J. Natn.Sci.Foundation Sri Lanka 2006 34(3): 155 - 158

SHORT COMMUNICATION

Bacterial isolates degrading acylated cornstarch-plastic in soil

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Revised: 09 December 2005; Accepted: 28 February 2006

Abstract: Among 13 bacterial isolates from physicochemically different soil samples, eight strains identified as *Alcaligens* sp., *Bacillus* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Arthrobacter* sp. and *Acetobacter* sp. showed a significant ($p<0.05$) degradation of a novel biodegradable acylated cornstarch-plastic in pure cultures. The culture medium contained plastic powder as the sole carbon source. The degradation was determined by the amount of starch and glucose produced after one and two weeks of incubation respectively. The study revealed the presence of plastic degrading bacteria in a variety of soil types, and also the ability of plastic degradation of the isolates in soil types collected in Sri Lanka and Japan.

Key words: Acylated cornstarch, bacteria, biodegradation, plastic, soil.

INTRODUCTION

Accumulation of plastic as waste material has become a major environmental problem. Disposable plastics such as wraps, shopping bags, cups, plates, and packaging materials contribute significantly for the plastic waste in the environment. Petrochemically derived plastic has become a pollutant because of their non-biodegradability and nature of remaining for a long time in the environment. Despite these problems, plastics have become indispensable in the modern world due to its versatility and cost effectiveness. The annual consumption of plastic products in Sri Lanka is around 5.0 kg per capita and is expected to rise to 12.7 kg in 2010.[1] The plastic content in Colombo municipal waste alone amounts about 1260 tonnes a month.[2]

Development of biodegradable plastics is being extensively promoted as one of the solutions to the plastic waste crisis. An effective degradable plastic must possess all of the physical properties expected by the consumer and then when placed in the environment, should degrade more rapidly than conventional plastics. Bacterial polyesters such as polyhydroxy butyrates and polyhydroxy valerates are considered as biodegradable and are used in the production of biodegradable plastics. Bacteria and fungi that degrade these plastic products were isolated from a wide variety of environments.[3, 4] Plastics like Polylacticacid, poly ε - caprolactone , and polytetramethylene succninate, produced by bacterial fermentation of agricultural waste products such as potato peels are considered as biodegradable.[5,6] The disadvantage of these biodegradables is that they cannot replace current polyethylene and polypropylene plastics due to their high production cost.

Natural polymers such as cellulose and starch are being used to render biodegradable properties to plastics.[7] But to achieve adequate biodegradation of the copolymer, the level of natural polymer should be 30-50%. [8] At this high level of natural polymer the loss of tensile strength is evident. Research on cornstarch based biodegradable plastic is increasing from recent years.[9-11] Since it has very good thermoplastic properties it can be turned into a plastic with less effort. But in all these plastics the incorporated starch has been either blended with other polymers or processed and casted into films in a way to make it biodegradable.

However, in the biodegradable plastic subjected to the present study, the starch polymer itself has been modified and turned into a plastic. Modification has been done by acylation with acetic acid and lawric acid. The degradation of different compositions of this particular modified plastic in physicochemically different soil types collected in Japan and the impacts of the repeated use of a mulch film of this plastic on soil microflora were assessed in our previous studies.[12,13] Several micro-organisms grown on this plastic were also isolated from plastic debris collected from soil

In the present study, we report the presence of bacteria that degrade this particular plastic in Sri Lankan

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soils, and the properties of plastic degradability by these bacteria in comparison with some bacteria isolated from Japanese soils during earlier studies.

METHODS AND MATERIALS

The biodegradable plastic used in this study was synthesized from cornstarch by Japan Corn Starch Company limited, Nagoya, Japan. Cornstarch was modified by acylation through substitution (75%) by acetic (2 C) and lawric acids (12 C). The modified crude cornstarch powder without the addition of plasticizers and other pigmenting agents was used in this study.

Soil samples were collected from two plastic dumping sites at Ambalangoda (soil A) and Kelaniya (soil K) in Sri Lanka. Both samples were sieved through 2 mm sieves to remove gravel and organic debris and incubated at room temperature (30°C) for 3 d prior to the experiment. The pH, Organic matter content and the texture of soil A and soil K respectively are 6.8, 0.8%, sandy clay loam and 7.2, 0.6%, sandy loam.

