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A COMPARATIVE STUDY OF IDENTIFYING POTENTIAL LOW DENSITY POLYTHENE DEGRADING BACTERIAL SPECIES FROM DIFFERENT DUMP SITES SOIL AROUND SRINIVASAPURAM, THANJAVUR DISTRICT

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***Abstract*:** There is a huge interest for this generation to developing a sustainable environment for the future. Plastic is one of the major pollutant to damage the environment so to eradicate the plastics and to create awareness of the harmful effects of plastics among the public is inevitable one. The usability of plastics and plastic products are vast since they are strong light-weighted durable and low cost. However, they are harmful to the environment as they are resistant to biodegradation and leading to pollution. Hence the removal of plastic waste from the environment is inevitable one. Generally plastic wastes are mitigated through physical and chemical methods, but these methods are cost effective and releasing harmful substances to the environment. So, the alternate method should be adopted to control the plastic pollution. In view of that the present study is focused to control the plastic wastes in natural way by using microorganism. The effective polyethylene degrading microorganisms were isolated from municipal solid waste dumping site soil around Srinivasapuram, Thanjavur District. The physicochemical parameters of the soil samples were analyzed and the native microorganisms of the soil samples were isolated by using the prescribed procedure. There are 10 different varieties of microorganisms were isolated and subjected to morphological, phonotypical and biochemical characterization. The potential polythene degrading microorganism among the 10 isolates were identified through clear zone assay. The microorganism showed good result during clear zone assay was subjected to molecular characterization and confirmed as Streptomyces lividans. Thus, the present study confirm that *Streptomyces lividans* isolated from the dumpsite soil can degrade the Low Density Polythene efficiently.

Keywords: Plastic waste, LDPE, biodegradation, soil microbes, *Streptomyces lividans*.

1. INTRODUCTION:

Pollution is the introduction of any substance (solid, liquid, or gas) or any form of energy (such as heat, sound, or radioactivity) into the natural environment that cause adverse change (Deepranjan Sarkar *et al*., 2017). Pollution is classified into different types they are air pollution, water pollution, land pollution, noise pollution, solid waste pollution, radioactive pollution etc. Modern world is also concerned about specific types of pollution namely noise pollution and solid waste pollution (M. Sudhakara *et al*., 2008). Solid waste generation has become an increasing public health and environmental problem in developing countries like India. India is one of the major country producing more amount of solid waste every day. Due to increasing population about 12 million tons of solid waste are generated in India from households (Neha Gupta *et al*., 2015). Solid waste consists of household waste, wastes from hotels, debris from building construction and demolition sites, and waste from hospitals. With increasing population and urbanization, the amount of solid waste has been increasing quickly and its composition has been changing. Ever growing population leads to high accumulation of non-biodegradable waste in the environment (G. Gnanavel *et al*., 2016). The solid waste comprising three different waste materials namely; Organic waste, Recyclable waste and Inert waste. Organic portion of the solid waste is biodegradable and it mainly consists of agro waste, vegetable waste, domestic waste and garden waste etc. The recyclable waste is non-biodegradable one and it consist of plastic, polythene, metals and paper. The inert waste consist of gravels and sand etc (I. Hussein *et al*., 2018).

The organic portion of the solid waste automatically get decomposed by microorganisms and the inert waste also get settled on land. But we should pay more attention to recyclable portion of the solid waste (Md. Abdur Rakib *et al*., 2014). The major part of the recyclable waste occupied by polythene. In India, conventional methods like physical and chemical ways are adopted to recycling the polythene but it may cause secondary pollution especially recycling of plastic through burning, usually produce some noxious gases like furans and dioxins which are dangerous greenhouse gases and play an important role in ozone layer depletion (T. Z. Quazi, 2015). Hence the alternate way of recycling polythene waste must be adopted to prevent the secondary pollution. In view of that the present study focused to provide alternate and ecofriendly solution to the polythene pollution.

2. MATERIALS AND METHODS:

2.1 Soil sample collection:

The soil samples were collected at a depth of 3-5cm from dump site of Srinivasapuram, Thanjavur District, Tamil Nadu. Polyethene is a major component of this dump site in which PE wastes had been buried for different periods. Hence this site was chosen as a study area for the present work. The soil samples were randomly collected from five different dump sites located within the Srinivasapuram, Thanjavur. The collected samples were designated as Sample-A, Sample-B, Sample-C, Sample-D and Sample-E. The agriculture soil from the organic farm used as the control. The soil samples were transferred into sterile conical flasks, labelled and transported to the laboratory for analysis.

