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Low Density Poly Ethylene Degradation (LDPE) Degradation Using Soil Bacteria from Plastic Dumped Sites

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KEYWORDS

Biodegradation, LDPE,

Bacillus sp,

plastics

ABSTRACT:

Low density polyethylene (LDPE) is one of the dominate usage of plastics in all applications which also leads to the plastic pollution. Biodegradation was an unharmed as well as tedious process. On this study, plastics can be able to degrade by competent microorganism by isolated the microbes from garbage area assuming that the potent strain can be accessible. The degrading bacteria can be isolated by enrichment method where the artificial media was prepared with LDPE strips as a sole carbon source. The isolated three strains EPSP1, EPSP2, EPSP3 were used for degrading studies. The bacterial biomass estimated of these strains done by the protein concentration which estimated EPSP1, EPSP2, EPSP3 has to be 0.18, 0.24, 0.2 µg /ml respectively. Biodegradation of LDPE can be observed by the weight loss of the LDPE films. Higher degradation occurs in EPSP2 strain (9.6%) which showed highest weight reduction. The potent strain EPSP2 undergoes for 16SrRNA sequencing which reveals that it was a Bacillus sp i.e. Priestia aryabhattai. Hence, this work paved that LDPE can be degraded by garbage sited microorganism and used for the further studies to determine the plastic pollution problems.

1. INTRODUCTION:

Plastics are polymers from petrochemicals which consist of carbon, hydrogen, sulphur, nitrogen, oxygen (Gnanavel *et al.*,2012). Plastics are categorised as polypropylene, polyethylene, polyvinyl chloride etc. Moreover, five Hundred billion to one trillion of plastic bags were used around the worldwide (Roy *et al.*, 2008). Plastics can be reduced by recycling, landfills and incineration (Sharma & Sharma 2004). Even though, plastics may be reduced by using these techniques but they cause other harmful problems. Clog of plastic wastes in landfills in anaerobic conditions leads to breakdown of chemical structure into micro plastics which enter into the water streams (He *et al.*, 2019). Accumulation of plastics in landfills, terrestrial areas and marine cause harmful damage to all ecosystem (Zylstra E.R 2013). Pernicious effect by polyethylene causes problems to marine organisms such as sea turtles, marine mammals, sea birds etc (Browne M.A *et al.*, 2011). Besides incineration or burning of plastics or polyethylene leads to release of toxic gases such as poly chloro dibenzofurans, PCBs (Poly Chlorinated Biphenyl), PAHs (Polycyclic Aromatic Hydrocarbons), NO2, CO2, SO2, heavy metals (Machado *et al.*, 2018).

Low Density Polyethylene (LPDE) is a type of polyethylene has 0.910-0.940 g/cm3. Low Density Polyethylene is non-degradable and hydrophobic in nature (Gupta and Devi 2019). The carbon atoms in a

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LDPE are less tightly packed and less crystalline so the density of the polythene is also lower (Sen and Raut 2015). LPDE breakdown easily when compare to other polyethylene. Most common type of LDPE are linear LDPE ad branched LDPE. They are differentiated by their branching, density and functional group. The exposure of sunlight in plastics produces greenhouse gases such as ethylene and methane.

Biodegradation is a natural process which converts polymers into monomers with the help of microorganisms such as bacteria, fungi, actinomycetes, algae (Gu 2003; Sivan *et al.*,2006). Microorganisms are known as decomposers which can degrade organic and inorganic compound. Degradation of plastics by using microbes may be slow process and this process does not produce any secondary toxic product so it is environmental-friendly (Fibriarti. *et al.,* 2021).

Degradation of polyethylene can be done by anaerobic and aerobic metabolism (Botre. *et al.,* 2015). Microorganism produces a certain type of enzyme in biodegradation process that can breakdown the polymers into monomers further used for microbial metabolism. The aerobic metabolism produces carbon dioxide and water whereas anaerobic metabolism produces water, carbon dioxide and methane as an end product (Usha. R *et al*.,2011). The biodegradation by microorganisms is influenced by various environmental factors which include humidity, temperature, moisture content, pH, biochemical and physiological nature of microorganism (Albertsson, 1987).

