and 2.1 percent weight reduction in MSM and MS (30 days).

3.5. Surface Change Analysis. The surface morphology changes on the PP mask pieces before and after biotic exposure were investigated with the help of a Field Emission Scanning Electron Microscope (FE-SEM). Figures 3(a), 3(b), and 3(c) show that the surface changes recorded during FE-SEM analysis were for *Pseudomonas aeruginosa* VJ 1 (T1A, T1B, T2A, T2B, T3A, and T3B) and untreated negative control (C1A, C1B, C2A, C2B, C3A, and C3B) after 30 days of treatment. It was observed that signs of surface deterioration appeared on the PP mask films treated with *Pseudomonas aeruginosa* VJ 1 after 30 days of incubation. On the other hand, the control film (untreated with *Pseudomonas aeruginosa* VJ 1 strain) kept a smooth surface under the same incubation conditions.

3.6. Structural Analysis Using FTIR. Structural changes in biologically treated PP mask films were further analyzed with the help of FTIR. This investigation of the degraded PP films has shown the stretching of numerous functional groups after incubation with the Pseudomonas aeruginosa VJ 1 strain. The differences were found in the FTIR spectra peaks of the control and test samples in all media used. Tables 4-6 summarize the functional group implicated in stretching by the role of Pseudomonas aeruginosa VJ 1, the wave number, and IR band position on the PP films. A considerable reduction in the carbonyl index (CI) was observed in the samples incubated with Pseudomonas aeruginosa VJ 1 for 30 days. Spectrophotometric variations of PP mask films and the value of CI determine the maximum degradation when compared to an untreated negative control.

In this study, FTIR analysis affords a close view of the N-H stretching of the aldehydes group at 3190.18 cm^{-1} . The C-C absorption peaks were shifted at 1255.18, 1302.34,

1794.87, 2427.49, and 2617.26 cm^{-1} . The conformational changes on PP mask film were supported by the changes in almost all functional groups (Figures 4(a)-4(l)).

4. Discussion

This study revealed that the isolated strain was *Pseudomonas aeruginosa* VJ 1 by biochemical and selective agar screening methods. Another author reported that the isolated bacteria were cultured in milk agar with cetrimide for the preliminary detection of *Pseudomonas* sp., which is similar to our findings [28]. Based on the results, it was reasonable to identify the isolated bacterium belonging to *Pseudomonas aeruginosa* VJ 1.

In this present investigation, the hydrophobicity was high in mid-log phase cells of Pseudomonas aeruginosa VJ 1. In most cases, a hydrophobic bacterium prefers a hydrophobic surface for adhesion, whereas the inverse is true for bacteria with hydrophilic qualities [17]. These results agree with previous reports [17], which observed that bacterial cells in the log phase are more hydrophobic. A previous investigation found similar results, with the isolates Kocuria palustris M16 and Bacillus subtilis H1584 showing a maximum increase in hydrophobicity of 24 percent turbidity reduction at 0.251 and a maximum decrease of 32 percent turbidity at 1501 of hydrocarbon like hexadecane. [27]. Another recent study found that the hydrophobicity of L. monocytogenes strain CICC 21332 was the lowest (12.5%), and the strain FSIS 57034 had the highest percentage of CSH (74.81%) at 1 ml of xylene concentration [29].

This investigation showed high affinity of *Pseudomonas aeruginosa* VJ 1 cells for the PP film. Similarly, in planktonic cells, the growth pattern of bacteria attached to the surface of PP film was investigated as well as the viability of surface-attached bacterial species [26, 30]. According to the concurrence model, the process of microbes forming biofilms begins when planktonic cell growth reaches a high density, allowing for the attachment of bacterial cells to a surface via cell signaling and the formation of microcolonies that will eventually frame the mature biofilms [31]. This biofilm population is diverse and stable and ideal for extended periods [32].

Pseudomonas sp. isolated from the soil of the Sisdol landfill site and the Sanothimi household garbage site in Nepal displays similar biochemical features [33]. *Pseudomonas* sp. ISJ14 was highly efficient at degrading low-density polyethylene (LDPE) in BHM [17]. On the other hand, this work is the first to show that a *Pseudomonas aeruginosa* VJ 1 can biodegrade a surgical face mask constructed of a rigid PP-like polymer.

Similar findings were recorded in earlier studies such as the biodegradation of untreated films of polyethylene by *Pseudomonas putida* IRN22, *Micrococcus luteus* IRN20, *Acinetobacter pittii* IRN19 [34], and other bacterial genera, including *Delftia*, *Stenotrophomonas*, and *Comamonas* [35], and *Galleria mellonella* isolated from the gut of the wax worm also have been establishing the capabilities of PE degradation [36]. Similar findings were reported by several other researchers on the LDPE surface [37–39]. The LDPE



(b) Figure 3: Continued.



FIGURE 3: (a) SEM images of PP films placed in nutrient medium: C1A and C1B, control; T1A and T1B, treated with *Pseudomonas* sp. NA and NB media, respectively. (b) SEM images of PP films placed in BHM: C2A and C2B, control; T2A and T2B, treated with *Pseudomonas* sp. in BHM agar and broth, respectively. (c) SEM images of PP films placed in MSM: C3A and C3B, control; T3A and T3B, treated with *Pseudomonas* sp. in MSM agar and broth, respectively.

TABLE 4: Comparison of IR band position in the PP films after incubation with Pseudomonas sp. in NB and NA (both control and test).

