
Table of Contents

I. Summary	VI
II. Zusammenfassung.....	VII
1 Introduction	1
2 Objectives	3
3 Theoretical Background.....	4
3.1 Quantitative Measurements in Microbial Metabolomics	4
3.2 Tandem Mass Spectrometry hyphenated with Liquid Chromatography.....	7
3.2.1 Liquid Chromatography for Metabolite Separation	7
3.2.2 Mass Spectrometry for Metabolite Detection.....	8
3.2.3 Application of LC-MS/MS for Microbial Metabolomics.....	12
3.3 Sample Processing for Metabolite Quantification.....	13
3.3.1 Quenching of the Metabolome during Sampling	14
3.3.2 Metabolite Extraction.....	15
3.4 Fundamentals of Metabolism	16
3.4.1 Central Carbon Metabolic Pathways.....	16
3.4.2 Regulation of Metabolism by the Intracellular Energy Level	19
3.4.3 Thermodynamic Constraints.....	21
3.5 Pathway repertoire of microorganisms	22
3.5.1 <i>Escherichia coli</i>	23
3.5.2 <i>Corynebacterium glutamicum</i>	24
3.5.3 <i>Pseudomonas putida</i>	25
3.5.4 <i>Yersinia pseudotuberculosis</i>	26
3.5.5 <i>Bacillus megaterium</i>	26
3.5.6 <i>Rhodopseudomonas palustris</i>	27
3.5.7 <i>Dinoroseobacter shibae</i>	28
4 Material and Methods.....	29
4.1 Strains	29
4.2 Chemicals.....	30
4.3 Growth Media	30
4.3.1 Complex Media	30
4.3.2 Minimal Media.....	31
4.4 Cultivation	35
4.4.1 Shake Flask Cultivation.....	35
4.4.2 Fed-batch and Chemostat Cultivations in Bioreactors.....	35
4.5 Analytical Techniques	36
4.5.1 Cell concentration	36

4.5.2	Glucose concentration	38
4.6	Metabolomics Workflow	38
4.6.1	Sampling.....	38
4.6.2	Metabolite Extraction.....	38
4.6.3	Generation of an U ¹³ C-labeled Internal Standard.....	39
4.6.4	LC-MS/MS Measurement.....	39
4.7	Limits of Detection and Quantitation.....	40
4.8	Validation of Metabolic Datasets by Energetic Constraints	41
5	Results and Discussion	42
5.1	Method Development and Optimization.....	42
5.1.1	Improvement of the Mass Spectrometric Set-Up for Metabolite Analysis.....	42
5.1.2	Chromatographic Separation of Standard Mixtures.....	44
5.1.3	Chromatographic Separation of Cellular Extracts.....	50
5.1.4	Verification of Ion Fragmentation Patterns	54
5.1.5	Detection and Quantification Limits	56
5.1.6	Optimization of Sample Pretreatment	58
5.1.7	Validation by thermodynamic constraints.....	62
5.2	Quantitative Analysis of the Energy Metabolism of <i>E. coli</i>	66
5.2.1	Dynamics of the Energy Level during Carbon Deprivation	66
5.2.2	Dynamics of Energy Metabolism during Chemostat Experiments.....	69
5.2.3	Inhibition of the Respiratory Chain and Impact on the Energy Charge.....	72
5.2.4	Adenylate Energy Charge during Fed-batch Cultivations	74
5.3	Central Carbon Metabolism of <i>Y. pseudotuberculosis</i>	84
5.3.1	Generation and Validation of Metabolic Profiles of <i>Y. pseudotuberculosis</i>	84
5.3.2	Comparison of the Core Metabolism of <i>Y. pseudotuberculosis</i> and <i>E. coli</i>	88
5.4	Stress Induced Changes in the Metabolome of <i>B. megaterium</i>	95
5.4.1	Generation and Validation of Metabolic Profiles of <i>B. megaterium</i>	95
5.4.2	Response of <i>B. megaterium</i> to Temperature Induced Stress	100
5.4.3	Response of <i>B. megaterium</i> to Salt Induced Stress	103
5.5	Impact of Nutrient Levels on the Central Carbon Metabolism of <i>D. shibae</i>	107
5.6	Integrated Analysis of Metabolic Datasets of Different Microbial Strains	113
6	Conclusions and Outlook.....	118
7	Abbreviations and Symbols	123
8	References.....	126
9	Appendix.....	145