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Fungal mediated degradation of low density polyethylene by a novel strain *Chamaeleomyces viridis* JAKA1.

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ABSTRACT

Increasing interest regarding the impact of the accumulation of plastic waste over on the environmental has led to the development of biodegradable plastic. Low density polyethylene (LDPE) is a major cause of persistence and long term environmental pollution. Of late, microbes have become the focus of interest for environmental friendly disposal of plastic and polymer-based waste. This manuscript presents the investigation on the biodegradability of LDPE by a novel isolate *Chamaeleomyces viridis*. Degradation of LDPE was determined by weight loss of the sample, CO_2 estimation, spectrum variations, analysis of LDPE morphological changes by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Our results reveal that *Chamaeleomyces viridis* has the potential to degrade LDPE film.

Keywords: LDPE; biodegradation; weight reduction; AFM; SEM; Fourier transform infra-red spectroscopy.

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INTRODUCTION

Low density polyethylene (LDPE) is one of the cost effective and an often used polymer in packaging industries and in our day to day life. They are non-degradable in nature due to hydrophobic backbone [1]. It was estimated that it takes about 300 years to degrade LPDE films with thickness of 60 µm [2]. These polymers are dumped into landfills or incinerated. Although incineration burns off the plastic waste entirely but at the same time causes air pollution [3,4]. Recycling is an expensive process and the quality of the recycled is lower than that of primary material [5,6]. Developing biodegradable plastic is the best approach for environmental cleanup. Degradability of a material is the property of a material to breakdown into simpler parts by microbes (biodegradable), thermal (oxidative) or ultraviolet (photodegradable) action. Recently, scientist and environmentalist are focusing on the biodegradation of LDPE due to the disadvantage of other methods in term of cost and pollution. Few microbes capable of degradation of the low density polyethylene have been isolated from soil, sea water, compost and activated sludge [7]. In most studies, fungi have been investigated for the biodegradation of LDPE because fungus produce degrading enzymes [8] and extracellular polymers such as polysaccharides which help to colonize the polymer surface [9]. Several fungus have been reported for degradation of plastic such as *Aspergillus niger* [9-11], *Aspergillus versicolor* [12,13], *Aspergillus flavus* [14,15], *Cladosporium cladosporioides* [15,16], *Chaetomium* sp. [17], *Fusarium redolens* [13,18,19], *Glioclodium virens* [10], *Mucor circinelloides* [12], *Penicillium simplicissimum* [20], *Penicillium pinophilum* [9,10], *Penicillium frequentans* [21], *Phanerochaete chrysosporium* [10,22,23], *Verticillium lecani* [13]. In the present study, enrichment technique was followed to isolate LDPE degrading fungus from landfill area and the isolated fungus was identified as *Chamaeleomyces viridis*. The ability of this novel fungus to degrade LDPE films was investigated. Several techniques were employed to elucidate the chemical and physical polymer structure. Changes in the chemical structure of polymeric films caused by biodegradation were interpreted on the basis of IR spectra analysis. The scanning electron microscope was used to examine these polymers morphologically (surface topography). This investigation enlightens on the use of fungus for biodegrading purposes.

MATERIAL AND METHODS

Polyethylene

LDPE films used in this study were collected from Vellore market, which were 20 µm thick in nature. For the experiments, LDPE were cut into small strips and they were sterilized with 70% ethanol.

Collection of soil sample

Soil sample was collected from solid waste dumping site around Vellore, Tamil Nadu, India. Sample was transported to laboratory, stored for further use.

Isolation of fungus

One gram of soil sample was added in 99 ml of sterile double distilled water. The soil solution was mixed thoroughly and serially diluted. For each dilution triplicate potato dextrose agar (PDA) plates were prepared to isolate the fungus. The PDA plates were incubated at 25°C for 3-4 days. The developed colonies were isolated and sub-cultured repeatedly to get the pure culture and preserved as slants at 4°C.

Screening of polythene degrading fungus

100 ml of M1 medium containing (g l^{-1}) $NaNO_3$ 2; KCl 0.5; $MgSO_4.7H_2O$ 0.5; glucose 10; $FeCl_3$ 10 **(mg)**; $BaCl_2$ 0.2 and $CaCl_2$ 0.5 at pH 6.8 was used for selecting the efficient polythene degrading fungus. The media was supplemented with 0.5 g of plastic strips (2X2cm) respectively as sole source of carbon and energy [24]. One ml of each fungal culture was inoculated in their respective flask. These flasks were incubated in a rotatory shaker at 120 rpm for 15days. After three cycles of enrichment, one ml of sample was serially diluted and plated on potato dextrose plate.