Many potent bacteria species have an ability to degrade LPDE. Some bacteria species are *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptomyces* spp., *Rhodococcus* sp., *Acinetobacter* sp., *Flavobacterium* spp., *Micrococcus* spp., *Ralstonia* spp.,*Microbacterium* sp. and *Nocardia* sp etc (Orr.I.G *et al.,* 2004).

The purpose of the current study was isolation of microorganisms from the plastic dumped site and to screen the competent bacteria which has high potential to degrade polyethylene or plastics. Observation of plastic degradation can be found by the weight loss of LPDE in the synthetic media.The potent LDPE degrading bacteria was identified by weight loss percentage in LDPE films and the organism was identified by 16S rRNA gene sequencing.

2.METHODOLOGY:

2.1. Sample collection:

The soil sample was collected from the garbage dumped area located in Kaliyakkavilai, Kanyakumari, Tamil Nadu. The soil was transferred immediately to the Ziplock cover and persevered in laboratory for further use.

2.2. Isolation of plastic degrading bacteria:

As the first step, serial dilution procedure was performed (Cappuccino & Sherman 1996). Enrichment method was used to isolate plastic degrading bacterial strains. LPDE films were cut into 3x3 pieces, 300g weighed and sterilized by 70% ethanol. Enrichment media was prepared containing polythene (LPDE pieces) as a sole carbon source. The synthetic media with LPDE films were incubated at 30°C for 30 days. Every seven days it is sub cultured and LDPE film were transferred.

To isolate the potent strain, the culture was spread in the nutrient agar plate and incubated for 37°C for 24 hrs and the colonies were streaked in agar plate and incubated to obtain pure culture and maintained it on slant for further use.

2.3. Identification of potent bacterial strains:

The potent plastic degrading bacteria can be identified according to morphological and biochemical characteristics by bergey's manual of determinative bacteriology (Holt *et al.,* 1994). Biochemical characterization includes Motility test, Catalase test, Indole production test, Methyl red test, Voges Proskauer test, Starch hydrolysis test, Gelatin liquefaction test, Casein hydrolysis test, Hydrogen sulphide test, Triple Sugar Iron test, Carbohydrate fermentation test.

2.4. Biodegradation studies:

The bacterial isolates were incubated in synthetic media with LPDE films individually for 30 days. During incubation the growth of bacterial isolates were studied.

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2.5. Growth curve of bacterial isolates:

When the bacterial culture enters in log phase, 10% of culture was inoculated in 250ml Erlenmayer flask with synthetic media and polythene films and incubated for 30 days. Non- inoculated culture in synthetic media with polythene films was considered as a control. The growth was measured by the absorbance of culture once in 7 days for 35 days by Spectrophotometer at 600nm (Rajeshree.P & Bagde U.S 2015).

2.6. Estimation of bacterial biomass:

LPDE films were takeout from the synthetic media and disinfected with 70% of ethanol and washed with sterile distilled water. These films were incubated with SM in a magnetic stirrer at 37°C.After incubated for 24 hrs; these films were boiled with 0.5 mol^{-1} of NaOH and centrifuge it for 10,000 rpm for 15 minutes. Saved the supernatant and the pellet undergoes same procedure again. The two supernatants were combined to determine the protein concentration by lowry's method (1951) (Pramila. R *et al* 2012).

2.7. Determination of dry weight of LPDE films:

After 30 days of incubation of culture, the LPDE films were taken out. LPDE films were treated with 2% of Sodium Dodecyl Sulfate (SDS) for 4hrs and washed with distilled water to remove impurities (Hadad *et al.*, 2005). The films were dried overnight at 60°C. The measurement of weight reduction was calculated by the following formula

Weight reduction in percentage (%) = ~~x 100~~

wi

W*i* – initial dry weight of LPDE films (g) e degradation.

W*f* – final dry weight of LPDE films (g) after degradation.

2.8. Molecular characteristics of potential bacterial strains:

The genomic DNA e was isolated, analysed by Agarose Gel electrophoresis, amplified by PCR and sequenced. These 16S rRNA gene sequences were compared with 16S rRNA database in NCBI using BLAST algorithm (Altschul *et al.*, 1997).

