

Contents

I Abbreviations	1
II Introduction	3
III Materials and Methods	11
1. Bacterial strains	11
2. Plasmids and oligonucleotides	12
3. Chemicals and enzymes	14
4. Media	15
5. Culture conditions of <i>G. oxydans</i> and <i>E. coli</i>	15
6. Determination of cell dry weight	17
7. Stock cultures	17
8. Molecular biological methods	17
8.1 Isolation of DNA	17
8.2 Recombinant DNA-techniques	18
8.3 Polymerase chain reaction (PCR)	18
8.4 Agarose gel electrophoresis	19
8.5 Transformation of <i>E. coli</i> and <i>G. oxydans</i>	19
8.6 Overexpression of the <i>G. oxydans</i> <i>ccp</i> gene encoding cytochrome <i>c</i> peroxidase	20
8.7 Construction of marker-free deletion mutants	21
8.8 RNA preparation	21
8.9 cDNA labeling and RT PCR	22
8.10 <i>G. oxydans</i> DNA microarrays	22
9. Biochemical methods	23
9.1 Cell disruption, preparation of crude extracts and membrane fractions	23
9.2 Determination of protein concentration	24
9.3 Polyacrylamide gel electrophoresis of proteins (SDS-PAGE)	24
9.4 Protein purification by column chromatography	24
9.5 Determination of oxygen consumption rates with a Clark electrode	26
9.6 Determination of enzyme activities	26
9.7 Conversion of inactive alcohol dehydrogenase to active enzyme in resting cells	30
10. Bioanalytical methods	30
10.1 Sampling and sample processing for LC-MS analysis	30
10.2 Determination of metabolites by high performance liquid chromatography (HPLC)	31
10.3 ¹³ C Metabolic flux analysis	31
10.4 MALDI-TOF-Mass spectrometry	32
IV Results	33
1. Characterisation of the deletion mutant <i>G. oxydans</i> 621H-Δ <i>qcrABC</i>	33
2. Genome-wide transcription analyses	51
3. ¹³ C-Metabolome analysis and flux analysis (MFA)	63
V Discussion	73
1. Analysis of physiological and metabolic functions of the cytochrome <i>bc₁</i> complex in <i>G. oxydans</i>	73
2. Differential gene regulation at oxygen limitation and at low pH	80
3. Characterisation of growth of <i>G. oxydans</i> 621H on glucose with micro-array-, ¹³ C-metabolome- and flux-analysis	84
VI References	89
VII Appendix	101