

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

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Degradation of Three Dimethyl Phthalate
Isomer Esters (DMPEs) by Mangrove
Sediment Fungi
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 K_m and V_{max} values for *p*-nitrophenol acetate (PNPA) were 0.47 mM and $4.62 \mu\text{M min}^{-1}$, 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°C and was stable below 30°C . 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.

Table of Contents

Abstract	i
Acknowledgements	iv
Table of Contents	v
List of Tables	x
List of Figures	xii
Abbreviations	xvi
 Chapter 1 Literature Review	 1
1.1 Physical and chemical characteristics of phthalate esters (PAEs)	1
1.2 Industrial usages of PAEs	7
1.3 Environmental distribution of PAEs	8
1.3.1 Occurrence in aquatic environments	11
1.3.2 Occurrence in terrestrial environments	13
1.3.3 Occurrence in the foods	15
1.4 Toxicity of PAEs	15
1.4.1 Aquatic toxicity	16
1.4.2 Mammalian toxicity	17
1.5 Degradation of PAEs	21
1.5.1 Abiotic degradation	21
1.5.2 Microbial degradation	23
1.5.2.1 Degradation of PAEs by bacteria	23
1.5.2.1.1 Aerobic degradation of PAEs by bacteria	24
1.5.2.1.1.1 Degradation of PAEs by axenic bacterial cultures	24
1.5.2.1.1.2 Degradation of PAEs by bacterial consortia	28

1.5.2.1.1.3 Biochemical mechanisms for PAE degradation in bacteria	30
1.5.2.1.1.4 Molecular basis of PAE degradation in bacteria	35
1.5.2.1.2. Anaerobic degradation of PAEs by bacteria	40
1.5.2.2. Degradation of PAEs by fungi	43
1.5.2.2.1 Biodegradation potential of fungi	43
1.5.2.2.2 Degradation of PAEs by fungal pure cultures	44
1.5.2.2.3 Degradation of PAEs by purified fungal enzymes	50
1.6 Rationale of the present study	54
1.6.1 Hypotheses raised in the present study	54
1.6.2 Plan of the present study	54
1.6.3 Plan of the thesis	55
 Chapter 2 Enrichment, Isolation and Screening of DMPE-degrading Fungi from Mangrove Sediments	 56
2.1 Introduction	56
2.2 Materials and methods	58
2.2.1 Sampling site	58
2.2.2 Sediment sampling	59
2.2.3 Enrichment and isolation of DMPE-degrading fungi	60
2.2.4 Identification of fungal strains	61
2.2.5 Screening and preliminary investigation of DMPE-degrading ability of fungal isolates	62
2.2.6. Chemical analysis	63
2.3 Results	63
2.3.1 Enrichment of DMPE-degrading fungi	63
2.3.2 Isolation and identification of fungal isolates	64

2.3.3 Preliminary investigation of DMPE degradation capability of fungal isolates	65
2.4 Discussion	69
2.5 Conclusion	70
 Chapter 3 Degradation of DMPEs by a Filamentous Fungus, <i>Fusarium</i> sp.	
DMT-5-3, Isolated from Mangrove Sediments	71
3.1 Introduction	71
3.2 Materials and methods	72
3.2.1 Fungal strain and culture conditions	72
3.2.2 Characterization of the fungal strain	72
3.2.3 Degradation of DMPEs and suspected intermediates by the fungal strain	73
3.2.4 Effect of pH on the degradation of DMPEs by the fungal strain	74
3.2.5 Chemical analysis	74
3.2.6 Cellular protein measurement	75
3.3. Results	75
3.3.1 Characterization of fungal strain	75
3.3.2 Biochemical degradation of three DMPE isomers	78
3.3.3 Effect of pH on the degradation of DMPEs	81
3.4 Discussion	84
3.5 Conclusion	86
 Chapter 4 Degradation of DMPEs by a Basidiomycetous Yeast, <i>Trichosporon</i> sp.	
DMI-5-1, Isolated from Mangrove Sediments	87
4.1 Introduction	87
4.2 Materials and methods	88

