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Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India

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ABSTRACT

Sixty marine bacteria isolated from pelagic waters were screened for their ability to degrade low-density polyethylene; among them, three were positive and able to grow in a medium containing polythene as the sole carbon source. The positive isolates were identified as *Kocuria palustris* M16, *Bacillus pumilus* M27 and *Bacillus subtilis* H1584 based on the 16S rRNA gene sequence homology. The weight loss of polyethylene was 1%, 1.5% and 1.75% after 30 days of incubation with the M16, M27 and H1584 isolates, respectively. The maximum (32%) cell surface hydrophobicity was observed in M16, followed by the H1584 and M27 isolates. The viability of the isolates growing on the polyethylene surface was confirmed using a triphenyltetrazolium chloride reduction test. The viability was also correlated with a concomitant increase in the protein density of the biomass. Polyethylene biodegradation was further confirmed by an increase in the Keto Carbonyl Bond Index, the Ester Carbonyl Bond Index and the Vinyl Bond Index, which were calculated from FT-IR spectra.

1. Introduction

The development and use of synthetic plastic has changed the nature of waste in last 3–4 decades (Sheavly, 2005). Over this period, it has replaced natural material in various aspects of human life and become an indispensable part of our society. Although the durability of plastic is ones of its most beneficial qualities, this same property is a major problem for our environment (Sivan, 2011). Plastics are chemically synthesized long-chain polymers (Scott, 1999) and are globally produced on a substantial scale. As per a recent estimate of the Central Pollution Control Board, New Delhi, India, 8 million tons of plastic products are consumed every year in India alone. A study on plastic waste generation in 60 major Indian cities revealed that approximately 15,340 tons per day of plastic waste is generated in the country (Central Pollution Control Board (CPCB) New Delhi, India, 2013). Low-density polyethylene (mainly used as carry bags) constitutes the major portion of this waste problem.

In the last two decades, the rate of plastic deposition has increased tremendously, and plastic has intruded into the marine environment. Plastic is found floating in oceans everywhere from the polar regions to the equator and has become one of the most common and persistent pollutants of seas and beaches worldwide (Frias et al., 2010; Moore, 2008; Teuten et al., 2009). Plastic debris is one of the largest contaminants of the marine environment. Polyethylene is the most commonly found non-degradable solid waste and has recently been recognized as a major threat to marine life. There are reports that suggest that polyethylene causes blockages in the intestines of fish, birds and marine mammals. In addition, entanglement in or ingestion of this waste has endangered hundreds of different species (Teuten et al., 2009; Secchi and Zarzur, 1999; Spear et al., 1995).

Polyethylene represents up to 64% of the synthetic plastics that are discarded within a short period after use (Byuntae et al., 1991). It is highly resistant to acids, alcohols, bases and esters. It is also biologically inactive and considered a recalcitrant material. Its inertness is due to the high molecular weight, hydrophobicity and lack of functional groups recognized by microbial enzymatic systems (Hamid, 2000). Polyethylene is a concern for waste management due to its accumulation in landfills and natural habitats (Thompson et al., 2009). Hence, a suitable method for disposal that is eco-friendly must be found. Recycling of polyethylene was considered a solution but has failed to provide safe disposal of these materials (Sivan, 2011); in this regard, microbial degradation is one of the best options. Some reports on the biodegradation of plastics indicate that it could be a viable proposition when suitable microorganisms are utilized (Singh and Sharma, 2008; Shah et al., 2008). Studies on polyethylene biodegradation (Albertson, 1980; Albertsson et al., 1987), including the biotic environment (Shah et al., 2008), have been reported. However, few studies have been

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conducted on non-amended polyethylene (Balasubramanian et al., 2010; Hadad et al., 2005).

In the present study, we screened sixty marine bacterial isolates cultured from the pelagic waters of the Arabian Sea, India, for their polyethylene degradation ability and found three potential strains. In addition, we characterized the cell-surface hydrophobicity and growth kinetics of these bacteria and the biodegradation of polyethylene.

2. Materials and methods

2.1. Screening of bacterial isolates for polyethylene degradation

Sea water samples were collected from different locations along the Arabian Sea coast, India. The map of different sampling locations and geographical coordinates is shown in Fig. 1. The bacterial cultures were isolated on Zobell marine agar 2216 (Himedia) plates using a dilution technique at room temperature (25 °C). All morphologically distinct colonies were purified and preserved in mineral oil.

Sixty bacterial isolates were assayed for their ability to utilize polyethylene as the sole source of carbon and energy. Each individual isolate was grown in Bushnell–Haas medium (1.0 g NH_4NO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.0 g K_2HPO_4, 0.1 g $CaCl_2 \cdot 2H_2O$ and 0.15 g KCl per liter of distilled water) supplemented with 3.5% NaCl. Then, 20 µl of 1% TTC solution was added to 10 ml of medium as an indicator of viability (Alef and Nannipieri, 1999).