2.2 Physicochemical analysis of soil samples:

The collected soil samples (A, B, C, D & E) were processed (O. J. Attoe, 1947) for identifying physical, chemical and microbial parameters as per the standard procedure. The important physical parameters such as soil color (N.P. Kirillovaa *et al*., 2018), soil texture (American Society for Testing and Materials, 1985), pH (E.O. McLean, 1982) & E.C (C.S. Piper, 1942) were analyzed. The important chemical parameters such as soil Organic Carbon, Nitrogen, Potassium, Calcium, Fe and Mn were analyzed at National Agro Foundation at Taramani, Chennai.

2.3 Isolation of microorganisms:

One gram of soil sample was transferred into a 250 ml conical flask containing 99 ml of sterile double distilled water. The soil solution was shaken properly and serially diluted from 10^{-1} to 10^{-6}. Pour plate method was adopted to isolate the microorganisms from different soil samples. To isolate the bacteria from the soil nutrient agar used as a medium whereas starch casein agar medium used for actinomycetes. For each dilution, three replicates were made. The plates were then incubated at 37°C for 2-7 days (S. Deepika and R. Jaya Madhuri, 2015).

2.4 Identification, characterization and purification of microorganisms:

The microbial isolates were subjected to morphological, phenotypical and biochemical characterization. The morphological characteristics like configuration, margin, elevation, color, opacity, size, cell shape, arrangement and spores were studied. Phenotypic characteristics such as Gram's reaction and motility were studied. The biochemical characterization test including Catalase test, Oxidase test, Urea hydrolysis, Gelatin hydrolysis, Starch hydrolysis,

H_2S Production, Nitrate reduction, Citrate utilization, Indole, Methyl red test, Vogesproskauer test and Gas production were performed as per Bergey's Manual of Systematic Bacteriology (2009). The identified isolates were sub cultured repeatedly to get pure colonies and then preserved in slant at 4°C for further studies.

2.5 Preparation of LDPE powder:

Low-density polyethylene was obtained from J.P. Plastic, Chennai – 600012, India. LDPE films were cut into small pieces, immersed into xylene and boiled for 15 min. Later, it was kept to evaporate the xylene and it was crushed with blender at 3,000 rpm. As obtained LDPE powder was further washed with ethanol to remove xylene residues and dried overnight in hot air oven at 50°C. The final product was stored at room temperature for further use (Merina Paul Das & Santosh Kumar, 2014).

2.6 Clear zone assay:

The potential polyethylene degrading microorganisms were screened through clear zone assay (R. Usha *et al*., 2011). Polyethylene powder was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixture was sonicated for 1hour at 120 rpm in shaker. After sonication the medium was wet sterilized at 120°C, 15lbs pressure for 15 min. Sterilized media was cooled to 45°C and about 15 ml sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 30-35°C for 2-4 weeks. The organisms producing zone of clearance were subjected to molecular level identification.

2.7 Molecular characterization and phylogenetic analysis:

Molecular identification of selected bacteria by 16S rRNA sequencing and Phylogenetic tree construction were performed by the method described by C. Elizabeth Rani *et al*., 2019.

3. RESULTS:

3.1 Physicochemical analysis of soil samples:

The color of the different soil samples A, B, C, D, E and control are presented in Table 1. The present study results revealed that the texture of the samples A to E are sandy clay loam in nature whereas texture of the control soil is sandy loam (Table 1).

Table 1: Results showing texture and color of the soil samples analyzed.

S. No.	Samples	Color	Soil texture			
			Sand	Silt	Clay	Class
1.	Sample A	Blackish brown	46	15	23	Sandy clay loam
2.	Sample B	Blackish brown	48	17	25	Sandy clay loam
3.	Sample C	Light brown	50	23	29	Sandy clay loam
4.	Sample D	Light brown	41	13	22	Sandy clay loam
5.	Sample E	Light Black	54	25	31	Sandy clay loam
6.	Control	Reddish brown	44	8	12	Sandy loam

Electrical conductivity of the soil samples C and D have high EC content compared to other soil samples and control (Table 2). In this work, the amount of nitrogen in the samples D, C and E are very high when compared to other sample and control. It may due to accumulation of organic pollutant in the dump site. Potassium is a macronutrient and it act as a regulator for metabolic activities. The exchangeable portion of potassium in the soil sample B is low when compared to other soil sample analyzed. Calcium is a secondary plant nutrient. It may regulate the cell function. The results of the present work showing that the level of calcium is high in samples C, D and E this may due to excess deposition of lime stone related product in the corresponding dump sites. The calcium level is moderate and minimal in samples A, B and control (Table 2).