Taxonomic identification of fungal strain

The isolated fungal strain was identified by 18S rRNA sequence analysis. The fungal genomic DNA was isolated by using AMpurE Fungal gDNA Mini kit. In this kit detergent and other non-corrosive chemicals are used to break open the cellulosic cell wall and plasma membrane to extract DNA from fungal cells. The 18S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal forward primer 5'-CGWCGRAANCCTTGTNACGASTTTTACTN-3' and reverse primer 5'- AWGCTACSTGGTTGATCCTSCCAGN-3'. Amplified PCR product was sequenced by using ABI3730xl genetic analyzer (Amnion Biosciences Pvt. Ltd. Bangalore, India). The sequencing result was submitted to the Gene bank National Centre for Biotechnology Information (NCBI) database.

Biodegradation studies

Determination of dry weight of residual LDPE

For the accurate measurement of dry weight of residual LDPE, LDPE films were recovered from the degradation media, and were washed with 2% (v/v) sodium dodecyl sulphate solution and further washed with distilled water [25]. The washed LDPE was dried overnight at 60ºC before weighing and the percentage weight loss was determined using the formula [1]:

$$\text{Weight loss (\%)} = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x\ 100$$

CO_2 evolution Test

The culture inoculum of the fungus was prepared in PDB broth and 5% inoculum of the isolate was used for the Sturm test. The pieces of polymer films were added to the test bottle, containing 100 ml of M1 medium devoid of carbon source. Polymer films served as the carbon source. Medium without any polymeric film served as the control. Sterilized air was supplied to keep conditions aerobic. The test was performed at room temperature for 4 weeks. After 4 weeks of incubation, the amount of carbon dioxide produced in the test and the control bottles, was calculated gravimetrically. The CO_2 evolved, as a result of degradation of polymeric chain was trapped in absorption bottles containing KOH (1M). Barium chloride solution (0.1M) was added to the bottles containing KOH and resulting in precipitation of barium chloride (using CO_2 released from the breakdown of polymer). CO_2 produced can be calculated gravimetrically by measuring weight of CO_2 precipitate evolved during the addition of $BaCl_2$. Changes in the result of test bottles were observed.

Confirmation of polyethylene degradation

Scanning Electron Microscopy

The surface morphology of the LDPE exposed to strain JAKA1 was analyzed through SEM to check out for any structural changes on the film. Polyethylene samples colonized by isolate was removed from the medium and examined by SEM (EVO LS 15; Carl Zeiss, Germany).

Atomic Force Microscopy

Changes in the surface topography of LDPE film was examined with AFM (Nanosurf 2 Easyscan). The degraded LDPE film samples along with the control LDPE film were analyzed in a scan speed of 1.0Hz. For the AFM studies degraded LDPE films were removed from the respective medium and were air dried on glass plates [26].

Fourier Transform Infra-Red (FTIR) Spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis was used to study the spectrum character of biodegraded sample associated with fungal exposure. The analysis was performed using FTIR (8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows) with a helium–neon laser lamp as a source of IR radiation.

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Pressed pellets were prepared by grinding the extracted samples with potassium bromide in a mortar with 1:100 ratio and immediately analyzed in the region of 4,000–400 cm $^{-1}$ at a resolution of 4 cm $^{-1}$ [27].

RESULTS AND DISCUSSION

Isolation and identification of isolate

Three different fungal strains were obtained from the solid waste dumping site and the most potent fungal strain was selected for degradation analysis. Based on 18S rRNA sequence analysis, strain isolates showed greatest similarity to the reference sequence of *Chamaeleomyces viridis.* 18S rRNA sequence of strain JAKA1 was deposited to GenBank which received accession number was KT148629. The characterization of the isolates by 18S rRNA gene sequencing followed by basic local alignment search tool (BLAST) was analyzed and their phylogenetic tree was constructed (Figure 1).

