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Effect of *Aspergillus versicolor* strain JASS1 on low density polyethylene degradation

A Gajendiran, S Subramani and J Abraham
Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

E-mail: jayanthi.abraham@gmail.com

Abstract. Low density polyethylene (LDPE) waste disposal remains one of the major environmental concerns faced by the world today. In past decades, major focus has been given to enhance the biodegradation of LDPE by microbial species. In this present study, *Aspergillus versicolor* with the ability to degrade LDPE was isolated from municipal landfill area using enrichment technique. Based on 18S rRNA gene sequencing confirmed its identity as *Aspergillus versicolor*. The biodegradation study was carried out for 90 d in M1 medium. The degradation behaviour of LDPE films by *Aspergillus versicolor* strain JASS1 were confirmed by weight loss, CO_2 evolution, Scanning electron microscopy (SEM) analysis, Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR) technique. From current investigation, it can be concluded that our isolated strain JASS1 had the potential to degrade LDPE films and it can be useful in solving the problem caused by polyethylene in the environment.

1. Introduction

Annually, five hundred billion to one trillion plastics bags are being used globally [1]. Increasing level of plastic discharge, decreasing land fill capacity and low percentage of plastic degradation leads to air, water and soil pollution [2] which demands an environmental friendly degradation method. Microbial mediated degradation of polyethylene is a better approach which leads to smaller macro and micro plastic particles [3]. The physiochemical characteristics of polyethylene impact the mechanisms of microbial degradation factors like the surface conditions of polyethylene play vital role in the biodegradation [4]. Recently, several bacterial and fungal species have been investigated and reported for plastic degradation. The high progress of fungal growth in soil and the growth development and penetration into other sites through the fungal hypea makes them more favourable than bacteria for degradation of polyethylene [5]. When compared with bacteria, fungi were preferred for polyethylene degradation [6-7]. Characterization of LDPE films degradation by waste source fungus is the main aim of this study because of their compatibility with a landfill and composting environment which consists of a mixture of discarded polyethylene. In this study, selected fungal strain was isolated from municipal landfill area and was identified as *Aspergillus versicolor*. The capability of this isolate to degrade polyethylene films in aqueous medium was investigated.

2. Materials and methods

2.1. Enrichment technique to isolate fungal strain from municipal landfill solid waste soil

Municipal landfill solid waste soil samples were collected in sterile polythene bag from Vellore district. Enrichment method was employed for isolation of fungus that utilizes polyethylene as carbon source. 10 g

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soil was added in 250 mL flask consist of mineral media (M1) was seeded with 0.5 g of LDPE films (2×2cm) as only nutrition mode of energy and carbon [8]. The flask was kept at 120 rpm for 15 d at 28 ºC. After the incubation period, 1 mL of enriched sample was used for isolation of fungus. PDA plate was incubated at 25ºC for 3 d.

Molecular identification of the isolated fungus was confirmed by 18S rRNA gene sequence analysis. 18S rRNA gene sequence was amplified using ITS1 and ITS4 primers. The process was performed with 35 cycles in a thermal cycler, following the method described by Gajendiran et al. [8].

2.2. Biodegradation studies

2.2.1. Weight loss. From the degradation medium, polyethylene films were recovered and washed using 2 % SDS solution and followed by purified water [8]. The washed polyethylene films were dried for 24 h at 60 ºC and then weight of LDPE films was calculated [9].

Determination of LDPE weight = [(Initial LDPE weight-Final LDPE weight)/ Initial LDPE weight] ×100

2.2.2. CO_2 evolution. CO_2 evolved during biodegradation of LDPE was determined by Sturm test [10]. The culture inoculum of the fungal isolate was prepared in potato dextrose broth. 5% inoculum of the isolate was inoculated in both test and control flask. Polyethylene films were supplemented to the test flask, containing M1 medium which was devoid of carbon source. Control flasks were maintained with only polythene films in M1 medium without fungus. The flasks were kept at 28 ºC for four weeks. The quantity of CO_2 formed in the flasks, were determined. CO_2 was trapped in flask consists of 1M of KOH. 0.1 M of $BaCl_2$ solution was supplemented into the KOH flask and which resulted in $BaCl_2$ precipitation. Amount of CO_2 produced was estimated gravimetrically by addition of $BaCl_2$.

3. Morphological confirmation

SEM and AFM examination provides the detailed information about the LDPE degradation mechanism.

