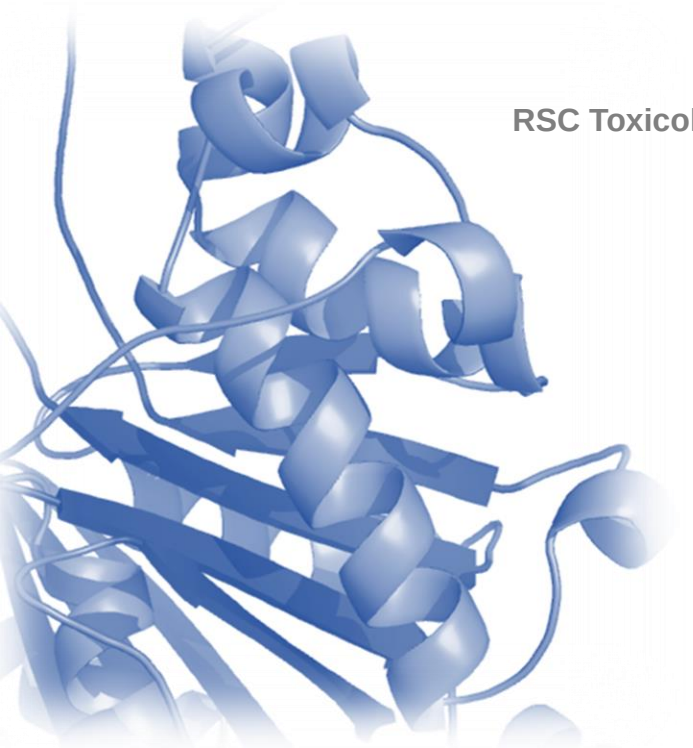
THE UNIVERSITY OF WARWICK

Discovery, Biosynthesis and Bioengineering of Novel Antibiotics from Bacteria



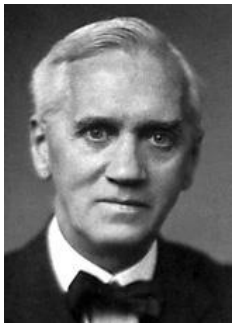
Dr. Matthew Jenner

RSC Toxicology Group: Tackling Antimicrobial Resistance

September 17th 2018

Traditional Approach for Bioactive Natural Product Discovery

'Grind and Find' Methodology

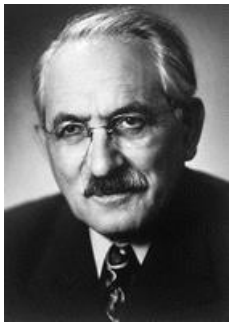


Alexander Fleming

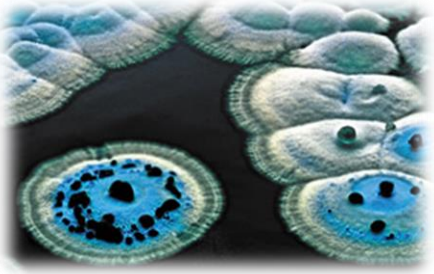
Howard Florey

Dorothy Hodgkin

Ernst Chain

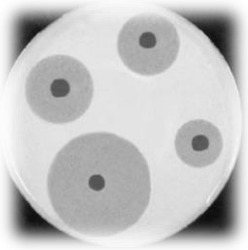


Selman Waksman



Extraction

Extract Bioactivity?

