Electronic Supplementary Material (ESI) for Natural Product Reports.

Electronic Supplementary Information

Metabolic engineering with systems biology tools to optimize production of prokaryotic secondary metabolites

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Table S1 Recent studies aimed at optimizing the production of secondary metabolites since 2012.

Secondary metabolite	Application	Production host	Approach	Production improvement	Refs
36-Methyl-FK506	Improving neurite outgrowth activity	*Streptomyces* sp. KCTC 11604BP *ΔtcbB* mutant strain (heterologous host)	Heterologous expression of isobutyrylmalonyl-CoA pathway from *Streptomyces* sp. CNH189	2-fold increase in titer, compared to the native host	1
5-Oxomilbemycins A3/A4	A precursor of an antiparasitic milbemycin oxime	*Streptomyces bingchenggensis*	Atmospheric and room temperature plasma (ARTP) mutation system, followed by knocking out a gene responsible for ketonization of 5-oxomilbemycins	Approximately 2-fold increase in titer, compared to the initial strain overproducing milbemycins	2
6-Deoxyerythronolide B	Macrolide core of an antibiotic erythromycin	*Bacillus subtilis* (heterologous host)	Optimized heterologous expression of 6-deoxyerythronolide B biosynthetic gene cluster from *Saccharopolyspora erythraea*, followed by removal of native secondary metabolite biosynthetic gene clusters and an operon for propionyl-CoA utilization, and feeding of propionate	43% increase in yield, compared to the initial engineered strain	3
A21978C (daptomycin)	Antibiotic against Gram-positive bacterial pathogens	*Streptomyces roseosporus*	Reporter-guided *rpsL* mutation selection method and ribosome engineering using streptomycin	2.2-fold increase in titer, compared to the parent strain	4
Actinorhodin	Antibiotic	*Streptomyces coelicolor*	Genome-scale metabolic modeling and its use to identify overexpression targets	52-fold increase in titer, compared to the wild-type	5
Actinorhodin	Antibiotic	*Streptomyces coelicolor*	Overexpression of the gene *abrC3* encoding a positive response regulator of antibiotic biosynthesis	33% increase in titer, compared to the wild-type	6
Actinorhodin	Antibiotic	*Streptomyces coelicolor*	Replacement of a promoter of the positive regulator *actII orf4* with synthetic promoters	Approximately 2.8 times increase in yield on biomass, compared to the wild-type	7
Actinorhodin	Antibiotic	*Streptomyces coelicolor*	Identification of gene overexpression targets using transcriptome-based metabolic simulation	Up to 2-fold increase in titer, compared to the wild-type	8
Amphotericin B	Antibiotic and antifungal	*Streptomyces nodosus*	Overexpression of a pathway-specific transcriptional activator (AmphRIV)	4-fold increase in yield on biomass, compared to the untransformed parent strain	9
Ansamitocin	Anticancer	*Actinosynnem pretiosum*	Increase of the precursor UDP-glucose pool by overexpressing phosphoglucomutase and UDP-	40% greater titer, compared to the control strain	10

