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Isolation and Selection of Stress Tolerant Plastic Loving Bacterial Isolates from Old Plastic Wastes

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Abstract: Fifty three samples of old polythene/ plastic wastes were collected from various organic matter rich sites of 4 districts of Chhattisgarh, India. Eighty six plastic loving bacterial isolates were isolated from these samples. Further, these isolates were tested for their stress tolerance behavior. The stress tolerant behavior study revealed that 35 out of 86 bacterial isolates showed thermal shock tolerance up to 60°C for 30 minutes. Similarly, 27 out of 86 isolates showed salt tolerance up to 50,000 ppm. Most of the samples showed the presence of *Pseudomonas, Xanthomonas, Flavobacterium, Agrobacterium,Bacillus spp* which might be the most associative bacterial flora with the samples of old polythene/ plastic wastes

Key words: Isolation, *Pseudomonas*, Temperature tolerance, Salt tolerance, Plastic degradation.

INTRODUCTION

In recent years, the quest for clean environment has stimulated interest in finding microbial methods of wastes disposal including degradation of hydrocarbons like petroleum and plastic wastes etc. A vast number of pollutants and waste materials containing hydrocarbons and heavy metals are disposed into the environment annually. Approximately 6 x 10^6 chemical compounds have been synthesized with thousands new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals including synthetic hydrocarbons like HDPE, LDPE plastic are in commercial use.

The growing demand and supply of fuel oil and new chemicals by the industrialized society of the twenty first century has placed increasingly higher stress on the natural environment [1]. Large amount of petroleum products enters the environment by various means.

Soil and water represent the first line of sink of petroleum product pollutants. The areas which are getting polluted by petroleum products are in danger because the land gets damaged and becomes infertile due to oil spills and this prevents growth of crops for varying period of time. The damaging effects are due to suffocation and toxicity of the crude oil [2]. Bacteria, which exist ubiquitously in the environment, have a great potential to degrade these crude oils and other petroleum products [3].

As we know the micro flora associated with any substance has the potentiality to degrade the material on which it grows to fulfill its nutritional requirement. Hence, in order to isolate and select bacterial isolates capable to degrade petroleum products, the samples were collected from various petroleum products polluted sites such as organic matter rich very old garbage dumps, places near transport agencies, petroleum refilling centers, motor workshops, garages, industrial areas, building construction sites, vehicle repair workshops etc.

MATERIALS AND METHODS

Sample collection sites: Fifty three samples of old plastic wastes were collected from various organic matter rich old garbage dumps sites near to transport agencies, petroleum refilling centers, motor workshops, garages, industrial areas, building construction areas, vehicle workshops, repair shops etc of Raipur, Durg, Mahasamund and Dhamtari districts of Chhattisgarh, India. These samples were kept as such in the sealed polythene bags to prevent loss of moisture and volatile petroleum products. Highly degraded part of samples were considered for microbial isolation studies.

Isolation of Microbes from Old Polythene/plastic Wastes: The collected polythene/plastic samples were washed and scrapped several times with care to remove the soil and

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soil microbes adhering on their surface with the help of sterilized water and blade, after that these samples cut into small pieces. In order to isolate plastic loving closely associated bacterial isolates, the small pieces of sample were then inoculated on Thornton's agar medium [4] 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.

Characterization Study:

Gram Staining: The isolated bacterial cultures were tested for their behavior towards Gram staining as described by Benson [5].

Colony Characters: Isolated bacterial isolates were grown on Petri plates and observed for their morphological characteristics [4].

Stress Tolerance Study:

Temperature Tolerance Study: Broth cultures of the 86 bacterial isolates were exposed to 40°, 50° and 60°C for 30 minutes at water bath. After thermal shocks when broth culture cooled down up to room temperature, each isolate was (Loop full) inoculated on Petri plates containing Thornton's agar medium with four replications. Control was also maintained for comparison. All the inoculated Petri plates were incubated in BOD incubator at 28±2°C up to 2-15 days [5]. Observation was recorded for survival and growth of inoculum. Promising isolates were repeated for their confirmations.

Salt Tolerance: The bacterial isolates were inoculated separately on specific agar medium containing 10,000, 20,000, 30,000, 40,000 and 50,000 ppm salt concentrations (NaCl). Four replications of the plates for each isolate were maintained along with control. After 48 hrs of incubation, observations for survival and growth of inoculum were started [5]. Promising isolates were repeated for their confirmations.

RESULT AND DISCUSSION

Eighty six bacterial isolates were isolated from 53 samples of old polythene/ plastic wastes, as presented in Table 1. All the isolates were characterized and tested for Gram staining and their tolerance capacity towards high temperatures and salt concentrations.