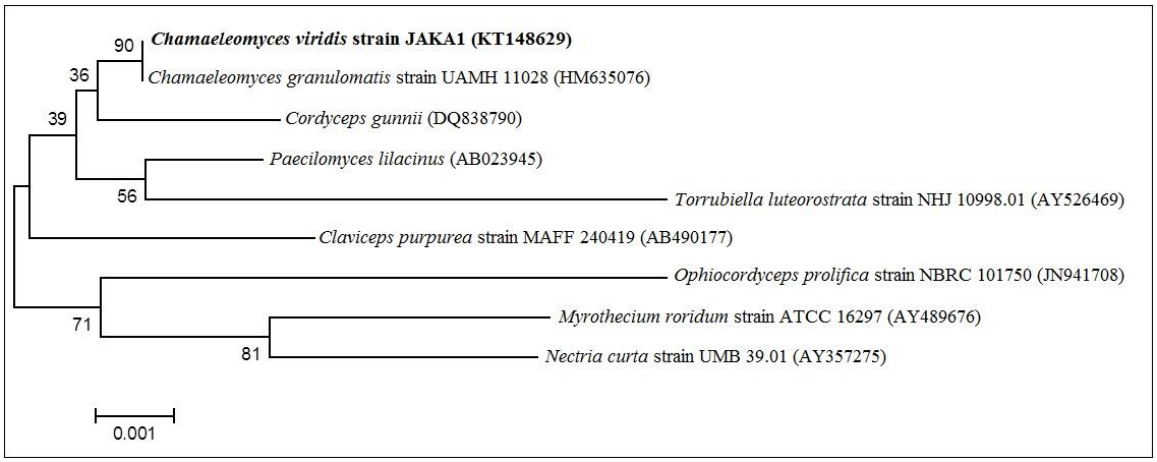

Fig.1 Phylogenetic relationship of *Chamaeleomyces viridis* strain JAKA1 based on 18S rRNA gene nucleotide sequences.

Several studies have also reported that microbes isolated from landfill soil and municipal waste dumpsite has the potential to degrade polyethylene. To the best of our knowledge, this is the first experimental report on degradation of LDPE films by a novel strain *Chamaeleomyces viridis* isolated from solid waste dumpsite. Mahalakshmi *et al*. [28] studied degradation of polyethylene using fungi and bacterial strains isolated from compost soil. Degradation analysis was carried out in mineral salt medium containing polyethylene as sole carbon source. In a similarity Sowmya *et al*. [29] isolated *Chaetomium globosum* fungus from local dumpsite soil which is able to use polyethylene as a sole carbon source for degradation.

Weight loss measurements

The degradation capability of *Chamaeleomyces viridis* was evaluated after 90 days. Weight loss of LDPE films was measured in 15 days interval. After incubation period of 90 days, *strain* JAKA1 showed a weight loss of 14.8% (Figure 2).

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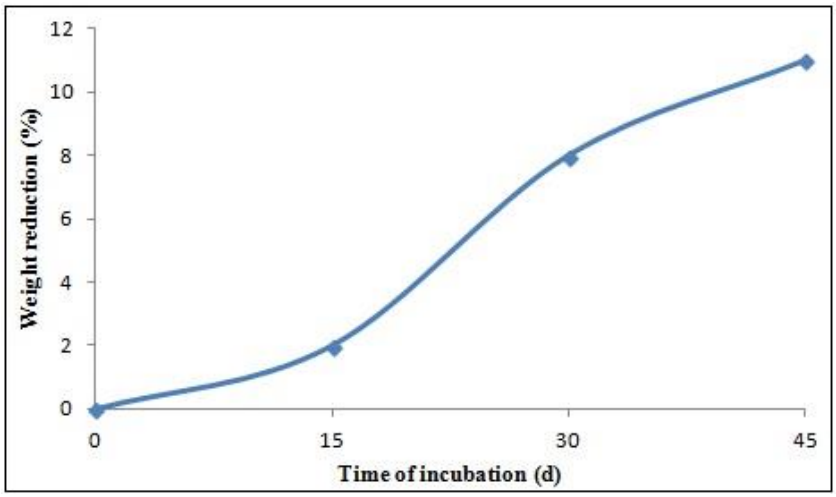

Fig.2 Graph represent the percentage weight loss of LDPE films incubated with *Chamaeleomyces viridis* strain JAKA1

The weight reduction of the LDPE films can be attributed to the breakdown of carbon backbone due to enzymatic degradation by isolated strain. Similar biodegradation assay has also been reported by Bhatia *et al*. [30]; Raaman *et al*. [31] and Kyaw *et al*. [1].

