



## Metabolic engineering of *Corynebacterium glutamicum* for production of the adipate precursor 2-oxoadipate

Markus Sebastian Spelberg

Forschungszentrum Jülich GmbH  
Institute of Bio- and Geosciences (IBG)  
Biotechnology (IBG-1)

# Metabolic engineering of *Corynebacterium glutamicum* for production of the adipate precursor 2-oxoadipate

Markus Sebastian Spelberg

Schriften des Forschungszentrums Jülich  
Reihe Gesundheit / Health

Band / Volume 70

---

ISSN 1866-1785

ISBN 978-3-89336-954-6

## CONTENT

1	Summary .....	1
1.1	Summary.....	1
1.2	Zusammenfassung .....	3
2	Introduction .....	5
2.1	Significance and application of adipate.....	5
2.2	Chemical production of petroleum-based adipate .....	6
2.3	Approaches for production of bio-based adipate.....	7
2.3.1	Microbial cyclohexane oxidation to adipate .....	7
2.3.2	Microbial production of the adipate precursor <i>cis, cis</i> -muconate .....	8
2.3.3	Microbial production of the adipate precursor glucarate .....	11
2.4	A new synthetic pathway for the bio-based production of 2-oxoadipate and adipate.....	11
2.4.1	Production of the intermediate 2-oxoadipate from glucose .....	12
2.4.2	A synthetic pathway for the conversion of 2-oxoadipate to adipate .....	14
2.5	<i>Corynebacterium glutamicum</i> as host for 2-oxoadipate and adipate production .....	15
2.6	Aim of this thesis .....	16
3	Material and methods .....	17
3.1	Strains and plasmids .....	17
3.2	Chemicals and culture media.....	23
3.3	Cultivation and conservation of bacteria .....	24
3.3.1	Cultivation of <i>E. coli</i> for plasmid isolation .....	24
3.3.2	Cultivation of <i>C. glutamicum</i> in shake flaks.....	25
3.3.3	Conservation of bacteria.....	25
3.3.4	Cultivation of <i>C. glutamicum</i> in a bioreactor .....	26
3.3.5	Cultivation of <i>S. cerevisiae</i> .....	27
3.4	Determination of growth parameters .....	27
3.5	Molecular biology methods .....	27

3.5.1	Isolation of nucleic acid .....	27
3.5.1.1	Isolation of plasmid DNA .....	27
3.5.1.2	Isolation of total RNA .....	28
3.5.1.3	Isolation of genomic DNA of <i>S. cerevisiae</i> .....	28
3.5.1.4	Purification of DNA fragments .....	28
3.5.1.5	Isolation of DNA fragments from agarose gels .....	29
3.5.2	DNA gel electrophoresis .....	29
3.5.3	RNA gel electrophoresis .....	29
3.5.4	Determination of nucleic acid concentrations .....	30
3.5.5	Recombinant DNA work .....	30
3.5.5.1	Restriction of DNA .....	30
3.5.5.2	Dephosphorylation of restricted plasmid DNA .....	30
3.5.5.3	Ligation .....	31
3.5.6	Generation and transformation of competent <i>E. coli</i> cells .....	31
3.5.7	Generation and transformation of competent <i>C. glutamicum</i> cells .....	32
3.5.8	Polymerase chain reaction (PCR) .....	33
3.5.9	Construction of expression plasmids .....	34
3.5.10	Construction of chromosomal gene replacements using the pK19 <i>mobsabB</i> system .....	34
3.5.11	Site-directed mutagenesis .....	35
3.5.12	DNA sequencing .....	36
3.6	Quantification of glucose and organic acids in the cell culture supernatant .....	36
3.7	GC-ToF-MS analysis of metabolites in the cell culture supernatant .....	36
3.8	Protein analysis .....	37
3.8.1	Determination of protein concentration .....	37
3.8.2	Homocitrate synthase enzyme assay .....	37
3.9	Global gene expression analysis using DNA microarrays .....	38
3.9.1	cDNA synthesis .....	38

## Content

3.9.2	<i>C. glutamicum</i> DNA microarray hybridisation .....	39
3.9.2.1	Array pre-hybridisation.....	39
3.9.2.2	DNA microarray hybridisation .....	40
3.9.2.3	Post-hybridisation .....	40
3.9.3	Measurement and analysis of the fluorescence signals .....	40
4	Results .....	42
4.1	Influence of 2-oxoadipate and adipate on growth parameters of <i>C. glutamicum</i> .....	42
4.2	Improved growth in the presence of growth-inhibiting adipate concentrations .....	46
4.3	Impact of 2-oxoadipate and adipate on global gene expression of <i>C. glutamicum</i> ...	48
4.4	Selection of enzymes for the conversion of 2-oxoglutarate to 2-oxoadipate .....	54
4.5	Homocitrate production with <i>C. glutamicum</i> .....	56
4.6	Enhancement of homocitrate production.....	61
4.6.1	Influence of modified cultivation conditions .....	61
4.6.2	Characterisation of the homocitrate synthase mutein Lys20 <sup>R276K</sup> .....	65
4.6.3	Metabolic engineering to improve precursor supply.....	67
4.7	Establishment of 2-oxoadipate production with <i>C. glutamicum</i> .....	75
4.8	2-Oxoadipate production under controlled conditions in a bioreactor .....	82
5	Discussion .....	85
5.1	Influence of 2-oxoadipate and adipate on growth and global gene expression of <i>C. glutamicum</i> .....	85
5.2	First generation of homocitrate and 2-oxoadipate producer strains .....	90
5.3	Improvement of production titers .....	95
5.4	On the route toward adipate.....	100
6	References .....	102
7	Appendix .....	115
7.1	Sequences of used enzymes.....	115



**Gesundheit / Health**  
**Band / Volume 70**  
**ISBN 978-3-89336-954-6**

 **JÜLICH**  
FORSCHUNGSZENTRUM