The present study results reported that the level of iron is optimum in samples B and C where as it little lesser in rest of the soil samples. The level of manganese in the test soil sample C is low and moderate in samples B, D and E where as it little high in sample A and control (Table 2).

Table 2: Results of physico-chemical analysis of different soil samples.

Parameter	Control	Sample A	Sample B	Sample C	Sample D	Sample E
pH	7.0 ± 0.03	8.3 ± 0.02	7.6 ± 0.01	8.4 ± 0.04	8.5 ± 0.05	8.3 ± 0.02
EC (mS/cm)	1.21 ± 0.04	1.39 ± 0.06	1.32 ± 0.02	2.24 ± 0.07	3.25 ± 0.09	1.29 ± 0.01
Carbon (ppm)	0.35 ± 0.05	0.39 ± 0.04	0.59 ± 0.02	0.49 ± 0.06	0.57 ± 0.08	0.49 ± 0.09
Nitrogen (ppm)	58.4 ± 0.01	57.6 ± 0.06	66.4 ± 0.03	54.0 ± 0.05	52.4 ± 0.02	51.0 ± 0.08
Potassium (ppm)	21.0 ± 0.07	23.0 ± 0.09	12.5 ± 0.06	26.3 ± 0.08	22.0 ± 0.05	12.5 ± 0.08
Calcium (ppm)	92.0 ± 0.03	95.0 ± 0.03	85 ± 0.02	110 ± 0.04	105 ± 0.05	135 ± 0.02
Fe (ppm)	9.31 ± 0.02	9.43 ± 0.01	10.68 ± 0.03	10.11 ± 0.04	9.16 ± 0.03	9.43 ± 0.04
Mn (ppm)	4.12 ± 0.04	4.17 ± 0.06	3.45 ± 0.05	2.50 ± 0.04	3.08 ± 0.06	3.33 ± 0.08

3.2 Isolation of microorganisms:

The microbial culture results revealed that there is huge heterotrophic bacterial colonies are there in soil samples A and B and rest of the samples shows very less number of colonies. The actinomycetes population is very high in sample B and less in samples A, C, D & E. There is no actinomycetes colony was found in control soil (Table 3).

Table 3: Total heterotrophic bacteria and actinomycetes count of different soil samples

Sample	Bacteria CFU × 10^{-6}/g	Actinomycetes CFU × 10^{-5}/g
Sample A	7	2
Sample B	6	8
Sample C	2	3
Sample D	1	2
Sample E	3	1
Control	3	0

CFU - Colony Forming Unit.

All the values are represented as mean ± standard deviation (n = 3).

3.3 Identification, characterization and purification of microorganisms:

Through the morphological, phenotypical and biochemical characterization studies there are 10 bacterial species were identified from the predominant colonies. They are: *Bacillus amylolyticus, Bacillus firmus, Pseudomonas putida, Pseudomonas fluorescence, Bacillus subtilis, Bacillus sp., Pseudomonas sp., Achromobacter sp. Staphylococcus sp. and Actinomycetes*. These isolates were subjected to low density polythene degradation assay to find out the potent microbes which involved in LDPE degradation.

3.4 Clear zone assay:

The identified microorganisms are subjected to LDPE clear zone assay. The results of LDPE degradation assay revealed that there is a large clear zone formed in the LDPE powder medium inoculated with actinomycetes (36 mm). The other isolates shows very lesser zone (Table 3). Hence this results strongly proved that Streptomyces species act as a potent degrader of LDPE. The species of the Streptomyces is confirmed further through molecular characterization studies.

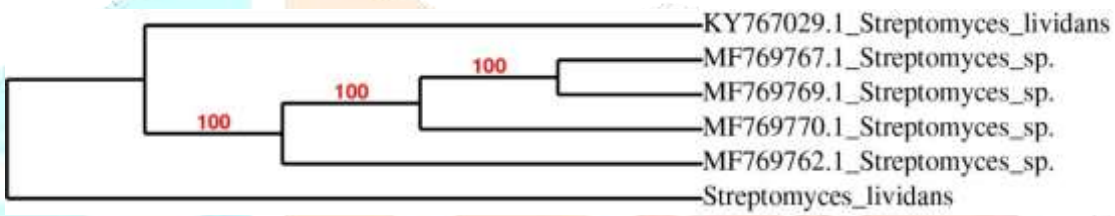
Table 3: Results showing the clear zone formation of the isolates

