JIRET Vol.: 01 ll Issue 01 ll Pages 01-35 ll April 2017

Exploration of Plastic Degrading Actinomycetes from Deccan Trap of Karnataka

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Abstract: Plastic has an increasing range of latent application and are driven by the growing use in packaging, storage, etc. Plastic wastes accumulating in the environment are facing an ever increasing ecological menace leading to a search of eco friendly type. Due to a lacunae in finding the safe Low Density Polyethylene (LDPE) and considering their abundance in the environment along with specificity in attacking plastics, biodegradation of plastics by microorganisms seems to be the most effectual process.

Actinomycetes in general and in specific being a free living, saprophytic bacteria and ubiquitous in nature have a great repository of extracellular secondary metabolites especially enzymes leading to the industrial and medical importance along with plastic degrading capacity. In the present study, five micro organisms being collected from 22 soil niche of varying habitats in and around Karnataka found to be quite promising in preliminary screening employing minimal salt media with incorporation of LDPE sheets (2cm X 5cm) followed by 1 month incubation period at 140 rpm in room temperature in an open shaker., In comparison to other Streptomyces plastic degradation ability (12% degradation for 2 months) recent result of the study showed 16% degradation after 1 month revealing a promising attribute to be achieved in near future by further studies which is in progress.

Keywords: Streptomyces, biodegradation, LDPE, screening, incubation

1 INTRODUCTION

Plastic accumulation in the environment is at a rate of 25 million tons per year. The fact that these organic polymers in the environment and the time required for the total mineralization to CO_2 is yet to be fully understood. Actinomycetes play an important role in the recycling of organic compounds and are able to degrade complex polymers (1). Plastic is a common term used to include all sorts of polythene (polyethylene) and polyvinyl chloride materials. Among these plastics, low Density Poly Ethylene (LDPE) is used mainly as carrier bags, zip bags, in agriculture and other industrial applications that constitutes the major portion of waste problem management. LDPE is chemically inert and microbial attack resistance as it is hydrophobic and absence of carbonyl and hydroxyl groups in it (9). The main effects of land pollution caused by plastics is the blockage of rain water from getting underground, thus reducing ground water level. These plastic wastes can be disposed by either recycling or incineration. Recycling is not widely used now because of economical allowance. Even incineration causes air pollution as combustion of plastic in mass will release highly toxic organic compounds in to the atmosphere. These issues raise alarm regarding the use of plastic materials. Since, the plastics have become an vital part of modern life. It is not possible to discontinue the use of plastic and therefore, there is an urge in need of biotechnological/ microbiological approaches to solve these environmental challenges related to plastic. Reports are there on plastic degrading microbes in general and Streptomyces in specific as observed by literature survey (Deepika and Jaya Madhuri,2015; Midhun kumar and Girija Shankar,2015; Shivan,2011; Ikhada and Tsuji,2000) Disintegration of LDPE and subsequent degradation of polymers by using Streptomyces is the main strategy in the present study. Furthermore, actinomycetes generally plays an important role in degradation of plastics because of their extracellular enzyme synthesis and unique metabolism to degrade complex materials (17).

The phylum actinomycetes is one of the most dominant phyla in the bacterial domain (2). They are gram positive bacteria having high Guanine and Cytosine content in their DNA (21) and were considered to be an intermediate between bacteria and fungi . Actinomyetes dominate the microbial life in soil. As many actinomycetes share characteristics with fungi, including shape and branching properties, spore formation and secondary metabolite production (21) most of the antibiotics are obtained

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from this group of organisms. Many plastic degrading actinomycetes strains were already identified. The ability of lignocellulose degrading *Streptomyces badius 252, Streptomyces setoni 75, and Streptomyces viridisporus T7A* strains (1).

Work on plastic degrading actinomycetes especially Streptomyces being screened from harsh ecological niche such as thermophilic vents, slatterns, swampy marsh etc. are quite unexplored habitats for the same study. Therefore, the present study is aimed at screening of a most potent Strptomyces having fast plastic biodegrading ability to suffice the need in land fields and composts.

2. MATERIALS AND METHODS

2.1. Materials

Low Density Poly Ethylene (LDPE) zip bags were collected and cut into pieces of measurements 5cm X 2cm (3)

1.1 Collection of soil samples

Samples were collected from different locations like organic waste dumped behind SIT boys hostel, Tumakuru, scrapings from *Millingtonia hortensis* (Local name: Aakasha mallige) tree near coffee shop SIT, Tumakuru and termite heap on the tree near Devarayanadurga, Tumakuru, Bendru Theertha hot springs, Mangaluru and many other places in Karnataka which is their profound habitat. These sampling sites are free from human activities. The samples were collected from 2-3cm depth and were kept for drying at room temperature (3) followed by continues stirring in an open shaker (Remi) at 100rpm for 5 days. The formation of a layer of mycelia on the wall of conical flasks contains the organisms (22).