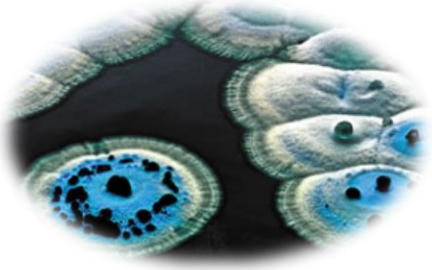


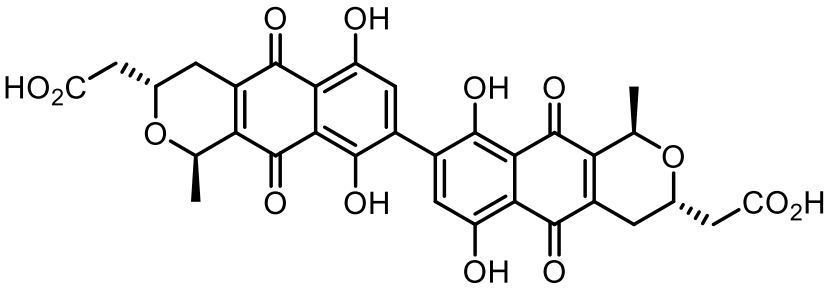
Metabolite Separation & Purification

Identify Bioactive Fraction → ANTIBIOTIC!

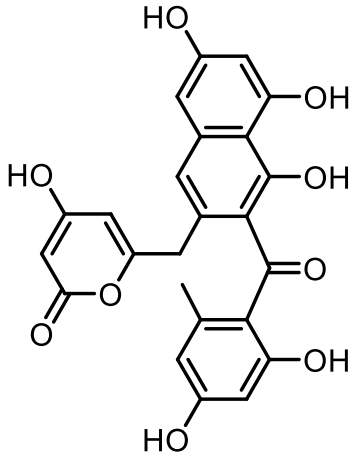
Streptomyces coelicolor – A Prolific Producer of Specialised Metabolites

'Grind and Find' Methodology

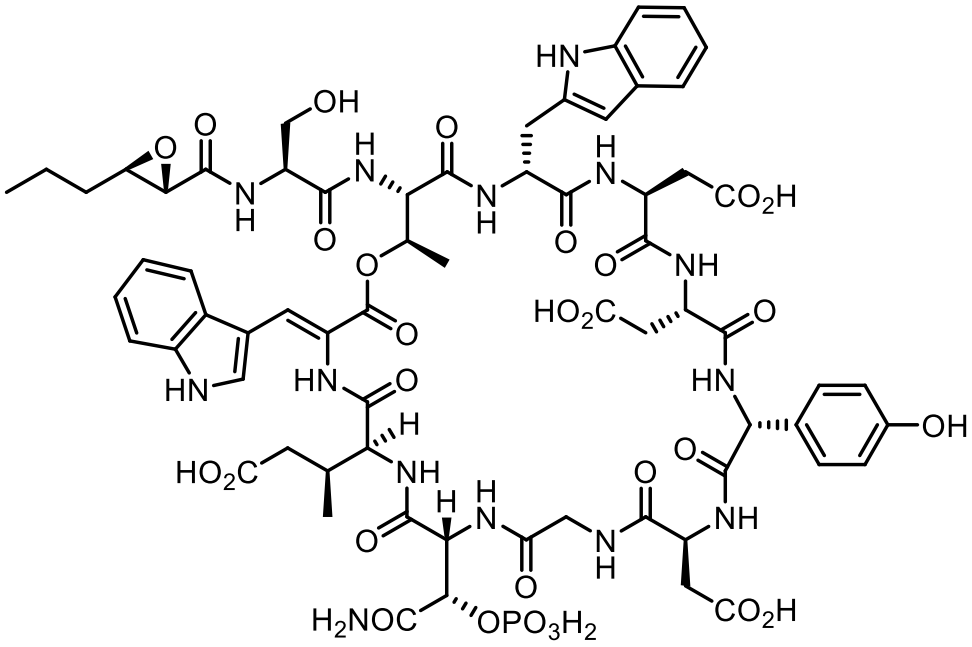




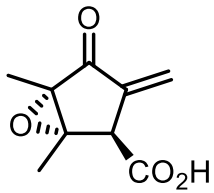
actinorhodins (*act*)



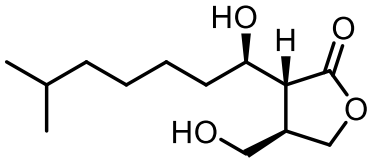
grey spore pigment (*whiE*)