Isolates	Size of the zone (mm)
Bacillus amylolyticus	15
Bacillus firmus	13
Pseudomonas putida	21
Pseudomonas fluorescence	23
Bacillus subtilis	12
Bacillus sp.	17
Pseudomonas sp.	19
Achromobacter sp.	8
Staphylococcus sp.	11
Actinomycetes sp.	36

3.5 Molecular characterization and phylogenetic analysis:

The potential LDPE degrading bacterial strain *Streptomyces sp*. was further confirmed by 16S rRNA gene sequencing. Based on DNA extracts of strain *Streptomyces sp*., 16S rRNA gene (1404bp) was amplified by PCR using 35 cycles. The bacterial 16S rRNA gene sequences were aligned with Blast search of NCBI databases and constructed phylogenetic tree (Fig. 1).

Fig. 1: Phylogenetic tree of potential LDPE degrading strain, *Streptomyces lividans*



4. DISCUSSION:

Soil quality is the ability of a soil to be functional and to promote the plant, animal and human life. Soil quality is the output of the interaction of the soil with plant, animal, microorganisms and micro climatic factors in a specific area or locality (S.S. Kekane *et al*., 2015). The soil keeps a unique balance among its physical, chemical and biological factors and this unique balance is determined by environmental and anthropogenic activities (Ku. Smita Tale and Sangita Ingole, 2015). Hence studying soil quality parameters help to find out its impact over the growth and multiplication of living organisms in it, especially microorganisms. (M.H. Bearea *et al*., 1997).

Color is one of the most important characteristics of the soil. The organic content, climate, mineral, microbial activity and pollution influence the soil color. The blackish color of the soil samples A and B are fertile and it may contain rich amount of organic matter and iron (J. A. Shields *et al*., 1968).

The structure and function of the soil is controlled by particle size and the size of the particles determine the soil texture. The amount of soil particles are dispersed in a liquid medium based on the size is called particle size distribution and it is used to find out the texture of the soil. The soil texture is controlling the water percolation and microbial activity of the soil (Miguel Ángel Martín *et al*., 2018).

The pH of the soil samples A, B, C, D and E are significantly differ with other control. The pH influence the macro and micro nutrients present in the soil sample and support the microbial growth (Pavan M. Kadam, 2016). The measure of the amount of salts in soil is called as Electrical conductivity (EC). It is an important indicator of soil health. The microbial activity of the soil is decreased by increased EC of the soil (Arushi Makkar *et al*., 2018). Soil sample D may shows lower microbial activity due to its high EC content.

Organic carbon is one of the important macro nutrient which support plant and microbial growth. The organic carbon content of the sample B is higher than other sample and control. The higher organic carbon may help the microbial activity of the soil.

Low amount of nitrogen in soil exist as available form and remaining nitrogen exist as organic form. The microorganisms present in the soil can convert organic nitrogen to available form of nitrogen (Chandak Nisha *et al*., 2017). Hence the amount of soli nitrogen highly influenced by the activity of soil microorganisms. The sample D may shows lower microbial activity than other soil samples due to the presence of low nitrogen content whereas soil sample B may show higher microbial activity due to its higher nitrogen content.

Soil micronutrients such as iron and manganese are essential for plant growth. The level of these micronutrients may high or low due to soil pollution or excess utilization by plants (Deepmala Satpathy, 2014; Arvind K. Shukla and Sanjib K. Behera, 2019). The soil macro and micro nutrient level may be increase or decrease based on the type of pollutants dumped in the site. These macro and micro nutrient level highly influenced in the growth and activity of soil microorganism. The results of physicochemical analysis of soil revealed that the soil sample B has the optimum quantity of nutrients and pH, this may help the higher microbial activity in soil sample B.

There are many bacterial and actinomycetes colonies present in the soil samples B. This result could give us clear idea that the optimum pH level and nutrient content of soil sample B may be the reason for excess population of actinomycetes in soil sample B. All isolates were subjected to clear zone assay to find out the potential LDPE degrading microbial species.

The clear zone assay result shows that there is a large clear zone (36 mm) is appeared in the petri plate inoculated by actinomycetes species isolated from soil sample B. In order to find out the species name Streptomyces strain was subjected to phylogenetic analysis and the result revealed that the sequence aligned has 97% similarity with *Streptomyces lividans*. Through these findings we could confirmed that the microbial strain isolated from the sample B is a *Streptomyces lividans* belongs to actinomycetes family and it may potentially degrade low density polythene under laboratory condition.

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