The Utilization of the Plasticizer Dimethyl Phthalate by an Isolated Strain of *Enterobacter aerogenes*

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INTRODUCTION

Recent research has shown that plastics undergo progressive deterioration permitting the leaching out of phthalic acid esters (PAEs), compounds to which plastics owe their pliability. PAEs are listed as persistant environmental contaminants, reaching the environment indirectly via waste plastics and directly through industrial manufacturers (AUTIAN 1973). Various studies have been concerned with the degradation of PAEs by microorganisms. Using the Semi-Continuous Activated Sludge Biodegradation Test it was found that PAEs and intermediates are rapidly biodegraded by the microbial community present in the environment (SEAGER, et al., 1973). However, limited work has been done in the actual isolation and identification of phthalate-utilizing microorganisms. This study was undertaken to isolate and identify a microorganism capable of utilizing dimethyl phthalate (DMP), one of the phthalic acid esters.

MATERIALS AND METHODS

Samples were taken, using a sterile loop, from old plastic tubing used soley as an outlet for deionized water. The darkened tint of the tubing's inner surface suggested the possibility microbial growth.

The samples were streaked and bacteria isolated in pure culture on Tryptic Soy Agar plates incubated at 37°C. Two types of colonies were the most common, a small white round colony (SW) and a large white mucoid (LM). Both isolates were inoculated into 250ml flasks containing 100ml of minimal salts medium (MSM) consisting of KH_2PO_4 (13.6g), $(NH_4)_2SO_4$ (2.0g), $MgSO_4$ (0.2g), $FeSO_4 \cdot 7H_2O$ (0.0005g) per liter of distilled water with the addition of 1000 ppm of DMP as a sole carbon source. MSM containing no carbon source was inoculated as a control.

The inocula for our experimental cultures were taken from a 24-hr culture grown on Tryptic Soy Agar. Following inoculation into the DMP-MSM and the control MSM, the SW and LM cultures were incubated for 41 days at 37°C and at an agitation speed of 100 r.p.m. with a 6 inch stroke in an American Optical Model #2156 Water Bath Shaker. Control flasks of uninoculated DMP-MSM were also incubated to measure the DMP loss due to the physical factors of incubation.

The cultures were monitored and growth determined by turbi-

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dometric measurements, reading optical density at 450 nm on a Baucsh and Lomb Spectrophotometer (Spectronic 20).

The DMP concentration of the isolate cultures was determined by gas chromatography, both prior to inoculation and following the 41 day incubation. The DMP in the aqueous media was extracted using a 2:1 ratio of hexane to media. The DMP extractions were done with the media plus the bacterial cells to insure that any phthalate adsorbed by the cell wall of the organism was also extracted and measured. The DMP extracts were measured using the Antek Model 340-LP Gas Chromatograph. Quantitation was calculated from a standard peak made of the 1000ppm DMP media.

The LM isolate was characterized by standard biochemical and morphological techniques and identified using Bergey's Manual of Determinative Bacteriology (1974).

RESULTS AND DISCUSSION

Of the two bacteria isolated from the plastic tubing, the LM isolate was found to utilize DMP as a sole carbon source. This bacterium was subsequently identified as a strain of Enterobacter aerogenes.

After 41 days of incubation, the E. aerogenes isolate culture on DMP-MSM exhibited a substantial amount of growth, with an increase in optical density from 0.00 to 1.05 . The E. aerogenes control culture, consisting of MSM with no carbon source exhibited a negligible amount of growth (Fig. 1).

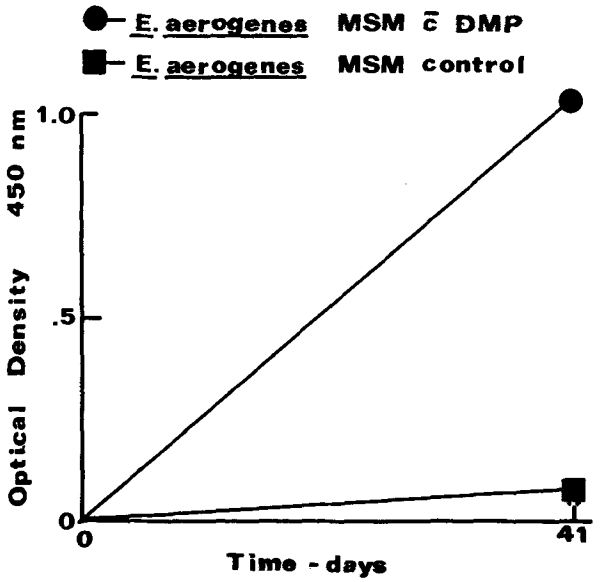

Fig. 1 Growth of E. aerogenes isolate on DMP as a sole carbon source.