4.2.1 Fungal strain and culture conditions	88
4.2.2 Characterization of fungal strain	88
4.2.3 Degradation of DMPEs and suspected intermediates by the fungal strain	88
4.2.4 Effect of pH on the degradation of DMPEs by the fungal strain	89
4.2.5 Chemical analysis	89
4.2.6 Cellular protein measurement	89
4.3. Results	89
4.3.1 Characterization of the fungal strain	89
4.3.2 Biochemical degradation of three DMPE isomers	92
4.3.3 Effect of pH on the degradation of DMPEs	95
4.4 Discussion	98
4.5 Conclusion	100
 Chapter 5 Purification and Characterization of an Intracellular Esterase from	
<i>Fusarium</i> sp. DMT-5-3 with DMT as the Inducing Substrate	101
5.1 Introduction	101
5.2 Materials and methods	103
5.2.1 Fungal strain and culture conditions	103
5.2.2 Extraction of extracellular and intracellular esterases	103
5.2.3 Enzyme and protein assay	104
5.2.4 Enzyme purification	105
5.2.5 Characterization of esterase	106
5.2.6 Estimation of molecular mass of esterase and activity staining	107
5.2.7 Substrate specificity analysis of esterase	107
5.3 Results	108
5.3.1 Comparison of esterase activity between intracellular protein and	

extracellular protein	108
5.3.2 Purification of intracellular esterase	110
5.3.3 Molecular mass of esterase	114
5.3.4 Characterization of the esterase	115
5.3.4.1 Kinetic parameters	115
5.3.4.2 Effects of temperature and pH on the enzyme activity	118
5.3.4.3 Effect of metal ions and EDTA on the enzyme activity	121
5.3.4.4 Substrate specificity	122
5.4 Discussion	123
5. 5. Conclusion	127
Chapter 6 General Discussion	129
6.1 Microbial degradation of DMPEs	129
6.2 Role of mangrove fungi on the fate of DMPEs in aquatic environment	130
6.3 Enzymes involved in degradations of DMPEs by mangrove fungi	135
6.4 Conclusions and future studies	137
References	140
Appendix 1 18S rDNA Gene Sequences of DMPE-degrading Fungi Isolated from Mangrove Sediments	156
Appendix 2 Conference Presentations and Publications	158
Appendix 3 Paper Published (Marine Pollution Bulletin): Degradability of the three dimethyl phthalate isomer esters (DMPEs) by a <i>Fusarium</i> species isolated from mangrove sediment	160
Appendix 4 Abstracts of Conference Presentations	164

List of Tables

Table 1.1	Physical-chemical properties of some phthalate esters ^a .	3
Table 1.2	Distribution and concentrations of PAEs in the environment.	8
Table 1.3	Degradation of PAEs by axenic bacterial cultures.	25
Table 1.4	Degradation of PAEs by bacterial consortia.	29
Table 1.5	Phthalate-degrading genes and their corresponding functions in several bacterial strains.	37
Table 1.6	Degradation of PAEs by fungal cultures.	44
Table 1.7	Enzymatic degradation of several PAEs by two purified fungal enzymes, <i>Fusarium oxysporum</i> f. sp. <i>psi</i> cutinase and <i>Candida cylindracea</i> esterase.	52
Table 2.1	Sediment samples in this study.	60
Table 2.2	Mycelial biomass in the 6 th subculturing enrichment cultures (+++, prosperous growth; ++, good growth; +, slight growth).	64
Table 2.3	Identification of DMPE-degrading fungi and growth of these fungal isolates in the MSM medium containing 100 mg l ⁻¹ DMPE as sole source of carbon and energy after 3 weeks of incubation (+++, abundant growth; +, slight growth; -, no growth).	66
Table 3.1	A comparison of removal percentages of DMPEs by <i>Fusarium</i> sp. DMT-5-3 under different pH levels after two incubation days using two-way ANOVA.	82
Table 4.1	A comparison of removal percentages of DMPEs by <i>Trichosporon</i> sp. DMI-5-1 under different pH levels after two incubation days using two-way ANOVA.	96
Table 5.1	Purification of phthalate esterase from <i>Fusarium</i> sp. DMT-5-3. Crude enzyme was extracted from 0.3 g of fungal biomass (dry weight).	113
Table 5.2	Linear regression equations for enzymatic hydrolysis of different concentrations of <i>p</i> -nitrophenyl acetate (PNPA) by phthalate esterase from <i>Fusarium</i> sp. DMT-5-3.	117
Table 5.3	Effect of metal ions and EDTA on esterase activity.	122
Table 5.4	Substrate specificity of phthalate esterase.	123
Table 5.5	Comparison of various phthalate esterases.	126
Table 6.1	Microbial degradation of DMPEs.	130