CO_2 evolution Test

Polymers are made up of carbon chain, during degradation of this polymer by microorganisms CO_2 and H_2O are obtained as byproducts. Metabolic CO_2 evolved during degradation was evaluated by Sturm test. After incubation for 90 days, it was found that the strain JAKA1 in the presence of polyethylene pieces produced 4.46 g l^{-1} amount of metabolic CO_2. The above result shows the capability of strain JAKA1 and in degrading of LDPE films.

Fourier transform infra-red spectroscopy

The structural analysis is significant parameters which confirm the changes that appear following degradation. **Figure 3 depicts the FTIR spectra of degraded LDPE films by strain JAKA1**. The FTIR spectra results showed that in the test sample, the peak in the region of 3535.52 and 3410.15 cm^{-1} corresponds to N-H stretching vibration of amine group. A peak at 3142.04 attributes to ≡C-H stretch of alkyne group. Asymmetric and symmetric stretching of alkanes group (H-C-H) respectively appeared at 2956.87 and 2922.16 cm^{-1}. Peak at 1614.42 cm^{-1} corresponds to N-H bend of amides. 1462.04 and 1379.10 cm^{-1} peak attributes to N=O stretch of nitro group. The peaks from 1261.45 to 970.19 cm^{-1} can be assigned to stretching vibrations of C-O. LDPE degraded spectra of similar pattern was observed by Da Luz *et al*. [32]; Bhatia *et al*. [30]; Labuzek *et al*.[33].

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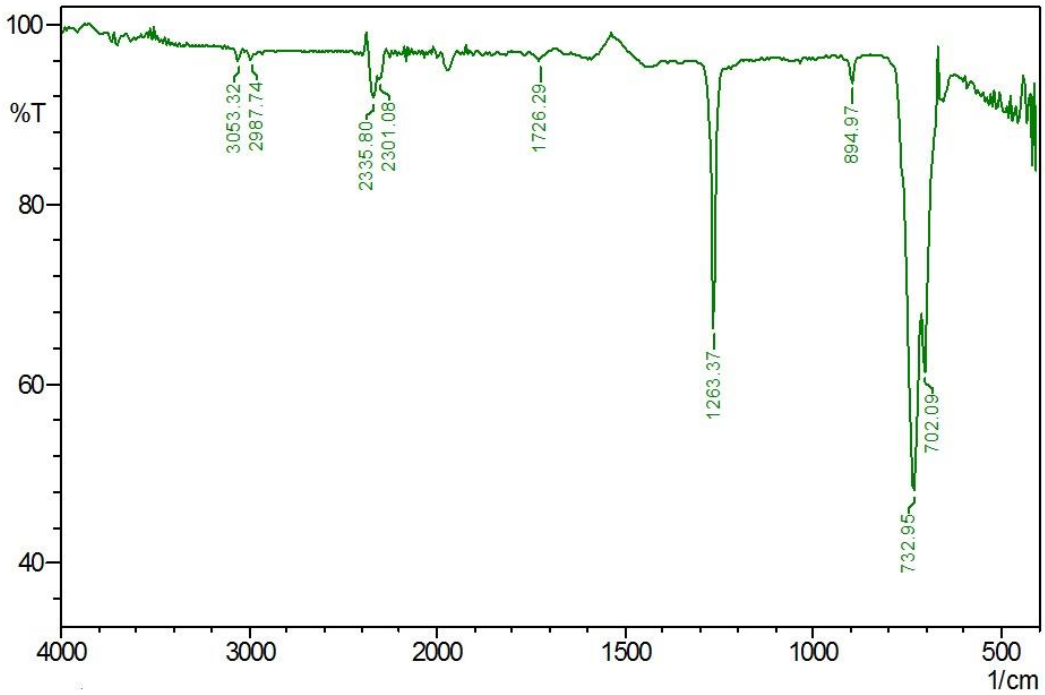

Fig.3 FTIR spectrum of biodegradation of LDPE film after 90 days of incubation in the presence of strain JAKA1.

Confirmation of LDPE films degradation

To further confirm the LDPE degradation ability of strain JAKA1, the change in the surface topography of the LDPE film was examined by both SEM and AFM analysis.

