“SELECTION AND SCREENING OF MICROBIAL CONSORTIA FOR EFFICIENT AND ECO-FRIENDLY DEGRADATION OF PLASTIC WASTES IN URBAN AND RURAL AREAS OF BANGALORE”

PROJECT REFERENCE NO. : 37S0835

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Keywords: Biodegradation of plastic, zone of clearance method, weight loss method, optimization, in silico studies

Introduction:

Plastics are an integral part of our day to day life and are being used in packaging, building materials and for many other purposes (Gnanavelet al, 2012). Due to their stable nature, they tend to accumulate in the environment causing severe pollution (Hemashenpagam et al, 2013; Tserkiet al, 2006; Kim et al, 2007; Ray et al, 2007). Improper recycling and waste management systems in developing countries are majorly responsible for plastic pollution (Jayasiriet al, 2013). Studies conducted indicate that several efforts have been made to eradicate plastic pollution in different part of India (Kathiressan, 2003; Hemashenpagamet al, 2013; Jayasiriet al, 2013). Bangalore considered as the cosmopolitan city of India, generates about 4,000 tons of garbage every day. Due to the lack of technologies available to degrade plastic in an eco-friendly manner, biodegradation is an upcoming trend in this field. Microorganisms which include bacteria and fungi can be employed to degrade plastic (Gu et al, 2000; Shimao, 2011). Sites which are contaminated are rich source of novel and adaptable microorganisms capable of biodegradation of plastic (Narancica, 2012). Enzymatic degradation is one of the most attractive plastic waste treatment methods currently available. These microbial enzymes are successful in increasing
the rate of biodegradation of plastics very effectively without having a detrimental impact on
the environment (Bhardwaj et al, 2012). Biodegradation is proven to be eco-friendly because
of its non-toxic end products like CO₂, H₂O and CH₄ (Shah et al, 2008). Moreover, the fact
that different microorganisms have different degradation capacities gave birth to the idea of
using a microbial consortium for stable and efficient degradation. This way, the plastic is
degraded in a more efficient way and it also saves time (Wu et al, 2010; Shah et al, 2008).
Plastic degradation as a process is extremely slow and this poses as a setback because in
order to confirm the presence of enzyme in the culture and study its nature under
experimental conditions, the experiment needs to be carried out for years to obtain
confirmatory results. In silico studies of the enzyme substrate relationship thus plays a very
important role as the confirmation of enzyme-substrate interactions can be concluded as a
crucial result. This study aims at providing a promising and eco-friendly solution by using
certain isolates, enzymatic degradation and microbial consortia for efficient elimination of
plastic from the environment thus, making Bangalore city a better place to live in.

Objectives:

- Isolation and screening of microbial consortia from the samples collected from
  selected plastic manufacturing industries in Rural and Urban districts of Bangalore.
- Enrichment and microbial characterization of plastic degrading bacteria isolated from
  collected samples.
- Optimization of the environmental parameters conditions for effective and efficient
  microbial plastic degradation.
- Identification and characterization of enzyme for plastic degradation.
- Study of the inhibitory activities of enzymes towards various polymeric substances
  present in plastic by computational simulation studies.

Methodology:

For the collection of samples, plastic polluted lakes in and around urban and rural areas of
Bangalore were selected. Soil and water samples were collected from these polluted areas.
The samples collected were enriched by culturing them in nutrient broth. For the first step of
the determination of plastic degradation we considered the degradation of polyethylene
glycol which is used as a plasticizer to mould plastic materials. This procedure was carried
out by zone of clearance method where PEG was used as the sole carbon source in minimal
media containing salts of ammonium and potassium and the zone of clearance around the colonies was observed by staining with coomassie blue. We further carried out the determination of plastic degradation by weight loss method where we incorporated plastic strips and pellets in liquid broth as the sole carbon source. The enriched samples were inoculated in the liquid broth and incubated for a period of 120 days. The weight loss was determined periodically. For comparative analysis, bacterial cultures which are previously reported to degrade hydrocarbons such as Pseudomonas putida, Pseudomonas stutzeri and Bacillus subtilis were obtained from MTCC and these were checked for plastic degradation by the same above procedures. This was followed by isolation and screening of plastic degrading bacteria from our samples where we grew our enriched samples on solid minimal media which contained plastic powder as the sole carbon source. Serial dilution method was performed for easier isolation of colonies. Bacteria which grew on this media were characterized by standard microbiology protocol. Optimization of culture conditions such as temperature and pH was performed for these isolates by incubating them on solid media in varying ranges of pH and temperature. The temperature range was 4°C to 45°C and pH was 5.0 to 9.0. This was further followed by formulating a consortium where the two characterized organisms were incubated together under optimized conditions for a period of 30 days, on a shaker incubator with plastic strips as the sole carbon source. The weight loss of the plastic strip was assessed periodically. We also speculated the enzyme responsible for plastic degradation to be lipase. For this, we grew our pure cultures with Tween 80 as the sole carbon source on solid media to confirm lipase presence which was followed by growth of pure cultures with olive oil as a sole carbon source in liquid minimal media, for enzyme isolation. Tests for the presence of protein was performed by Lowry’s method in the broth supernatant before and after standard enzymatic purification techniques like centrifugation, ammonium sulphate precipitation (20-40% for lipase) and dialysis. However, enzyme activity could not be confirmed due to very low quantities of enzyme present. Finally, in silico studies were performed to study the enzyme substrate relationships between the polymer molecules and lipase enzyme. The polymer molecules of oxidized polyethylene and polystyrene were docked with lipase open conformation and extracellular lipase crystal structures which were retrieved from PDB database by using docking software PatchDock and PyMOL visualization tool.
Results And Conclusions:

