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Biodegradation of PBSA Fungi Isolated from Soil

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ABSTRACT

Fungi isolated from soil samples were screened for their ability to degrade biodegradable plastics. Strain Alternariaporri, which has newly isolated from a soil sample, was selected as the best strain. From taxonomical studies, the strain was tentatively described to belong to the genus Alternariaporri. Strain could degrade both solid and emulsified poly (butylene succinate co adipate) .Identification of the fungal strains was performed by using the 18srRNA sequence analysis.

Keywords : Degradation, Soil fungi, PBSA (Poly butylene succinate co adipate)

Introduction

In recent times, the excessive consumption of synthetic plastics derived from petroleum has had an adverse impact on the environment because the majority of these synthetic plastics do not degrade in the environment, and incineration of plastics generates co_2 and dioxin [1,2]. These molecules increase the warming of the earth and environmental pollution. In view of this,some aliphatic polymertypes have been developed as biodegradable plastics [3].

Biodegradable plastics have several excellent properties and may provide solutions to global environmental problems. First, biodegradable plastics are degraded by microorganisms in the natural environment [4,5].Second, they can be composted, and burn with a lower calorific value than that of synthetic plastic materials. Poly (butylene succinate co adipate) (PBSA) are the most promising materials among commercially available synthetic biodegradable plastics.

PBSA is produced through the copolymerization of a glycol, such as 1,4-butanediol, with an aliphatic dicarboxylic acid, such as succinate. It is a white crystalline thermoplastic, with melting points in the range of 90-120°c,and has excellent processability;therefore, It can be processed into injection molded products, films, paper laminates, and sheets [6].PBSA also biodegrades in compost, moist soil, fresh water with activated sludge, and seawater[7,8].Further, various microorganisms have been isolated as PBS-degraders[9,10,11].

Most of the organic wastes undergo microbial degradation and contribute to the biological productivity either directly or indirectly. Since microorganisms are capable of degrading most of the organic and inorganic materials, there is a lot of interest in the microbial degradation of plastic and polythene materials[12].This study was aimed to determine the fungi their ability to degrade the PBSA.

Methodology

Preparation of emulsified PBSA

PBSA (Bionolle # 3020:showa Highpolymer co Ltd) Emulsions were prepared by using the detergent Plysurf A210G (Daiichi Kogyo Seiyaku Co., Kyoto, Japan).2 g of PBSA was dissolved in 40 ml of dichloromethane and transferred into 250 ml of distilled water containing 40 mg of Plysurf A210G and blended in a homogenizer (10,000 rpm for 4 min). The emulsified solution was then incubated at 80°C for 3 hrs under a lab hood to remove dichloromethane.

Screening of PBSA degrading fungi

Soil samples were collected from various sites from South India (Tamil Nadu and Kerala), for screening of PBSA-utilizing bacteria. 0.2 g of each soil samples were transferred to different test tubes which contained 10 ml of the basal medium with PBSA as the sole carbon source(mg l^{-1}): KH_2PO_4, 200: K_2HPO_4, 1600: $(NH_4)_2SO_4$, 1000: $MgSO_4.7H_2O$, 200: $FeSO_4.7H_2O$, 10: $MnSO_4.4H_2O$: 0.5; $ZnSO_4.7H_2O$, 1: $CuSO_4$., 0.1 and $CaCO_3$, 5000. The final pH was 7.0 for the solid medium , emulsified PBSA was added and 15 g l -1 agar was added.). The medium was incubated at 30°C with shaking condition. After a week, 0.5 ml of culture broths was transferred into the tubes containing fresh basal medium. This procedure was repeated for five times.Isolates which showed a halo zones were stored for further work.

Degradation assay

PBSA pellets (0.2 g) were added to an Erlenmeyer flask containing 100 ml of the basal medium. The medium was inoculated, and was incubated on a rotary shaker at 30°C. Experiments were performed in triplicate. Uninoculated cultures were used as control. PBSA degradation was monitored by measuring the weight of the PBSA pellets before and after the incubation.

