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Biodegradation of phthalates and phthalate esters

T B KAREGOUDAR and B G PUJAR*
Department of Chemistry, Karnatak University, Dharwad 580003, India.

Abstract. Several microorganisms utilising terephthalate and diethylphthalate have been isolated from garden soil. Protocatechuate was one of the intermediates in the degradation of terephthalic acid and diethylphthalate. Accumulation of protocatechuate during the growth of these strains has been described.

Keywords. Biodegradation; terephthalate; diethylphthalate.

1. Introduction

Large scale manufacture over the past two decades of phthalates and phthalic acid esters for the textile and plastic industries has recently provoked concern as to their possible role as pollutants in water and soil. A number of reports on the biodegradation of phthalates and phthalate esters have appeared in literature (Ribbons and Evans 1960; Kurane *et al* 1977, 1980; Engelhardt and Wallnofer 1978; Saeger and Tucker 1976; Keyser *et al* 1976). Much information is available on the degradation of *o*-phthalate by the genus *Pseudomonas*. (Ribbons *et al* 1960). The isolation and study of bacteria that grow at the expense of *o*-phthalate led to the discovery of a catabolic pathway that has a common intermediate, 4,5-dihydroxyphthalate and protocatechuate. Biodegradation of isophthalate has been studied in *Micrococcus* (Hari Babu and Vaidyanathan 1982). Recently, we have isolated four different micro-organisms growing on terephthalate and two strains growing on diethylphthalate as the sole carbon sources. The present study gives the characterization of these strains and describes the accumulation of protocatechuic acid during the growth of these organisms.

2. Methods and materials

Microorganisms were isolated from garden soil by enrichment shaking culture at 30°C using Kurane's mineral salt medium (Kurane *et al* 1977). Liquid cultures were grown in 500 ml Erlenmeyer flasks containing 100 ml of mineral base with additions of 0·05 to 0·2% substrates. Growth of bacteria was determined by measuring the optical density of the culture at 660 nm. All these strains were identified and characterised according to Bergey's manual of determinative bacteriology by applying broad spectrum criteria (Buchanan and Gibbons 1974). The organisms were tested for cleavage of catechol or protocatechuate by the *ortho* or *meta* pathway (Stanier *et al* 1966). Protocatechuate and terephthalate were estimated by extraction of compounds from the media followed by quantitative UV analysis at λ_{max} 292 nm and 241 nm respectively.

* To whom all correspondence should be addressed.

3. Results and discussion

Terephthalate was utilised by three gram-positive organisms and one gram-negative organism. The three gram-positive strains were identified as *Bacillus cirroflagellosus*,

Table 1. Aromatic compounds which acted as the source of carbon for growth of some bacteria isolated from garden soil by enrichment with terephthalate as carbon source.

Strain	Source of carbon				
	Protocatechuic acid	Benzoic acid	*o*-phthalic acid	Diethyl phthalate	Dimethyl terephthalate
Bacillus cirroflagellosus	+	+	+	+	–
Nocardia resticta	+	+	+	+	–
Arthrobacter terregens	+	+	+	+	–
Pseudomonas alcaligenes	+	+	+	+	–
Micrococcus varians	+	+	+	+	–