1. **RESULTS:**

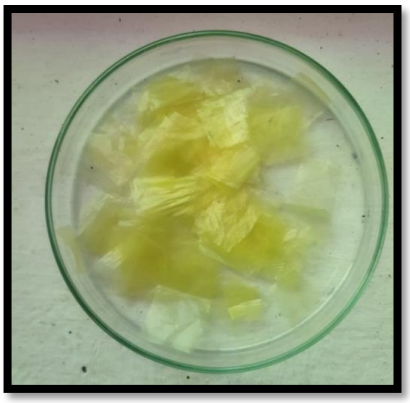

Fig 1: Low Density Polyethylene sheets observed in plate

Fig 2: Incubation for 35 days

Fig 3: Biodegradation of LPDE films

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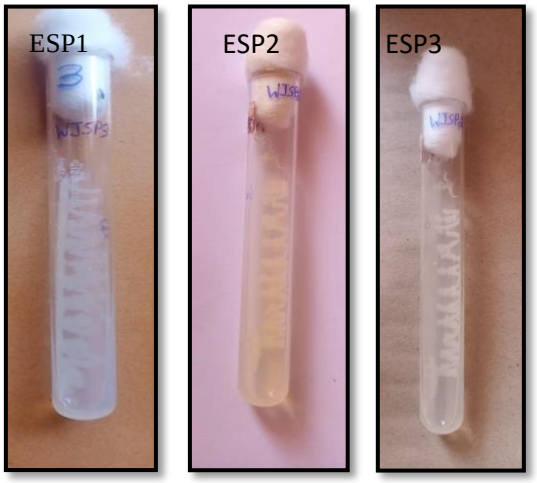

Fig 4: Maintenance of pure culture

Table 1: Biochemical characterization of the isolates

Description	INFERENCE		
	EPSP1	EPSP2	EPSP3
Colony morphology	Whitish creamy opaque, jagged edges and rough colonies	Whitish smooth edges, creamy large flat colonies	Yellowish opaque, uneven edges granular colonies
MICROSCOPIC EXAMINATION			
Gram's staining	+	+	+
Spore staining	+	+	-
BIOCHEMICAL TESTS			
Motility test	+	+	+
Catalase test	+	+	-
Indole production test	-	-	+
Methyl red test	-	-	-
Voges- proskeur	+	+	+
Starch hydrolysis	-	+	+
Gelatin liquefaction	+	+	+
Casein hydrolysis	+	+	+
Hydrogen sulphide test	+	+	-
TSI test	Yellow	Pink	Pink
Carbohydrate fermentation test			
Sucrose	-	+	+
Glucose	+	+	-
Dextrose	-	+	+

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Table 2: Growth curve studies

Name of the isolates	No. of days of incubation					
	3	7	14	21	28	35
EPSP1	0.18	0.31	0.35	0.24	0.13	0.11
EPSP2	0.15	0.34	0.29	0.22	0.16	0.10
EPSP3	0.20	0.39	0.33	0.26	0.12	0.11