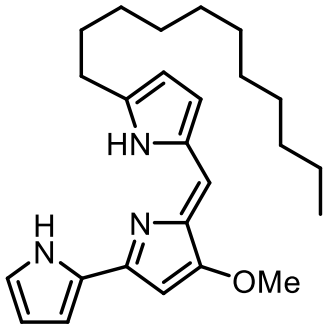
calcium-dependent antibiotics (*cda*)



methylenomycins (*mmy*)



***S. coelicolor* butyrolactones (*scb*)**



undecylprodigiosin (*red*)

Streptomyces coelicolor – A Prolific Producer of Specialised Metabolites

'Grind and Find' Methodology

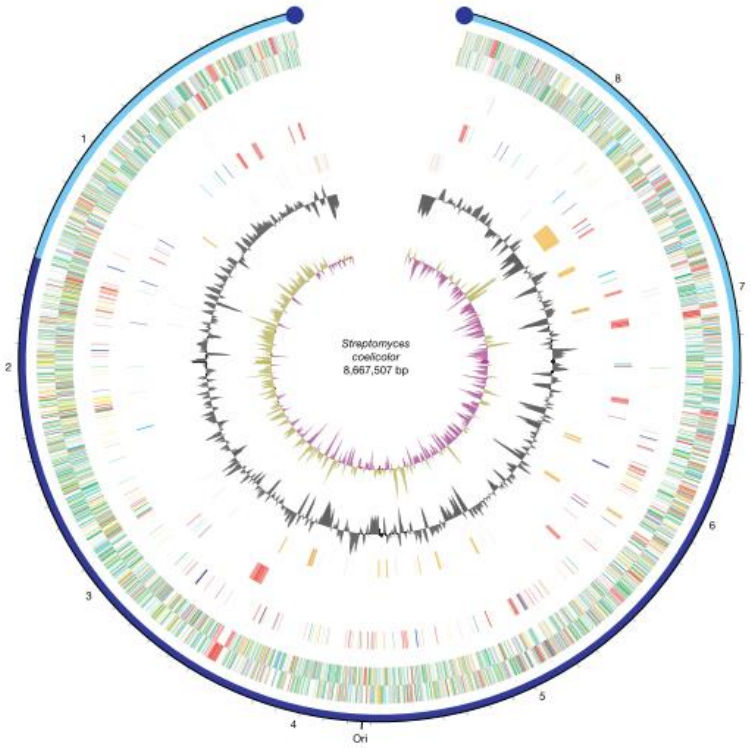
articles

Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2)

S. D. Bentley*, K. F. Chater†, A.-M. Cerdeño-Tárraga*, G. L. Challis†‡, N. R. Thomson*, K. D. James*, D. E. Harris*, M. A. Quail*, H. Kieser†, D. Harper*, A. Bateman*, S. Brown*, G. Chandra†, C. W. Chen§, M. Collins*, A. Cronin*, A. Fraser*, A. Goble*, J. Hidalgo*, T. Hornsby*, S. Howarth*, C.-H. Huang§, T. Kieser†, L. Larke*, L. Murphy*, K. Oliver*, S. O'Neil*, E. Rabbinowitsch*, M.-A. Rajandream*, K. Rutherford*, S. Rutter*, K. Seeger*, D. Saunders*, S. Sharp*, R. Squares*, S. Squares*, K. Taylor*, T. Warren*, A. Wietzorrek†, J. Woodward*, B. G. Barrell*, J. Parkhill* & D. A. Hopwood†

* *The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK*
† *John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK*
‡ *Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK*
§ *Institute of Genetics, National Yang-Ming University, Shih-Pai, Taipei 112, Taiwan*

