

Table of Contents

Abbreviations	i
Table of Figures	v
List of Tables	vii
1 Introduction	1
1.1 Diversity and impacts of natural products	1
1.2 Advancements in discovery of lead-structures from natural products	6
1.2.1 Technological advancements in bioanalytics	6
1.2.2 Bioinformatics accelerate target identification	8
1.3 Biosynthetic origins for terpene and peptide secondary metabolites	10
1.3.1 Terpenes: the probably largest class of natural products	12
1.3.2 Non-ribosomally synthesized peptides: structural and biosynthetic complexities	13
1.4 Significance of Bacterial Secondary Metabolites	18
1.4.1 The genus <i>Xanthomonas</i> : phytopathogens aiding drug discovery	19
1.4.2 The genus <i>Streptomyces</i> : the principal providers of bioactive molecules	20
2 Aim of the Work	21
3 Materials	23
3.1 Equipment and software	23
3.2 Chemicals, enzymes and materials	26
3.3 Plasmids and oligonucleotides	30
3.4 Microorganisms and antibiotics	31
3.4.1 Microorganisms	31
3.4.2 Antibiotics	31

3.5	Culture media	32
3.6	Kits, buffers and solutions	34
3.6.1	Kits	34
3.6.2	Buffers	34
3.6.3	Solutions	35
4	Methods	37
4.1	Microbiological methods	37
4.1.1	Cultivation of strains	37
4.1.2	Precursor and isotope-labelled precursor feeding	38
4.1.3	Preparation of CaCl ₂ -competent <i>E. coli</i> DH ₅ α cells	38
4.1.4	Preparation of a <i>Streptomyces</i> spore suspension	38
4.1.5	Isolation of <i>Streptomyces</i> mutants	38
4.1.6	Agar diffusion assays	39
4.2	Isolation and purification of bacterial metabolites	39
4.2.1	XAD extraction of liquid <i>Xanthomonas</i> cultures	39
4.2.2	Solid-phase extraction	39
4.2.3	Extraction of solid <i>Streptomyces</i> cultures	40
4.2.4	Size-exclusion chromatography	40
4.2.5	Flash chromatography	40
4.2.6	Preparative HPLC	41
4.2.7	Semi-preparative HPLC	41
4.3	Analytical methods	42
4.3.1	Analaytical HPLC	42
4.3.2	LC-(HR)-ESI-MS ⁿ experiments	42

4.3.3	Molecular network analysis	43
4.3.4	NMR analysis	44
4.3.5	Amino acid analysis using chiral GC-MS	45
4.3.6	MALDI imaging MS	45
4.4	Bioinformatics	47
4.4.1	Genome mining	47
4.4.2	Sequence alignments of NRPS modular domains	47
4.4.3	Modeling of A-domains	48
4.5	Biochemical methods	48
4.5.1	ATP-(³² P)-PP _i exchange assay	48
4.6	Molecular biology methods	48
4.6.1	PCR amplification of genomic DNA segments	48
4.6.2	Agarose gel electrophoresis	49
4.6.3	Genomic DNA, PCR products, plasmid DNA purification and analysis	50
4.6.4	Cloning of plasmid insert into CaCl ₂ -competent <i>E. coli</i> DH ₅ α cells	51
4.6.5	Plasmid transformation into <i>E. coli</i> ET12567/pUZ8002 cells by electroporation	52
4.6.6	Conjugation of <i>Streptomyces</i> spores with <i>E. coli</i> ET12567/pUZ8002 clones	52
5	Results and Discussion	55
5.1	Discovery of a pABA-containing metabolite from <i>Xanthomonas citri</i> pv. <i>mangiferaeindicae</i>	55
5.2	Isolation and purification of the target compound	56
5.3	Structure elucidation of the pABA-containing metabolite	59
5.4	Cytotoxic activity of xanthomonic acid	64
5.5	Production of streptofactins by <i>Streptomyces tendae</i> Tü 901/8c	68
5.6	Partial amino acid sequence of streptofactin B using LC-(HR)-ESI-MS ³ analysis	68

5.7	Bioinformatics	70
5.7.1	Identification of the putative biosynthetic gene cluster	70
5.7.2	Sequence alignments of the NRPS domains	73
5.7.3	Specificity-conferring codes of the adenylation domains	73
5.8	Specificity of the A8 domain	75
5.9	Molecular biology	77
5.9.1	PCR amplification and ligation in conjugation plasmids	77
5.9.2	Cloning of pK18mob_apra-sfnD into CaCl ₂ -competent <i>E. coli</i> DH ₅ α and <i>E. coli</i> ET12567/pUZ8002 cells	79
5.9.3	Conjugation of <i>Streptomyces</i> spores with <i>E. coli</i> ET12567/pUZ8002 clones	79
5.9.4	Genomic DNA isolation and analysis	80
5.10	MALDI-IMS for the production streptofactins	81
5.11	Isolation and purification of streptofactin A	82
5.12	Structure elucidation of streptofactin A	84
5.12.1	Amino acids analysis using chiral GC-MS	84
5.12.2	LC-(HR)-ESI-MS/MS analysis	87
5.12.3	NMR spectroscopy	90
5.13	Biosynthesis of streptofactins	95
6	Outlook	99
7	Appendix	103
8	References	123