Identification of fungal strain

The identification of fungi was performed on the basis of macroscopic and microscopic examination.The fungi was identified after staining them with cotton blue by following the keys [13]. Moreover 18S rRNA gene sequencing was carried out to find the species level.

Results

Screening of PBSA degrading Fungi

Fungal strains were primarily isolated from soil samples by using basal medium containing PBSA as a sole carbon source. By performing subsequent experiments, isolated colony was showed its highest ability to form haloes on the emulsified PBSA agar plates. and it was confirmed by weight reduction method (Plate 1).

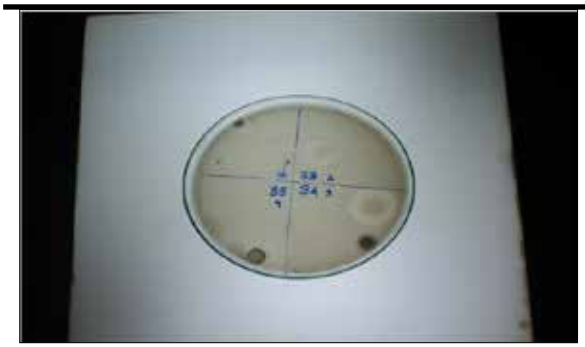

Plate 1. PBSA degrading Fungi in solid agar

Identification

Soil fungi were isolated and identified and characterized using staining such as Lactophenol cotton blue.