Table 6.2	Hydrolysis ability of test fungi on different isomers of DMPEs and MMPEs.	136
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List of Figures

- Figure 1.1** Chemical structures of three isomers of dimethyl phthalate esters (dimethyl phthalate (DMP), dimethyl isophthalate (DMI), and dimethyl terephthalate (DMT)) and six PAEs listed as priority pollutants by the United States Environmental Protection Agency, including DMP, diethyl phthalate (DEP), di-*n*-butylphthalate (DnBP), di-*n*-propylphthalate (DnPP), di-*n*-octylphthalate (DnOP), and di-2-ethylhexylphthalate (DEHP). 1
- Figure 1.2** Photo-Fenton process to produce hydroxyl radicals (Al-tawabini, 2003). 23
- Figure 1.3** Photo-generation of hydroxyl radicals by Fe(III) aquacomplexes (Mailhot et al., 2002). 23
- Figure 1.4** Transformation pathways of PAEs to protocatechate by aerobic bacteria (Wang, 2005). **A.** transformation pathway by Gram-positive bacteria (Eaton, 2001); **B.** transformation pathway by Gram-negative bacteria (Chang and Zylstra, 1998). **I.** *ortho*-phthalate ester; **II.** Monophthalate ester; **III.** *Ortho*-phthalic acid; **IV.** *cis*-3,4-dihydroxy-3,4-dihydrophthalate; **V.** 3,4-dihydroxyphthalate; **VI.** *cis*-4,5-dihydroxy-4,5-dihydrophthalate; **VII.** 4,5-dihydroxyphthalate; **VIII.** protocatechuate. 33
- Figure 1.5** Degradation pathways of protocatechuate by aerobic bacteria (Wang, 2005). **A.** *ortho*-cleavage pathway (Keyser et al., 1976); **B.** *meta*-cleavage pathway (Keyser et al., 1976; Eaton, 2001). **I.** protocatechuate; **II.** 3-carboxy-*cis*, *cis*-muconate; **III.** β -ketoadipate; **IV.** 2-hydroxy-4-carboxymuconic semialdehyde; **V.** 2-hydroxy-4-carboxymuconic semialdehyde-hemiacetal; **VI.** 2-pyrone-4,6-dicarboxylate; **VII.** 4-oxalomesaconate; **VIII.** 4-oxalocitramalate; **IX.** oxaloacetate; **X.** pyruvate. 34
- Figure 1.6** Comparison of operons encoding the metabolism of phthalate from several bacterial strains (Stingley et al., 2004). *phtAa*, gene coding for phthalate dioxygenase large subunit; *phtAb*, gene coding for phthalate dioxygenase small subunit; *phtAc*, gene coding for ferredoxin subunit; *phtAd*, gene coding for ferredoxin reductase; *phtB*, gene coding for *cis*-3,4-dihydro-3,4-dihydroxyphthalate dehydrogenase; *phtC*, gene coding for 3,4-dihydroxyphthalate decarboxylase; *phtR*, gene coding for the transcriptional regulator; *pht2* and *ophA1*, genes coding for phthalate dioxygenase reductase; *pht3* and *ophA2*, genes coding for phthalate dioxygenase; *pht4* and *ophB*, genes coding for 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase; *pht5* and *ophC*, genes coding for 4,5-dihydroxyphthalate decarboxylase; *phtI*, *orfI*, and *ophD*, genes coding for putative phthalate transporter. 36
- Figure 1.7** Degradation pathway of DMT by fungi. **A.** by *Sclerotium rolfsii* (Sivamurthy et al., 1991). **B.** by *Aspergillus niger* (Ganji et al.,

