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ISOLATION AND CHARACTERIZATION OF A BACTERIUM THAT DEGRADES PBSA

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ABSTRACT

Microorganisms isolated from soil samples were screened for their ability to degrade biodegradable plastics.Among them, the most active strain S-32, was selected as the best strain for degrading these plastics. From its phenotypic and genetic characteristics of S-32 was closely related to *Pseudomonas aeruginosa* which could degrade both solid and emulsified poly butylene succinate co adipate (PBSA).

KEYWORDS: Poly Butylene Succinate Co adipate (PBSA), Degradation assay, GC, HPLC *Pseudomonas aeruginosa*,

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INTRODUCTION

Conventional plastic have been widely used as the basic materials for various applications because of their excellent physical properties and cost competitiveness. However, plastics tend to accumulate in the environment, causing severe pollution problems due to their stability and weather ability (Tserki *et al.,*2006; Kim *et al.,* 2007;Ray *et al*.,2007). Biodegradable polymers are complementary to recycling plastic for the environment protection against waste plastic. The most important family of currently developed biodegradable polymers consists of aliphatic polyester such as Poly butylene scccinate (PBS) and poly butylene succinate-co-adipate (PBSA). Poly butylene succinate-co-adipate (PBSA) is produced on a semi- commercial scale through the transesterification and polycondensation of 1,4-butanediol, succinic acid and adipic acid. The biodegradability of PBSA depends strongly on the composition of PBSA, as well as the mechanical, thermal and crystalline properties (Honda *et al* .,2003;Zhao *et al*.,2005;Tseki *et al*.,2006). Its has stronger mechanical properties and relatively low production cost in comparison to the other biodegradable polymers, and there by it is expected to be used widespread as an alternative to the ordinary commodity plastics, such as polyethylene and polypropylene. Bionolle is the commercial name of PBSA (no.1000 series) and PBSA (no.3000 series) Bionolle has a melting point of 90ºC to 120ºC and a glass transition temperature range of about -45 to -10°C: it has density of about 1.25 gm^{-3} and heat combustion below 25 kjg^{-1}(Takashi 1998): It has potential application as food containers, foamed sheets, textiles and its use an alternative material to ordinary plastics is expected to increase. Although many PBS-degrading mesophilic and thermophilic microorganisms have been extensively studied (Pranamuda *et al*.,1995; Suyama *et al*.,1998a;Kleeberg *et al*.,1998;Transengo *et al*.,1998a) there are only few studies on their degradation enzymes. In this study was aimed isolation and identification of PBSA degrading bacteria from various environmental sources and further studies on biodegradation behaviour of the isolates using HPLC, GC-MS and characterization of enzymes involved in this degradation.

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). 0.5g of PBSA was dissolved in 20ml of dichloromethane and transferred into 65ml distilled water containing 20mg of plysurf A210G. The emulsified was then incubated at 80ºC for 3hrs under a lab hood to remove dichloromethane.

Screening of PBSA degrading microorganism

Soil samples were collected from various sites from South India (Tamil Nadu & 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 composition 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 mg/L 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. Single-colony isolation was done using PBSA-emulsified solid medium. 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 bacterial strain

Isolated soil bacteria were characterized and identified using standard biochemical methods according to Bergey's manual of systematic bacteriology. Moreover 16S rRNA gene sequencing was carried out to find the species level.

Analysis of degradation products by HPLC & GC

To determine the degradation products from PBSA, strain was cultivated in Basal medium containing emulsified PBSA. Un inoculated emulsified PBSA in basal medium used as control. The degradation products derived from PBSA in culture supernatant were analyzed by HPLC at 30 and 90 days intervals. The mobile phase was 5mM H_2SO_4. GC was using HP 5700 series with FID detector equipped with packed column as a carrier gas helium and a combustion

gas hydrogen and synthetic air was used. The injection volume was 1 µl. The temperature of the injector was adjusted to 250ºC and those of the FID detector to 300ºC.

RESULTS

Screening of PBSA degrading microorganisms

Total of 100 different soil samples have been collected from southern states in Tamil nadu and Kerala and stored in sterile air tight bags. The no. of sample from different regions were as follow Coimbatore(12), Tirupur (8), Erode (6), Salem (8), Ooty (5), Metupalayam (5), Maduri (12), Theni (3), Kambam (2), Palakad (3), Cochin(14), Chennai(9) & Trichy (7). 32 strains were primarily isolated from 100 samples by using basal medium containing PBSA as a sole carbon source. By performing subsequent experiments, 5 isolates was showed its highest ability to form haloes on the emulsified PBSA agar plates and it was confirmed by weight reduction method. (Plate 1).