The DMP concentration in the E. aerogenes isolate culture, as determined by gas chromatography, showed that 674ppm or 67.4% of the DMP had been utilized. Taken into account was the 40ppm of DMP lost to the environment as calculated from the control flasks of uninoculated DMP-MSM also incubated for 41 days (Fig.2).

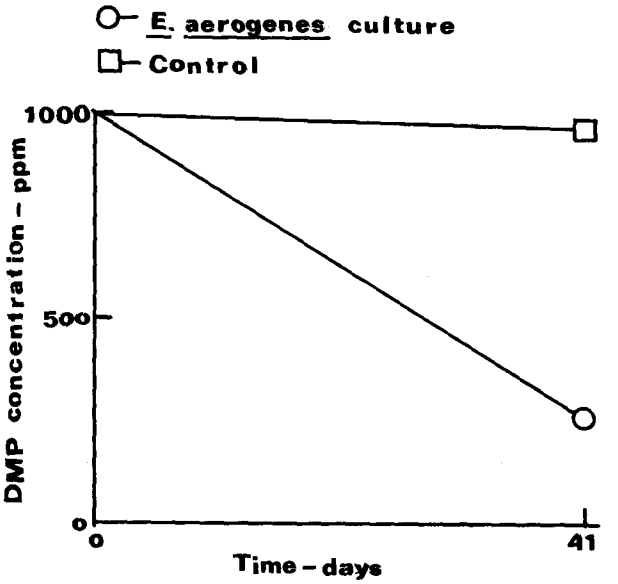

Fig. 2 DMP concentration in E. aerogenes culture

The data clearly shows that the E. aerogenes isolate effectively utilizes the phthalic acid ester - DMP as a sole carbon source. Being a virtually ubiquitous saprophyte found in sewage, soil, water, and dairy products, this microorganism could play an important role in solving the problem of the pollutant plasticizers

Of interest and importance is the fact that E. aerogenes is the second bacterial species of the tribe Klebsielleae (family Enterobacteriaceae) to be identified as a PAE utilizer. Serratia marcescens has been shown to utilize the PAE di-2-ethylhexly phthalate (DEHP) as a sole carbon source (MATHUR, et al., 1975). The genera Klebsiella, Enterobacter, Hafnia, and Serratia which make up the Klebsielleae tribe are very closely related and often confused (Bergey's Manual of Determinative Bacteriology 1974). Therefore, since two genera have been shown to utilize PAEs, it seems the next step would be to investigate the possible phthalate-utilizing ability of the Klebsiella and Hafnia genera.

Also of importance is the relationship between Enterobacter aerogenes and Klebsiella pneumoniae, species which many investigators have suggested be classified as a single group (Bergey's Manual of Determinative Bacteriology 1974). Both species are commonly found in the human intestinal tract and both are reportedly

responsible for sinusitis, pharyngitis, meningitis, endocarditis, septicemia, peritonitis, liver abscess, salpingitis, and urinary tract infections. The results of this study showing that E. aerogenes is capable of contaminating and proliforating in plastic tubing, utilizing PAEs as the only apparant carbon source, suggests that an added precaution be taken in hospital environments where humid plastic articles such as tubing and oxygen masks are sometimes used indiscriminantly.

Althought the isolate reported in this study should be helpful in facilitating an answer to the pollutant plasticizer problem and perhaps in assessing the susceptibility of certain plastics to microbial attack, it should be remembered that another area of concern cannot be ignored: that of controlling the contamination of hospital and other high risk environments by these PAE-utilizing microorganisms.

ACKNOWLEDGEMENT

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