2.2. Identification of the bacteria

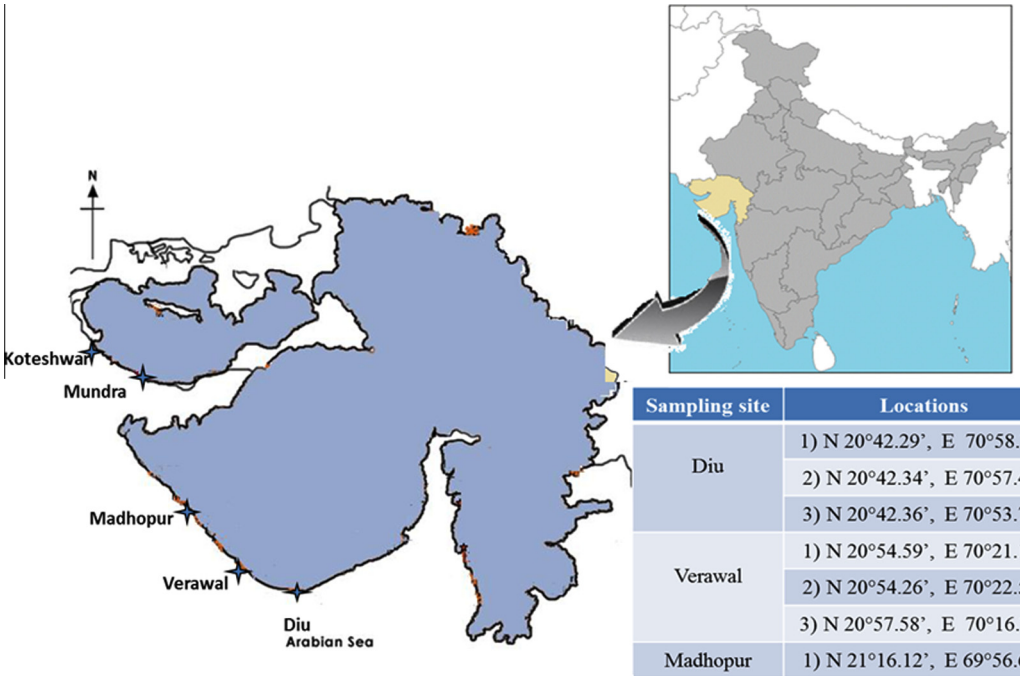
The genomic DNA was extracted from the bacterial culture using a standard phenol chloroform extraction procedure. Amplification of the 16S rRNA gene was conducted using universal primers (Weisburg and Barns, 1991): 27F (5′-GAGAGTTTGATCCT GGCTCAG-3′) and 1495R (5′-CTACGGCTACCTTGTTACGA-3′). The reaction mixture (50 µl) contained 0.5 µl of each primer, 400 µM of dNTPs, 2.5 U of Taq polymerase, 1.5 mM of $MgCl_2$, 50 ng of DNA template and 5 µl of 10 × PCR buffer. The PCR was conducted at an initial denaturation temperature of 94 °C, 8 min; followed by 28 cycles of 94 °C, 1 min; 58 °C, 1 min; 72 °C, 2 min; and a final extension at 72 °C for 10 min. The PCR product was purified, analyzed and sequenced (M/s Microgen Inc., Seoul, South Korea). The similarity search was conducted *in-silico* using the BLAST database of NCBI. The phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011). Bootstrap analysis with 100 replicates was performed to estimate the confidence of the tree topologies.

2.3. Polyethylene film biodegradation assay

The biodegradation tests were performed on samples of low-density polyethylene film (i.e., pieces of polyethylene bags) that had been dried overnight at 60 °C, weighed, disinfected (autoclaved at 105 °C for 1 h) and added to each flask (approximately 50.0 mg of polyethylene film per flask) containing 50 ml of BH medium. The flasks were inoculated with 2 ml of a mid-exponential phase culture that were maintained in Zobell marine broth (ZMB). Before inoculation, the culture was washed with Bushnell–Haas (BH) medium to remove medium and cellular soluble debris. The cell densities of the inoculums were adjusted to 1.5×10^6 colony-forming units (CFU) per ml. The flasks containing non-inoculated BH medium supplemented with polyethylene film served as the control.

2.4. Determination of the dry weight of residual polyethylene

To facilitate accurate measurement of the residual polyethylene weight, the bacterial film colonizing the polyethylene surface was removed by supplementing the cultures with a 2% (v/v) aqueous sodium dodecyl sulfate (SDS) solution. The flasks were then



Sampling site	Locations
Diu	1) N 20°42.29', E 70°58.71'
	2) N 20°42.34', E 70°57.45'
	3) N 20°42.36', E 70°53.75'
Verawal	1) N 20°54.59', E 70°21.12'
	2) N 20°54.26', E 70°22.50'
	3) N 20°57.58', E 70°16.75'
Madhopur	1) N 21°16.12', E 69°56.67'
Mundra	1) N 22°45.82', E 69°42.25'
Koteswar	1) N 23°41.34', E 68°31.57'

Fig. 1. A geographical map showing the sampling sites along the coastline and their locations.