Table of Contents
Introduction

1. Major environmental pollutants
   1.1 BTEX compounds
      (i) Benzene
      (ii) Ethylbenzene
      (iii) Toluene
      (iv) Xylene
   1.2 Phenol
   1.3 Nitrophenols
   1.4 Chlorophenols
   1.5 Cresols
   1.6 Polycyclic aromatic compounds
      (i) Carbazole
      (ii) Biphenyl and polychlorinated biphenyls
      (iii) Naphthalene

2. Microbial utilization of aromatic compounds

3. Aromatic compounds degradation pathways in bacteria
   3.1 Ortho-cleavage pathway
   3.2 Meta-cleavage pathway

4. Alpha/beta hydrolase fold enzymes

5. Origin of the present work
1. **Bacterial strains and plasmids**
   1.1 Bacterial strains
   1.2 Plasmids

2. **Materials**
   2.1 Antibiotics
   2.2 Biochemicals
   2.3 Restriction and other enzymes
   2.4 Protein Markers
   2.5 Gel Filtration Markers
   2.6 DNA Markers
   2.7 Primers
   2.8 Membrane filters

3. **Methods**
   3.1 Procedures for preparation of stock, working standard solutions and buffers.
   3.2 Media and growth conditions
      (a) LB medium
      (b) M9 medium
      (c) Minimal salts medium
   3.3 Protein estimation
   3.4 Enzyme assays
      (a) Parathion hydrolase assay
      (b) Assay of Orf243 hydrolase activity
4. DNA manipulation

4.1 Isolation of plasmid DNA by Alkaline lysis method
4.2 Isolation of plasmid DNA by KADO method
4.3 Isolation of plasmid DNA using QIAgen Mini preparation kit
4.4 Gel extraction of DNA using QIAgen quick gel extraction kit
4.5 PCR amplification.
4.6 Purification of PCR amplified DNA fragments using QIAgen kit.
4.7 Restriction Digestion
4.8 Ligation

5. Transformation

6. Bacterial conjugation

7. Analytical methods

7.1 Agarose gel electrophoresis for DNA separation.
7.2 SDS-PAGE for protein separation.
7.3 Polyacrylamide gels for separation of small DNA fragments

8. Protein purification

8.1 Overexpression studies
8.2 Preparation of cell free extracts
8.3 Purification, Solubilization and Refolding of Orf243 from Inclusion Bodies (IBs)
8.4 Protein purification by Affinity Chromatography
8.5 Purification of PH and MfphF (Orf243) complex from Pseudomonas aeruginosa (PAO1161):
   (a) Ammonium sulphate fractionation
Gel filtration chromatography

9. Studies on kinetic properties of MfphF (Orf243)
   9.1 Effect of substrate(s) concentration
   9.2 Effect of pH
   9.3 Effect of Temperature
   9.4 Effect of metal ions and chelating agents
   9.5 Effect of site specific reagents

10. Molecular weight determination

11. Western Blotting

Chapter-I

Cloning, Expression and Purification of Orf243  56-85

Results

1. Sequence analysis of Orf243

2. Heterologous expression and purification of Orf243
   2.1. Cloning of orf243 in pET15b
   2.2. PCR amplification and Cloning of orf243
   2.3. Expression of Orf243
   2.4. In vivo assessment of p-nitrophenol (PNP) degradation by Orf243 in E.coli
   2.5. Cloning of orf243 in pRSETA
   2.6. Expression of his-tagged Orf243
   2.7. Localization of Orf243 in E.coli BL21 (DE3)
   2.8. Inclusion Bodies of Orf243
   2.9. Isolation, Solubilization and Refolding of Orf243 from Inclusion Bodies
2.10. Cloning, Expression and Purification of Orf243 from *Pseudomonas aeruginosa* PAO1161

2.11. Cloning of orf243 in pMMB206

2.12. Mobilization of pSM14 into *Pseudomonas aeruginosa* PAO1161

2.13. Expression and localization of his-tagged Orf243 in *Pseudomonas aeruginosa* PAO1161

2.14. *In vivo* assessment of PNP degradation by Orf243 in *Pseudomonas aeruginosa* PAO11061

2.15. Purification of Orf243 from *Pseudomonas aeruginosa* PAO1161 (pSM14)

3. Native molecular weight determination of Orf243

4. *In vitro* assessment of *p*-nitrophenol (PNP) degradation by Orf243

5. *In vitro* assessment of Orf243 activity towards *meta*-fission products of catechol and substituted catechols

**Discussion**

1. **Characterization of Orf243**
   
   (i) *p*-nitrophenol degradation
   
   (ii) Orf243 was found to be a *meta*-fission product hydrolase

**Chapter II**

**Kinetic properties of MfphF (Orf243)** 86-108

**Results**

1.1. Effect of substrate concentration
1.2. Effect of pH

1.3. Effect of Temperature

1.4. Effect of metal ions and chelating agents

1.5. Effect of Group specific inhibitors

Discussion

1. Unique properties of MfphF and its significance in evolution of MFP- hydrolases

2. Genetic location of mfphF was found to be unique among meta-fission product hydrolase genes

Chapter III

Metabolic engineering: Expression of Opd and MfphF in *Pseudomonas aeruginosa* PAO1161 to achieve complete mineralization of methyl parathion 109-125

Results

1. Expression of opd and mfphF in *E.coli* JM109 (DE3)

2. Co-expression of Parathion Hydrolase (PH) and MfphF in *Pseudomonas aeruginosa* PAO1161

3. Construction of opd-operon

4. Mobilization of pSM16 into *Pseudomonas aeruginosa* PAO1161

5. Expression and localization of MfphF and Parathion hydrolase in *Pseudomonas aeruginosa* PAO1161

6. Studies on Parathion hydrolase (PH) and MfphF interactions
7. Purification of Parathion hydrolase and MfphF complex from Pseudomonas aeruginosa PAO1161

8. Kinetic properties of Parathion Hydrolase (PH) and MfphF complex

9. Determination of the molecular mass of PH:MfphF complex

Discussion

Conclusions 126-127

References 128-142