3.1. SEM

LDPE film colonized by strain JASS1 was analyzed through SEM to examine the surface morphology of the polyethylene films. LDPE films were washed with graded ethanol. The dehydrated sample was coated with a gold layer polyethylene samples colonized by the isolate was removed from the medium and examined by SEM (EVO LS 15; Carl Zeiss, Germany).

3.2. AFM

Surface morphology of the test (treated with strain JASS1) and control (untreated with strain JASS1) LDPE film was observed by AFM in 1.0 Hz scan speed and a high resolution. To remove the adhered fungal strain from the polyethylene films, it was washed with 2% sodium dodecyl sulphate and air-dried for 24 h [11].

3.3. FTIR of degraded LDPE

Infrared spectrometer was employed to identify the chemical groups present in the degraded polyethylene. Fungal inoculated LDPE films was analysed with a FTIR (8400 Shimadzu, Japan) by pressed pellet method [12].

4. Results and Discussion

4.1. LDPE degrading fungal strain

The potent LDPE degrading fungal strain was obtained through enrichment technique. And the isolate was designated as strain JASS1.18S rRNA gene sequence analysis was performed to identify the fungal isolate. The result of 18S rRNA gene sequence was submitted in GenBank NCBI database and the accession number KT148628 was assigned. BLAST results of 18S rRNA gene sequence of strain JASS1 showed high similarity with *Aspergillus versicolor*. Based on phylogenetic and 18S rRNA gene sequence, the strain JASS1 was identified as *Aspergillus versicolor* JASS1 (Figure 1).

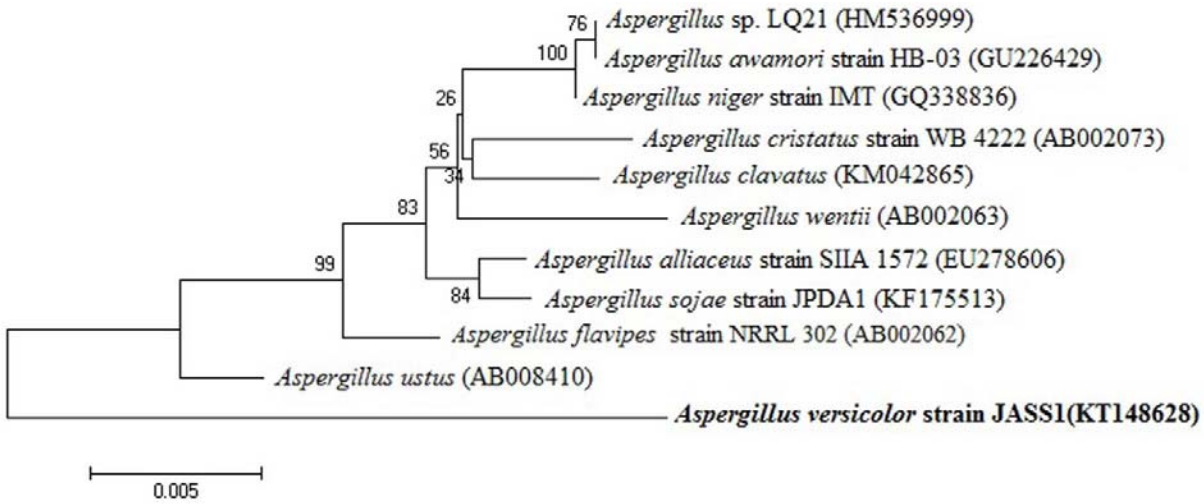

Figure 1. Phylogenetic tree of *Aspergillus versicolor* strainJASS1

Usha et al. [13] isolated *Aspergillus nidulans* and *Aspergillus flavus* by enrichment technique, polyethylene powder served as a carbon source. Similarly, Das and Kumar [14] isolated four *Aspergillus* sp. and one *Fusarium* sp. from soil sample by spread plate technique using mineral salt medium.

4.2 Mycodegradation studies of polyethylene

4.2.1 Weight loss measurements. Weight reduction of polyethylene films was taken at regular time intervals and the percentage of weight loss was depicted in Figure 2. *Aspergillus versicolor* strain JASS1 showed 40.6% of degradation potential. Lucas et al. [15] reported that loss of polyethylene weight during biodegradation may be because of disappearance of soluble impurities.