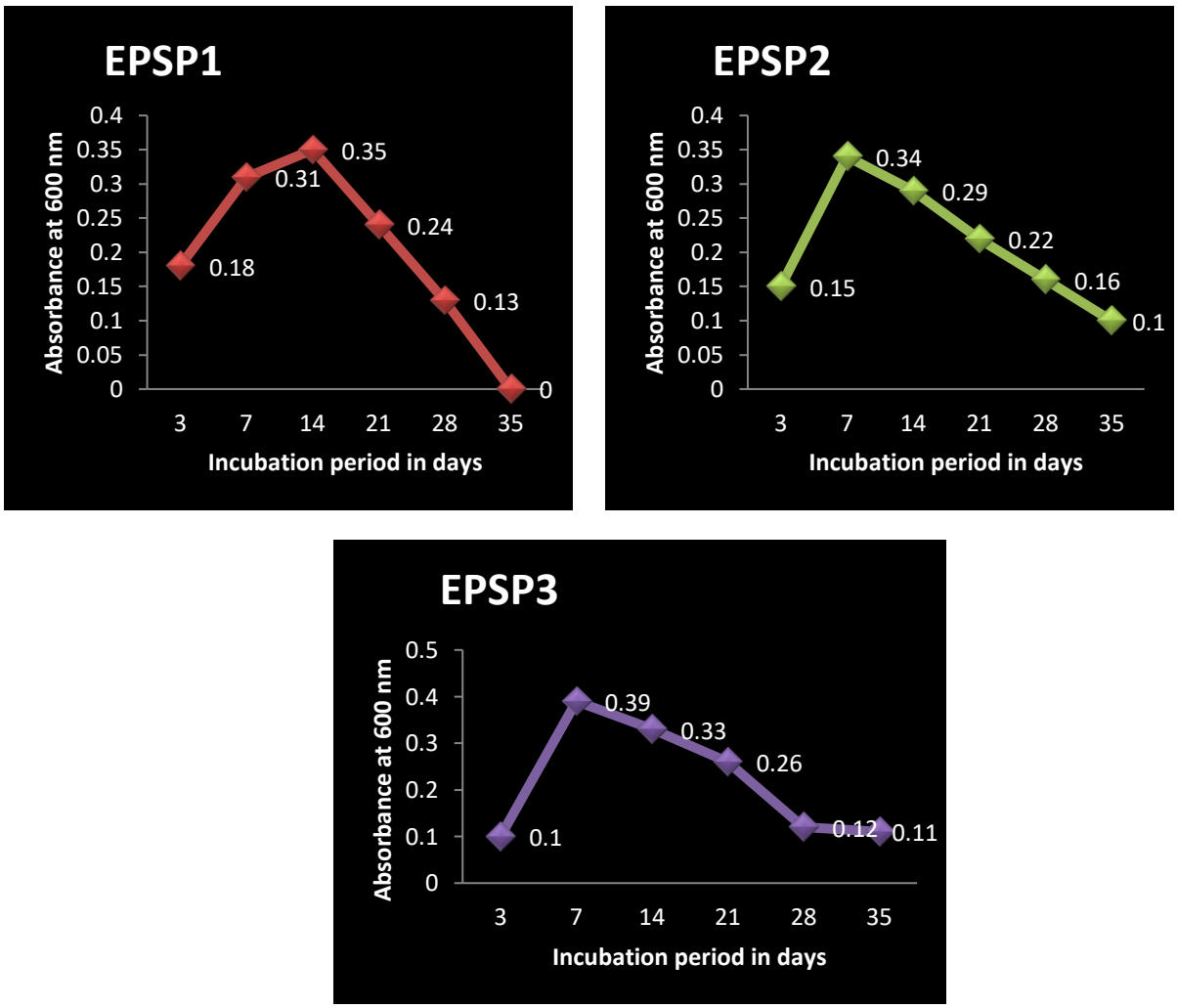

Fig 5: Growth curve studies

Table 3: Quantification of bacterial biomass

SI.NO	Bacterial isolates	OD value at 670 nm	Biofilm protein content (µg)
1.	EPSP1	2.1	0.18

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2.	EPSP2	3.3	0.24
3.	EPSP3	2.8	0.20