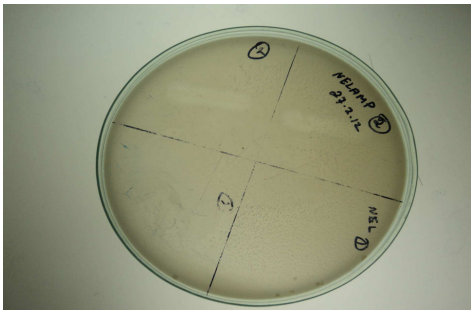


Plate 1
PBSA degrading organism in solid agar

Degradation assay

Table 1 shows PBSA pellet degradation at different spectrum using various strains. About 135 mg of PBSA pellet in the initial stage was degraded to 62 mg within 30 days by strain S-32.

Table 1
Degradation of PBSA pellets by different strains

S.No	Strains No	PBSA (Before) in mg	PBSA (After) in mg	Different in weight(mg)
1	**1**	**58**	**52**	**6**
2	2	61	57	**4**
3	5	67	64	**3**
4	**9**	**104**	**87**	**18**
5	14	108	105	**3**
6	**17**	**88**	**81**	**7**
7	21	85	82	**3**
8	23	105	101	**4**
9	**29**	**107**	**102**	**5**
10	**32**	**135**	**62**	**73**

Identification of PBSA degrading strain

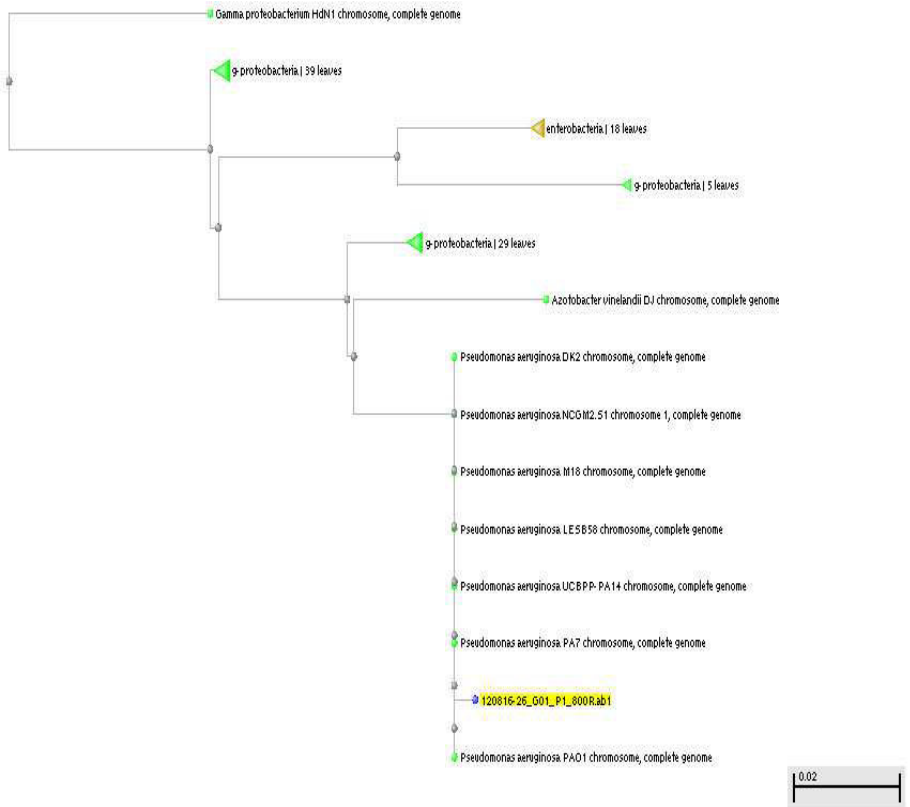
Strain S-32 was a rod – shaped aerobic G^{-ve} bacterium, Oxidase – positive, Catalase – positive, nitrate reduction – positive, Indole production – negative, Urease – negative and Gelatin hydrolysis – positive. Based on the biochemical characteristic, the strain was identified as *Pseudomonas* sp. 16S rRNA gene sequencing was performed and submitted to Gen bank (The Gen Bank accession No. is KC522650). The 16S rRNA gene sequence of S_{32} was close to *Pseudomonas aeruginosa.*

Sequence Alignment of Psuedomonas aeruoginosa

CCCTCTTTTCGCCCCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGC
CACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATT
CCACCACCCTCTACCGTACTCTAGCTCAGTAGTTTTGGATGCAGTTCCCA
GGTTGAGCCCGGGGATTTCACATCCAACTTGCTGAACCACCTACGCGCGC
TTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGG
CTGCTGGCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACA
GCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAAT
CCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCA
TTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCT
CAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCC
TTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTG
ATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCTCAGGACGTATGCGG
TATTAGCGCCCGTTTCCGGACGTTATCCCCCACTACCAGGCAGATTCCTA
GGCATTACTCACCCGTCCGCCGCTGAATCCAGGAGCAAGCTCCCTTCATC
CGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGCCAG
AATTCAAACTCTAAAATTTCTAAGTTTCCTA

Figure 1

Phylogenetic Tree of P.aeruginosa showing 99% sequence similarity.



