International Journal of Research in Engineering, Science and Management
Volume-2, Issue-3, March-2019
www.ijresm.com | ISSN (Online): 2581-5792

Isolation and Screening of Plastic Degrading Bacteria from Garbage Soil Samples Collected from Bank of Krishna River

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***Abstract*: Plastics are the most commonly used polymer for routine application. These plastics are less degradable in the environment. So the plastics accumulated drastically in the environment and badly effects on environment. Low density polyethylene (LDPE) is a major cause of persistence and long term environment pollution. The main objective of the present study is isolation and screening of bacterial species having capability to degrade low density polyethylene (LDPE). The four bacterial isolates identified from garbage soil collected from bank of Krishna river near Karad city of state Maharashtra in India. These four bacterial isolates identified by M9 medium incorporated with LDPE. The selected isolates are comparatively screened by an agar cup method to find out strong LDPE degraders. This isolates are further tested for the extent of degradation using a film degradation assay. Out of four isolates two species showing high LDPE degrading ability and these are selected for further study. The percentage degradation was found out using the film degradation assay and species S1 showing higher percentage degradation than the species S2.**

***Keywords*: Biodegradation, LDPE, Plastic.**

1. Introduction

A plastic is the general term given to polymerized xenobiotic compounds that are resistant to degradation (Archana et al., 2017). Plastics are polymer that consist of monomers linked together by chemical bonds (R. Vignesh et al., 2016). They accumulate in an environment and creating many problems (Archana et al., 2017). A general estimate of worldwide plastic waste generation is annually about 57 million tone (Shristi Kumar et al., 2007). The microbial degradation of plastic is carried out by enzymatic activities which led to the breakdown of polymer into monomers and oligomers. In this study the biodegradation of plastic(LDPE) bag was analyzed 3 weeks of incubation in liquid culture. Total four isolates are identified in garbage soil collected from bank of Krishna river near Karad city of state Maharashtra in India. Out of four isolates two isolates showing higher percentage degradability than other two. The M9 (Pramila. R & Vijaya Ramesh. K 2012) agar is used for isolation of plastic degrading bacteria. The screening of bacterial isolates done by agar cup method. The bacterial isolates were characterized morphologically by performing gram staining and biochemical test like MR-VP, sugar fermentation etc. The percentage of degradation was evaluated by comparing the initial and final dry weights of polyethylene before and after incubation. (Archana, B & Rajesh, M. 2017)

2. Research methodology

A. *Material:*

Materials are used for research works are: Soil samples-1) Garbage soil (S1) 2) Petroleum soil (S2), M9 agar, M9 broth, Iodine solution, Glassware, Glucose phosphate broth, Tryptone broth, Peptone nitrate broth, Starch agar, Nutrient agar, Nutrient broth, Peptone water, Biochemical test reagent.

B. *Methods*

- *Sample collection:* Soil Sample such as garbage soil were collected from different locations of Krishna river bank in Karad city of state Maharashtra in India and stored in airtight polythene bags.
- *Preparation of LDPE powder:* 1. The LDPE sheets was cut into small bits and immersed in 20ml of xylene 2. It was boiled for 15 min as xylene dissolves the LDPE film and residue was crushed. 3. The LDPE powder was obtained and washed by ethanol to remove residual xylene. 4. The powder was dried in hot air oven at 60 degree centigrade.
- *Isolation of microorganisms:* 1. Soil sample are taken from different location. 2.The different dilution were prepared of each sample in autoclaved distilled water. 3. The different dilution were spread on M9 agar plates containing 0.1%LDPE powder as the only sources of carbon. 4.plates were incubated for 72 hours at 37 degree centigrade. 5. After incubation bacterial colonies was isolated and these isolates were preserved on LB slants
- *Secondary screening for high capacity LDPE degraders:* 1. Secondary screening can be carried out by using agar cup method. 2.Wells are bored in the M9 agar plates and culture suspension was adjusted at O.D. at 0.3(620 nm). 3. Plates were incubated at 37