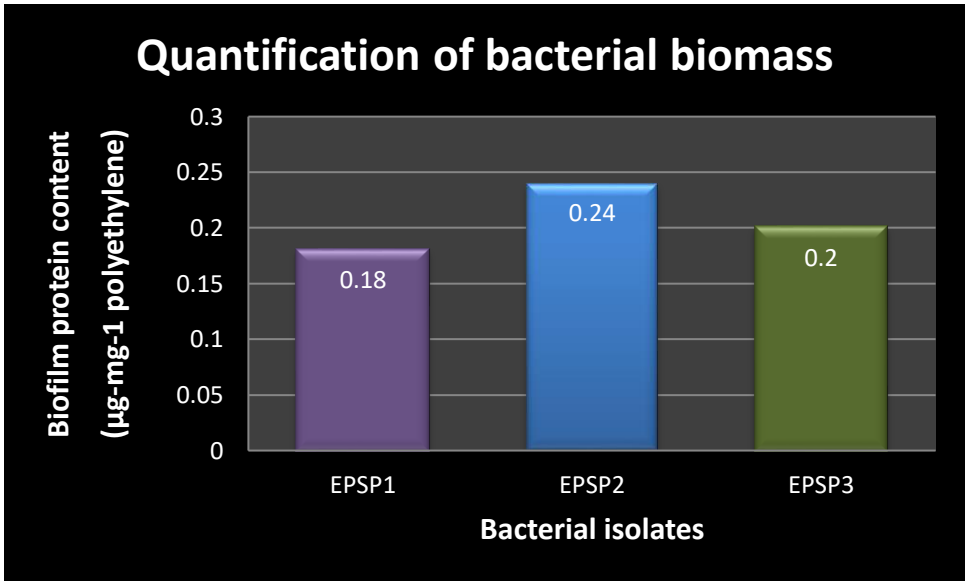

Fig 6: Quantification of bacterial biomass

Table 4: Weight reduction of LDPE films in 35day time period

Name of the isolates	Weight reduction after 35 days (g)	Weight loss percentage (%)
EPSP1	0.295	1.6
EPSP2	0.271	9.6
EPSP3	0.283	5.6

*Initial Weight of LDPE film = 0.3 g

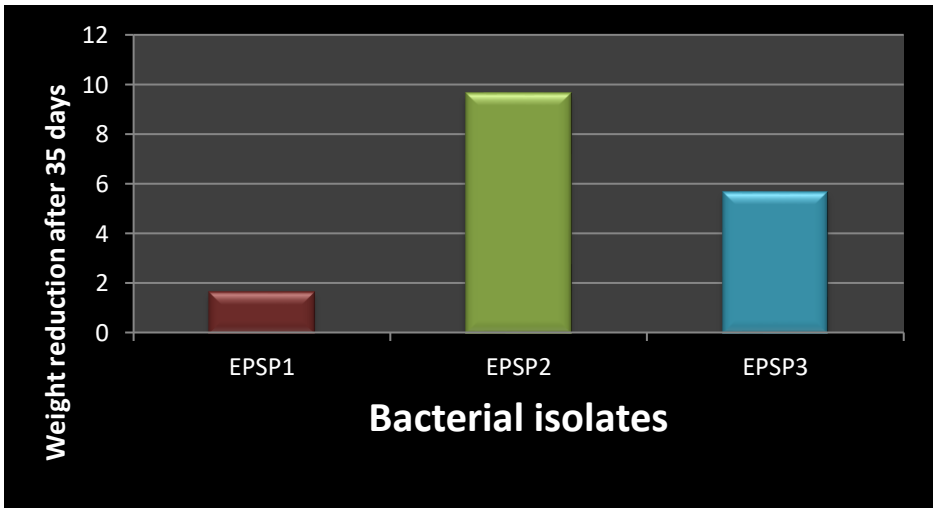

Fig 7: Weight reduction of LDPE films in 35days

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16SrRNA gene sequence ACCESSION NO: OR456346

>EPSP2

GCCCTTTGTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCC
CACCTTCC
TCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTAAATGCTGGCAACTAAGATCAAGGGTTGCGCT
CGTTGCGGG
ACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCCAAAGGG
AAACGCTCT
ATCTCTGGAGTTGTCAGAAGATGTCAAGACCTGGTAGGGTTCTTCGCGTTGCTTCAAATTAAACCA