2.2. Isolation and Identification of actinobacteria

Serial dilution (3) of the top clear layer was performed from the sample flasks kept on open shaker for incubation. Standard dilutions 10^{-3} and 10^{-4} of 0.1ml (13) were plated on starch casein agar (SCA) medium (solublestarch-10g, casein-0.3g, KNO_3-2g, $Mg_2SO_4.7H_2O$-0.05g, $CaCo_3$-0.02g, $FeSo_4$-0.01g, K_2HPO_4-2g, NaCl-2g, distilled water-1000ml, agar-20g with pH-7.5) (1) and incubated at 35 C for 7-15 days (14).Followed by incubation, distinct colonies of actinomycetes were identified on the plates based on morphological observations (gram staining)and colony characteristics (23).The selected colonies were coded as AMOc, AMD1c, AMD1s, AMNJ, AMTG pertaining to names of group members, guide and habitat.lly identified colonies of Streptomyces were specially selected from cultured serial diluted petriplates for further sub culturing in SCA slants and kept at -4 C for further studies (13). The isolates AMOc, AMD1c, AMD1s, AMNJ, AMTG obtained were further identified for genus level study employing different ISP media (23) such as:

1) Inorganic salt starch agar (ISP Medium no 4) (HiMedia)

Soluble starch-10gms, Di potassium phosphate-1g, Sodium chloride-1g, Ammonium sulphate-2g, Calcium carbonate-2g, Ferrous sulphate-0.001g, Manganous chloride-0.001g, Zinc sulphate-0.001g, Magnesium sulphate-1g, $D.H_2O$ -1000ml, Agar-20g with pH-7.2.

2) Oatmeal agar (ISP Medium no 3) (HiMedia)

Oat meal-20gm, Trace solution-1ml, $D.H_2O$-1000ml, Agar-18gms with pH-7.2

3) Glycerol Asparagine agar (ISP Medium no 5) (HiMedia)

L- Asparagine-1g, Di potassium phosphate-1g, Trace solution-1ml, $D.H_2O$-1000ml, Agar-20gm with pH-7.4

2.4. Screening of Plastic degrading Streptomyces

Potent strains of Streptomyces as identified tentatively from preliminary studies were screed for LDPE biodegradation ability by standard "Weighing method" (3).

2.4.1. Biodegradation of LDPE by weighing method

The fresh LDPE plastics were collected as mentioned earlier and were dimensionally cut into 5cm X 2cm for about 30 pieces with scissors in aseptic manner. The cut plastic sheets were sterilized with 70% ethanol and dried followed by weighed in digital weighing balance(Omega, Bengaluru). In a series 3 erlen Meyr conical flaks containing 250ml of Minimal salt media (Sodium phosphate-64g, Potassium phosphate-15g, Sodium Chloride-2.5g, Ammonium Chloride-5g, Magnesium sulphate solution-1M-2ml, Calcium chloride solution-1M-0.1ml, 20% glucose-20ml, $D.H_2O$-1000ml with pH-2.0) (10). About 30 pieces of plastics in each flasks were inserted and inoculated with a loop full of primarily identified, selected strains (AMOc, AMD1c, AMD1s) of Streptomyces. These flasks were kept in an open shaker at 140 rpm in room temperature for 30 days and weekly photos were taken to see

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change in turbidity and transparency of media in flasks containing plastics to confirm about plastic degradation by the strain. A control was kept without inoculum to compare the degradability. Followed by incubation, LDPE plastics were taken out, washed with 70% ethanol and dried. The percentage of degradation was calculated by the formula as mentioned below (3).

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

3. RESULTS AND DISCUSSIONS

3.1. Collection of soil samples and isolation of actinobacteria

For successful isolation of actinomycetes from different habitats serial dilution technique is still now preferable although being a conventional one. In the present study, all total from 22 samples (Table 1) being collected 5 strains of Streptomyces were selected based on morphological, as mentioned in Table 2. All the strains are found to be typically dry, powdery with smooth circular margin and with varied aerial and substrate mycelial features representing the genus tentatively as Streptomyces (Gram positive with high aerial mycelial branching).The nature of the collected soil samples are found to be recorded from Table 1 and 2 as blackish brown to white sandy with rough clumpy to smooth texture showing an extreme variation of pH and temperature of the different niche. Also the Gram staining view and sketch representation of the mycelial branching pattern with sporulation features are highlighted in Table 2 with the selected strains of Streptomyces.

3.2.Selection of potent biodegradable Streptomyces strains

Biodegrdation of plastic due to the production of major extra cellular lignocellulose degradation was the main criteria for many of the studies to select a potent strain. There are reports depicting the similar kind of studies as in report of (1) *Streptomyces babicus* 252, *Sreptomyces setoni* 75 , *Micromonospora* sp. (Polyester degrading actinomycetes) (15), Thermophilic actinomycetes like mentioned in (24) .

