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RESEARCH ARTICLE

DEGRADATION OF POLYTHENE WITH BACTERIAL ISOLATES OF RHIZOSPHERE OF *AVICENNIA MARINA* (FORSK.) VIERH., BASED ON PERCENT WEIGHT LOSS

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ABSTRACT

Due to high durability, cheap cost and ease of manufacture, billion tons of plastic based products are manufactured, which leads to generation of millions tons of plastic waste annually around the globe. Of the total plastic waste, polythene shared 64%. The plastic waste finally enters into the sea through different water bodies and leads to deaths of million sea animals. Among the various methods used to dispose plastic waste, biodegradation is considered as the most accepted, ecofriendly and cost effective method. In the present study, rhizosphere soil of *Avicennia marina* was collected from Vashi, Maharashtra, India. Total numbers of bacterial isolates recorded from the rhizosphere soil were 22 only. Screening of polythene degradation was carried out based on percent weight loss (% WL) after 4 months of regular shaking at room temperature. After 4 months period maximum % WL (10.63±0.77%) was recorded with VASB10 at pH 7. The characterization of polythene degrading bacteria is under progress.

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INTRODUCTION

Polythene is one of the most important polymers around the globe based on its properties. Due to its abundant usage, the plastic waste is generating at an alarming rate annually around the globe. Among the plastic waste polythene has the maximum share. The complete degradation of polythene in nature is still a nightmare. As it needs around 1000 years to get degraded under natural condition (Sangale *et al*., 2012). Many workers tried to dispose polythene waste (plastic) using various strategies such as landfill, incineration, degradation, recycling of the plastic, conversion of plastic waste into fuel and using plastic waste in construction of buildings and roads. The degradation of the polythene by using microbes is the most accepted and environmental friendly method. There are various sources of polythene degrading bacteria (Sangale *et al*., 2012) and one among them is the rhizosphere of the *Avicennia marina*. In the rhizosphere soil of *A. marina* various types of bacteria were reported such as *Streptococcus*, *Staphylococcus*, *Micrococcus* (Gram +ve), *Moraxella,* and *Pseudomonas* (Gram –ve) *Pseudomonas fluorescens* (Kathiresan, 2003; Tariq *et al*., 2008; Thiripurasundari *et al*., 2010).

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Among them only *Streptococcus*, *Staphylococcus*, *Micrococcus, Moraxella* and *Pseudomonas,* are reported to have polythene degradation potential (Kathiresan, 2003). *Avicennia marina* (Forsk.)Vierh.is a mangrove plant, from family Avicenniaceae (Khafagi *et al*., 2003). Among the mangroves *A. marina* is an important species because it is the most salt tolerant mangrove and can grow in all climatic and tidal conditions (Khan *et al*., 2001; Duke *et al*., 1988). *A. marina* enjoys worldwide distribution along the sea coasts in tropical and sub-tropical regions. In India, populations of *A. marina* are distributed along the western and eastern sea coast. At global level they are reported to grow along the sea coasts of various countries such as Arabia, Australia, Africa, China, Egypt, Hong Kong, Iran, Taiwan, Philippine and New Zealand (Duke, 1983; Crumbie, 1987). *A. marina* is a shrub to medium sized tree 2-5m tall (Pengand Xin-men, 1983). The key characteristics of this species of *Avicennia* are its elliptic oblong or elliptic ovate leaves and very short beaked & bean-like fruits. Around the red sea its leaves are used as camel fodder. In India and Australia its branches are looped and are fed to cattle (Duke, 1983). It flowers during April to June, while the fruit time is between June to August (Shu, 1994). Besides being the green sentinels of the coasts (Sengupta, 2010), it is also used to treat rheumatism, small pox, ulcers and other ailments in traditional systems of medicine (Bandaranayake, 2002). According to List and Horhammer

(1969-1979) the root and bark are used as aphrodisiac while wood is used for snakebite and the unripe fruits are poulticed onto wounds and leaves onto skin ailments. Its heavy even-textured wood is used for poles and ribs of boats (Duke, 1983). In the present study an attempt has been made to isolate and screen polythene degrading bacteria isolated from the rhizosphere soil of *Avicennia marina* at pH 7 with regular shaking at room temperature after 4 months of incubation.

