



Thibault Godard (Autor)
Rainer Krull (Herausgeber)

Systems biology of stress in *Bacillus megaterium* and its potential applications



<https://cuvillier.de/de/shop/publications/7331>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany

Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>



Table of contents

| | |
|---|-------------|
| Danksagung | III |
| Summary | VII |
| Zusammenfassung | VIII |
| 1 Introduction and aim of the study | 1 |
| 2 Theoretical background | 5 |
| 2.1 Systems biology and omics technologies | 5 |
| 2.1.1 Systems biology and its recent development..... | 5 |
| 2.1.2 Genomics and Transcriptomics | 7 |
| 2.1.3 Proteomics | 10 |
| 2.1.4 Metabolomics | 13 |
| 2.1.5 Fluxomics | 16 |
| 2.2 Stress emergence and response in bacteria..... | 20 |
| 2.2.1 Living in hostile environments..... | 20 |
| 2.2.2 From cold to heat - Survival mechanisms in bacteria..... | 22 |
| 2.2.3 Osmo-adaptation in moderate halophile bacteria..... | 25 |
| 2.3 Polyhydroxyalkanoates and their synthesis in <i>Bacillus megaterium</i> | 29 |
| 2.3.1 Bio-based economy and industrial relevance of biopolymers..... | 29 |
| 2.3.2 <i>Bacillus megaterium</i> as a working horse for PHA production..... | 30 |
| 3 Materials and methods | 35 |
| 3.1 Strains and plasmids | 35 |
| 3.2 Chemicals | 35 |
| 3.3 Growth media..... | 36 |
| 3.4 Cultivation techniques | 37 |
| 3.4.1 Shake flasks | 37 |
| 3.4.2 Bioreactors | 37 |
| 3.5 Analytical techniques..... | 37 |
| 3.5.1 Biomass determination | 37 |
| | IX |



| | | |
|--------|--|----|
| 3.5.2 | Sugars and organics acids | 38 |
| 3.6 | Transcriptomics | 39 |
| 3.6.1 | Sampling and RNA processing | 39 |
| 3.6.2 | Microarray analysis..... | 40 |
| 3.7 | Proteomics | 40 |
| 3.7.1 | Protein extraction and quantification | 40 |
| 3.7.2 | Protein digestion and purification..... | 41 |
| 3.7.3 | Protein identification and quantification by LC-IMS ^e | 42 |
| 3.8 | Metabolomics | 44 |
| 3.8.1 | Sampling and extraction procedure | 44 |
| 3.8.2 | Quantification by LC-MS/MS | 44 |
| 3.9 | Fluxomics..... | 45 |
| 3.9.1 | Sampling and labelling analyses of proteinogenic amino acids..... | 45 |
| 3.9.2 | Metabolic network and flux calculation..... | 46 |
| 3.10 | Biomass composition and specific precursor demand | 47 |
| 3.10.1 | Protein content and its amino acid composition | 47 |
| 3.10.2 | DNA | 49 |
| 3.10.3 | RNA | 50 |
| 3.10.4 | Polyhydroxybutyric acid (PHB) | 50 |
| 3.10.5 | Intracellular amino acids and potassium | 50 |
| 3.10.6 | Lipid fraction and its composition..... | 51 |
| 3.10.7 | Peptidoglycan layer | 52 |
| 3.10.8 | Glycogen | 53 |
| 3.11 | Genetic engineering | 53 |
| 3.11.1 | Isolation of genomic DNA from <i>B. megaterium</i> | 53 |
| 3.11.2 | DNA amplification by polymerase chain reaction (PCR) | 54 |
| 3.11.3 | DNA digestion and fragment separation by gel electrophoresis..... | 55 |
| 3.11.4 | Purification of DNA fragments and ligation reaction | 55 |



| | | |
|-----------|--|------------|
| 3.11.5 | Production and transformation of competent <i>E. coli</i> cells using CaCl ₂ | 55 |
| 3.11.6 | Preparation of plasmid DNA from <i>E.coli</i> | 56 |
| 3.11.7 | Production and transformation of <i>B. megaterium</i> protoplasts..... | 57 |
| 4 | Results and discussion | 59 |
| 4.1 | System-wide analysis of adaptation to harsh temperatures | 59 |
| 4.1.1 | Physiological modifications induced by cold and heat stress in <i>B. megaterium</i> | 59 |
| 4.1.1.1 | Growth characteristics and by-product secretion..... | 60 |
| 4.1.1.2 | Cellular composition and membrane alterations | 64 |
| 4.1.2 | Adaptation of <i>B. megaterium</i> carbon core metabolism during sustained temperature stress..... | 68 |
| 4.1.3 | Global adaptation to harsh temperatures..... | 78 |
| 4.1.3.1 | Statistical approach to temperature stress | 78 |
| 4.1.3.2 | Specific response to heat..... | 83 |
| 4.1.3.3 | Specific response to low temperatures | 94 |
| 4.2 | System-wide analysis of adaptation to osmotic stress | 101 |
| 4.2.1 | Impact of ionic osmotic stress on cellular physiology in <i>B. megaterium</i> | 101 |
| 4.2.2 | Adaptation of <i>Bacillus megaterium</i> carbon core metabolism during sustained osmotic stress..... | 106 |
| 4.2.3 | Specific responses elicited by sustained osmotic stress..... | 114 |
| 4.2.4 | Biotechnological production of osmotically relevant compounds..... | 120 |
| 5. | Conclusion | 131 |
| 6. | Outlook | 133 |
| 7. | Abbreviations and symbols..... | 135 |
| 7.1 | Abbreviations | 135 |
| 7.2 | Symbols | 137 |
| 8. | References..... | 139 |
| 9. | Appendix..... | 171 |
| 9.1 | Tables | 171 |
| 9.2 | Figures..... | 212 |