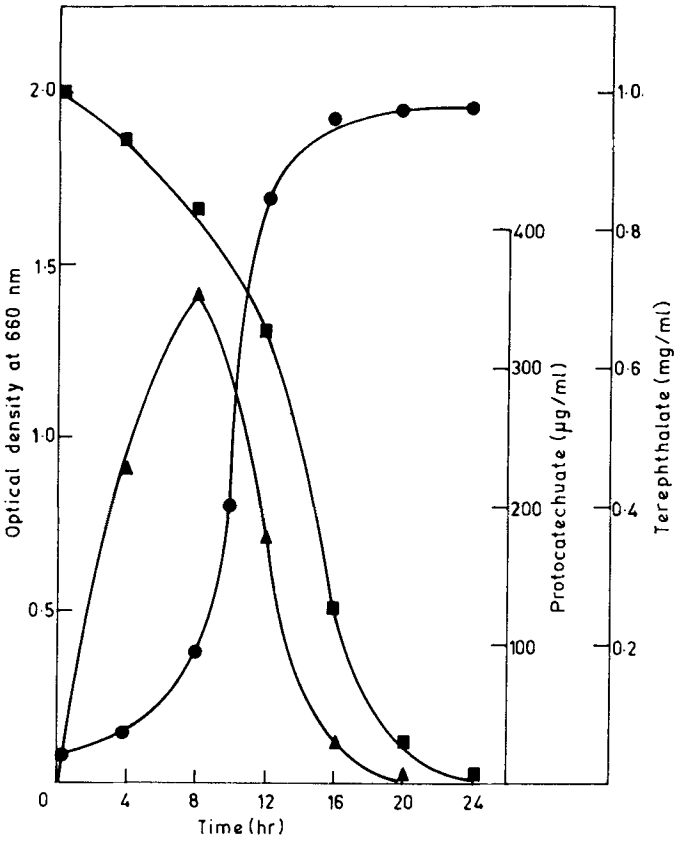

Figure 1. Transient accumulation of protocatechuic acid and disappearance of terephthalate from culture solution during growth of *Bacillus cirroflagellous* in 0·2% terephthalate-mineral base. Accumulation of protocatechuic acid was measured by UV analysis at 292 nm and degradation of terephthalate was measured at 241 nm. Symbols: ●—● growth; ■—■ disappearance of terephthalate; ▲—▲ transient accumulation of protocatechuate.

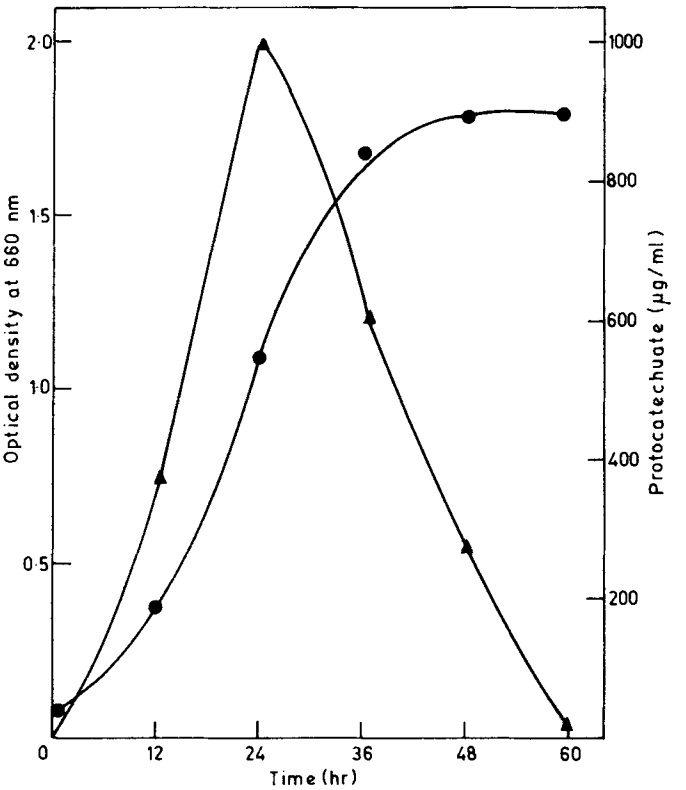

Figure 2. Accumulation of protocatechuic acid during the growth of *Micrococcus varians* in (0·07%) diethyl phthalate-mineral base. Protocatechuic acid was measured by UV analysis at 292 nm. Symbols: ●—● growth; ▲—▲ transient accumulation of protocatechuate.

Nocardia resticta and *Arthrobacter terregens*. The lone gram-negative strain was identified as *Pseudomonas alcaligenes*. Diethylphthalate supported the growth of two gram-positive organisms, *Micrococcus varians* and *Bacillus sphaericus*. All these strains followed the *ortho* cleavage pathway as shown by the Rothera reaction (Rothera 1908).

Besides terephthalate, some other aromatic compounds which are unrelated to the phthalate pathway, were also tested for their ability to support growth. Protocatechuic acid, benzoic acid and *o*-phthalic acid supported the growth of these organisms (table 1). Dimethylterephthalate failed to support the growth of these organisms.

Protocatechuate was detected as one of the intermediates during the growth on terephthalate and diethylphthalate. Degradation of terephthalate and transient formation of protocatechuate during the growth of *Bacillus cirroflagellosus* are shown in figure 1. Extinctions were linear with substrate concentrations. Maximum growth of this organism took place in about 20 hours. Observations were made on *Nocardia* sp. where the maximum growth was observed at 60 hours (Engelhardt *et al* 1976). Similar observations were made with *Micrococcus varians* growing on diethylphthalate as the sole carbon source. Formation of protocatechuate took place during the growth on diethylphthalate which is shown in figure 2. In all these cases protocatechuate was

found to be one of the intermediates. These results suggest that such organisms may be abundantly present in the environment and that these organisms utilise potentially hazardous chemicals like phthalate and phthalate esters.

Acknowledgement

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