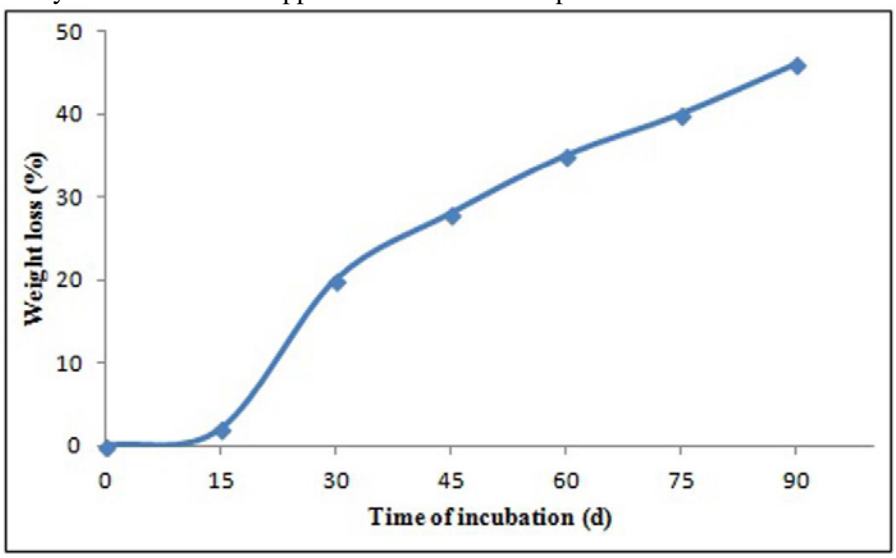

Figure 2. The percentage weight loss of polyethylene films exposed with strain JASS1.

An unaided visual examination of LDPE films incubated with strain JASS1 revealed a visible colour change of the sheet resulting in brownish white discolouration with foul smell. A white powder like substance was noticed on the polyethene pieces, presumed to be microbes adhering to the polyethylene pieces.

4.2.2. Sturm's test. Modified Sturm test was used to evaluate the degradation rate of polymer by calculating the total amount of CO_2evolved. After the incubation period, it was found that *Aspergillus versicolor* strain JASS1 produced and 11 g L^{-1} amount of CO_2. Pramila and Ramesh et al. [16] reported on biodegradation of LDPE with fungal strain *Aspergillus flavus* and *Mucor circinelloides* and the CO_2 evolution after 30 d was 10 g L^{-1} and 1.05g L^{-1} as respectively.

4.2.3. Morphological analysis. SEM examination supporting the degradation by revealing the presence of porosity and fragility of fungal degraded LDPE surface. Structural modification was noticed with cavities, and fissures on the polymer films. Micrographs of films exposed to strain JASS1 is presented in Figure 3.