degree centigrade for 72hours

- *Biochemical characterization of bacterial strain:* 1. Bacterial isolates which degrades the plastic were morphologically and biochemically characterized. 2.Performthe Gram staining of the bacterial isolates. 3.Performthe biochemical tests such as catalase, IMVIC test etc.
- *Film degradation assay or Microbial degradation assay:* 1. Pre weighted plastic strip of size 2*2 cm is taken washed with distilled then by acetone. 2.The strip are dried and dry weight are taken. 3.The strip are transferred in the M9 broth containing isolates from secondary screening. 4. A control flask was maintained with film without any culture suspension. 5. The flask put on the shaker for the 37 degree centigrade 150 rpm for 3weeks. 6. After 3 weeks the LDPE film were weighed again and the percentage of plastic degradation by the microbes was determined.
- *Formula:* The weight loss is measured byusing the following formula: Percentage of weight loss = Initial weight –Final weight / Initial weight *100.
- End product detection test: Sturm test for CO_2 evolution: 1. CO_2 evolution as a result of LDPE biodegradable was determined by sturm test. 2.The sample was added to the culture bottle with medium. 3. Pure bacterial culture obtained was used for degradation of the sample. 4.Bottles are incubate at RT for 4 weeks. 5. After 4weeks of culturing the changes was observed with rising of amount of samples because of co2 evolution.

3. Observation

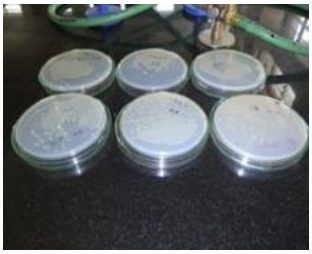

Fig. 1. Isolation and screening of bacterial isolates

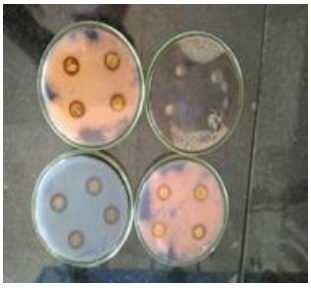

Fig. 2. Secondary screening of bacteria by using agar cup method

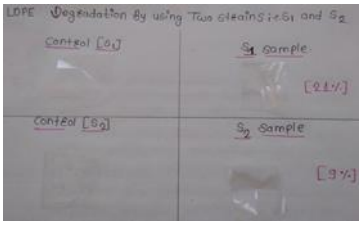

Fig. 3. LDPE degradation by using two bacterial strain S1 & S2

Fig. 4. LDPE degradation by S1 with different innoculum concentrations

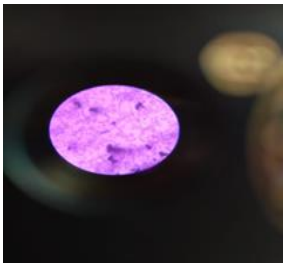

Fig. 5. Gram staining of S1 Strain

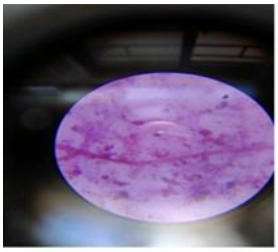

Fig. 6. Gram staining of S2 Strain

Table 1
LDPE degradation after 3weeks

S. no.	Isolated strain	Initial weight (gm)	Final weight (gm)	Percentage of degradation
1.	Control	0.029	0.029	0
2.	S1 sample	0.023	0.018	21%
3.	S2 sample	0.021	0.019	9%

Table 2
LDPE degradation by S1 sample with different quantity of concentration

S.no.	Strain (quantity)	Initial Weight (gm)	Final Weight (gm)	Percentage of degradation
1.	Control	0.030	0.030	0
2.	S1 (2ml)	0.031	0.024	22.25%
3.	S1 (4ml)	0.031	0.018	41.93%
4.	S1 (6ml)	0.031	0.016	48.38%
5.	Sewage sample	0.030	0.019	36.66%

4. Result

A. Isolation and screening of bacterial isolates:

Two bacterial strains were isolated from the collected samples from bank of Krishna river near Karad. These isolates were purified by frequently re-streaking them on M9 agar plates. The primary screening as well as secondary screening can be done.

B. Morphological and Biochemical characterization of bacterial isolates:

Two bacterial strains which were isolated and subjected to morphological and biochemical characterization. From characterization the S1 strain belongs to Micrococcus sp. and S2 belongs to Streptococcus sp.

C. LDPE degradation by S1 sample with different quantity of concentration

Observation from LDPE degradation by S1 sample with different quantity of concentration showing higher degradation at higher concentration.

5. Conclusion

Plastic degrading microorganisms were isolated from garbage soil samples and degradation of plastic strips by these isolated microorganisms was determined. The isolates which shows high opacity was selected and used for further study. The organisms identified was further inoculated into different culture media and their biodegradative ability was determined by loss of weight after a period of 21 days. The plastic strip was degraded by using S1 sample is approximately 21% percentage and by S2 sample is 9%. From this project we conclude that the bacterial strains isolated from different soil samples can have ability to degrade the plastic and we can use the concentrated culture of isolates for degradation of plastics.

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