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Association of Microbes with Plastic Degradation & its Thermal Resistance from the Environment of Karachi

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Abstract: Plastic has been used in various applications in daily routine human life. It is durable and its durability is a cause of environmental pollution so it's time for the development of those materials that do not burden the environment. There are various methods used for the removal of plastic wastes but all of these have some side effects and costly so there is a need to devise a cheap method with sustainable and environment friendly technologies. Pakistan is the developing country and there is lot of issues to be resolved including the environmental pollution. In this connection our study emphasized on the isolation of plastic degrading microbes from environmental plastic of Karachi with reference to the relative resistance towards temperature at 40, 50 and 60 °C was assayed. 80 old garbage plastic were analyzed numerous plastic degrading bacteria were isolated including *Pseudomonas* which was about 18.75%. It was observed that all of these (100%) isolates showed resistance at the temperature ranging from 40-50 °C, 57.5% species were resistant at 50-60°C and 53.42% isolates were resistant at 60°C. This conductive study implies great importance in the field of environmental biotechnology for the improvement of environment especially in developing countries because of its cost affectivity towards the elimination of hazardous compounds.

Key words: Environmental Pollution · Biodegrading bacteria · Hazardous compounds · Plastic · Sustainable technologies

INTRODUCTION

Most beneficial synthetic manmade substances obtained from resources of fossil fuel, is plastic. Plastic are cheap, light weight, unbreakable and extremely durable that's why widely used for many applications including food, clothing, shelter, transportation e.t.c. from the past thirty years [1]. Synthetic plastic are used worldwide approximately 30%. In Pakistan the growth rate of plastic industry is 12% per annum and approximately 600-700 plastic processing units are present in all over the country [2]. Annually large number of polluting material including hydrocarbons and heavy metals are dumped into the environment [3]. The first line of sink for petroleum product pollutants are soil and water and these areas are in danger. Land become infertile due to oil spills and gets damaged because of suffocation and toxicity of crude oil [4].

Plastic is a broad name given to many polymers [5] for example high levels of organic pollutants, toxic chemicals such as polychlorinated biphenyls (PCBs), nonylphenol (NP), organic pesticides, including dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), poly brominated diphenyl ethers (PBDEs) and bis phenol A (BPA) are present in plastic particle [6-8]. Polyethylene can be degraded by different molecular mechanism including the chemical degradation, photo degradation and biological degradation [9].

At least 75 different genera of bacteria accumulate Poly(3-hydroxybutyrate) [P(3HB)] as an intracellular granule, provided carbon and energy resources under growth limiting conditions (1). Polyhydroxyalkanoates are considered to be biodegradable in the environment and posses thermoplastic behavior. In accordance with the substrate, some bacteria's are able to give yield

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of copolymers of various hydroxyalkkanoates. Fluorescent *Pseudomonads* are capable of synthesizing poly (3-hydroxyalkanoates) (PHAs) with longer side chains [10]. Previous studies showed that with pure culture studies of various bacterial and fungal species has been able for the biological degradation of polyethylene films such as *Alcaligenes, Amycolatopsis sp., Bacillus, Comamonas acidovorans, Flavobacterium,, Micrococci, Pseudomonas, Streptococcus, Streptomyces sp., Staphylococcus, Xanthomonas* and the fungal species are *Alternaria, Aspergillus, Aureobasidium, Fusarium, Phanerochaete sp., Penicillium, Spicaria sp.* and extracellular enzymes of *Poecilomyces* [11].

Biodegradable plastic consists of long chain of carbon, oxygen, nitrogen, sulfur and phosphorous atom. There are some bacteria's that have the enzyme, oxygenase that break the plastic by disrupting the local electric charges [12]. Degradation of existing plastic by microorganisms as well as development of biodegradable plastic is under consideration. Most of the natural organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses are degraded by most of the microorganisms [13].

Different studies were conducted for effective degradation of plastic by microorganism and hence biodegradable plastics are environmentally friendly [14]. World demand of plastic is increasing continuously and, because of more durability and visibility than any other solid waste public and media pays more attention on its removal from the environment [2]. For this purpose this study was conducted in which plastic degrading microbes were isolated from organic rich soil of different areas of Karachi, Pakistan and also the effect of extreme temperature on growth of the isolated microbes were considered.

MATERIALS AND METHODS

Isolation of Microbes from Old Polythene/plastic Wastes: Washed and scrapped the collected plastic samples several times with the help of sterilized water to remove the soil and soil microbes adhering on the plastic. After that these sample cut into small pieces with blade. For the isolation of plastic degrading microbes these small pieces of sample were inoculated on Thorton's agar medium [15] at 28±2°C for 2 to 7 days till the appearance of new specific bacterial colonies. The colonies were further streaked on the agar medium to get pure culture of polythene/plastic loving bacterial isolates. The isolated bacterial cultures were tested for their behavior towards gram staining as described by Benson [16]. For further identification of gram positive and gram negative bacteria we have performed different biochemical tests including; catalase, oxidase, sugar fermentation test, TSI utilization, citrate utilization, IMVIC test, Urease test and hemolysis on blood agar by gram positive bacteria.