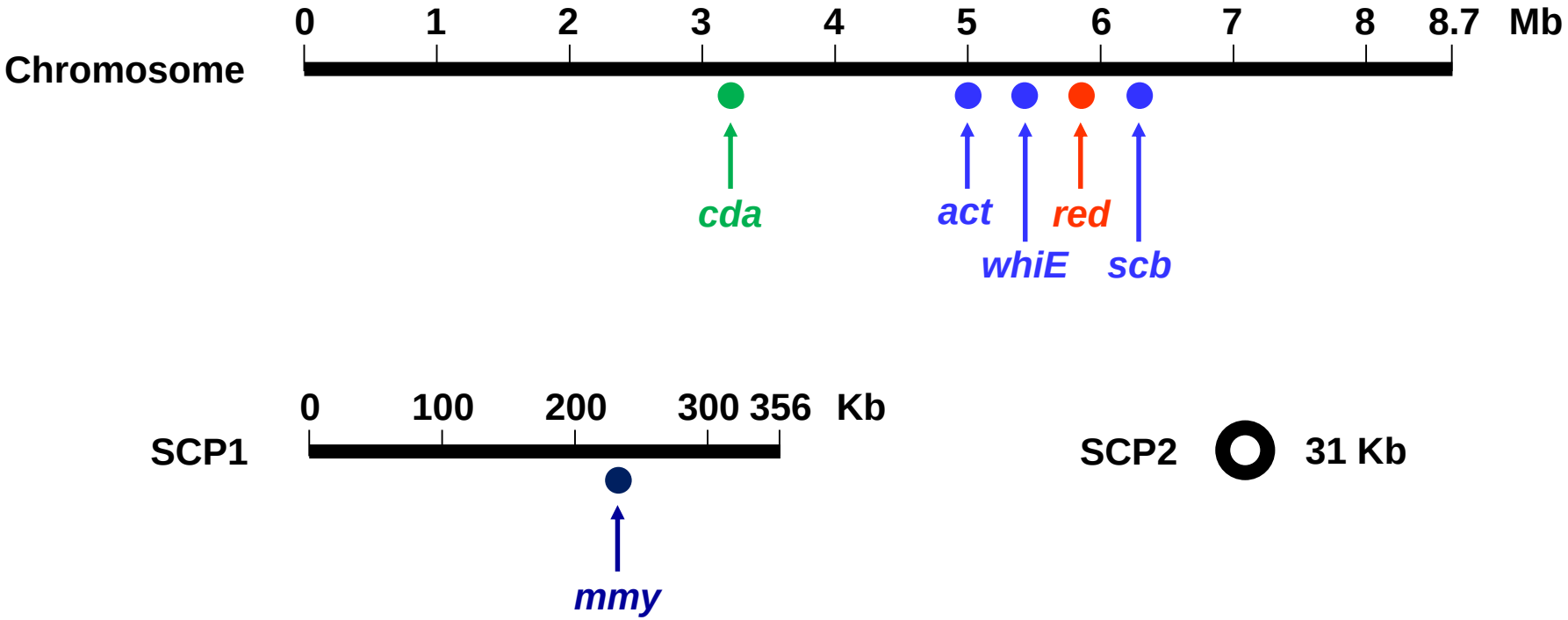
Streptomyces coelicolor **is a representative of the group of soil-dwelling, filamentous bacteria responsible for producing most natural antibiotics used in human and veterinary medicine. Here we report the 8,667,507 base pair linear chromosome of this organism, containing the largest number of genes so far discovered in a bacterium. The 7,825 predicted genes include more than 20 clusters coding for known or predicted secondary metabolites. The genome contains an unprecedented proportion of regulatory genes, predominantly those likely to be involved in responses to external stimuli and stresses, and many duplicated gene sets that may represent 'tissue-specific' isoforms operating in different phases of colonial development, a unique situation for a bacterium. An ancient synteny was revealed between the central 'core' of the chromosome and the whole chromosome of pathogens *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*. The genome sequence will greatly increase our understanding of microbial life in the soil as well as aiding the generation of new drug candidates by genetic engineering.**



Nature **2002**, *417*, 141
Microbiology **2003**, *149*, 505
Mol. Microbiol. **2004**, *51*, 1615

