



Georg Martin Richter (Autor)  
**Development and Application of a Method for Quantitative  
Metabolome Analysis of Various Production Strains**



<https://cuvillier.de/de/shop/publications/7984>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany  
Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>

---

## 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 <sup>13</sup> 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