Secondary metabolite	Application	Production host	Approach	Production improvement	Refs
			glucose pyrophosphorylase		
Avermectin	Anthelmintic and insecticide	*Streptomyces avermitilis*	Overexpression of two genes targeted by the TetR family transcriptional regulator SAV151	Approximately 2-3 folds increase in titer, compared to the wild-type	11
Bottromycin	Antibiotic	*Streptomyces coelicolor* (heterologous host)	Heterologous expression of a gene cluster from *Streptomyces* sp. BC16019 and overexpression of an efflux pump	20-fold increase in titer, compared to the unoptimized transformant of *S. coelicolor*	12
Bisabolene	Fragrance and food additive	*Streptomyces venezuelae*	Heterologous expression of a codon optimized bisabolene synthase from *Abeis grandis*, removal of competing pathways and overexpression of genes encoding farnesyl pyrophosphate synthase	350 times increase in titer, compared to the cumulative terpene titers from the wild-type	13
Chlortetracycline	Antibiotic	*Streptomyces aureofaciens*	Overexpression of the gene *ctcP* encoding an $FADH_2$-dependent halogenase responsible for the last step of chlortetracycline biosynthesis	1.73-fold increase in titer, compared to the wild-type	14
Daptomycin	Antibiotic against Gram-positive bacterial pathogens	*Streptomyces roseosporus*	Ribosome engineering using a diterpenoid antibiotic pleuromutilin	30% increase in titer, compared to the wild-type	15
Daptomycin	Antibiotic against Gram-positive bacterial pathogens	*Streptomyces roseosporus*	Overexpression of the *dptJ* gene encoding tryptophan-2,3-dioxgenase	More than 2-fold increase in titer, compared to the wild-type	16
Daptomycin	Antibiotic against Gram-positive bacterial pathogens	*Streptomyces roseosporus*	Medium optimization using Plackett-Burman design and response surface methodology	2.2-fold increase in titer, compared to the unoptimized medium	17
Daptomycin	Antibiotic against Gram-positive bacterial pathogens	*Streptomyces roseosporus*	Overexpression of DepR1, a TetR-family transcriptional regulator	41% greater titer, compared to the wild-type, in 50 L bioreactor	18
Deoxyviolacein	Antibacterial, antitumor and antiviral	*Escherichia coli* (heterologous host)	Division of deoxyviolacein biosynthetic pathway into two modules with L-tryptophan as a key intermediate, and sequential optimization of each module independently using a newly developed biosensor that detects the intracellular pool of L-tryptophan	20% increase in titer, compared to the previously reported recombinant *E. coli* showing the best production performance	19
Divergolides	Antibacterial and antitumor	*Streptomyces* sp. W112	Overexpression of a positive regulator of the divergolides biosynthetic pathway	More than 2-fold increase in the titer, compared to the wild-type	20

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Doramectin	Anthelmintic	*Streptomyces avermitilis*	Genome shuffling and ribosome engineering with streptomycin	11.2-fold increase in titer, compared to the parent strain NEAU1069, and production of 930.3 ± 3.8 mg L^{-1} from 50 L bioreactor fermentation	21
Erythromycin	Antibiotic	*Saccharopolyspora erythraea*	Genome-scale metabolic modeling and its use to identify supplementary amino acid to increase the production yield	50% increase in yield on biomass, compared to the unoptimized media	22
Erythromycin	Antibiotic	*Escherichia coli* (heterologous host)	Enhancing intracellular supply of a precursor propionyl-CoA by removing *ygfH* gene in addition to heterologous expression of erythromycin biosynthetic gene cluster	7-fold increase in titer, compared to the control strain	23
Erythromycin	Antibiotic	*Saccharopolyspora erythraea*	Constitutive overexpression of SACE_5599, a positive regulatory protein of erythromycin biosynthesis newly identified using comparative proteome analysis	32% increase in titer, compared to the wild-type	24
Erythromycin	Antibiotic	*Escherichia coli* (heterologous host)	Reduction in the number of genes and expression plasmids to minimize metabolic burden and plasmid instability	5-fold increase in titer, compared to the control strain	25
Erythromycin	Antibiotic	*Saccharopolyspora erythraea*	Overexpression of SACE_7301, TetR family regulator	27% greater titer, compared to the industrial strain (WB)	26
Erythromycin	Antibiotic	*Saccharopolyspora erythraea*	Inactivation of a TetR family transcriptional regulator (SACE_3986) that negatively controls the biosynthesis of erythromycin	54.2% increase in titer, compared to the parent (industrial) strain	27
FK506	Immunosuppressant	*Streptomyces tsukubaensis*	Enhancing pathway-specific precursor supply via gene amplification and optimization of glucose supply	150% increase in titer, compared to the parent strain	28
FK506	Immunosuppressant	*Streptomyces tsukubaensis*	Genome-scale metabolic modeling and its use to identify gene knockout and overexpression targets, followed by examination of the effects of combined gene manipulations	1.47-fold increase in titer, compared to the wild-type, from fed-batch fermentation	29
FK506	Immunosuppressant	*Streptomyces tsukubaensis*	Overexpression of biosynthetic genes associated with FK506 production and external feeding of additional nutrients	146% increase in titer after the combined gene overexpressions, compared to the wild-type, and	30