Determination of PBSA Degradation products by HPLC

Metabolites produced by the degradation of emulsified PBSA by strain s32 were investigated by HPLC analysis. Fig 2 shows a chromatogram of PBSA, used as a control. After 30 days incubation, the peaks were observed for the degradation activity (Fig 3)

Figure 2
HPLC degradation activity in control

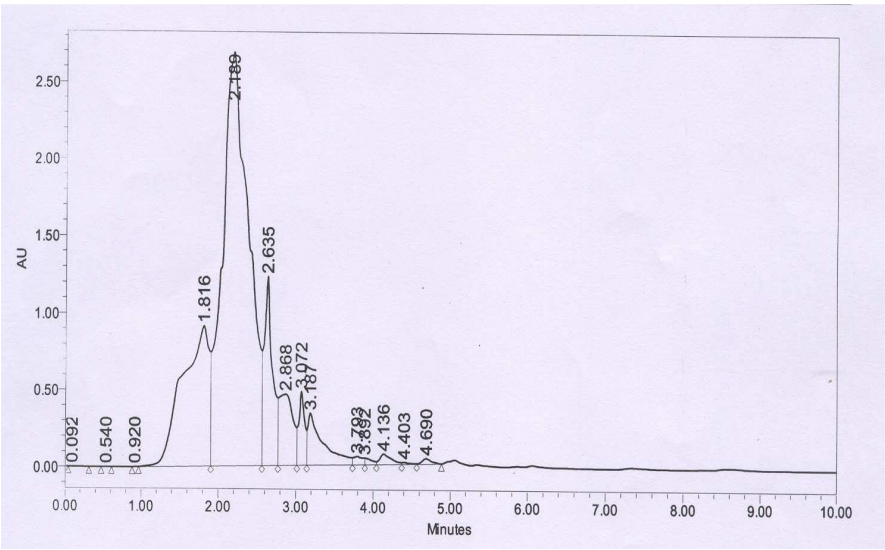
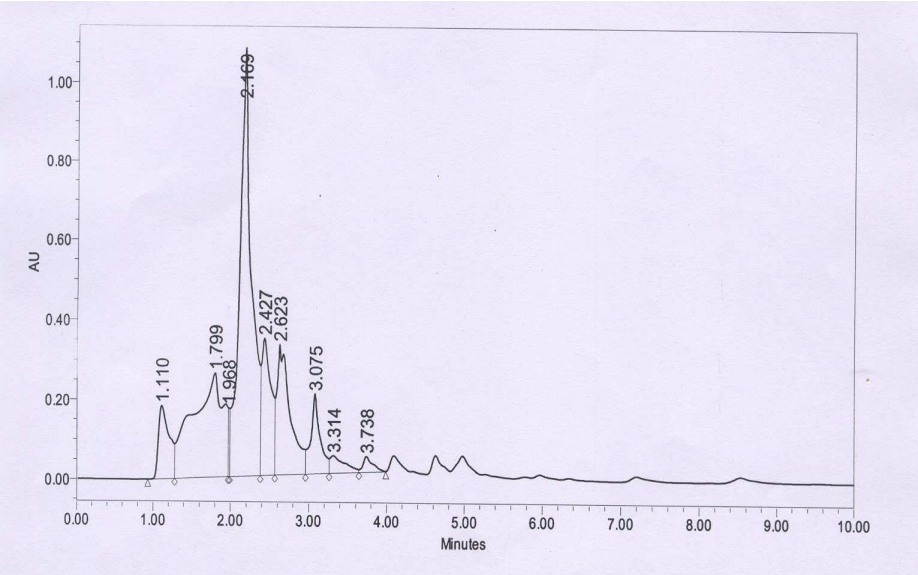


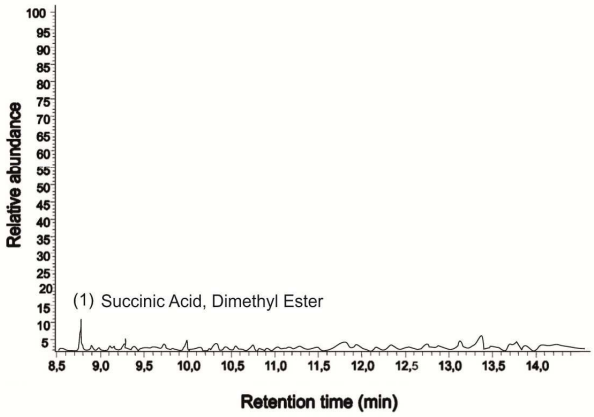
Figure 3
HPLC degradation activity in sample within 30 days