Scanning Electron Microscopy

LDPE films colonized by fungus were analyzed by scanning electron microscopy for any structural changes caused in the LDPE films. Several studies have been reported about SEM analysis in LDPE biodegradation **[12, 34-37].** SEM analysis showed the presence of fungal spores on the polymeric films surface after 90 days of incubation **(Figure 4)**. Formation of crack and pits were observed on the surface of the film. The above mentioned changes in the surface morphology of LDPE confirm the successful degradation by the fungal strain.

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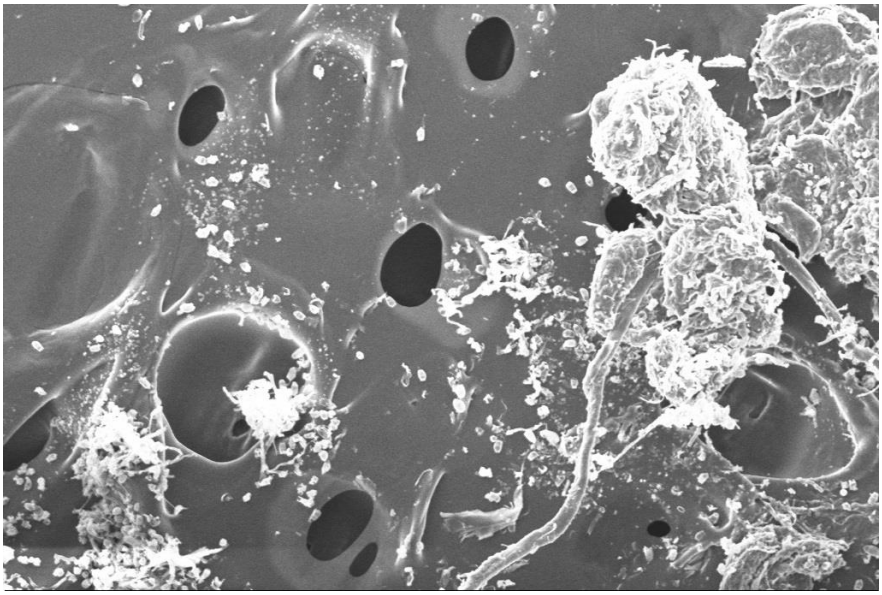

Fig.4 Scanning electron microscopy of LDPE films after 90 days of incubation *Chamaeleomyces viridis*.

Atomic force microscopy analysis

Surface topography of the LDPE films treated with the strain JAKA1 after 90 days of incubation was examined by AFM **(Figure 5)**. Tribedi and Sil, [26] has analyzed the localized degradation of LDPE film treated with strain *Pseudomonas* sp. AKS2 through AFM micrographs.

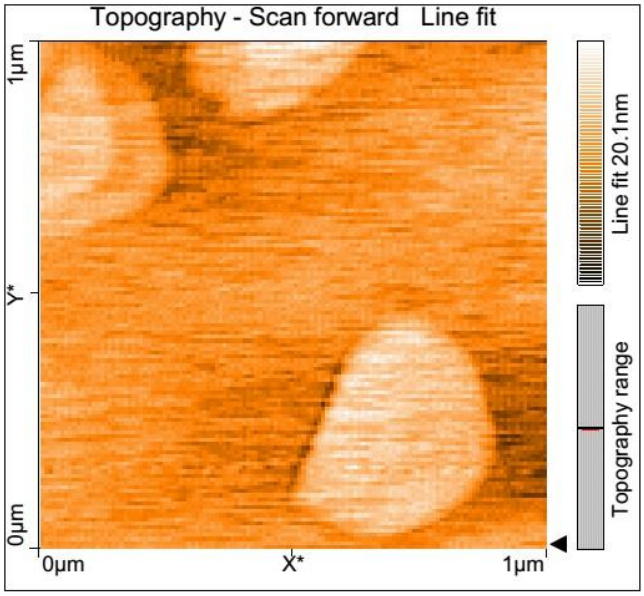

Fig.5 Surface morphological changes of LDPE films after 90 days of incubation with strain JAKA1

CONCLUSION

An indigenous microbe was employed in degradation of LDPE films in laboratory condition on synthetic media. *Chamaeleomyces viridis* strain JAKA1 has found possess to degradation ability when compared with other fungus and bacteria of earlier studies.

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