APPLICATION OF NOVEL THERMOPHILIC BACTERIAL CONSORTIA SCREENED FROM COW DUNG AS AN ECO-FRIENDLY APPROACH FOR THE DEGRADATION OF PLASTIC GARBAGE IN BENGALURU CITY

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INTRODUCTION: Uncontrolled use of plastics, primarily low density and high density polyethylene (LDPE and HDPE) for various purposes such as packaging, transportation, in industry and in agriculture in rural as well as urban areas is a rising concern as the plastic usage surpasses 260 million tonnes per annum globally. The applications and advantages of plastics are many, yet the drawbacks cannot be overlooked. Although there are conventional strategies of plastic disposals such as incineration, recycling and landfills, they have their own shortcomings. Incineration releases hazardous end products, availability of land is a chief issue for landfills, while recycling poses a challenge during the collection of wastes. Biotechnological strategies can effectively degrade polyethylene garbage better when compared to the current conventional approaches and can contribute for long term bioremediation. Studies that have been previously carried out have revealed the use of plastic degrading bacteria isolated from the soil contaminated with plastics. However, the current study focuses on the biodegradation of two forms of plastics, LDPE and HDPE, by means of novel thermophilic bacteria isolated from cow dung gathered from plastic contaminated environments, and formulating a novel consortia constituting different thermophiles for obtaining an efficient degradation of plastics. The present study paves an insight into the utilization of novel thermophilic microbes from cow dung and also proposes that the possible scale-up of these microbes may deliver better waste management techniques for plastics as well as similar types of garbage.

The project is an extension work with modification which was sponsored by KSCST, 37th SPP Programme entitled “Ref. No. 37S0835, Selection and Screening of Microbial Consortia for Efficient and Eco-Friendly Degradation of Plastic Wastes in Urban and Rural Areas of Bangalore carried out by Ms. Megha M, Ms. Meghna N Kini, Ms. Kamath Manali Mukund, Ms. Alya Rizvi and the 38th SPP Programme (Ref No. 38S0142) entitled “Formulation and
Characterization of Novel Plastic Degrading Microbial Consortia Isolated From Various Places of Bangalore City and Study of their Biodegradation Potential carried out by Mr. Vishal M, Ms. Subiya Sultana, Ms. Chandana Jois and Ms. Vidhya Bai K under the guidance of Prof. Sinosh Skariyachan”.

OBJECTIVES:

1. Isolation and characterization of plastic degrading bacteria from cow dung samples habituated to various plastic polluted areas in Bengaluru city.
2. Study of the biodegradation efficiency of isolated bacteria towards various forms of plastics and analysis of ideal environmental parameters for effective degradation.
3. Formulation of the novel thermophilic bacterial consortia and analysis of their degradation potential in various combinations in comparison with previous studies.
4. Evaluation of the biodegradation end products and partial characterization of the enzyme responsible for biodegradation.
5. Molecular characterization of the microbial consortia and study of evolutionary relationship of the isolates and prediction of probable degradation mechanism by Bioinformatics analysis.

METHODOLOGY:

The cow dung samples which were highly contaminated with plastic garbage were collected from nine major hotspots in Bangalore city. The plastic degrading bacteria were screened and enriched. The degradation potential of bacteria was determined by zone of clearance method. The percentage of degradation was monitored by weight loss method. The isolated bacteria were characterized by standard microbiology approaches. The best isolates were used to form a microbial consortia and the degradation efficiency of the consortia was compared with individual isolates. The degradation end products were characterized by FTIR analysis and NMR studies and the degradation efficiency was studied. The surface attributes were studied by SEM and EDS analysis. The best isolates were characterized by 16S rDNA characterization. The partial enzyme identification responsible for degradation was also performed. Further, the RNA secondary structure prediction for analysis of gene stability during degradation activity was studied by Bioinformatics approaches.

RESULTS AND CONCLUSIONS:

Novel thermophilic bacteria from cow dung which has the capability of degrading plastic materials (LDPE and HDPE) were identified by zone of clearance method. When the strips and pellets were incubated for 120 days with the bacterial consortia, the LDPE strips showed a degradation rate of 55% while the pellets degraded by 50%. Likewise, the HDPE strips degraded by 48% while the pellets by 38%. The pure isolates degraded LDPE by 10-14% while HDPE degraded by 13-15%. This is suggestive that bacterial degradation using different consortia collectively, is superior to using a single isolate for degradation. The microbial characterization of the best isolates were carried out and when these isolates were plated on blood agar, all the isolates were established to be non-hemolytic strains. Therefore, the bacterial isolates are regarded as generally recognized as safe (GRAS) and industrial scaling up of such bacteria can be considered as a possible option for large scale removal of plastic garbage. From the biochemical characterization outcomes, the organisms were found to be Pseudomonas spp, Stenotrophomonas spp and Bacillus spp. In the present study, the FTIR spectrum of the test samples were compared
with their corresponding controls and the advent of new absorption bands were due to the formation of C=C moieties in LDPE and C-C moiety in HDPE sample. Further, SEM images of the test samples exhibited the development of cracks, erosion, cavities as well as the adherence of bacterial colonies. Moreover, the formation of a bacterial biofilm and ruptures observed on the surface of strips indicates that microorganisms were involved in the mechanism of biodegradation. EDS analysis showed the decrease in carbon content of the plastics by 5%. The end products analysis by NMR showed that the samples formed methyl group and an aldehyde moiety (as end products) post degradation. The 16S rDNA sequencing and BLAST analysis revealed the novel thermophilic organisms to be *Pseudomonas protegens*, *Stenotrophomonas maltophilia* and *Bacillus vallismortis*. Further, the extra-cellular enzyme responsible for biodegradation was identified as lipase. The RNA secondary structure of the bacteria further proved the stability of the gene sequences during biodegradation mechanism. Thus, the present study paves an insight into the scope and applications of novel thermophilic microorganisms for eco-friendly and rapid removal of plastic garbage.

**SCOPE FOR FUTURE WORK**

The degradation should be carried out for longer time periods and the end products must be further characterized. Field trials must be conducted and industrial scale up of the identified organisms may assist in obtaining permanent solution to plastic pollution.