Streptomyces coelicolor – A Prolific Producer of Specialised Metabolites

'Grind and Find' Methodology



Nature **2002**, *417*, 141
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Streptomyces coelicolor – A Prolific Producer of Specialised Metabolites

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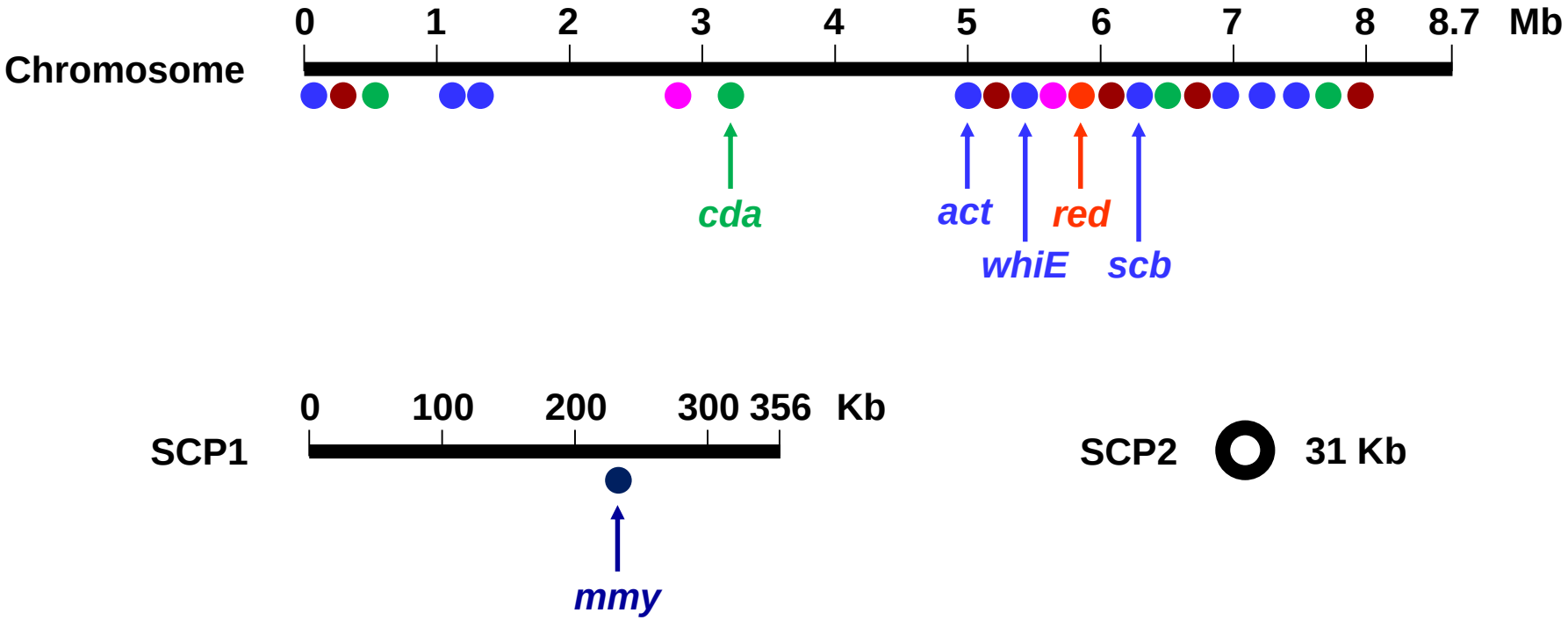
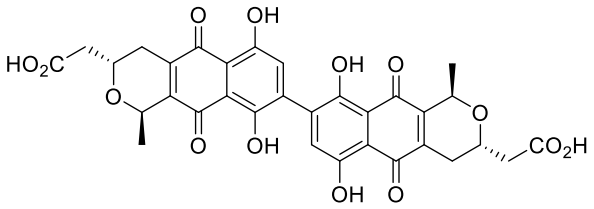


