Biodegradation Of Polyhydroxyalkanoates

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Citation

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Abstract

The biodegradation of Polyhydroxybutyrate (PHB) and its copolymer Polyhydroxybutyrate-co-hydroxyvalerate (PHB-co-HV) was studied. Bacterial as well as fungal isolates were isolated from 9 different ecosystems. All these isolates were tested for the degradation of the above polymers in assay agar medium as well as in liquid cultures. The culture biomass and the clear zone around the colonies were measured to evaluate the activity of these isolates. In all, the fungal isolates were found to degrade these polymers more rapidly when compared to bacteria, due to their versatile depolymerase activities.

INTRODUCTION

Poly-3-hydroxybutyric acid (PHB) is the most well known member of the family of Polyhydroxyalkanoates (PHA). PHB is similar to polypropylene in its physical properties but has the advantage of being biodegradable. PHB has been shown to be biodegradable by bacteria into water and carbon dioxide (and methane under anaerobic conditions) in natural environments including water, soil and compost. Polyhydroxyalkanoates are bacterial storage polyesters currently receiving much attention due to their synthesis from renewable resources and their applicability as biodegradable and biocompatible plastics (Steinbuchel,1991). Most aliphatic polyesters are readily mineralized by a number of aerobic and anaerobic microorganisms that are widely distributed in nature. However, aromatic polyesters are more resistant to microbial attack than aliphatic polyesters. The fungal biomass in soils generally exceeds the bacterial biomass and thus it is likely that the fungi may play a considerable role in degrading polyesters, just as they predominantly perform the decomposition of organic matter in the soil ecosystem (Kim and Rhee, 2003). The majority of synthetic polymers are extremely resistant to microbial attack due to their excessive molecular mass, high number of aromatic rings, unusual bonds or halogen substitutions (Alexander, 1981). The extent of polymer degradation in an ecosystem is affected by material processing, the inherent characteristics of the substrate to be degraded, and various microbiological and environmental factors. These factors are all interdependent (Brandl et al.,1995). Degradation of P(3HB) and its copolymers has been investigated in different natural environments such as soils (Doi et al., 1992b; Mergaert et al.,1992,1993), composts (Mergaert et al.,1994), and natural waters (Doi et al.,1992a ; Brandl and Pucner, 1992; Matavulj et al., 1993; Mukai et al., 1993; Mergaert et al.,1995).The microorganisms involved in the degradation have been isolated and identified.

Employing a basal culture medium made turbid by the addition of the co-polymer, a clear zone test was used to screen bacterial and fungal isolates from a variety of habitats. The appearance of clear zones on a turbid plate or vial indicates the degradation of the polymer .

MATERIALS AND METHODS

ISOLATION OF FUNGAL AND BACTERIAL POLYMER DEGRADERS

A. FUNGI

Soil samples were collected from 9 natural habitats from the campus area of Osmania University for the study of polymer degradation capabilities of fungi and bacteria. The fungi were isolated on potato dextrose agar medium (PDA).

Samples were suspended by vortexing in sterile distilled water and allowed to stand for several minutes. The supernatants were then serial diluted. A 0.1 ml portion from each dilution was plated onto the PDA plates and incubated at room temperature for 2-7 days. The isolated fungi were maintained on PDA slants.

B. BACTERIA

Serial dilutions were made in sterile distilled water and the samples were directly pour plated on to Luria-Bertani (LB)

agar plates. The plates were then incubated at 30°C for 2-3 days. Colonies with different morphology, colour, pigmentations etc were isolated in pure form and maintained on slants.

SCREENING FOR P(3HB-CO-HV) AND P (3HB) DEGRADATION

The polymer degrading ability of the isolated bacteria and fungi was determined by different techniques.

For the fungal isolates the basal medium was used which was supplemented with 0.01% peptone and 0.01% yeast extract, containing about 0.02% (w/v) of dry polymer (either PHB or PHB-co-HV) as a sole carbon source and solidified with 1% agar at pH 6.0.(Lee et al., 2005). The plates were incubated at room temperature for 1-3 weeks.

The bacterial isolates were screened by using minimal media containing 0.03% (w/v) of the polymer (either PHB or PHB-co-HV) as a sole carbon source, which was solidified with 2% agar at pH 7.0. The isolates were transfered on to plates with this minimal media and were incubated at 30°C ± 2 °C for 7-12 days.

In case of degradation of the polymers by fungal isolates, it was detected by the formation of a clear zone surronding the growth and the extent of degradation was measured from the width of the clear zone formed. The bacterial isolates were seen as clear colonies on media with the polymer as a sole carbon source.