Temperature Resistance: Broth cultures of bacterial isolates were exposed to 40 °C, 50 °C and 60°C for 30 minutes at water bath. After treatment with extreme temperatures broth culture cooled down at room temperature, each isolate was (Loop full) inoculated on thornton's agar medium. Plates were incubated at 28±2°C up to 2-15 days [16]. Observation was recorded for survival and growth of inoculums. Surviving isolates were repeated for their confirmations.

RESULTS AND DISCUSSION

Although numerous studies has been done for the isolation of plastic degrading microbes from different areas of the world; as Pakistan is the developing country facing number of issues and one of them is the environmental pollution whose major cause might be the non-degradable plastic. For consideration of this issue a study has been carried out in which 80 different old plastic samples collected from different sites of Karachi, Pakistan; 63.75% plastic degrading microbes were isolated. All the isolates were characterized and tested for gram staining for identification. The microbes that were isolated from plastic samples including *Pseudomonas (18.75%)* that was obtained with maximum percentage apart from it other microorganisms which were isolated including *Agrobacterium, Bacillus, Flavobacterium, Micrococcus, Streptococci, staphylococcus, Xanthomonas* and fungus *Candida*. The data revealed from gram staining represents 73 bacterial cultures, 35 showed Gram negative and 38 showed gram positive behavior and there percentages were mentioned in Table 1.

The second step of this study was to evaluate the tolerance ability of isolated plastic degrading microbes against increased temperature (Table - 2). Previous studies showed that plastic degradation abilities of microorganism can be enhanced by extreme temperatures [10] as the results indicates that the 53.42% of isolated microorganisms were resistant to extreme temperature of 60°C. Discrimination of microbes with

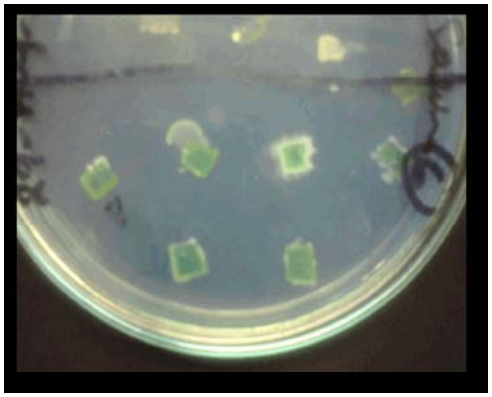

Fig. 1: Growth of plastic loving bacteria on Thorton's Agar Medium

Fig. 2: Growth of Microorganism at 40°C

Fig. 3: Growth of Microorganism at 50°

Fig. 4: Growth of Microorganism at 60°C

Table 1: Prevalence of Plastic Degrading Microbes from the Environment

S. No.	Name of Isolates	Prevalence
01	*Bacillus*	12.5%
02	*Agrobacterium*	5%
03	*Flavobacterium*	10%
04	*Pseudomonas*	18.75%
05	*Streptococci*	16.25%
06	*Micrococcus*	11.25%
07	*Staphylococci*	7.5%
08	*Xanthomonas*	10%
09	*Candida*	08%

Table 2: Effect of Temperature on Plastic Degrading Microbes

S.No	Temperature Range °C	Growth of Microorganism	Percentage
1	40	All 73 isolates	100%
2	50	42 isolates	57.53%
3	60	39 isolates	53.42%

extreme temperatures is beneficial to generate effective culture which sustain and withstand such climatic conditions for longer period of time [17].

Biodegradation of plastic can be monitored by different characteristics one of which is the accumulation of biomass indicating the utilization of plastic as a sole source of carbon which is observed by the growth of microorganisms including bacteria and fungi [18]. These type of microorganisms have strong enough to degrade the plastic material [19]. Past studies revealed that the *Pseudomonas* could be one of the active hydrocarbon degrading microorganisms as in this study 18.75% isolated microorganisms were *Pseudomonas*. Currently for the disposal of plastic on large scale three methods have been used landfill, incineration and recycling. Landfills require large portions of land space for disposal of plastic as well as dangerous secondary pollutants has been released in the environment from landfills and incineration. Recycling is relatively inefficient. Biodegradation is an attractive option for efficient disposal of plastic waste [20].

CONCLUSION

Biodegradable plastic could be novel means of solving the plastic disposal issue. Recent study showed the biodegradation of plastic waste with bacterial species specifically the *Pseudomonas* and the *Streptococci.*

In order to overcome plastic waste, biodegradation could be the safe option but further studies are needed to devise a commercial protocol and its confirmation at commercial level.

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