- terpene synthase
- nonribosomal peptide synthetase (NRPS)
- NRPS-independent siderophore synthetase
- polyketide synthase (PKS)
- hybrid NRPS / PKS / α-oxamine synthase
- hybrid PKS / butenolide synthase

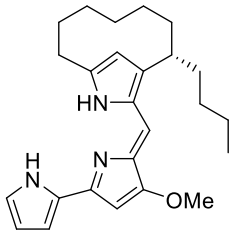
Nature **2002**, *417*, 141
Microbiology **2003**, *149*, 505
Mol. Microbiol. **2004**, *51*, 1615

Streptomyces coelicolor – A Full Metabolic Profile

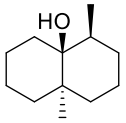
Application of Genomics-Based Discovery



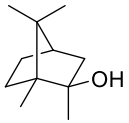
actinorhodin



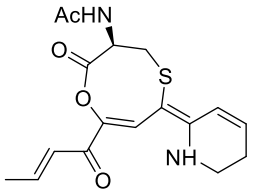
streptorubin B



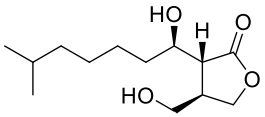
geosmin



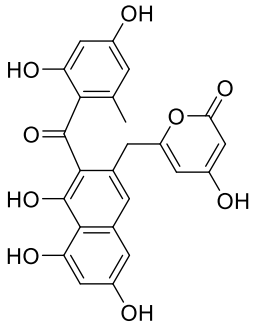
methyl-*iso*-borneol



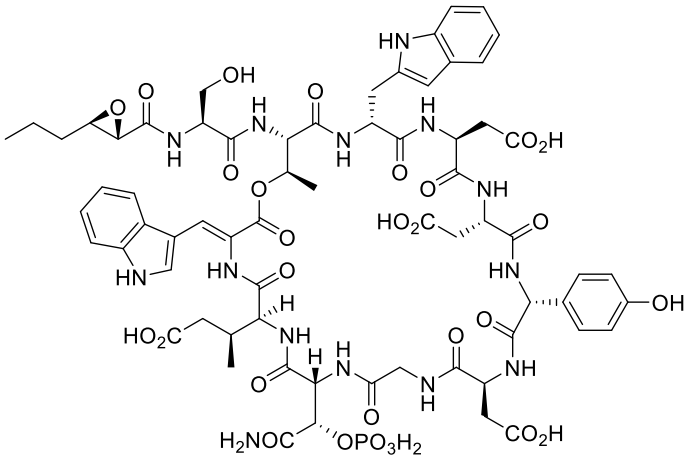
coelimycin P1



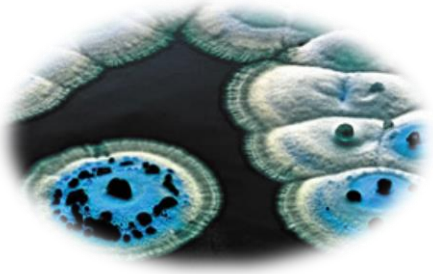
S. coelicolor **butyrolactone 1**