DEGRADATION IN LIQUID MEDIA

The degradation of the polymers in the liquid media was determined by growing the bacterial and fungal isolates in the assay medium supplemented with 0.02% of the polymer. After inoculation, the samples were incubated on rotatory shaker for 7 to 10 days at 120 rpm and 30°C. The leftover residual amount of the polymer after incubation was determined using Law and Slepecky method (1961), after extraction with chloroform and evaporation to dry. The amount of the dry biomass was determined to calculate the growth of the isolates.

RESULTS

ISOLATION AND SCREENING OF BACTERIA AND FUNGAL ISOLATES

Samples were collected from 9 different ecological niches for the isolation of bacterial and fungi. Diluted samples incubated on the specific plates (i.e. LB media or PDA) produced a variety of fungal and bacterial colonies after incubation.Colonies with different morphological appearances were selected and maintained on respective agar slants.

All the fungal isolates were screened for P(3HB) degradation with basal medium supplemented with 0.01% peptone and 0.01% yeast extract containing 0.066g wet PHB inclusions (about 0.02g of dry PHB) and solidified with 1.0% agar and pH 6.0.

A total 77 bacterial isolates and 49 fungal cultures (Table 1) were isolated in pure form. The results were presented as shown in table-1 which indicates the diversity of both bacterial and fungal isolates in different samples. The polymer degrading bacterial isolates were 28 in number accounting to about 36.3% of the total bacterial isolates. Of the total 66 fungal isolates, 40 isolates showed polymer degradation capabilities amounting to 60% of the total fungal isolates. The fungal isolates showed much higher capabilties than bacteria in degradation of PHA polymers which was in agreement with previous findings, described by Kim and Rhee (2003).

Figure 1

Table 1: Isolation and screening of polymer degrading bacteria and fungi.

Samples tested	No. of bacterial isolates	No.of polymer degrading bacterial isolates	No. of fungal isolates	No. of polymer degrading fungal isolates
Soil compost	08	04	10	08
Garden soil	10	06	11	09
Farm soil	18	09	07	06
Plant root soil	06	01	06	03
Rock soil	04	-	05	01
Pond water	11	05	09	03
Pond sediment	08	02	03	04
Litter	05	01	06	04
Fallen leaves	07	-	07	02

Figure 2

Figure 1 : Photograpgh of minimal media plate with bacterial isolates.

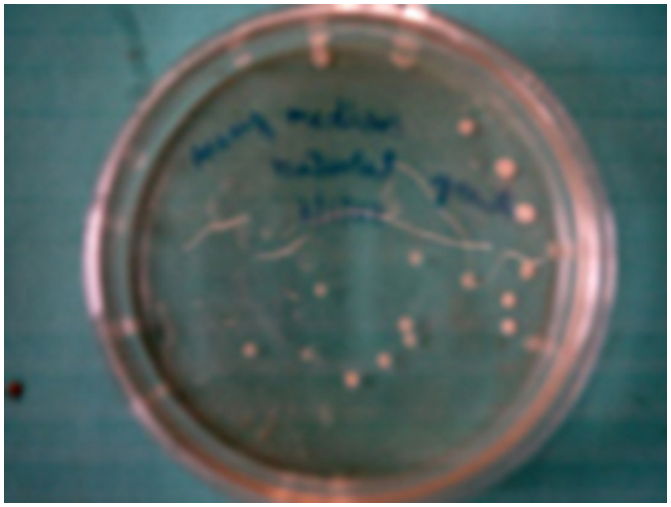
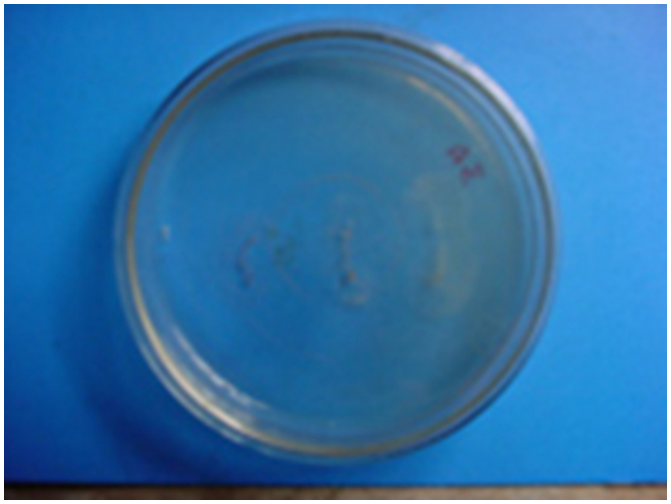


Figure 3

Figure 2 : Photograpgh of minimal media plate with fungal isolates.



