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Studies on PHB (Polyhydroxybutyrate) degradation by some species of Aspergillus

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Abstract: PHB degradation capabilities of seven species of *Aspergillus* namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus fumigatus* and *Aspergillus parasiticus* on the polymer produced by *Rhodospseudomonas palustris* KU003 were assayed . Among all the fungi investigated, the ability of A.niger to utilize the biopolymer was the highest and produced a biomass of 0.22 g/L. This was followed by *A.nidulans, A.flavus and A.terreus*. The other three fungi *A.ochraceus, A.fumigatus* and *A.parasiticus* could not utilize the polymer as carbon source. Significance of the above in the light of existing literature is discussed in this communication.
Keywords: PHB, Aspergillus, degradation.

Introduction:

PHA-producing bacteria can be generally classified into two groups[2]. The first class of bacteria, including *Ralstonia eutropha,* produces short chain length PHA with monomer units ranged from C_3 to C_5, while the other class, including *Pseudomonas oleovoran*s, produces medium chain length PHB with monomer units from C_6 to C_{14}[2]. Rohini *et al.*[3] characterized PHB from *Bacillus thuringienesis* R1 strain isolated from soil sample. Bacteria such as *Ralstonia eutropha, Alcaligenes latus* and *Azotobacter vivelandii* may be induced to synthesize PHB by imposing a chemical stress. This is normally done by depriving the organism of a nutrient such as nitrogen or phosphorus or sulfur, which are required for cell growth[4] . PHB production from phototrophic bacteria under different cultural conditions were reported by Merugu *et al.*[5,6] Fungi plays a considerable role in degrading polyesters as they are predominantly involved in the decomposition of organic matter in the soil[7] . Considering the role of fungi in the degradation of the polymer, a preliminary investigation was done on the ability of Aspergillus species in the degradation of the polymer.

Material and Methods:

Phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the Biebl and Pfennig's medium and incubated anaerobically in the light.

Bacterial pellet was suspended in 5ml of hypochlorite and incubated for 10 minutes. The suspension was centrifuged at 8000 rpm for 10 minutes. The pellet was washed with diethylether and was then assayed for PHB. PHB extracted by the above method was assayed by Law and Slepecky method[8]. PHB sample was treated with 5 ml of concentrated H_2SO_4 and a placed in a boiling water bath for 20 min. On cooling absorbance was recorded at 236 nm on a UV-Vis spectrophotometer. Standard was run using poly hydroxy butyrate.

All the fungi used in were isolated from soil. Polymer degradation was determined by growing the fungi in the Asthana and Hawkers medium (100ml) supplemented with 0.04% of the polymer. After inoculation, the samples were incubated on rotatory shaker for 4 to 8 days at 30± 2° C. The leftover residual amount of the polymer after incubation was determined using Law and Slepecky method[8]. The amount of the dry biomass was determined to calculate the growth of the isolates.

Results and Discussion:

The spectral values of the isolated polymer are given below:

IR spectra values: 1290(C-O), 1680(C=O aliphatic), 2670(CH_2),2910-2960(C-H streching)

NMR values: 1.08 (3H,d,-CH_3), 2.35 (2H,d,-CH2-), 5.15(1H,m,-CH-)

Table 1 shows the dry biomass as well as the residual polymer in medium of selected fungal isolates. the ability of *A.niger* to utilize the biopolymer was the highest and produced a biomass of 0.22g/L. This was followed by *A.nidulans, A.flavus and A.terreus*. Lowest biomass production was observed in *A.terreus*. The other three fungi *A.ochraceus, A.fumigatus and A.parasiticus* could not utilize the polymer as carbon source. Degradation of PHB by fungal isolates has been investigated in different natural environments such as soils, composts and natural waters[9,10,11]. The fungal isolates showed much higher capabilities than bacteria in degradation of PHA polymers (Kim and Rhee, 2003). Lee *et al*. [12] and Sayal *et al.*[13]have also shown the degradation of the polymer by some Aspergillus species. The present study shows that *A.niger* can be further investigated for the degradation of the polymer. Degradation using different cultural conditions and media should be studied for improving the degradation abilities of this organism.

Table 1: PHB utilization as carbon by some *Aspergillus* species.

Fungi	Initial polymer conc (%)	pH	Biomass (g/100ml)	Residual polymer (%)
Aspergillus niger	0.04	5.0	0.22	0.018
Aspergillus flavus	0.04	5.0	0.14	0.024
Aspergillus ochraceus	0.04	5.0	------	------
Aspergillus nidulans	0.04	5.0	0.16	0.022
Aspergillus terreus	0.04	5.0	0.10	0.028
Aspergillus fumigatus	0.04	5.0	-----	------
Aspergillus parasiticus	0.04	5.0	------	------

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