MATERIALS AND METHODS

Collection of the rhizosphere soil of the *Avicennia marina*

The rhizosphere soil of *A. marina* was collected randomly by uprooting the young plants or the aerial pneumatophores along with the adhering soil from SagarVihar, Vashi (longitude: 19° 04'31.38" N; latitude: 72° 59'02.14"; altitude: 0m), Mahrashtra, India in a sterile bags.

Screening of the polythene (PE) degrading bacteria

The 20 micron thick, banned polythene carrier bags (Fig. 1) were procured from the local market of the Pune city, Maharashtra, India. We purchased PE pickup bags from the vegetable vendors because the local plastic shop keepers afraid to sell the banned PE carry bags legally. PE carrier bags less than 50 micron thickness are banned in Maharashtra and most states of India. With the aid of sharp surgical blade 2cm×2cm strips of the polythene (PE) were made. Before subjecting bacterial infection PE strips were weighed (Precision balance, Sartorius, Germany), sterilized (Autoclave, Steel mate, India) followed by UV treatment for 15-20 minutes in laminar air flow (Microfilt, Mumbai). To each sterile LB broth test tube (Borosil) 3 PE strips were transferred aseptically. 1ml (7.2×10^3 CFU/ml) of 2 day old bacterial culture was transferred to each test tube (3 test tubes/isolate). After inoculation all the cultures were placed on orbital rotary shaker (Steel mate, India) at the speed of 140±20 rpm at room temperature for a period of 4 months (Fig. 2).

Fig.1. Sample of polythene carrier bag used For polythene biodegradation

Fig. 2. Representative image of the polythene degradation assay with regular

After 4 months of incubation at pH 7 with regular shaking at room temperature the screening of the polythene degrading bacterial isolates was carried out based on the percent weight loss of the polythene.

Screening of the polythene degrading bacterial isolates based on percent weight loss (% WL)

At the end of four months incubation, the degraded PE strips were harvested from each test tube aseptically in separate petri dishes followed by washing with absolute alcohol (once) and tap water (twice). After washing all the PE strips were dried in oven overnight at 40°C. The dried PE strips were weighed and the percent weight loss was calculated using the formula:

$$\text{Percent weight loss} = \frac{X - Y}{X} \times 100$$

Where X: initial weight (mg) and Y: final weight after 4 months

Statistical analysis

All the experiments were performed in triplicate and standard deviation was calculated using Microsoft excel 2007.

RESULTS AND DISCUSSION

Total 22 bacterial isolates from the rhizosphere soil of *A. marina* (Fig. 1) were recorded. Luria Bertani (LB) media was found best for the cultivation and growth of the bacterial isolates. All the 22 bacterial isolates were utilized for assessing their polythene degradation potential based on percent weight loss at pH 7. After 4 months of regular shaking at room temperature, the %WL of the polythene was calculated.

Table 1. Percent weight loss of polythene after 4 months of regular shaking at room temperature with the bacterial isolates