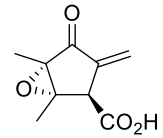


TW95a

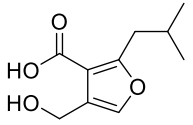


calcium-dependent antibiotic 2a

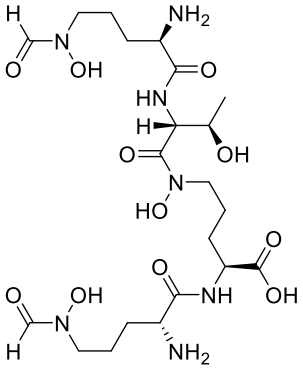




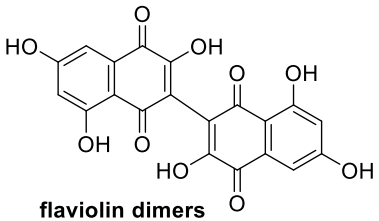
methylenomycin A



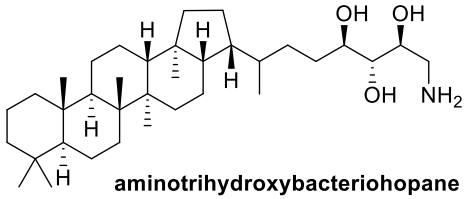
methylenomycin furan 1



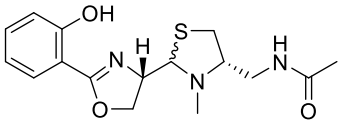
coelichelin



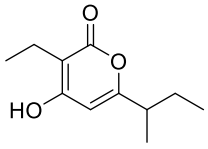
flaviolin dimers



aminotrihydroxybacteriohopane



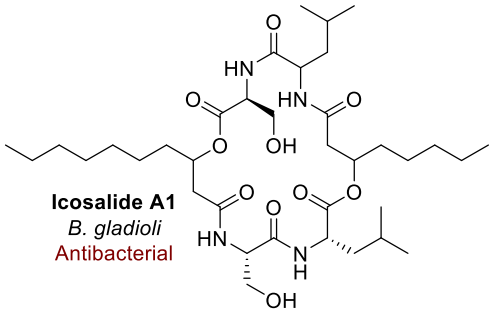
coelibactin *aka* spoxazomicin



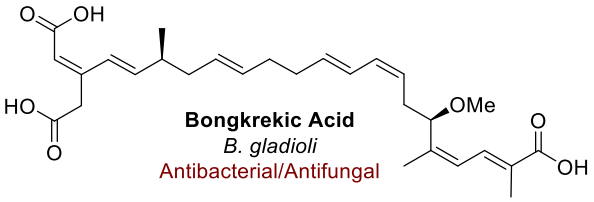
germicidin A

Natural Products from *Burkholderia*

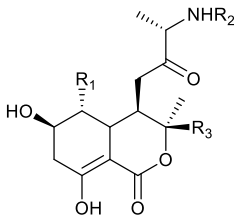
Diverse Metabolic Arsenal



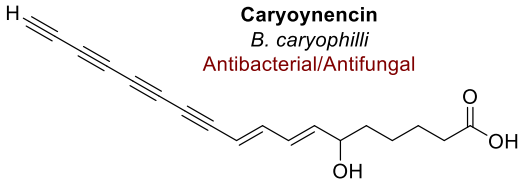
Icosalide A1
B. gladioli
Antibacterial



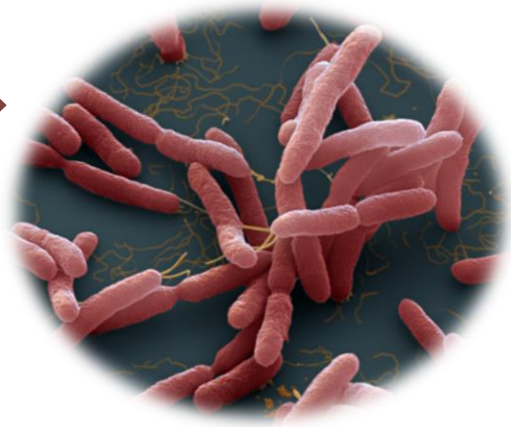
Bongkrekic Acid
B. gladioli
Antibacterial/Antifungal

