



René Bücker (Autor)

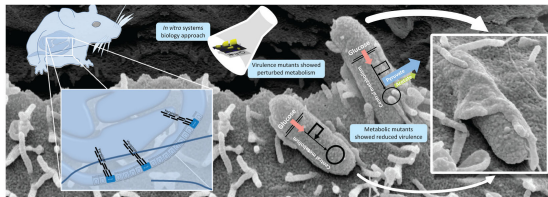
A multi-omics view on the pathogen *Yersinia pseudotuberculosis* – bridging metabolism and virulence



UNIVERSITÄT
DES
SAARLANDES



Band **2**



René Bücker

A multi-omics view on the pathogen *Yersinia pseudotuberculosis* – bridging metabolism and virulence

Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

<https://cuvillier.de/de/shop/publications/7018>

Copyright:

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

**TABLE OF CONTENTS**

| | |
|--|-----------|
| Summary | X |
| Zusammenfassung | XI |
| 1 Introduction | 1 |
| 2 Objectives | 3 |
| 3 Theoretical Background | 4 |
| 3.1 Clinical relevance of <i>Yersinia pseudotuberculosis</i> | 4 |
| 3.2 <i>Yersinia pseudotuberculosis</i> - Life style and infection process | 5 |
| 3.3 Virulence promoting metabolism of <i>Yersinia pseudotuberculosis</i> | 9 |
| 3.4 Metabolism as part of the resistome – the intrinsic bacterial resistance | 12 |
| 3.5 Systems-level analysis of biological systems | 13 |
| 3.6 Concept of ¹³ C metabolic flux analysis | 16 |
| 4 Materials and Methods | 19 |
| 4.1 Bacterial strains and mutant construction | 19 |
| 4.2 Cultivation | 23 |
| 4.2.1 Strain conservation | 23 |
| 4.2.2 Batch cultivation for metabolic flux analysis | 23 |
| 4.2.3 Continuous cultivation for temperature shift experiments | 23 |
| 4.3 Analytical methods | 25 |
| 4.3.1 Glucose, organic acids, and amino acids | 25 |
| 4.3.2 Cell concentration | 25 |
| 4.3.3 GC-MS labeling analysis | 26 |
| 4.3.4 Quantification of RovA by Western blot analysis | 27 |
| 4.3.5 Quantification of RovA by fluorescence-activated cell sorting | 28 |
| 4.4 Analysis of cellular composition of <i>Y. pseudotuberculosis</i> | 28 |
| 4.4.1 Protein content and amino acid composition | 28 |
| 4.4.2 RNA content and nucleotide composition | 29 |
| 4.4.3 DNA content and nucleotide composition | 29 |
| 4.4.4 Glycogen and Lipids | 30 |
| 4.5 Gene expression profiling | 30 |
| 4.5.1 Hierarchical clustering analysis | 31 |
| 4.6 Metabolic flux analysis | 32 |
| 4.6.1 Metabolic network and biomass requirements | 32 |



TABLE OF CONTENTS

| | | |
|----------|---|-----------|
| 4.6.2 | Metabolic flux calculation and statistical evaluation | 32 |
| 4.7 | <i>In vivo</i> mouse studies | 33 |
| 4.7.1 | Mouse infections | 33 |
| 4.7.2 | Ethics statement | 34 |
| 5 | Results and Discussion | 35 |
| 5.1 | Integration of virulence and metabolism - A systems biology approach | 35 |
| 5.1.1 | Glucose-grown <i>Y. pseudotuberculosis</i> secretes high amounts of pyruvate under fully aerobic conditions..... | 35 |
| 5.1.2 | Influence of the global regulators RovA, CsrA, and Crp on the growth behavior and overflow metabolism of <i>Y. pseudotuberculosis</i> | 37 |
| 5.1.3 | Metabolic and isotopic steady state – two important prerequisites for metabolic flux analysis | 38 |
| 5.1.4 | Cellular composition of <i>Yersinia pseudotuberculosis</i> | 40 |
| 5.1.5 | Intracellular fluxes of glucose-grown <i>Yersinia pseudotuberculosis</i> strongly differ from those of its relative <i>Escherichia coli</i> | 42 |
| 5.1.6 | The lack of RovA, CsrA, and Crp perturbs the intracellular carbon fluxes in <i>Y. pseudotuberculosis</i> | 44 |
| 5.1.7 | Mutants deficient in RovA, CsrA, and Crp reveal an altered expression pattern of virulence-associated, stress adaptation, and metabolic genes | 46 |
| 5.1.8 | The pyruvate-TCA cycle node as metabolic switch point of virulence... 51 | |
| 5.1.9 | Perturbations of the metabolic core machinery at the pyruvate-TCA cycle node reduce <i>Yersinia virulence</i> | 54 |
| 5.1.10 | Post-transcriptional control of flux as a crucial strategy for virulence.... | 57 |
| 5.1.11 | The secretion of high amounts of pyruvate by <i>Y. pseudotuberculosis</i> is unique among pathogens | 59 |
| 5.1.12 | The pyruvate node and the TCA cycle are focal points of virulence control | 60 |
| 5.2 | Antibiotic treatment..... | 63 |
| 5.2.1 | Response of core metabolism to antibiotic treatment | 63 |
| 5.2.2 | Inherent erythromycin resistance of <i>Yersinia pseudotuberculosis</i> is accompanied by maintaining a highly active glycolysis..... | 67 |
| 5.2.3 | The high susceptibility for tetracycline is accompanied by the absence of major flux rerouting in <i>Yersinia pseudotuberculosis</i> | 67 |
| 5.3 | Control of virulence by temperature..... | 70 |
| 5.3.1 | Impact of temperature on virulence of <i>Y. pseudotuberculosis</i> | 70 |
| 5.3.2 | Development of a reactor setup for precise temperature profiles..... | 70 |

VIII



| | | |
|----------|--|------------|
| 5.3.3 | Determination of an appropriate dilution rate for RovA synthesis | 71 |
| 5.3.4 | Temperature up-shift mimicking the entrance of <i>Y. pseudotuberculosis</i> into the host | 72 |
| 5.3.5 | Temperature down-shift mimicking release from the host..... | 74 |
| 5.3.6 | Fluorescence-activated cell sorting correlates with Western blot analysis | 75 |
| 5.3.7 | Silencing and activation of <i>rovA</i> transcription follow different kinetics... | 75 |
| 5.3.8 | Bistability can explain the observed RovA expression pattern..... | 77 |
| 5.3.9 | Does RovA expression fit with known models of bistability? | 77 |
| 6 | Conclusion and Outlook | 81 |
| 7 | Appendix | 84 |
| 7.1 | Abbreviations..... | 84 |
| 7.2 | Symbols..... | 87 |
| 7.3 | Data from ¹³ C metabolic flux analysis..... | 88 |
| 7.4 | Data from gene expression analysis..... | 92 |
| 8 | References | 105 |