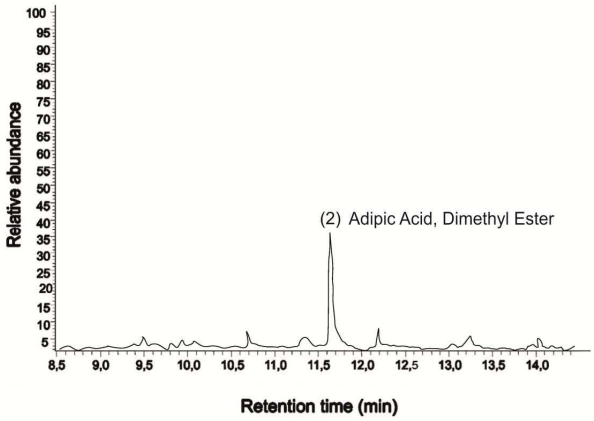


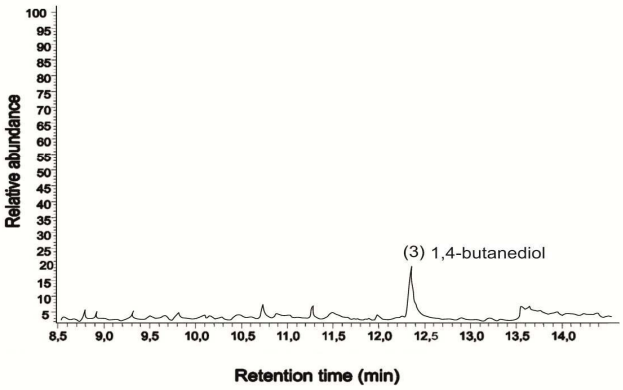
Identification of unknown compounds by GC

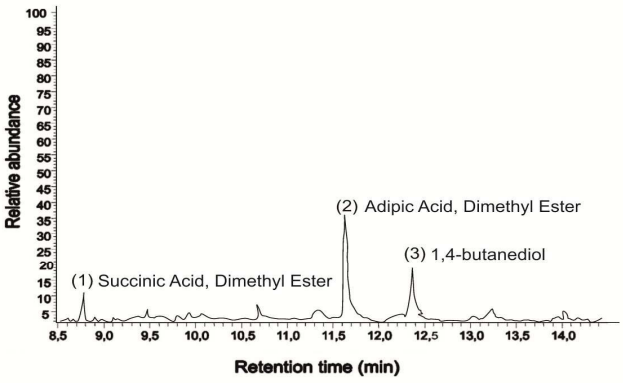
In the case of PBSA, an unknown peak was detected by the GC analysis. After hydrolysis additional peaks were detected this observation indicates that at least one watersoluble oligomer which consist of succinic acid adipic acid and 1,4 butanediol is produced as an intermediate by this strain (Fig4).

Figure 4
Identification of unknown compound by GC technique

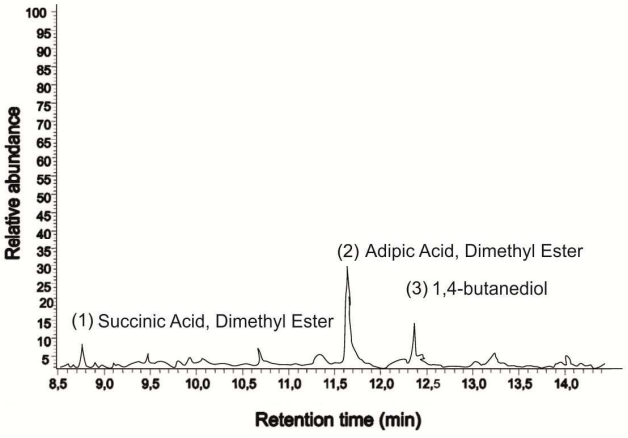








Degradation of PBSA (Standard)



Degradation of PBSA (1 Month)

DISCUSSION

Although there have been many reports on the biodegradation of PBSA bacteria (Uchida *et al.,*2000) . *Pseudomonas aeruginosa* isolated in this study had not been reported previously and is a novel PBSA degrading bacteria. *Pseudomonas aeruginosa* showed degradation activity against both solid and emulsified PBSA (pellet).But the degradation activity of S32 against emulsified PBSA seems to be greater than that of solid PBSA.

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