- 1995). **I.** dimethyl terephthalate; **II.** monomethyl terephthalate; **III.** terephthalic acid; **IV.** protocatechuate. 47
- Figure 1.8** Degradation pathway of DBP by *Polyporus brumalis* (Lee et al., 2007). **I.** dibutyl phthalate; **II.** diethyl phthalate; **III.** monobutyl phthalate; **IV.** phthalic acid anhydride. 47
- Figure 1.9** Degradation pathway of DEHP by *Fusarium sporotrichioides* NFRI-1012 (Chai et al., 2008). **I.** di-(2-ethylhexyl) phthalate; **II.** dimethyl phthalate; **III.** 2-ethylhexanol; **IV.** 2-ethylhexanoic acid. 49
- Figure 1.10** Degradation pathway of DMP by *Aspergillus niger* (Pradeepkumar et al., 2000). **I.** dimethyl phthalate; **II.** monomethyl phthalate; **III.** phthalic acid; **IV.** protocatechuate. 49
- Figure 2.1** Sampling location map: **A**, a raw sewage outlet; **B**, a random floor site in a natural mangrove forest; **C**, a river outlet (Fengtang River). 59
- Figure 2.2** Fungal enrichment cultures with three DMPE isomers as the sole carbon and energy source. Mycelial biomass is indicated by arrows. 64
- Figure 2.3** (a) Degradation of DMI by *Trichosporon* sp. DMI-5-1. (b) Degradation of DMI by *Penicillium* sp. DMI-5-2. (c) Degradation of DMT by *Fusarium* sp. DMT-5-3. Curves include DMI (■), MMT (▲), and control (◇). Error bars show standard deviations amongst the triplicate samples. 68
- Figure 3.1** Optical micrograph showing macroconidium of *Fusarium* sp. DMT-5-3 grown on CMA medium. 76
- Figure 3.2** The phylogenetic 18S rDNA-based tree showing the relationship between the fungal strain DMT-5-3 and selected members of genus *Fusarium*. 77
- Figure 3.3** Degradation of DMPEs and suspected intermediates by *Fusarium* sp. DMT-5-3 over 24 days: (a) DMI, (b) DMT, and (c) MMT. Curves include DMI (◀), MMI (▷), DMT (■), MMT (▲), TA (▽), protein (●), and control (◇). Error bars show standard deviations amongst the triplicate samples. 80
- Figure 3.4** Proposed biochemical degradation pathways for DMPEs by *Fusarium* sp. DMT-5-3: (a) DMP, (b) DMI, and (c) DMT. 81
- Figure 3.5** Effect of pH levels on the degradation of DMPEs by *Fusarium* sp. DMT-5-3 after 4 days and 10 days of incubation: (a) DMI degradation, and (b) DMT degradation. Bars with different letters are significantly different at $p < 0.05$ (Tukey Test). Error bars show standard deviations among the triplicate samples. 83
- Figure 4.1** Optical micrograph showing budding cells and arthroconidia of

