DEGRADATION OF THREE DIMETHYL PHTHALATE ISOMER ESTERS (DMPEs) BY MANGROVE SEDIMENT FUNGI

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紅樹林真菌對三種鄰苯二甲酸二甲酯同分異構體的降解

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Abstract

Phthalate esters (PAEs) are important industrial compounds mainly functioning as plasticizers and additives to increase flexibility and softness of plastic products. PAEs are of major concern due to their widespread use, ubiquity in the environment, and endocrine-disrupting activity. Degradation pathways of PAEs using bacteria as agents have been well elucidated including molecular studies of enzymes involved. By contrast, fungal degradation of PAEs has received surprisingly little attention. Although limited reports have demonstrated the potential of fungi on PAE degradation, the degradation mechanisms of PAEs by fungi remain largely unknown. Therefore, this study aims to isolate dimethyl phthalate esters (DMPEs)-degrading fungi from mangrove sediments and to explore the degradation pathways and key enzymes involved.

Two fungal strains, with the ability to degrade DMPEs, were isolated from mangrove sediments contaminated with industrial pollutants in the Futian Nature Reserve of Shenzhen, China, by enrichment culture technique. Based on spore morphology and molecular typing using 18S rDNA sequence, these fungi were identified as *Fusarium* sp. DMT-5-3 (a filamentous fungus) and *Trichosporon* sp. DMI-5-1 (a basidiomycetous yeast).

Comparative investigations on biodegradation of three isomers of DMPEs, namely dimethyl phthalate (DMP), dimethyl isophthalate (DMI), and dimethyl terephthalate (DMT), were carried out by these two fungi. It was found that both fungi could not completely mineralize DMPEs but transform them to the respective monomethyl phthalate or phthalate acid. Biochemical degradation pathways for different DMPE isomers by both fungi were different. Both of these two fungi could transform DMT to monomethyl terephthalate (MMT) and further to terephthalic acid

(TA) by stepwise hydrolysis of two ester bonds. However, they could only carry out one step ester hydrolysis to transform DMI to monomethyl isophthalate (MMI). Further metabolism of MMI did not proceed. Only *Trichosporon* sp. DMI-5-1 was able to transform DMP to monomethyl phthalate (MMP) but not *Fusarium* sp. DMT-5-3. The optimal pH for DMI and DMT degradation by *Fusarium* sp. DMT-5-3 was 6.0 and 4.5 respectively, whereas for *Trichosporon* sp. DMI-5-1, the optimal pH for the degradation of all the three DMPE isomers was at 6.0. These results suggest that the fungal esterases responsible for hydrolysis of the two ester bonds of PAEs are highly substrate specific.

Esterase activity was detected in both the cell-free supernatant and fungal mycelium when incubating *Fusarium* sp. DMT-5-3 in mineral salts medium with DMT as the inducing substrate. Intracellular esterases showed a much higher hydrolytic activity than extracellular esterases. An intracellular esterase was isolated from fungal mycelium and was purified by ion-exchange chromatography and gel-filtration chromatography in sequence. The molecular mass of the enzyme was about 240 Kda. The enzyme consisted of six identical subunits, of which the molecular mass was about 40 Kda. The Km and Vmax values for *p*-nitrophenol acetate (PNPA) were 0.47 mM and 4.62 μM min^{-1}, respectively. Effects of temperature, pH, and metal ions on esterase activity were investigated with PNPA as the substrate. The enzyme showed maximum esterase activity at 50℃ and was stable below 30℃. The optimal pH was 8 and the enzyme was stable at pH 6-10. The esterase activity was strongly inhibited by Cr^{3+}, Hg^{2+}, and Cu^{2+}; and slightly inhibited by Zn^{2+}, Ni^{2+}, Cd^{2+}, and EDTA. Substrate specificity analysis showed that the enzyme was only able to hydrolyze DMT but not DMP, DMI, MMP, MMI, MMT, which was consistent with the degradation pathways of DMPEs by the test fungus, and supported the notion that the fungal esterases involved in the cleavage of two carboxylic ester linkages of DMPEs are highly substrate specific.

In summary, this is the first detailed study demonstrating the degradation of PAEs by mangrove sediment fungi. The pathways of degradation of DMPEs as demonstrated by the test fungi are similar to the reported degradation pathways shown by bacteria. The catalytic characteristics of esterase isolated from test fungi support the notion that esterases are a diverse group with distinct enzymes catalyzing different part of the hydrolytic reactions but involving two identical carboxylic ester bonds of PAEs. Further investigation can focus on the cloning and expression of the esterase gene to elucidate the molecular basis of PAE degradation in fungi.

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