Five grams of each soil were added into separate flasks containing 50 mL of autoclaved liquid mineral medium prepared according to the protocol reported earlier, [14] with some modifications by supplementing with 1% (w/v) plastic powder. The flasks were incubated on a shaker at 100 rpm at 30 °C. After every 2 wks, 1 mL of the culture was transferred into new flasks containing the same medium. The plastic powder was sterilized with 6% H_2O_2, followed by three serial washings with sterilized distilled water, before adding into the medium. After 6 wks, 1 mL sample of each culture was plated on mineral agar supplemented with starch plastic powder.

Five bacterial strains that were isolated from the enrichment cultures and eight bacterial isolates from a previous study done by burying the same acylated cornstarch-plastic films in two types of soil, a sandy clay loam with a pH of 6.4 and an organic matter content of 0.5%, and a volcanic andosol with a pH of 6.0 and an organic matter content of 5.2%, collected from Japan, were tested for their degradability. The bacteria were grown in 1% nutrient broth for 2 d and centrifuged at 12000 g for 5 min to get a pellet of the cells. The pellets were washed with sterilized distilled water and three loops of each bacterial culture were transferred into a tube containing 3 mL of sterilized distilled water and votexed. Each culture suspension was adjusted into equal turbidity, so that the inoculum contains approximately equal number of bacterial cells. One mililitre of each culture suspension was inoculated into flasks containing 50 mL of 0.1% plastic supplemented mineral medium,

Table 1: Starch and glucose production in culture media inoculated with bacteria

Bacterial Strain	Country isolated	Starch (g/100mL) (M±SE)	Glucose (mM) (M±SE)
Arthrobacter sp. (B26)	Japan	0.24 ih ±. 005	7.12 a.± .095
Bacillus sp. (EB3)	Sri Lanka	0.34 cb± .003	6.95 a± .017
Alcaligens sp. (EB2)	"	0.31 ced± .002	6.40 a± .083
Acetobacter sp. (EA2)	"	0.35 b± .003	5.26 b± .192
Pseudomonas sp. (NB1)	"	0.33 cbd± .003	5.18 b± .221
Acenitobacter sp. (M2)	Japan	0.30 fge± .005	5.13 b± .019
Acetobacter sp. (M6)	"	0.28 fge ± .005	4.75 b± .055
Arthrobacter sp. (M4)	"	0.32cbd ± .009	3.31 c± .024
B30[NI]	"	0.27 fgh ±. 005	2.37 d± .035
M7	"	0.42 a± .008	2.13 d± .011
B27	"	0.28 fgeh± .003	1.63 d± .017
M1	"	0.27 gh ± .003	0.23 e± .035
B12	"	0.23 ij ±. 003	0.14 e± .019
CTL		0.20 j ± .006	0.81 e± .003
LSD (α = 0.05)		0.0343	0.7887
CV(%)		6.89	13.15

Means followed by the same letter are not significantly different at 5% probability level.
NI = Not identified.

and incubated in a shaker at 100 rpm and at 30° C for 2 wks.

After 1 wk of incubation, the amount of starch present in the culture medium was determined by mixing a 3 mL sample of each culture with 0.2 mL of KI/I solution. Absorbance was measured at 620 nm using a spectrophotometer. The starch content was calculated using a standard curve plotted with known concentrations of starch solutions.

The amount of glucose present in the culture medium was determined after 2 wks of incubation by dinitrosalicylic colorimetric method, [15] where 3 mL of 3, 5, dinitrosalicylic acid reagent was added to 3 mL of each culture, and the tubes containing the mixture was heated for 15 min at 90° C until the red brown colour developed. After cooling, 40% potassium sodium tartrate was added and the absorbance was measured at 575 nm.

Eight bacterial strains that showed significantly high capability of degrading the plastic were identified by morphological and biochemical characteristics using a bacteriological key according to the Bergey's manual.[16] All the assessments were done in triplicate samples in the completely randomized design, and data were analyzed by one way ANOVA, SAS (1999). Means were separated using least significant difference (LSD) at $p<0.05$.