In plates with PEG as the carbon source, white coloured, opaque and translucent circular colonies were observed. Upon staining, a very small yet distinct zone of clearance around most of the colonies was observed indicating the utilizing of PEG. We could conclude that PEG could be utilized as the sole carbon source and degraded in all the samples we had collected. Growth of colonies was observed within 72 hours of incubation. In weight loss method, we were able to record a 20% to 50% decrease in the weight of plastic strips and a 3% to 5% decrease in weight of plastic pellets over an incubation period of 120 days. From comparative analysis with cultures obtained from MTCC, it was found that all the three bacteria showed degradation activity albeit the degradation was observed to be very slow when compared to our samples.

Plastic powder incorporated in solid minimal media as sole carbon source showed the growth of colonies upon 72 to 96 hours of incubation. Growth of colonies in our cultures indicated that the bacteria presented in our samples were capable of utilizing plastic as their sole carbon source. Many studies have revealed that plastic is most commonly degraded by Pseudomonas spp. which is a Gram negative rod (Kyawet al, 2012; Nanda et al, 2010; John et al, 2012; Ushaet al, 2011). Characterization of our isolates was performed by standard microbiology protocols such as morphological, physiological and biochemical methods. All our isolates were found to be Gram negative. Among the 12 isolates, only 2 showed affirmative results for all the biochemical tests and these isolates were of Hebbal soil and water samples. These two were confirmed to be Pseudomonas spp. We observed that these two isolates showed maximum growth under extremely alkaline conditions of pH 9.0 and an optimum temperature of 37°C proved to be the best temperature to achieve maximum growth. Further, the weight reduction of plastic strips under these optimum conditions was found to be around 30% over 30 days of incubation period. We were able to confirm the presence of extracellular lipase in the presence of Tween 80 as a zone of clearance was observed around the colonies. Presence of protein of broth supernatant with olive oil as carbon source was determined to be 960 ug/ml in crude extract whereas, it was found to be 120 ug/ml after 20-40% ammonium sulphate precipitation and dialysis indicating very low quantities of lipase. Enzyme activity could not be confirmed due to this reason. The consortium, having been incubated at the optimized conditions showed 20% degradation upon 30 days of incubation
period. In silico studies showed promising enzyme substrate relationships. Docking of oxidized PE with lipase open conformation showed a score of 3544 and its best minimum binding energy was determined to be -209 kcal/ J. Following this, polystyrene was docked with this enzyme and the best minimum binding energy obtained was -397.91 kcal/ J and the score of the complex was found to be 4890. The interaction between the ligand and binding pocket of the enzyme was found to be covalent and not due to hydrogen bonding thereby accounting for the low minimum energy value. When oxidized polyethylene was docked with Pseudomonas extracellular lipase enzyme, the best minimum binding energy and the score was obtained to be -166.94 kcal/ J and 4022 respectively and seven amino acids were found to interact with the ligand. Polystyrene was then docked with the extracellular lipase enzyme to obtain a minimum energy of -359.04 kcal/ J with a score of 4644.

From this study, it is evident that bacteria found in the areas of plastic contamination are capable of degrading hydrocarbons. These bacteria if formulated in a consortium seem to be a reliable method for plastic degradation. Also, the direct application of enzymes for the degradation of plastic might be a possibility. Thus, we can conclude that biodegradation of plastic can serve as a promising method for the processing and subsequent elimination of plastic from the environment.

**Scope for Future Work:**

Although our study paves a significant insight towards plastic degradation, more studies would be appropriate to achieve scientific standards and clarity. We would like to propose some of the concepts as future perspectives:

1. More number of bacteria capable of degrading plastic can be characterized from these polluted areas in and around Urban and Rural areas of Bangalore in order to target the problem of plastic pollution.

2. Various other sample collection strategies can be employed for isolating bacteria under micro-aerophilic or anaerobic conditions which might have the property of faster degradation. If possible, a consortium of symbiotic bacteria, fungi and actinomycetes can be formulated to achieve a very high rate of degradation.

3. 16S RNA characterization of the isolated species of bacteria in this study needs to be carried out to identify the organisms at species level.
4. Validation of the in silico studies of the enzyme-substrate interactions of lipase and plastic are to be done under experimental conditions.

References:


