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DISSERTATION SUMMARY

Investigation of the copper-regulated expression of methane monooxygenases in *Methylococcus capsulatus* (Bath)

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Methanotrphic bacteria or methanotrophs are Gram negative aerob bacteria. They are unique in their ability to utilise methane as a sole energy and carbon source. Methane is the most stable carbon compound in anaerobic environments and is a very important intermediate in the reaction that eventually led to the mineralization of organic matter. Methane escapes from the anaerobic environments to the atmosphere when it is not oxidised by methanotrophs. The release of the methane to the atmosphere results in an increased rate of global warming and causes other changes in the chemical composition of the atmosphere.

The use of enzymes known as methane monooxygenases to catalyse the oxidation of methane to methanol is a defining characteristic of methanotrophs. The methanotroph *Methylococcus capsulatus* strain Bath (*M.c.*) contains two genes for two methane monooxygenases, a particulate (pMMO) and a soluble (sMMO) enzyme. Only one of the two MMOs is expressed at a time, and the single factor regulating enzyme expression was found to be the copper-to-biomass ratio. When the level of copper ions is high, the pMMO is expressed, whereas at very low levels of free copper ions, the bacteria switch to the synthesis of the sMMO. Transcription of the MMO genes is known to be regulated by copper. The soluble methane monooxygenase exhibits a striking lack of substrate specificity, resulting in the fortuitous metabolism of a very large number of compounds including xenobiotic chemicals, especially chlorinated aromatic hydrocarbons. Because of the ability of sMMO to catalyse a large number of biotransformations, it has attracted the interest of scientists involved in the development of biological methods for degradation of toxic chemicals and in the use of bacteria containing MMOs for the production of chemicals with commercial value, for example, primary alcohols and epoxides. Considering the growing number of microbial genome sequencing projects, in-depth molecular biological examination of a diverse range of bacteria is expected in the future.

To analyse the functions of the genes of the increasing number of prokaryotes with sequenced genomes we need to adapt the classical genetic and molecular techniques of the model organisms such as *E. coli* to these, often "difficult" bacteria. Investigation of methanotrophs in the past few decades indicated the high biotechnological potential of these organisms. *M.c.* is a well known, moderately thermophylic methanotroph and its genome is being sequenced at TIGR.

My work concentrated on the optimisation of a conjugation system for *M.c.* which is then used to introduce different vectors into the cells such as broad host range cloning vehicles, transposon delivery and suicide vectors. Practical use of the developed techniques was demonstrated in the investigation of copper regulated expression of the MMOs. Downstream from the sMMO structural genes we have found putative regulatory genes, which highly homologous to the so-called two-component regulatory system genes (*mmoQ-mmoS*), a s^{54} dependent transcription factor homologue (*mmoR*) and a chaperonon homologue (*mmoH*). Mutants were created by marker exchange mutagenesis for these genes. The mutants were characterised concentrated on their influence on the expression of the sMMO. We found that each gene required for the production of active sMMO. The promoter of the sMMO gene cluster was mapped with a transcriptional fusion vector containing the green fluorescent protein as reporter gene.

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