DEGRADATION OF THE POLYMER IN LIQUID CULTURES

The fungal and bacterial isolates were grown as liquid cultures in minimal media with the polymers as a sole carbon source. The dry biomass attained by these isolates after 3 days (bacteria)-7 days (fungi) of growth was measured by drying constantly at 80 °C for bacteria and at room temperatures for fungi. The residual polymer left over in the medium after growth was extracted by using chloroform technique and was quantitatively estimated by the UV spectrophotometric method. The table 2 shows the dry biomass as well as the residual polymer in medium of selected fungal and bacterial isolates. In this table the clear zones obtained after growth on assay agar by fungal isolates were also presented.

From the table 2, it was established that fungal isolate 09/A from soil compost grew well in the assay media showing its good polymer degradation capabilities. The bacterial isolate 17/G from pond sediment has the best polymer degradation capabilities, since it produces highest biomass leaving less residual polymer in the medium.

Figure 4

Table 2: Dry biomass and residual polymer after growth in assay medium.

Isolate	Dry biomass (g/l)	Residual polymer (g/l)	Clear zone (cm)(on assay agar)
11/A#	0.119	0.11	NA
09/A*	0.3	0.09	7
12/C#	0.07	0.14	NA
17/G#	0.11	0.09	NA
2/B#	0.08	0.12	NA
1/C*	0.2	0.14	6
2/E*	0.10	0.15	3
1/G*	0.09	0.16	2

Key: NA = not applicable; * = fungal isolates from different samples; # = bacterial isolates from different samples.

DISCUSSION

Degradation of both P(3HB) and P(3HB-co-13% 3HV) was affected significantly when the PHA containing media were supplemented with easily consumable carbon sources. Results of supplementation studies have indicated that the degradation of P(3HB) as well as P(3HB-co-13% 3HV) was significantly retarded by all of the carbon sources supplemented in the degradation medium. PHA depolymerase synthesis by the Aspergillus spp., therefore, appeared to be regulated by the presence of easily consumable carbon sources in the degradation medium. The reduction of clear-zone formation in carbon source supplemented media in comparison to control (with polymer alone) indicated repression of depolymerase production and activity, the extent of such repression appeared to be influenced by the type of carbon source(s) supplemented in the medium. Synthesis of extracellular PHA depolymerase, the enzyme responsible for hydrolytic cleavage of PHA

polymer is known to be repressed by soluble carbon sources in bacteria (Jendrossek et al., 1993) as well as in terrestrial isolate of Aspergillus ustas. After exhaustion of the readily available nutrients, the synthesis of PHA depolymerases is derepressed in most of the strains (Sayal et al.,2006). In accordance with the results of the above authors, the fungal clear-zone formation was observed as large zones around the colonies, though no supplement of carbon sources in the degradation medium was studied in the present work.

The fungal isolates were identified as largely Asperigillus strains from their spore morphology. All the environment samples showed evidence of PHB degraders. This is predictable, in view of the biodiversity of the polymer-producing prokaryotes in these environments (Brandl et al., 1990) and the consequent availability of PHB as a nutrient source. It appears likely that PHB-containing bacteria became non-viable or were victims of predation, leading to the availability of this nutrient source and ecological sucess for microbes that can use it (Lee et al., 2005). Fungi that degrade PHB and copolymer were mostly isolated from soil compost, garden soil and farm soil. All the other environmental samples showed fungal polymer degraders only to a little extent (Table 1).

Bacteria are considerable degraders of PHB through their capability of production of intracellular depolymerase enzyme. Hence, their presence in these environmental samples was studied through a basal minimal media containg 0.03% of the polymers as a sole carbon source. The results indicate that soils from garden and an agricultural farm exihibited highest number of PHB degrading bacteria, followed by pond water and compost samples. Rock soil and fallen leaf samples yielded no PHB degrading bacteria (Table 1).

The cultivation of a few selected fungal and bacterial isolates in liquid cultures was carried out to study the amount of polymers utilized by these isolates during their growth. The fungal isolates showed a high capability of utilizing PHB and P(3HB-co-3HV) as growth substrate than bacterial isolates (Table 2). The fungal isolate 09/A from soil compost produced a 7 cm clear-zone on the assay and produced a highest biomass of 0.3 g/l in liquid cultures.

PHA degrading bacteria secrete specific PHA depolymerases which hydrolyze the polymer to water soluble monomers or oligomeric esters. The hydrolytic products are taken up by the cells and metabolized (Jendrossek et al., 1993). However, ecological studies on PHA degradation are rare.

The main objective in this work was to determine the biodegradability of the PHB and co-polymers, by various bacterial and fungal isolates from different ecosystems. Since, the above tabulated (table 2) fungal as well as bacterial isolates shown good results in degradation of the produced polymers this objective has been achieved. As seen in the table 1, the number of fungal degraders of PHB and copolymers were much more higher than that of bacterial degraders of these polymers. This may be due to the varsatile carbon source utilizing capabilites of the fungal isolates.

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