



Metabolic engineering of *Corynebacterium glutamicum* for production of L-leucine and 2-ketoisocaproate

Michael Vogt

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

Metabolic engineering of *Corynebacterium glutamicum* for production of L-leucine and 2-ketoisocaproate

Michael Vogt

Schriften des Forschungszentrums Jülich
Reihe Gesundheit / Health

Band / Volume 73

ISSN 1866-1785

ISBN 978-3-89336-968-3

Content

Summary	1
Zusammenfassung	2
1 Introduction	3
1.1 Bioeconomy: Sustainable production processes using renewable resources	3
1.2 Biotechnological relevance of <i>C. glutamicum</i> and applications of branched-chain amino acids.....	4
1.3 Biosynthesis of BCAAs and its regulatory mechanisms in <i>C. glutamicum</i>	6
1.4 <i>C. glutamicum</i> strains for the production of BCAAs and their keto acid precursors: Metabolic engineering as an alternative to random mutagenesis	9
1.5 Aims of this work	12
2 Material and Methods	13
2.1 Bacterial strains and plasmids.....	13
2.2 DNA oligonucleotides	19
2.3 Chemicals and growth media	23
2.3.1 Chemicals	23
2.3.2 Growth media	23
2.4 Cultivation of microorganisms	24
2.4.1 Maintenance of microorganisms	24
2.4.2 Cultivation in shake flasks	25
2.4.3 Cultivation in bioreactors	25
2.4.4 Determination of bacterial growth	26
2.5 Molecular biological work	26
2.5.1 Preparation of plasmid DNA from <i>E. coli</i> cells.....	26
2.5.2 Preparation of genomic DNA from <i>C. glutamicum</i> cells	26
2.5.3 Polymerase chain reaction	27
2.5.4 Purification of DNA fragments	27
2.5.5 DNA agarose gel electrophoresis	27
2.5.6 Extraction of DNA fragments from agarose gels	27
2.5.7 Determination of nucleic acid concentration.....	28
2.5.8 DNA sequencing	28
2.5.9 Restriction digest and modification of DNA.....	28
2.5.10 Ligation of DNA	28

2.5.11	Preparation and transformation of competent <i>C. glutamicum</i> cells	28
2.5.12	Preparation and transformation of competent <i>E. coli</i> cells	29
2.5.13	Integration and deletion of genes using the pK19 <i>mobsacB</i> system	29
2.5.14	Plasmid construction	30
2.5.15	Preparation of RNA	32
2.5.16	RNA agarose gel electrophoresis	32
2.5.17	Synthesis and labeling of cDNA.....	33
2.5.18	DNA microarray analysis	33
2.6	Quantification of metabolites	34
2.6.1	Quantification of amino acids.....	34
2.6.2	Quantification of organic acids and glucose.....	35
2.6.3	Calculation of glucose uptake rate.....	35
2.7	Protein biochemical methods	36
2.7.1	Preparation of crude cell extract	36
2.7.2	Determination of protein concentration	36
2.7.3	Enzyme assay for 2-isopropylmalate synthase	36
2.7.4	Enzyme assay for 3-isopropylmalate dehydratase.....	37
3	Results.....	39
3.1	The role of <i>leuA</i> -encoded 2-isopropylmalate synthase for increased L-leucine production.....	39
3.1.1	Analysis of the L-leucine producer B018.....	39
3.1.2	Characterization of the <i>leuA</i> -encoded IPMS from wild-type ATCC 13032 and B018.....	39
3.1.3	Construction of further feedback-resistant IPMS.....	40
3.2	Relevance of the repressor LtbR for L-leucine synthesis	42
3.3	Construction of strains for increased <i>leuA</i> expression	43
3.3.1	Exchange of the native <i>leuA</i> promoter and its attenuator.....	43
3.3.2	Integration of a second <i>leuA</i> copy.....	43
3.4	Balancing precursor supply and metabolic flux towards L-leucine.....	43
3.4.1	Use of feedback-resistant acetohydroxyacid synthase	43
3.4.2	Analysis of limitations of L-leucine production in strain MV-Leu55	44
3.4.3	Analysis of the growth behavior of MV-Leu55.....	44
3.5	Genome wide gene expression analysis	46
3.6	Optimizing productivity of MV-Leu55	48
3.6.1	Increase of glucose uptake.....	48
3.6.2	Integration of a third <i>leuA</i> copy	48

3.7	Reduction of citrate synthase activity.....	50
3.8	Relationship between growth rate and L-leucine titer	51
3.9	Batch and fed-batch cultivations.....	54
3.9.1	Test fermentations.....	54
3.9.2	Optimizing fermentation conditions.....	54
3.9.3	Characteristics of fed-batch cultivations of MV-LeuF1 and MV-LeuF2	56
3.10	Production of 2-ketoisocaproate	61
3.10.1	Consequences of <i>ilvE</i> deletion at elevated L-leucine production	61
3.10.2	Reduction of transamination activity in L-leucine producers	62
3.10.3	Batch fermentation of MV-KICF1	64
4	Discussion.....	67
4.1	IPMS is the key enzyme for L-leucine production	67
4.2	Increasing precursor supply, detecting further limitations, and performing transcriptomics.....	69
4.3	Balancing titer, yield, and productivity.....	71
4.4	Fed-batch fermentations	72
4.5	Production of 2-ketoisocaproate	74
4.6	Outlook: Possible improvements for L-leucine and 2-ketoisocaproate production...	76
	References.....	79



Gesundheit / Health
Band / Volume 73
ISBN 978-3-89336-968-3