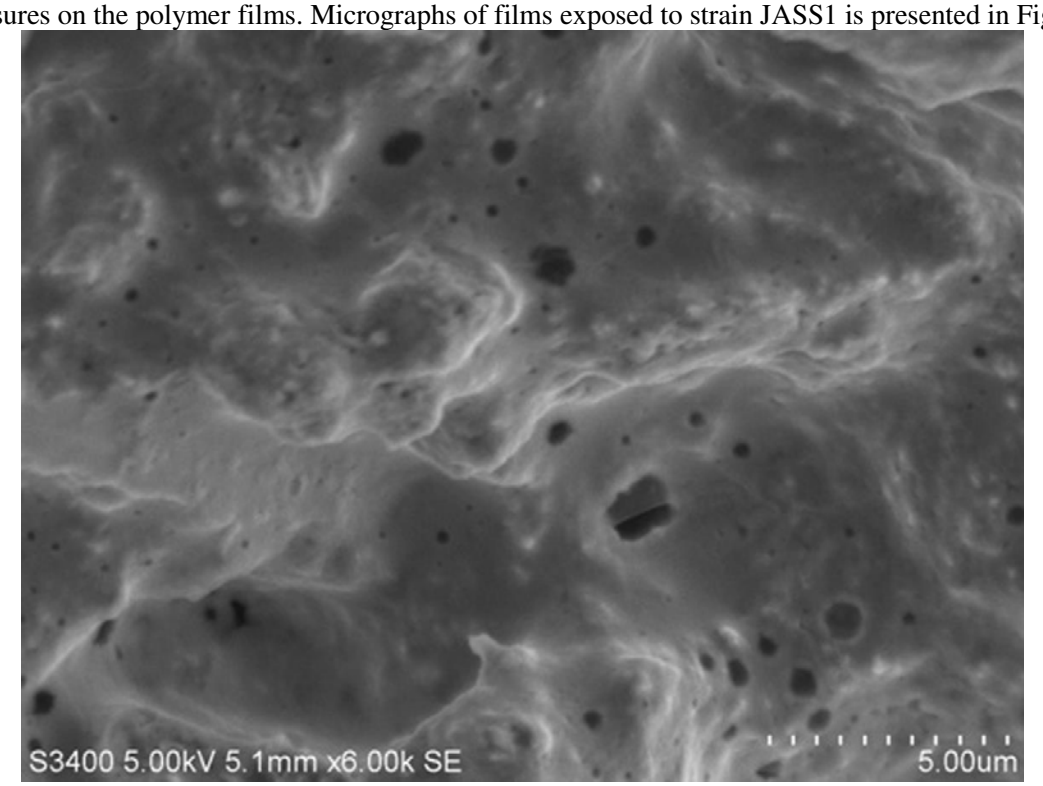

Figure 3.SEM micrograph of polyethylene films exposed to strain JASS1

The surface topology of the polyethylene films exposed by strain JASS1 has also been examined from AFM study. From Figure 4 it is evident that the surface of the polyethylene exposed with the isolate showed crevices and cracks, with the overall surface turning rough in texture. Sowmya et al. [18] observed the formation of holes and disruption of polyethylene structure through SEM and confirmed the degradation ability of isolate *Trichoderma harzianum*, *Aspergillus flavus* and *Mucor circinelloides* showed the morphological destruction on LDPE films which is evident through SEM analysis [17].

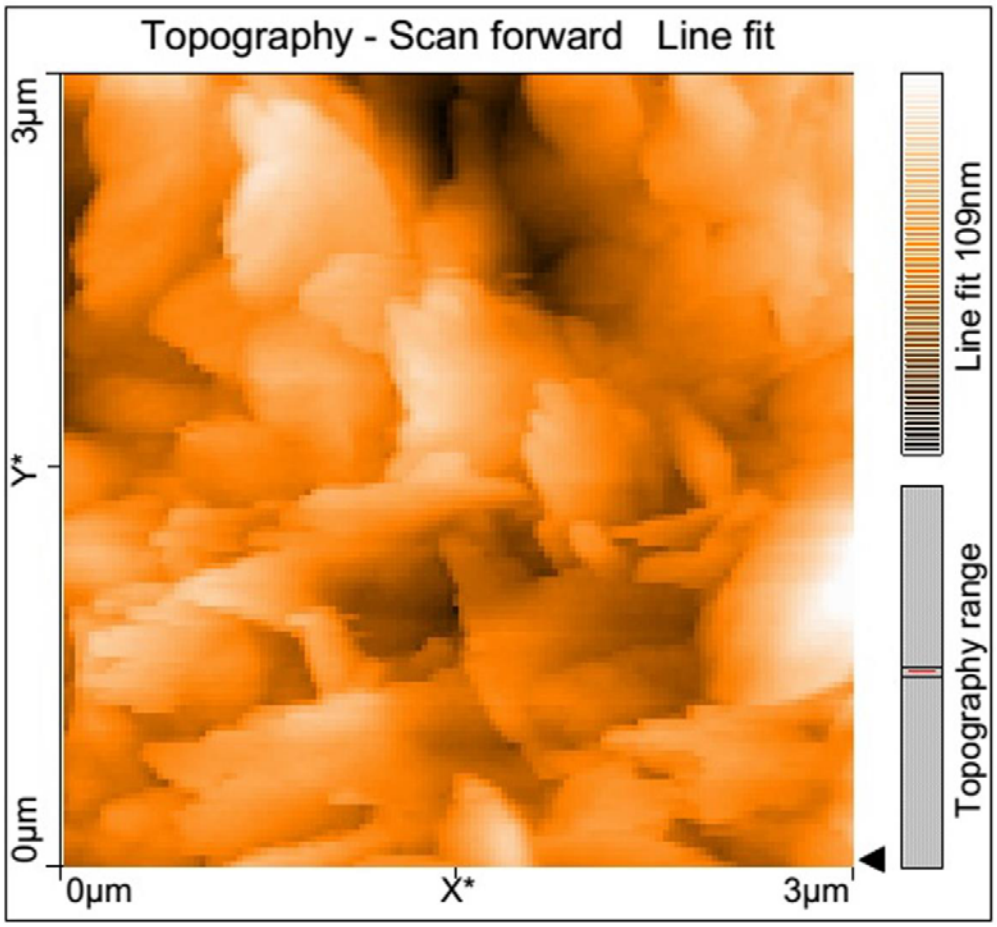

Figure 4. Surface morphological changes of polyethylene films exposed to strain JASS1