18S rRNA gene sequencing

Sequence Alignment of Alternariaporri

CCCCTGGGGATCCTACCTGATCCGAGGTCAACG-
TAAAAATGTAGTCTTGATGGATTGCCGTCCTTGT-
GCTGATTGCAAGCGCAAAAATGTGCTGCGCTGC-
GAAACCAGTAGGCCGGCTGCCAATCGTTTTAAGGC-
GAGTCTTTGGGCGAGGCCAAAGACAAAAGACGC-
CCAACACCAAGCAAAGCTTGAGGGTACAAAT-
GACGCTCGAACAGGCATGCCCTTTGGAATACCAAA-
GGGCGCAATGTGCGTTCAAAGATTCGATGATTCACT-
GAATTCTGCAATTCACACTACGTATCGCATTTCGCT-
GCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTT-
GTTGAAAGTTGTAAATGATTTACATTTGTTATACT-
GACGCTGATTGCAACTGCATAAAAAAGGTTTATGGTGTG-
GTCCTGGTGGCGGGCGAACCCGCCCAGGAAACAACAA-
GTGCGCAAAAGACAAGGGTGATAAAAATAATCCAGC-
CGAAGCCTTCATATTTTAATTGTGTAATGATCCCTC-
CGCAGGTTCACCTACGGAGACCTTGTACACTTTTTTTA
TTTCAAAANNNNNNTTNNTNNNNNTNNNNNNNTNN
NNTTNTTTNTTTTCCNTTTTTTTTTTTNTTNTTTNTTTNTTT
TTTTTTTTTTTTTTTTTTTNTTTNTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTNTTTTNNTTNTTT
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TTTTTTTTTTNTTNTTTTTTNNTTTTTTTNTTTTNNNNNTTT
TTTTTTTTTTTTNNTTNTTNTTTTTNNTNTTTNTTTNNTN
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TNNNNTTNTTTTTNTNNNTNNTTTTTTTNNTTNTNTTNTN
TTNNNNTNTNNTTTTTTTTTTNTTTTTTTNNNTNNTNTNN
NTTTTTTNNTTTTTTTTTTTTTTTTTTTNNNNTTTTNTNT
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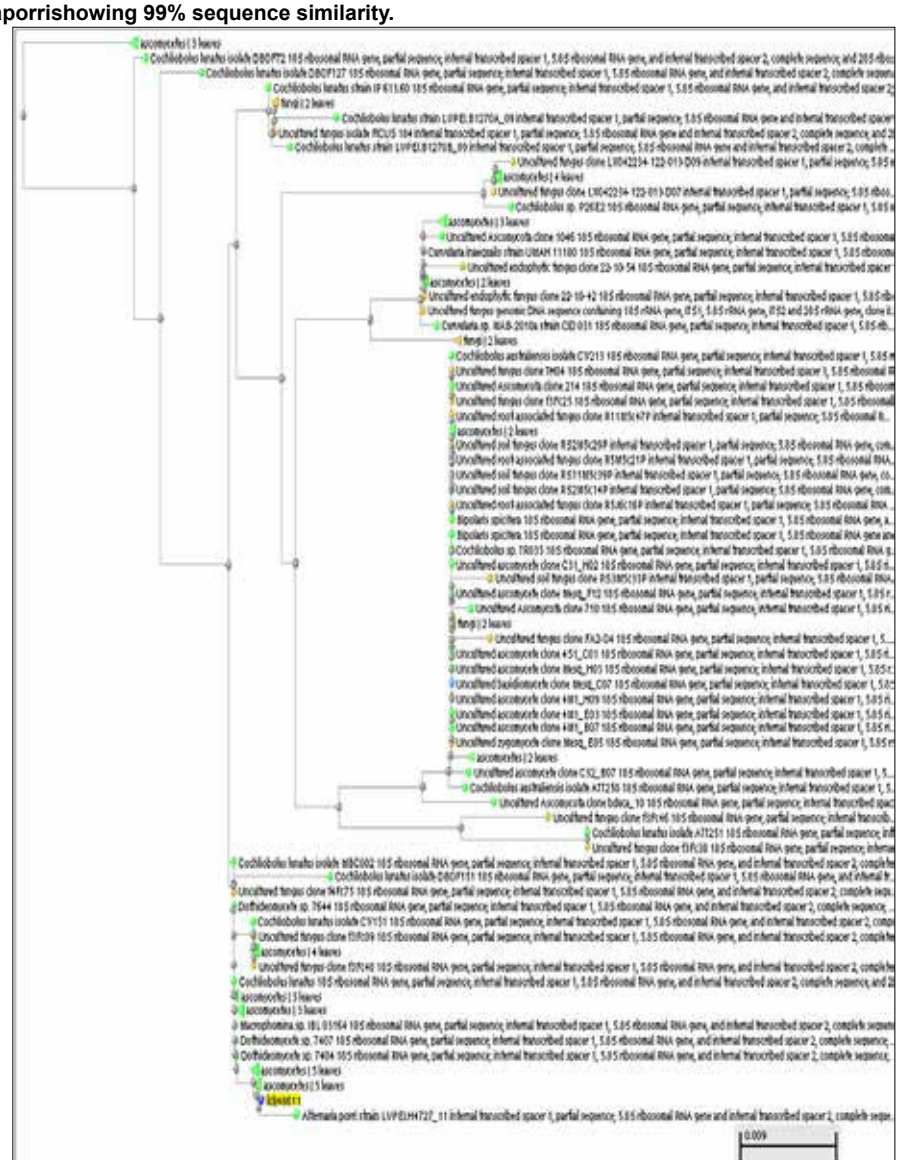
TTTTTNN

Phylogenetic Tree ofAlternariaporrishowing 99% sequence similarity.



Discussion

Microorganisms play a significant role in decomposition of materials,including poly (butylene succinate co adipate) (PBSA) and synthetic polymers in natural environments. High density and low density polyethylene are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. In this regard, there is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms([14,15]).Hence, further study on microbial enzymes or organic acids in degradation of the PBSA and plastic will pave way for finding technology for degrading these environmentally hazardous plastic materials.

Conclusion

Fungal strain were selected for poly (butylene succinate co adipate)(PBSA) degradation under laboratory conditions. The isolated microbes were native to the site of poly (butylene succinate co adipate) (PBSA) disposal and might show some degradability in natural conditions and also biodegradation in laboratory conditions on synthetic media.

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