S.No.	Bacterial isolates	Percent weight loss of PE			
		Rep.1	Rep.2	Rep.3	Average
1	Control	2.94	2.86	2.94	2.91±0.05
2	VASB1	6.91	7.69	6.49	7.03±0.61
3	VASB2	3.68	3.18	2.44	3.10±0.63
4	VASB3	2.38	4.76	2.38	3.17±1.37
5	VASB4	11.45	9.91	10.53	10.63±0.77
6	VASB5	3.31	2.94	6.46	4.24±1.93
7	VASB6	5.18	4.22	4.05	4.48±0.61
8	VASB7	3.46	4.84	5.85	4.72±1.20
9	VASB8	5.09	4.05	4.86	4.66±0.55
10	VASB9	6.87	5.78	6.32	6.32±0.54
11	VASB10	5.96	6.10	3.33	5.13±1.56
12	VASB11	7.04	4.05	7.26	6.12±.80
13	VASB12	4.59	2.81	2.29	3.23±1.20
14	VASB13	5.67	5.22	2.38	4.42±1.78
15	VASB14	4.44	3.26	3.18	3.63±0.71
16	VASB15	2.17	3.43	4.02	3.21±0.94
17	VASB16	6.10	5.46	5.19	5.59±0.47
18	VASB17	8.26	4.57	7.98	6.93±2.05
19	VASB18	4.84	4.48	2.63	3.99±1.19
20	VASB19	6.12	3.63	2.56	4.11±1.83
21	VASB20	3.82	5.51	3.62	4.32±1.04
22	VASB21	2.87	4.25	1.92	3.01±1.17
23	VASB22	4.69	6.15	5.46	5.44±0.73

Fig. 3. *Avicennia marina* (Forsk.)Vierh.: A: Habit; B: flowering twig; C: Fruits; D: pneumatophores

Fig. 4. Representative image of thecollection site of the rhizosphere soil of *A. marina*

Among the 22 bacterial isolates only 7 isolates (VASB-1, VASB-4 VASB-9, VASB-11, VASB-16, VASB-17, VASB-22)reports more than 5% WL at pH 7 (Table 1). Maximum %WL (10.63±0.77) was recorded with VASB-4. The least %WL (3.01±1.17) of the polythene was reported with VASB22 followed by VASB2 (3.10±0.63% WL). As per literature the key sources of polythene degrading microbes are rhizosphere soil of mangroves (Kathersian, 2003, Kumar *et al*., 2007), plastic waste dumping sites (Nayak, 2001; Hadad *et al*., 2005; Chandra and Rustgi, 1997; Fontanella *et al*., 2010; Gautam *et al*., 2012; Balasubramanian *et al*., 2010; Reddy, 2008) and marine water (Pramila and Ramesh, 2011; Rutkowska *et al*., 2002). In the present study we have selected the collection site from the Vashi, which represent almost all the sources of polythene degrading microbes (Figure 4). Kathersian (2003) isolated polythene degrading microbes from the rhizosphere soil of mangroves (*Avicenna* and *Rhizophora*). From Central India (Jabalpur) plastic dumping sites were used for the collection of soil and plastic samples for isolation of polythene degrading bacteria *Pseudomoansstutzeri* (Sharma and Sharma, 2004). From the Southeastern tip of India (Gulf of Mannar) partially degraded polythene along with the adhering soil were collected for isolating of plastic degrading bacteria (Balasubramanian *et al*., 2010). Harshvardhan and Jha, (2013) isolated the polythene degrading bacteria from the pelagic water of Arabian Sea Coast of Gujarat. No one has tried to collect the samples of the soil with all these sources of polythene degrading microbes from the West Coast of Maharashtra to get the elite polythene degrading bacteria till date.

Degradation of plastic waste is becoming a great challenge and the longest test period used to assess the degradation of the plastic is 32 years (Otake *et al*., 1995) followed by 12 years (Potts, 1978) and 10 years (Albertsson and Karlsson, 1988). After 32 years low density polythene (LDPE) buried in the soil records only 1% Carbon mineralization (Otake *et al*., 1995). After 12 years of dumping polythene sheet in the moist soil no signs of deterioration was documented (Potts, 1978). Compared to the above reported, we got good result with more than 10 percent weight loss in just 4 months duration. Further efforts are needed to screen more bacteria responsible for the degradation of polythene and screening from all the sources to get the most efficient bacteria to commercialize polythene biodegradation process. The characterization of the most efficient polythene degrading bacteria is under process.

Conclusion

Based on the study carried out, VASB10 reported to degrade more than 10% of the polythene in-terms of weight loss with 4 months duration at regular shaking at room temperature.

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