Secondary metabolite	Application	Production host	Approach	Production improvement	Refs
				additional 70% increase in titer after nutrient feeding	
FK506	Immunosuppressant	*Streptomyces* sp. RM7011	Random mutagenesis, followed by overexpression of propionyl-CoA carboxylase (PCC) pathway and external feeding of nutrients	11.63-fold increase in titer (RM7011 strain), compared to the wild-type, through random mutagenesis, 1.75-fold increase in titer from overexpression of PCC pathway, compared to the RM7011 strain, and further increase in titer by providing vinyl propionate and Tween 80	31
Gougerotin	Antibiotic	*Streptomyces graminearus*	Engineering of the biosynthetic gene cluster by removing a pathway-specific regulatory gene and replacing native promoters with constitutive promoters, integration of the engineered gene cluster into the host genome and external feeding of glycine	2.5-fold increase in titer, compared to the wild-type	32
Jadomycin B	Antibiotic	*Streptomyces venezuelae*	Use of heterologous constitutive promoters locating upstream of jadomycin B biosynthetic gene cluster	2-fold increase in titer, compared to the strain with a widely used constitutive promoter *ermE**p	33
Milbemycins A3/A4	Pesticide and veterinary medicine	*Streptomyces bingchenggensis*	Removal of other byproducts via gene knockouts	74% increase in titer, compared to the milbemycins-overproducing control strain of *S. bingchenggensis*	34
Mithramycin	Antitumor	*Streptomyces argillaceus*	Enhancing intracellular supply of two precursors malonyl-CoA and glucose-1-phosphate by preventing their conversions to other metabolites via gene knockouts, and upregulation of their biosynthetic reactions	3.3 and 4.7 times increases in titer, compared to the wild-type strain, in liquid and solid media, respectively	35
Nargenicin A_1	Antibiotic	*Nocardia* sp. CS682	Optimization of transformation condition and overexpression of heterologous genes encoding positive regulator of secondary metabolite biosynthesis (*S*-adenosylmethionine synthetase) or acetyl-CoA carboxylase	2.8- and 3.8-fold increases in titer for overexpression of *S*-adenosylmethionine synthetase and acetyl-CoA carboxylase, respectively, compared to the wild-type	36

Secondary metabolite	Application	Production host	Approach	Production improvement	Refs
			in order to enhance precursor supply		
Natamycin	Antifungal	*Streptomyces chattanoogensis*	Overexpression of one of genes encoding phosphopantetheinyl transferases (SchPPT)	40% increase in titer and 24 h faster production, compared to the industrial natamycin producer *S. chattanoogensis* L10	37
Neomycin	Antibiotic	*Streptomyces fradiae*	Medium optimization using Plackett-Burman design and response surface methodology	2.7-fold increase in yield from dry coconut oil cake, compared to the unoptimized medium	38
Nosiheptide	Antibiotic	*Streptomyces actuosus*	Medium optimization using Plackett-Burman design and response surface methodology	1.56-fold increase in titer, compared to the unoptimized medium	39
Phenazine-1-carboxylic acid (Shenqinmycin)	Antibiotic and biopesticide	*Pseudomonas aeruginosa*	Enhancing fluxes towards phenazine-1-carboxylic acid (PCA) biosynthesis by removing or downregulating competing reactions, and amplifying reactions that increase biosynthesis of PCA and its precursors	53.6-fold increase in titer, compared to the wild-type	40
Pikromycin	Antibiotic	*Streptomyces venezuelae*	Medium optimization using Plackett-Burman design, method of steepest ascent and response surface methodology	1.5- to 2-fold increase in titer, compared to the unoptimized media	41
Pretubulysin A	Structural core of an anticancer tubulysin	*Myxococcus xanthus* (heterologous host)	Heterologous expression of a gene cluster from *Cystobacter* sp. SBCb004 and supplementation of L-pipecolic acid	100-fold increase in titer (0.19 mg L^{-1}), compared to another heterologous host *Pseudomonas putida*	42
Pristinamycin II	A component of pristinamycin that is antibiotic	*Streptomyces pristinaespiralis*	Overexpression of the pristinamycin II biosynthetic gene cluster, knockout of a repressor gene, overexpression of two activator genes, and addition of macroreticular resin	5.26-fold increase in titer, compared to the parental strain, from 5 L bioreactor	43
Spinosad	Antibiotic and pesticide	*Saccharopolyspora spinosa*	Overexpression of genes associated with biosynthesis of precursors	5-fold increase in titer, compared to the wild-type	44
Spinosad	Antibiotic and pesticide	*Saccharopolyspora spinosa*	Genome-scale metabolic modeling and its use to examine the effects of supplementary amino acids and transhydrogenase activity on the production yield	86.5% increase in titer, compared to the wild-type	45
Spinosad (spinosyns	Antibiotic and pesticide	*Saccharopolyspora*	UV mutagenesis, followed by	7 to 8-fold increase in titer,	46