Characterization Study: The data related to Gram staining behavior of the bacterial isolates revealed that among 86 bacterial cultures 51 showed Gram negative and 35 showed Gram positive behavior as mentioned in Table-2. It is clear from the present investigation that most of the isolates were found Gram negative they were further classified into 20 groups on colony morphological aspect (Table-3).

Behavior of Isolates under Stress Conditions: Study related to stress tolerant behavior of the bacterial isolates revealed that 35 out of 86 bacterial isolates showed temperature tolerance up to 60°C for 30 minutes (Table-4). Similarly, 27 out of 86 isolates showed salt tolerance up to 50,000 ppm, salt (Table-5). It was also emphasized that identification of stress tolerant microbes is certainly useful in order to formulate effective cultures which can survive and persist for longer period and work more efficiently under such climatic conditions [6].

Pseudomonas spp. is commonly recognized as being capable of degrading hydrocarbons including polythene/ plastic. Number of *Pseudomonas* strains capable of degrading petroleum were isolated by several scientists from areas receiving petroleum products as waste discharge. Apart from *Pseudomonas spp.* most of the samples also showed the presence of *Xanthomonas, Flavobacterium, Agrobacterium,Bacillus spp* which might be the most associative bacterial flora next to *Pseudomonas spp* with the samples of old polythene/ plastic wastes. Further these microbial isolates are being screened and tested to find out their efficiencies to degrade plastics.

Table 1: Surveyed area from where polythene/ plastic waste samples were collected for plastic loving bacterial isolation

Surveyed Districts	No. of Samples collected	Types of soil associated with samples	No. of bacterial isolates	Important bacterial genera associated with samples
Raipur, Durg, Mahasamund,Dhamtari	53	Vertisol, Alfisol, Inseptisol Entisol	86	*Pseudomonas,*Xanthomonas, Flavobacterium,Micrococci Streptococcus Staphylococcus Bacillus

Table 2: Gram's staining reaction of plastic loving bacterial isolates associated with polythene/ plastic wastes

Gram +ve bacterial Isolates	Gram-ve bacterial Isolates
35	51

Table 3: Morphological characteristics of colonies of the plastic loving bacterial isolates, isolated from plastics/ polythene waste samples

		Colony Morphology				
S.N.	Bacterial Isolates:PB No.	Colour	Form	Margin	Elevation	Density
1	1, 11, 16,	yellow	circular	entire	raised	opaque
2	2, 17	white	circular	serrate	flat	translucent
3	3, 13, 20, 21, 22, 38, 84	yellow	circular	entire	convex	opaque
4	4	yellow	rhizoid	filamentous	umbonate	translucent
5	5, 41, 64, 66	white	circular	entire	umbonate	translucent
6	6, 7, 58, 82	waxy white	circular	entire	convex	opaque
7	9, 23, 27, 32, 45	yellow	irregular	undulate	umbonate	opaque
8	10, 44, 75	white	circular	undulate	convex	opaque
9	12, 76, 78, 84	orange	circular	entire	convex	opaque
10	14, 77	yellow	circular	serrate	umbonate	opaque
11	8, 15, 19, 30, 31, 33, 34, 35, 39, 40, 46, 5053, 55, 57, 65, 69, 73, 81, 83	white	circular	entire	convex	opaque
12	47, 49, 56	white	circular	entire	flat	opaque
13	18, 36, 37, 63	white	circular	entire	flat	translucent
14	24, 25, 26	yellow	irregular	undulate	raised	opaque
15	28, 61	yellow	circular	entire	convex	translucent
16	29, 72, 79, 80, 85	white	circular	entire	raised	translucent
17	42, 62	yellow	irregular	lobate	flat	opaque
18	43, 48, 51, 52, 54, 59, 60	white	irregular	lobate	flat	opaque
19	67, 68, 71	Cream	circular	entire	convex	translucent
20	70, 74, 86	Cream	circular	entire	convex	opaque

Table 4: Heat tolerance study of selected polythene/plastic loving bacterial isolates

Heat Concentration	Isolates showing tolerance
40°C	All the 86 isolates
50°C	81 isolates
60°C	35 isolates

Table 5: Salt tolerance study of selected polythene/plastic loving bacterial isolates

Salt Concentration	Isolates showing tolerance
10,000 ppm	All the 86 isolates
20,000 ppm	84 isolates
30,000 ppm	75 isolates
40,000 ppm	48 isolates
50,000 ppm	27 isolates

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