



Novel insights into the transcriptional regulation of cell division in *Corynebacterium glutamicum*

Kim Julia Kraxner

Schlüsseltechnologien / Key Technologies

Band / Volume 241

ISBN 978-3-95806-560-4

Forschungszentrum Jülich GmbH
Institut für Bio- und Geowissenschaften
Biotechnologie (IBG-1)

Novel insights into the transcriptional regulation of cell division in *Corynebacterium glutamicum*

Kim Julia Kraxner

Schriften des Forschungszentrums Jülich
Reihe Schlüsseltechnologien / Key Technologies

Band / Volume 241

ISSN 1866-1807

ISBN 978-3-95806-560-4

Table of Contents

Table of Contents	I
Abbreviations.....	III
1. Summary	1
1.1. English Summary	1
1.2. Deutsche Zusammenfassung	2
2. Introduction	3
2.1. <i>Corynebacterium glutamicum</i>	3
2.2. 2-Oxoglutarate dehydrogenase and its regulation in <i>C. glutamicum</i>	4
2.3. Cell division in <i>C. glutamicum</i>	6
2.4. Aim of this work.....	10
3. Materials and Methods.....	11
3.1. Bacterial strains, plasmids, and growth conditions.....	11
3.2. Recombinant DNA work and construction of insertion and deletion mutants	14
3.3. Fluorescence microscopy.....	18
3.4. Purification of FtsR.....	18
3.5. Electrophoretic mobility shift assays (EMSA)	19
3.6. Promoter studies with P _{ftsZ} fused to mVenus	20
3.7. Chromatin affinity purification with subsequent Sequencing (ChAP-Seq)	20
3.8. DNA microarrays	21
3.9. DNA affinity purification and MALDI-ToF-MS analysis	21
3.10. Genome re-sequencing	22
3.11. Quantitative PCR	22
3.12. Coulter counter measurements	24
4. Results	25
4.1. Phylogenetic conservation of <i>odhl</i> (cg1630) and adjacent genes.....	25
4.2. Deletion of <i>ftsR</i> affects growth behavior	29
4.3. Complementation experiments with <i>C. glutamicum</i> ATCC13032Δ <i>ftsR</i>	29
4.4. The morphological phenotype of <i>C. glutamicum</i> caused by <i>ftsR</i> deletion and overexpression	31
4.5. Transcriptome comparison of the Δ <i>ftsR</i> mutant with its parent wild type	33
4.6. Genome re-sequencing of the <i>ftsR</i> deletion mutant and amplification of the trehalose cluster	36
4.7. DNA affinity purification for unraveling transcriptional regulation of <i>odhl</i>	38

4.8. Focusing on investigation of the uncharacterized regulator FtsR.....	39
4.9. Deletion and overexpression of the <i>ftsR</i> gene in the MB001 background	40
4.10. Complementation of the MB001 Δ <i>ftsR</i> phenotype with native FtsR and homologs of <i>C. diphtheriae</i> and <i>M. tuberculosis</i>	41
4.11. Quantitative PCR to further investigate trehalose cluster amplification	42
4.12. Effect of <i>ftsR</i> deletion on <i>ftsZ</i> promoter activity and FtsZ distribution	43
4.13. Genome-wide profiling of <i>in vivo</i> FtsR binding sites	45
4.14. <i>in vitro</i> binding of purified FtsR to the proposed binding motif in the <i>ftsZ</i> promoter region.....	51
4.15. DNA affinity chromatography with the <i>ftsZ</i> promoter	53
4.16. Analysis of the transcriptome of a Δ <i>ftsR</i> mutant in the MB001 background	55
4.17. FtsR-independent expression of FtsZ.....	56
4.18. Influence of FtsR on <i>ftsZ</i> promoter activity in strains with FtsR-independent <i>ftsZ</i> -expression	60
5. Discussion	62
5.1. Regulation of Odhl in <i>C. glutamicum</i>	62
5.2. Do secondary effects contribute to the Δ <i>ftsR</i> mutant phenotype?	63
5.3. The switch to MB001 as background strain.....	65
5.4. FtsR, the first transcriptional regulator of FtsZ identified for the <i>Corynebacteriales</i> order.....	65
5.5. The importance of fine-tuning	66
5.6. FtsR's mode of action and binding site	66
5.7. FtsR must have additional targets besides FtsZ	68
5.8. The physiological function of FtsR	70
6. References	71

Schlüsseltechnologien / Key Technologies
Band / Volume 241
ISBN 978-3-95806-560-4