Secondary metabolite	Application	Production host	Approach	Production improvement	Refs
A and D)		*spinosa*	heterologous expression of genes from *Streptomyces peucetius*, all involved in spinosad biosynthesis	compared the wild-type	
Surfactin	Biosurfactant and plant disease control	*Bacillus amyloliquefaciens*	Genome shuffling	10.3-fold increase in titer, compared to the wild-type	47
Teicoplanin	Antibiotic	*Actinoplanes teichomyceticus*	Selection of the *hrdB* gene encoding principal sigma factor from *Actinoplanes missouriensis* 431, *Micromonospora aurantiaca* ATCC 27029 and *Salinispora arenicola* CNS-205 according to molecular evolutionary analysis, and their expression in *A. teichomyceticus* L-27 after their random mutagenesis and DNA shuffling	2-fold increase in titer, compared to the industrial producer strain *A. teichomyceticus* L-27	48
Teicoplanin	Antibiotic	*Actinoplanes teichomyceticus*	Overexpression of a pathway-specific regulatory gene *tei15**	3.5-fold increase in titer, compared to the wild-type, from 3 L bioreactor	49
Thailanstatin A	Anticancer and an analog of spliceostatin	*Burkholderia sp.* FERM BP-3421	Fermentation media optimization, removal of a reaction converting the target compound into another metabolite, and overexpression of the target compound biosynthetic reaction	More than 40-fold increase in titer (2.5 g L^{-1}), compared to the wild-type	50
Toyocamycin, tetramycin P, tetrin B and tetramycin A	Antifungal	*Streptomyces diastatochromogenes*	Ribosome engineering using erythromycin, gentamicin, paromomycin, rifampin and streptomycin	4.1-, 7.8-, 5.1- and 13-fold increases in titer of toyocamycin, tetramycin P, tetrin B and tetramycin A, respectively, using paromomycin, compared to the wild-type	51
Tylactone	A precursor of an antibiotic tylosin	*Streptomyces venezuelae* (heterologous host)	Enhancing a polyketide precursor ethylmalonyl-CoA by preventing its conversion to other metabolites via a gene knockout, ethylmalonate supplementation in the medium and upregulation of the tylosin biosynthetic pathway	Near 10-fold increase in titer, compared to the parent strain	52
Validamycin A	Antibiotic and antifungal	*Streptomyces hygroscopicus*	Overexpression of the validamycin biosynthetic gene cluster	34% increase in titer, compared to the wild-type	53

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Validamycin	Antibiotic and antifungal	*Streptomyces hygroscopicus*	Dual knockouts of two γ-butyrolactone receptor genes *shbR1* and *shbR3*	55% increase in titer, compared to the wild-type	54
Valinomycin	Antibiotic	*Escherichia coli* (heterologous host)	Heterologous expression of valinomycin biosynthetic genes from *Streptomyces tsusimaensis*, followed by production optimization through high cell density cultivation and coexpression of type II thioesterase	13 mg L^{-1}, a 43-fold increase in titer, compared to the recombinant *E. coli* strain before the production optimization	55
Valinomycin	Antibiotic	*Escherichia coli* (heterologous host)	Optimization of fed-batch cultivation	33-fold increase in titer without external feeding of specific precursors, compared to the batch cultivation	56
WAP-8294A and HSAF (dihydromaltophilin)	WAP-8294A, antibiotic; HSAF, antifungal	*Lysobacter enzymogenes*	Site-specific integration of ORF8 gene that positively regulates the production of these two compounds	2-fold increase in titer for WAP-8294A and 7-fold increase in titer for HSAF, both compared to the wild-type	57

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§ References in press are indicated with DOI.

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