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Complete degradation of dimethyl phthalate by biochemical cooperation of the *Bacillus thuringiensis* strain isolated from cotton field soil†

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Dimethyl phthalate (DMP), a phthalate ester, is widely used in cosmetics, perfumes, and plasticizers. It has been classified as a suspected endocrine disruptor by many countries. The present study describes the biodegradation of DMP by a new aerobic bacterium, isolated from soil samples of a cotton field by an enrichment culture technique utilizing DMP as the sole source of carbon and energy. The isolate was identified as *Bacillus thuringiensis* based on the morphological and biochemical characteristics as well as gene sequence analysis. *Bacillus thuringiensis* grows best in a mineral salt medium of pH 7.0 at 30 °C incubation for 48 hours. The effects of temperature, inoculum size, substrate concentration and incubation time on DMP degradation were also studied. *Bacillus thuringiensis* is able to biodegrade 400 mg L⁻¹ of DMP under aerobic conditions with 99% degradation potential. A combination of GC and GC-MS analysis revealed a complete DMP biodegradation pathway. The results indicate that *Bacillus thuringiensis* may prove a promising source for DMP bioremediation at a commercial scale.

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Introduction

Dimethyl phthalate (DMP) is widely used as a plasticizer in the plastic industry during the manufacturing of compounds such as latex, cellulose acetate films and plastic. DMP leaches into the environment from tubing, dishes, paper, and containers via the general use of plastic.¹ DMP molecules are bound physically to the plastic structure; they are easily released from plastic products and leached into the environment.² Moreover it is a relatively stable compound in the natural environment with a half-life of ≈ 20 years.³ Therefore, DMP has been frequently identified in diverse environmental samples including groundwater, river water, drinking water, open ocean water, soil humates, lake and marine sediments.⁴ DMP promotes chromosome injuries in human leucocytes and known to be an endocrine disrupting chemical (EDC) that interferes with the reproductive system and normal development of animals and humans.⁵

Natural processes such as hydrolysis and photo decomposition have been previously reported for degradation of phthalates from environment.^{6–8} Unfortunately degradation of DMP under abiotic conditions has been reported to be slow and insignificant process.³

Breakdown by microorganisms plays a major role in DMP degradation and bacterial activities bring about actual assimilation of various compounds under eco-friendly conditions. Several investigators have demonstrated successful degradation of DMP by microbes under aerobic conditions in soil, natural water and wastewater.⁹ Different bacterial strains have been isolated with the ability to degrade DMP and their isomers from activated sludge, mangrove sediment, wastewater, *etc.*^{9–11} The microbial strains such as *Sphingomonas paucimobilis*, *Pseudomonas fluorescens*, *Pseudomonas aureofaciens*, *Xanthomonas maltophilia*,¹² *Rhodococcus rubber*,¹³ *Pseudomonas fluorescens FS1*,¹⁴ *Flavobacterium* sp.,¹⁵ *Candida rugosa*¹⁶ and *Arthrobacter* sp.¹⁷ have reported as to degrade DMP. Studies have shown that bioremediation can offer a potential solution for the conversion of phthalic acid esters into harmless end products such as CO₂ and H₂O.¹⁸ However; many authors have not described the degradation pathways, although few have reported the conversion of DMP into monomethyl phthalate (MMP) and phthalic acid (PA).

Hence in the present study, an aerobic bacterial strain *Bacillus thuringiensis* was enriched from cotton field soil. *Bacillus thuringiensis* is a soil dwelling bacterium and is deadly to insects but it is non-pathogenic to human beings. The

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