In the present study, the selected 3 strains (AMOc, AMD1c, AMD1s) of *Streptomyces* sp.(Table 3) are found to degrade plastics 0.21%, 13.75%,16.25% respectively. Reports on plastic degrading Streptomyces by (1) were found to be 20% and 14% by 2 strains of *Streptomyces* sp. Also some of the interesting reports were found on effect of plastic degradation by different periods of incubation such as report by *Pseudonocardia dioxanivorans*, Streptomyces sp. (20,22) etc. The results in the present study are rightly befits with the reports as mentioned in (3) in which they reported 12% degradation in 2 months from *Streptomyces sp.* Other reports mentioned in (6) reported 17.060±1.778% plastic degrdation in 1 month by *Streptomyces* sp.,and in (7) repored 12.04 µg of maximum weight loss for 55days by *Streptomyces sp.,* in (5) 7.8% degradation for 1 monthwere also satisfactiorily befits with present results. Thus, this study shows that the *Streptomyces* sp. has an efficient ability to degrade the LDPE plastics and with an increased percentage when compared with cited reports in the present paper. The reason may be attributed to the choice of potent ecological niches for isolation of strains for the particular study from different habitats of myriad physiological features .

Table 1 : Properties of samples collected from different habitat for isolation of actinomycetes

SAMPLE NO.	PLACE	COLOUR	TEXTURE	PHOTOGRAPH	SPECIAL PROPERTY
1	SIT organic waste dumping slot, behind MG boys hostel.	Blackish red	Rough clumpy		Long time ago dumped soil. pH-8.0
2	SIT coffee shop opp. *Millingtonia hortensis tree* bark inside	Red	Smooth mixed with wood particles		pH was near to basic .
3	Near Devarayanadurga,scraped from the tree.	Blackish red	Rough clumpy		pH was about 8 .
4	Narasimha jharna natural hotsprings, Bidar	White sandy	Rough and smooth		pH was near to 7

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Table 2: Detailed profile of selected stains of actinomycetes (Streptomyces) from different soil samples collected and isolated

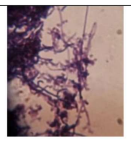
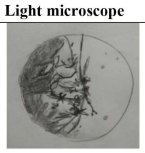
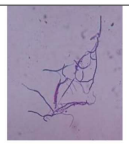
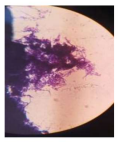
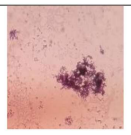
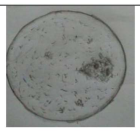
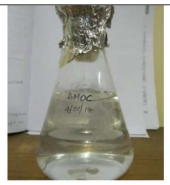
Sl no	Sample code	Area of collection	Type of soil	Nature of soil	Longitute	Latitude	pH and Temp. (^{0}C)	Media used	Light Microscopic view (under 100X)	Sketch of branching pattern and sporulation features under 100X in Light microscope
1.	AMOc	Waste dumped slot behind MG block boys hostel SIT.	Blackish red	Rough clumpy	77.7.35	13.19.32	8.0; 40	Starch Casein Agar and tyrosine gelatin agar		
2.	AMD1c	Near Devarayanadurga,scraped from the tree.	Brown	Rough	85.20.45.	19.35.50	7.0;45	Starch Casein Agar and tyrosine gelatin agar.		
3.	AMD1s	Near Devarayanadurga scraped from the tree.	Blackish red	Rough clumpy	77.7.35	13.19.32	7.0; 40	Starch Casein Agar and tyrosine gelatin agar		
4.	AMNJ	NarasimhaJharna Natural Hot Spring	White sand	Rough and smooth			8.0; 40	ISI Media (Inorganic salt starch Agar)		

Table 3: Selection of potent LDPE degrading Streptomyces strains by visual display in flask study

Sl No.	Strain name	1st day	1st week	2nd week	3rd week	4th week	% degradation (1 month)
1	**AMOC (500ml-media, 30-strips, 5loops culture, 140 rpm)**						
	Initial& Final weight	**1.404g**				**1.401g**	**0.21**
2	**AMD1c (250ml-media, 15-strips, 3loops culture, 140 rpm)**						
	Initial& Final weight	**0.8g**				**0.69g**	**13.75**
3	**AMD1S (250ml-media, 15-strips, 3loops culture, 140 rpm)**						
	Initial& Final weight	**0.8g**				**0.67g**	**16.25**

CONCLUSION

Environmental factors such as light, , moisture, chemical conditions and biological activity showes physical or chemical change in polymer is termed as degradation of plastics (11). LDPE has a wide range of usage in daily human life and so accumulation in the environment. Its nature of inertness that resist degradation is causing serious environmental issues. This naturally growing soil microbe (Actinomycetes) showed great value in degrading LDPE zip bag sheets. This degradation study being *in vitro* showed the potentiality of *Streptomyces* isolate AMD1s based on its capability

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of growing by utilizing LDPE as main carbon source. Hence, further study on degradation of the LDPE by other *Streptomyces* sp. exhibiting high rate of degradation will overlay way to find new technology for degrading the environmentally perilous plastic materials. The study has been showed to conclude that *Streptomyces* isolate AMD1s degrade the plastic materials more fast compared to the available strains upon which work has been carried out.

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