S. no.	Incubation period	IR band position in NB control-test (cm^{-1})	IR band position in NA control-test (cm^{-1})	Functional group involved
1		459.12-458.59	458.78-455.96	C-X stretching
2	30 days	1104.03-1102.10	1102.65-1101.96	C-H stretching
3		2722.14-2721.10	2722.18-2721.97	C-H stretching
4		3763.83-3761.66	3763.18-2911.74	O-H stretching

TABLE 5: Comparison of IR band position in the PP films after incubation with *Pseudomonas* sp. in BHM broth and BHM agar (both control and test).

S. no.	Incubation period	IR band position in BHM broth control-test (cm ⁻¹)	IR band position in BHM agar control-test (cm^{-1})	Functional group involved
1		572.18-460.23	1002.24-808.93	C-X stretching
2	20 dave	2950.10-2838.92	2918.97-2917.61	C-H stretching
3	50 days	2916.61-2722.13	2947.73-2838.92	C-H stretching
4		3762.08-3189.68	3761.52-3189.68	O-H stretching

film treated with *Pseudomonas* sp. showed a 20% reduction after 120 days of treatment, which has also been reported [40]. Several other studies on the LDPE surface have reported similar results [37–39]. The LDPE film treated with

Pseudomonas sp. showed a 20% reduction after 120 days of treatment, which has also been reported [40]. However, a recent study provided strong evidence for PP microplastic degradation by *Rhodococcus* sp.36 with 6.4% degradation

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TABLE 6: Comparison of IR band position in the PP films after incubation with	Pseudomonas sp. in MSM broth and MSM agar (both control
and test).	

S. no.	Incubation period	IR band position in MSM broth control-test (cm^{-1})	IR band position in MSM agar control-test (cm ⁻¹)	Functional group involved
1		562.12-458.98	460.00-459.35	C-X stretching
2	20 dava	2839.02-2722.15	2839.57-2838.65	C-H stretching
3	50 days	2916.76-2838.83	2918.00-2917.14	C-H stretching
4		3760.91-3346.85	3351.15-3190.32	O-H stretching





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FIGURE 4: Continued.



FIGURE 4: Continued.







FIGURE 4: Continued.



FIGURE 4: (a) IR spectra of PP films in NB (control). (b) IR spectra of PP films in NB (treated). (c) IR spectra of PP films in NA (control). (d) IR spectra of PP films in NA (treated). (e) IR spectra of PP films in BHM broth (control). (f) IR spectra of PP films in BHM broth (treated). (g) IR spectra of PP films in BHM agar (control). (h) IR spectra of PP films in BHM agar (treated). (i) IR spectra of PP films in MSM broth (treated). (j) IR spectra of PP films in MSM broth (treated). (k) IR spectra of PP films in MSM agar (control). (l) IR spectra of PP films in MSM broth (treated). (i) IR spectra of PP films in MSM broth (treated). (k) IR spectra of PP films in MSM agar (control). (l) IR spectra of PP films in MSM agar (treated).

and *Bacillus* sp.27 with 4%, demonstrating the excellent degradation capacity of bacterial strains [41].

Similar findings were reported in the surface morphology of the LDPE films treated with *Pseudomonas* sp. by SEM after 40, 80, and 120 days of incubation [40]. Another 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 [17]. In a crossreference to the earlier research studies on LDPE biodegradation, many authors have reported similar morphological changes in LDPE degradation by *Aspergillus* spp. [42], as well as *A. clavatus JASK1* [43]. The LDPE film incubated with *P. aeruginosa* PAO1 showed a maximum reduction in CI, as reported by [40, 44]. Based on our findings, a significant reduction of the carbonyl index was detected in the samples incubated with *Pseudomonas aeruginosa* VJ 1 for 30 days. A similar observation was reported by several authors [45]. Our results were supported by various previous research studies that noticed the formation of functional groups and the loss of these groups in the LDPE degradation using the strain *Bacillus amyloliquefaciens* [46]. In our study [43], we noticed visible modifications in the synthetic polymers that undergo biodegradation before and after exposure to microbes by FTIR analysis. Thus, our results

suggest that the *Pseudomonas aeruginosa* VJ 1 has a notable ability to degrade the PP mask films.

5. Conclusion

This work offered a versatile biological process to evaluate the degradation of disposable face masks used in this SARS-CoV-2 pandemic. The in vitro biodegradation of the Pseudomonas aeruginosa VJ 1 in three different solid and liquid mediums reflects the eco-friendly approach. We observed a tremendous biodegradation efficiency of our isolates towards the PP-based disposable face mask, whose molecular weight was as high as 228,000. The isolate can form biofilm on the PP surface and utilize it as a sole nutrient source for growth. The weight reduction of PP mask films relative to untreated control films reflects within 30 days (up to 5.37 percent weight reduction). Based on the FE-SEM and FTIR analysis results, the Pseudomonas aeruginosa VJ 1 is suitable for PP degradation without UV treatment. However, further studies on the enzyme-based metabolic passages of Pseudomonas aeruginosa VI 1 are also recommended to better understand its tremendous role in biodegradation.

Data Availability

The data supporting the findings of this study are included within the article.

Disclosure

A preprint of this manuscript is available on Research Square and the link is https://www.researchsquare.com/article/rs-1002388/v1.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Selvakumar Vijayalakshmi contributed to conceptualization, methodology, data curation, original draft writing, and review and editing; Preethi Gopalsamy contributed to methodology, data curation, initial draft writing, and review and editing; Karnan Muthusamy contributed to formal analysis; Dinesh Kumar Sundarraj contributed to software provision, data curation, and review and editing; Steffi Pulikondan francis, Ly Thi Thuy Duong, Tuyet Thi Anh Truong, Huu Tap Van, Shankar Karuppannan, and Thiyagarajan Ramesh contributed to review and editing; Deog-Hwan Oh contributed to conceptualization, methodology, supervision, project administration, and review and editing.

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