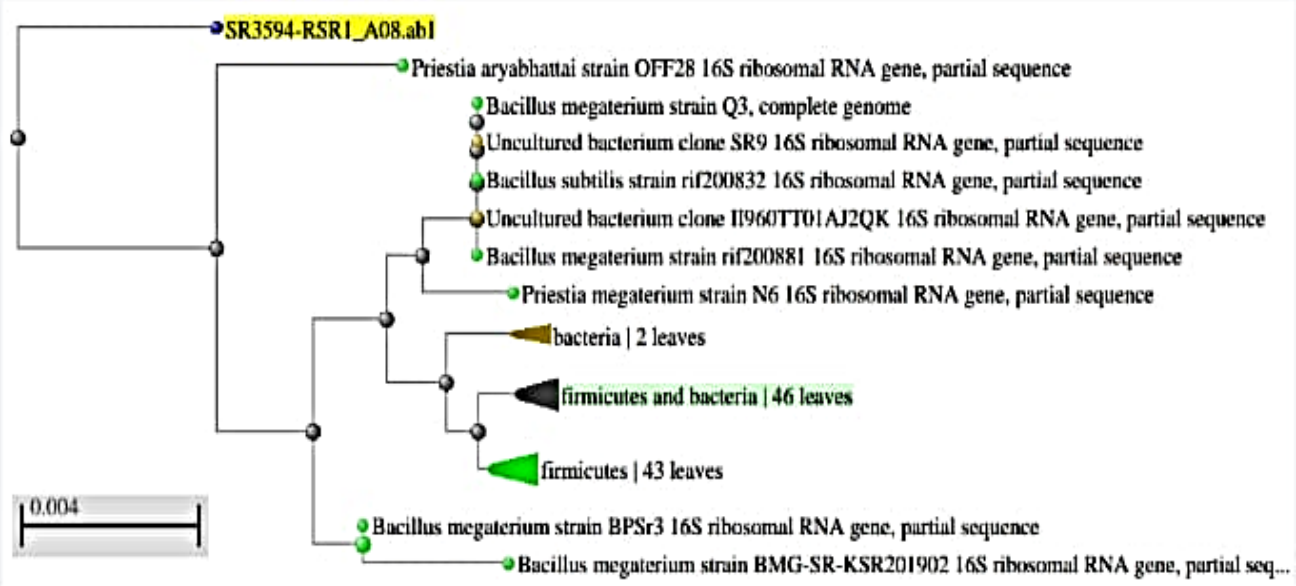

Fig 8: Phylogenetic Tree

DISCUSSION:

Plastic consumption became a part of day to day life. Low Density Polyethylene is petroleum-based polymers and hydrophobic in nature. They are durable, lightweight and low cost and also appropriate for many applications. This type of polythene used in various industries for packing, agriculture, food industries, automobile industries etc. (Sanchez *et al.*, 2020). These application leads to the high usage of plastics by humans. The higher the use of plastics leads to the higher threat to the environment. In India, annually 3.5 million metric tons of plastics wastes are generated during 2019-2020. In 2022, NGO analysed that every day 25,940 tons of plastics wastes are generated (The Central Pollution Control Board, India).

Low density polyethylene (LDPE) can be degraded by microbes, although it's a slow process, but it cannot produce harmful byproducts. Microorganism plays an important role in biodegradation process. There are two types of depolymerisation enzymes which are used to degrade polymers. They are extracellular and intracellular depolymerase (Gu *et al.,* 2000). These exo-enzymes degrade polymers and breakdown into smaller monomers which can penetrate into semi-permeable membrane of microorganism for carbon and energy source (Frazer, 1994; Hamilton *et al.*, 1995).

Due to the presence of –CH2 group the polymers has high hydrophobicity, and it has molecular weight which is more than 30 kDa. The microorganism which has an extracellular enzyme can degrade the polymer chain and change its properties. This

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mechanism makes reasonable for the biodegradation of polymers (F.W.Bilmeye 1971).

Similarly, thermal treatment or radiation treatment on polyethylene makes variation in the polymeric chain and produce oxidize group like carbonyl, hydroxyl and carboxyl. These treatments alter the properties (morphological changes, crystalline level) of polymers and promote the degradation level (H.Rajandas *et al.,* 2012).

Many reports or articles described about the polythene biodegradation by many species of microorganisms till now, but it has an insufficient knowledge about the metabolic pathway, and the enzymes which are involved (Y.Otake *et al.,* 1995; D.K.Barnes *et al.,* 2009).

In the current research conducted, Competent polythene degrading bacterial soil sample are isolated and the efficiency of degrading ability for further studies were explored. These strains should have the higher degradation potentiality. This study examined about the LDPE degradation by the capable strains after 35 days of incubation period.

