



TABLE OF CONTENTS

SUMMARY	VIII
ZUSAMMENFASSUNG.....	IX
1 INTRODUCTION AND OBJECTIVES.....	1
1.1 Introduction.....	1
1.2 Objectives.....	4
2 THEORETICAL BACKGROUND	5
2.1 <i>Basfia succiniciproducens</i> – a novel industrial workhorse	5
2.1.1 The distinct metabolism and physiology of <i>Basfia succiniciproducens</i> ...	5
2.2 The C ₄ platform chemical succinate – an industrial production accessible using <i>Basfia succiniciproducens</i>	9
2.3 C ₃ chemicals – industrial chemicals of interest to be derived by <i>Basfia succiniciproducens</i>	12
2.3.1 Pyruvate.....	12
2.3.2 Lactate	12
2.3.3 L-Alanine	13
2.3.4 β-Alanine.....	14
2.3.5 3-Hydroxypropionate (3-HP)	15
3 MATERIAL AND METHODS	17
3.1 Bacterial strains and plasmids.....	17
3.2 Genes used for metabolic engineering	17
3.3 Chemicals and media.....	19
3.3.1 Chemicals	19
3.3.2 Media for genetic engineering.....	19
3.3.3 Cultivation media	20
3.4 Genetic engineering	22
3.4.1 Isolation of nucleic acids	22
3.4.2 Amplification, ligation and assembly of nucleic acids.....	25
3.4.3 Gel electrophoresis	27
3.4.4 Enzymatic digestion	28
3.4.5 Transformation.....	28
3.4.6 Strain validation	29
3.4.7 Strain preservation.....	30

TABLE OF CONTENTS

3.5	Cultivation and strain characterization.....	31
3.5.1	Aerobic cultivation in shake flasks	31
3.5.2	Anaerobic cultivation in serum bottles.....	31
3.5.3	Cultivation in lab scale bioreactors.....	32
3.6	Analytical methods	33
3.6.1	Cell and biomass concentration.....	33
3.6.2	Substrates and products	33
3.6.3	GC-MS labeling analysis.....	34
3.6.4	Enzyme activities	35
4	RESULTS AND DISCUSSION	36
4.1	Growth physiology of <i>Basfia succiniciproducens</i> DD3 under aerobic and anaerobic conditions.....	36
4.2	Development of genetic tools for <i>Basfia succiniciproducens</i>	39
4.2.1	Development of a blue-white screening system for genomic engineering	39
4.2.2	<i>Basfia succiniciproducens</i> contains two β-galactosidase genes	40
4.2.3	Modification of the integrative pClik ^{CM} vector for blue-white screening .	43
4.2.4	Application of blue-white screening to gene deletion	46
4.2.5	Deletion of the gene <i>DD0789</i> impairs growth on sucrose	50
4.2.6	Validation of functionality of <i>neo</i> gene integration into the genome	51
4.2.7	Evaluation of native promoter strength to support heterologous gene expression in <i>Basfia succiniciproducens</i>	54
4.3	Application of <i>Basfia succiniciproducens</i> for L-alanine production.....	58
4.3.1	Aerobic physiology of a novel alanine overproducer.....	58
4.3.2	Metabolic overflow at the pyruvate-node impairs efficient alanine production	60
4.3.3	Screening of industrial relevant substrates under different process conditions.....	62
4.3.4	Optimization of xylose-based L-alanine production using different gas atmospheres	64
4.3.5	Batch production of L-alanine from xylose under N ₂ atmosphere.....	67
4.3.6	Fed-batch production of L-alanine from xylose.....	69
4.3.7	<i>Basfia succiniciproducens</i> ALA-1 is a promising strain for L-alanine production.	71
4.4	Metabolic engineering for β-alanine production.....	72
4.4.1	Wild type <i>Basfia succiniciproducens</i> does not form β-alanine	73

4.4.2	Aspartate 1-decarboxylase depicts a possible entry point to β-alanine synthesis.....	74
4.4.3	Screening for suitable heterologous enzymes for β-alanine synthesis..	75
4.4.4	Determination of the optimal ammonium sulphate concentration.....	77
4.4.5	Codon optimization of the <i>panD</i> gene.....	79
4.4.6	Harnessing the <i>panP</i> gene from <i>Vibrio natriegens</i> for β-alanine production	81
4.5	Metabolic engineering for 3-hydroxypropionate production	87
4.5.1	Conversion of β-alanine to 3-hydroxypropionate – concept and plasmid construction.....	87
4.5.2	There is no native 3-hydroxypropionate metabolism in <i>Basfia succiniciproducens</i>	89
4.5.3	The 3-hydroxypropionate production module is functional in <i>Basfia succiniciproducens</i>	90
4.5.4	Combination of the β-alanine and 3-HP production module.....	91
4.5.5	Characteristics of the β-alanine carbon loss in the 3-HP production strain	93
4.5.6	The codon-optimized <i>panD_{cgl}</i> ⁺ gene enables <i>De-novo</i> 3-HP synthesis from glucose	96
5	CONCLUSION AND OUTLOOK	98
6	APPENDIX	101
6.1	Figure index.....	118
6.2	Table index	119
7	BIBLIOGRAPHY	120