Index

Abstract: 1

Introduction: 3-7
Metabolism of n-alkane in Gordonia SoCg

Chapter I: 8-53
An alkane hydroxylase system of Gordonia sp. strain SoCg is involved in degradation of solid n-alkanes.

Introduction: 8-22
Alkane hydroxylases in Gram negative and Gram positive bacteria

- Aerobic bacterial degradation of *n*-alkane 9
- The AlkB family of alkane hydroxylases 10-13
- Cytochrome P450 alkane hydroxylases 14
- Alkane hydroxylases for long-chain *n*-alkanes 15
- The role of the rubredoxin-rubredoxin reductase system 16
- Metabolism of the alcohols and aldehydes derived from the oxidation of alkanes 17-18
- Regulation of genes involved in aerobic *n*-alkane utilization in bacteria 19-22

Aims 22

Materials and Methods: 23-31
- Bacterial strains, culture conditions and vectors 23
- PFGE analysis 24
- Southern hybridization analysis. 24
- Cloning and sequence analysis of *Gordonia SoCg alk* genes 25
- Heterologous expression of *alkB* in *S. coelicolor* and *E. coli* *26*
- Time course of growth on *n*-alkanes. 26
- Time course of *n*-alkane consumption 27
- Analysis of the metabolic intermediates from *n*-alkanes oxidation pathway 28
- Total RNA isolation, RT-PCR analysis, and absolute qRT-PCR 29
- *Gordonia SoCg* electrocompetents cells 30
- Construction of *Gordonia* SoCg *alkB* disruption mutant 30-31

Results: 32-49
- Identification and properties of strain SoCg 32-33

- Growth on long chain *n*-alkanes and biotrasformation kinetics. 34-35
- Cloning and sequence analysis of SoCg *alk* genes 36
- Characterization of AH system of *Gordonia SoCg* 37-49
  - *alk* genes expression analysis 37-38
  - Long chain *n*-alkanes biotransformation 38-39
  - The *alkB* disruption mutant 40-43
  - AlkB heterologous expression in *S. coelicolor* and *E. coli* 44-48
  - *S. coelicolor* M145-AH expressing SoCg *alkB* grows on *n*-triacontane 49

Discussions 50-53

Chapter II: 54-77

Converting excess carbon into storage materials.

Introduction: 55-64

- Microbial strategies for accessing long chain n-alkanes 57
- Membrane Alterations 58-60
- Converting excess carbon into storage materials 61
- Physiological role of the neutral lipids 62
- Pathways for Wax ester synthesis 62
- Atf enzymes 63-64

Aims 64

Materials and Methods: 65-74

- Analysis of neutral lipid 65
  - TLC analysis 65
  - SPME-GC-MS analysis 65
- Detection of *atfa* 66
- In vivo analysis of *atfa*-like gene expression. 67

Results: 68-74

- Analysis of storage compounds 68-70
- Identification of *atfa*-like gene in *Gordonia SoCg* 71-72

- Screening for estimation of copy number of the *atfa*-like gene 73
- In vivo expression analysis of atfa-like gene 74

Discussions 75-77

Chapter III: 78-97
Proteomic insights into metabolic adaptation.

Introduction: 79-84

- Behavioral and physiological responses to hydrocarbons 79-82
- The genus *Streptomyces* 83-84

Aims 84

Materials and Methods: 85-87

- Heterologous expression of *alkB* in *S. coelicolor* 85
- Analysis of the metabolic intermediates from *n*-alkanes oxidation pathway. 85
- *In vivo* analysis of heterologous expression of *alkB* 85
- Total protein extraction and DIGE analysis 86-87

Results: 88-97

- Experimental design 88-90
- General results 90
- Protein identification 90-94
- Main remarks 7 94-97

Conclusions: 98-102

Tables: 103-110

- Table 1 104-105
- Table 2 105-106
- Table 3 106
- Table 4 107-110

Acknowledgements 111

References 112-120