RESULTS AND DISCUSSION

As shown in the Table 1 among the 13 bacterial isolates, 8 strains produced a significantly high level of glucose in the culture medium after two weeks compared with the uninoculated control. This revealed that the bacterial isolates possess the enzyme systems that can degrade this acylated starch-plastic into glucose. The rates of glucose production of the strains (degradation) were in the following order; B26>EB3>EB2>EA2> NB1>M2>M6>M4>B30.

Very little is currently known about the interactions of amylolitic bacteria and starch containing plastics. Im am and Gould[17] reported about an *Arthrobacter* sp. with high amylase activity that degrade more than 80% of the starch within 30 days from starch-polymethylacrylic graft polymers and starch polyethylene-coacrylic plastics. In the present study, the bacterial strains B26 and M2 that showed the highest and substantial glucose production respectively were identified to be *Arthrobacter* sp.

It appears that in bioplastics, high levels of starch in polymer blends tend to deteriorate physical properties of the plastic, and on the other hand, when the starch volume is lower, the plastic shows resistance to degradation. However, the modified starch plastic in this study is advantageous, because it is purely cornstarch and the percentage of acylation can be altered in order to achieve required physical strength.[12]

Starch is a possible intermediate product of the process of degradation of this plastic, because once the ester bonds between the hydroxyl moieties of the amylase and the carboxylic moieties of the fatty acids (acetic and lawric acids) are digested, starch and fatty acids could be released. As presented in Table 1, the rates of degradation of the plastic by the strains into starch at a significant level were in the following order; M7>EA2> EB3> NB1> M4> EB2>M2>M6> B27>B30>M1. When these results are compared with the results of glucose production assay, it reveals that the mechanisms of degradation are different between the bacterial isolates. The highest level of starch was detected in the tube containing the strain M7, which on the other hand gave a comparatively low glucose production. The strain B26 that produced the highest level of glucose showed a very low amount of starch in the culture medium after one week. Thus, it can be assumed that the degraders of this plastic produce esterases that cleave the ester bond between the starch polymer and fatty acids, and then the enzymes amylases and lipases act on the amylose and fatty acids respectively and break them down to glucose. A *Pseudomonas* sp. and *Variovorax* sp. in a similar study degraded a biodegradable polyhexamehtylene carbonate plastic by secreting lipolytic enzymes.[18] The bacterium *Lactobacillus amylovorus*, isolated from a corn waste fermentation secreted amylases that degrade starch granules in a starch –plastic blend.[19]

The behaviour of B26, and M7 can be explained in two ways. The strain B26 could have either rapidly degraded all the starch produced as an intermediate product or produced glucose in an alternative pathway where starch is not involved as an intermediatory product. In contrast, the strain M7 could have lacked or had very low amylase enzyme activity, but possessed high esterase activity so that it could cleave the ester bonds between the fatty acids and amylase molecule in the plastic. With respect to glucose production the strains EB2 and EB3 are also not significantly different from the strain B26, and the starch production of these strains also substantial. Therefore it can be proposed that in addition to amylase activity the enzymes esterase

and lipase could be considerably active in the strains EB2 and EB3.

The strain EA2, the strains EB2, EB3, and NB1 were isolated by enrichment of the culture medium with plastic from two different soil types, soil A and soil K collected from two areas in Sri Lanka; Ambalangoda and Kelaniya respectively. The strains B12, B26, B27, B30 and the strains M1, M2, M4, M7 were isolated from two different types of soil from two different places in Japan using partially degrading plastic films buried in soil. The results in this study suggest that the bacteria isolated in enrichment cultures are capable of high rates of degradation when compared to those isolated directly from degrading plastic films.

However, strains B26, M2, M6, M4 and M7 show evidence of the existence of better degraders of this plastic in Japanese soils. The results also reveal the presence of bacteria that degrade this plastic in a variety of physicochemically different soil types regardless of the locations from where the soil samples are collected. Nevertheless, the performance of the strains B26, EB2, EB3 and M7 is interesting and further research is required for proper understanding of their behaviour in the degradation of this plastic.

Acknowledgement

The authors gratefully acknowledge Prof. D. M. Sirisena for providing facilities to carry out this research in the Department of Botany, University of Kelaniya.

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