The current work is based on degradation of low density of polyethylene films. LDPE films (fig 1) are petroleum-based thermoplastic (Shah *et al.,* 2009) which hard to degrade and it causes plastic pollution. For this study, LDPE films were customized. To attain the capable plastic degrading bacteria, the soil sample was taken from the plastic dumped area. A gram of soil sample undergone for serial dilution and 1ml of diluted sample was inoculated in synthetic media with pieces of LDPE films. It was incubated for 35 days and the weight reduction noted at regular intervals. After 35 days of incubation, (fig 2,3) the sample was spread in the nutrient agar plate to isolate the potent LDPE degrading bacteria individually. For pure culture, the colonies were streaked repeatedly. The native strains were selected and named as EPSP1, EPSP2, EPSP3. These strains were maintained and preserved in refrigerator (fig 4) for further uses. Biochemical characterization of the cultures was performed and found that all the three cultures were gram positive. Table 1 interprets the results of biochemical test results.

The three indigenous strains were incubated for 35 days in synthetic media with LDPE films. Growth of the culture was evaluated by observing the optical density by spectrophotometer periodically. Only the LDPE degrading bacteria can survive in the media because the only source of carbon is LDPE films (fig 2,3).

Growth of an organism were viewed by the turbidity of the medium. The increase in the growth of the culture also increases the turbidity level. In these studies, the three strains had somewhat different growth rate. For about 14 days there was steady increase in growth and then gradually increased in ESP1. For EPSP1, the growth increased upto 14 days and decreased gradually. In case of ESP2, there was steady increase in growth rate upto 14 days and after that the growth rate decreased moderately. Likewise, EPSP3 showed the steady increase and decrease in growth rate after some days. The growth curve studies were interpreted and given in table 2 and figure 5. Adding small volume of fresh synthetic media to the culture flask can reduce the decline phase of growth.

The determination of degrading bacterial biomass was examined by various traditional methods such as cell counting or plating. But these methods could not be used to figure out the density of bacteria accurately. The bacteria are attached to the polythene film surface, so the protein concentration in the polythene films were used to measure the density. Lowry's method was used to estimate the protein concentration. The protein concentration of the strains EPSP1, EPSP2, EPSP3 were determined to calculate the biomass. The concentration of protein for the strains EPSP1, EPSP2, EPSP3 were found to be 0.18, 0.24, 0.2 µg/ml respectively (Table 3, fig 6). Hence, EPSP2 has a high protein rate and thus has more increase in biomass content. It is in turn indicated that the strain ESP2 is an actively growing strain and had higher LDPE degrading efficiency.

The degradation of LDPE can be noticed by many methods. One of the simple and quickest method to figure out the degradation of LDPE films is by determination of weight loss. The bacteria degrade the polythene surface leading to the weight loss of polymers (Sudhakar *et al.,* 2008). The strain EPSP2 exhibited the

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highest weight loss of 9.6% representing the greatest capable of degradation. While EPSP3 exhibited 5.6% as a moderate degradation capacity and EPSP1 exhibited low degradation ability (Table 4, fig 7). Thus, EPSP2 has great efficiency of degradation and also this strain was worthy for further degradation studies.

To recognize the competent strain EPSP2, the strain undergoes DNA isolation, PCR and 16S rRNA DNA sequencing. The DNA sequenced results obtained and run under BLAST N. Therefore, the EPSP2 strain divulges as *Priestia aryabhattai* with 100 % identity i.e homologous sequences. These sequences were submitted in the GENBANK and was given ACCESSION NO OR456346 (fig 8). Phylogenetic tree was created and found out that was similar to *Bacillus* sp (fig 8). This confirmed that the *Bacillus* species is capable of degradation and furthermore for greater degradation incubation time can be increased.

CONCLUSION:

This study manifested that microorganisms have a potential to degrade LDPE films. Isolated microorganism from plastic dumped regions can react with polythene, modify its mechanical properties, including its tensile strength, as well as its optical changes, disintegration and decolorization. From the studies it was concluded that the isolated strain Priestia aryabhattai (ESPS2) is efficient in degradation of LDPE film by using it as sole source of carbon. Further with long time incubation, percentage of degradation could be increased.

Statements & Declarations:

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Contribution by authors:

All the authors contributed equally to this research work, including conceptualization, design and discussion. Data collection and experimental procedures were carried out by Ms.Evangelin Priya S. Data analysis, interpretation of results, discussion and conclusion were collectively done by all the authors.

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