4.2.4. Fourier transform infra-red spectroscopy (FTIR). FTIR spectra of the polymer film after degradation by strain JASS1 is presented in Figure 5. FTIR determination of test LDPE sheets showed a band at 3273.20 cm^{-1} corresponds to ≡C-H stretch of alkyne group. Peak at 1631.78 cm^{-1} which represent to C-C=C stretch of alkenes. Peaks at 1263.37 and 1078.21 cm^{-1} attributes to C-O stretch of esters. Peak at 987.55 cm^{-1} corresponds to $=CH_2$. Earlier studies have reported that visible changes in polyethylene before and after microbial treatment by IR analysis [19-21].

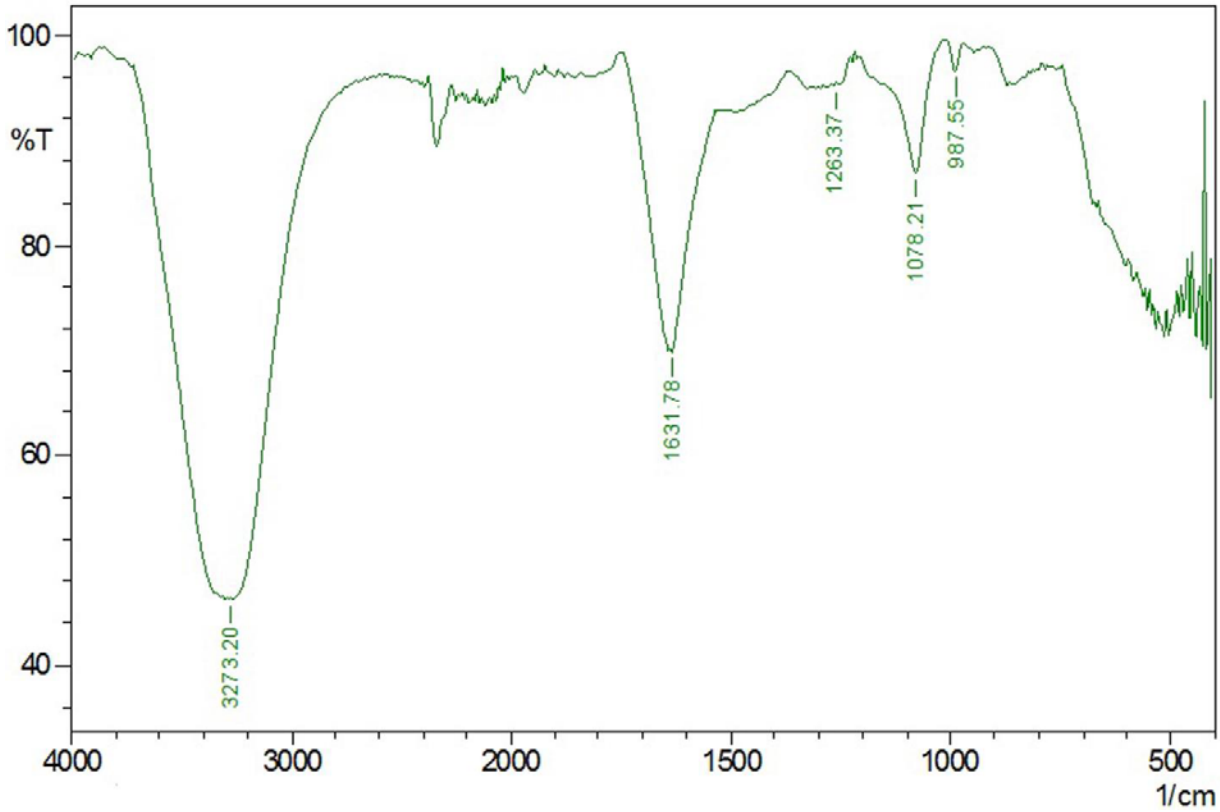

Figure 5. FTIR spectrum of degraded polyethylene film exposed to strain JASS1

5. Conclusions

From this study it can be concluded that *Aspergillus versicolor* JASS1 which was isolated from municipal landfill solid waste site are capable to utilize LDPE as a sole carbon source. The extent of biodegradation was carried out by weight loss, Sturm test, FTIR analysis, SEM, AFM observation revealed that strain JASS1 is efficient in degrading 20 micron polyethylene bags. The result of this study showed promising results for LDPE degradation under laboratory condition.

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