- Trichosporon* sp. DMI-5-1 grown in PDB medium. 90
- Figure 4.2** The phylogenetic 18S rDNA-based tree showing the relationship between the yeast strain DMI-5-1 and selected members of genus *Trichosporon*. 91
- Figure 4.3** Degradation of DMPEs and suspected intermediates by *Trichosporon* sp. DMI-5-1: (a) DMP, (b) DMI, (c) DMT, and (d) MMT. Curves include DMP (■), MMP (▲), DMI (▼), MMI (◄), DMT (►), MMT (△), TA (▽), protein (●), and control (◇). Error bars show standard deviations amongst the triplicate samples. 94
- Figure 4.4** Proposed biochemical degradation pathways for DMPEs by *Trichosporon* sp. DMI-5-1: (a) DMP, (b) DMI, and (c) DMT. 95
- Figure 4.5** Effect of pH levels on the degradation of DMPEs by *Trichosporon* sp. DMI-5-1 at after 4 days and 10 days of incubation: (a) DMP degradation, (b) DMI degradation, and (c) DMT degradation. Bars with different letters are significantly different at $p < 0.05$ (Tukey Test). Error bars show standard deviations among the triplicate samples. 97
- Figure 5.1** Production of extracellular esterase in the *Fusarium* sp. DMT-5-3 culture with DMT as the inducing substrate. Error bars show standard deviations amongst the triplicate samples. 109
- Figure 5.2** Comparison of esterase activity between intracellular and extracellular protein in *Fusarium* sp. DMT-5-3 culture with DMT as inducing substrate. 109
- Figure 5.3** Elution profile for the purification of esterase using a Hitrap DEAE FF column (eluted with stepwise ionic strength gradients). Symbols: (■), total esterase activity; (◇), total protein. 111
- Figure 5.4** Elution profile for the purification of esterase using a Hitrap DEAE FF column (elution with continuous ionic strength gradient (0-0.1M NaCl)). Symbols: (■), esterase activity; (◇), protein concentration. 112
- Figure 5.5** Elution profile for the purification of esterase using a Hi-Prep Sephacryl S-200 column. Symbols: (■), esterase activity; (◇), OD₂₈₀. 113
- Figure 5.6** SDS-PAGE showing the purification of phthalate from *Fusarium* sp. DMT-5-3. Lane 1, molecular weight markers; lane 2, crude extract; lane 3, the purified esterase after DEAE FF chromatography (0-0.05M NaCl elution); lane 4, the purified esterase after DEAE FF chromatography (0-0.1 M NaCl linear gradient elution); lane 5, the purified esterase after Sephacryl S-200 chromatography. 114
- Figure 5.7** Native-PAGE (a) and SDS-PAGE (b) of phthalate esterase from *Fusarium* sp. DMT-5-3. (a): lane 1, molecular weight markers; lane 2, purified esterase by native-PAGE; lane 3, purified esterase by

- activity staining. (b): lane 1, molecular weight markers; lane2, purified esterase by SDS-PAGE. 115
- Figure 5.8** Enzymatic hydrolysis of different concentrations of *p*-nitrophenyl acetate (PNPA) by phthalate esterase from *Fusarium* sp. DMT-5-3. The concentrations of PNPA were 2 mM (■), 1 mM (◆), 0.05 mM (▲), .0.03 mM (□), 0.02 mM (◇), and 0.01 mM (△). 116
- Figure 5.9** Lineweaver-Burk plot of phthalate esterase from *Fusarium* sp. DMT-5-3, the linear regression equation was $1/V_0 = 102.53 (1/[S]) + 216.38$, $r^2=0.9993$. 117
- Figure 5.10** (a) Effect of temperature on the activity of phthalate esterase. (b) Effect of temperature on stability of phthalate esterase. 119
- Figure 5.11** Thermal stability of phthalate esterase at 50 °C. 119
- Figure 5.12** (a) Effect of pH on the activity of phthalate esterase. (b) Effect of pH on the stability of phthalate esterase. Symbols: (■), 20 mM citrate buffer; (□), 20 mM sodium phosphate buffer; (◆), 20 mM Tris-HCl buffer; (◇), 20 mM Glycine-NaOH buffer. 120
- Figure 6.1** The proposed flow chart of mineralization of DMPEs in mangrove sediments. 134