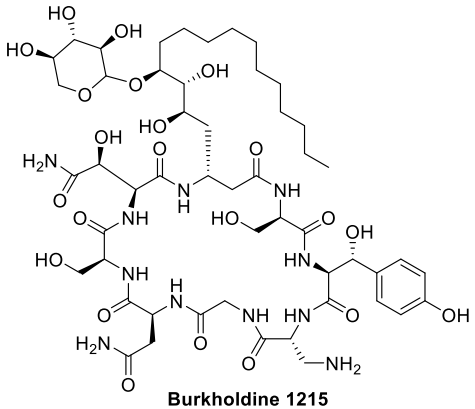


Bactobolins A-H
B. thailandensis
Antibacterial

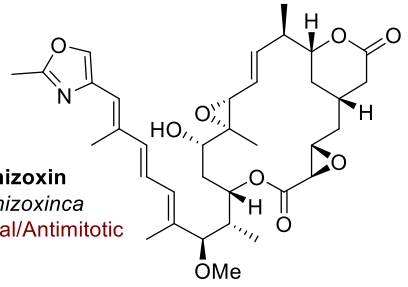


Caryoynencin
B. caryophilli
Antibacterial/Antifungal

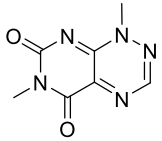




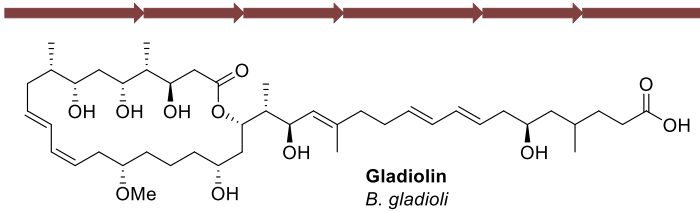
Burkholdine 1215
B. ambifaria/vietnamiensis
Hemolytic/Antifungal



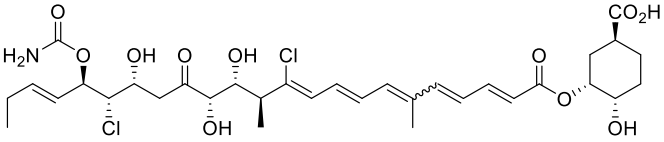
Rhizoxin
B. rhizoxinca
Antifungal/Antimitotic



Toxoflavin
B. gladioli
Antibacterial/Cytotoxic



Gladiolin
B. gladioli
Antibacterial



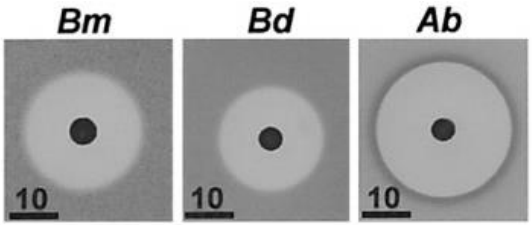
Enacyloxin IIa
B. ambifaria
Antibacterial

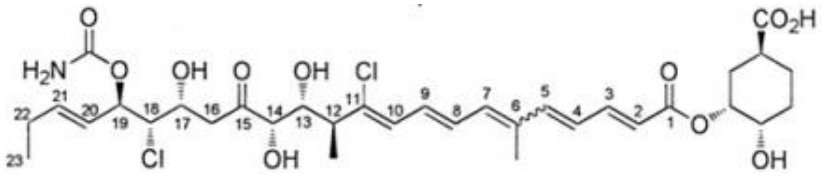
Exploiting *Burkholderia* for Novel Antimicrobial Compounds

'An untapped source of novel bioactive metabolites'

Enacyloxins Are Products of an Unusual Hybrid Modular Polyketide Synthase Encoded by a Cryptic *Burkholderia ambifaria* Genomic Island

Eshwar Mahenthiralingam,[1,*] Lijiang Song,[2] Andrea Sass,[1] Judith White,[1] Ceri Wilmot,[1] Angela Marchbank,[1] Othman Boaisha,[1] James Paine,[3] David Knight,[3] and Gregory L. Challis[2,*]





enacyloxin IIa (Δ-4,5 = *E*)
***iso*-enacyloxin IIa (Δ-4,5 = *Z*)**

Acinetobacter baumannii (MIC 2 µg/mL)

Chem. Biol. **2011**, *18*, 665.

Exploiting *Burkholderia* for Novel Antimicrobial Compounds

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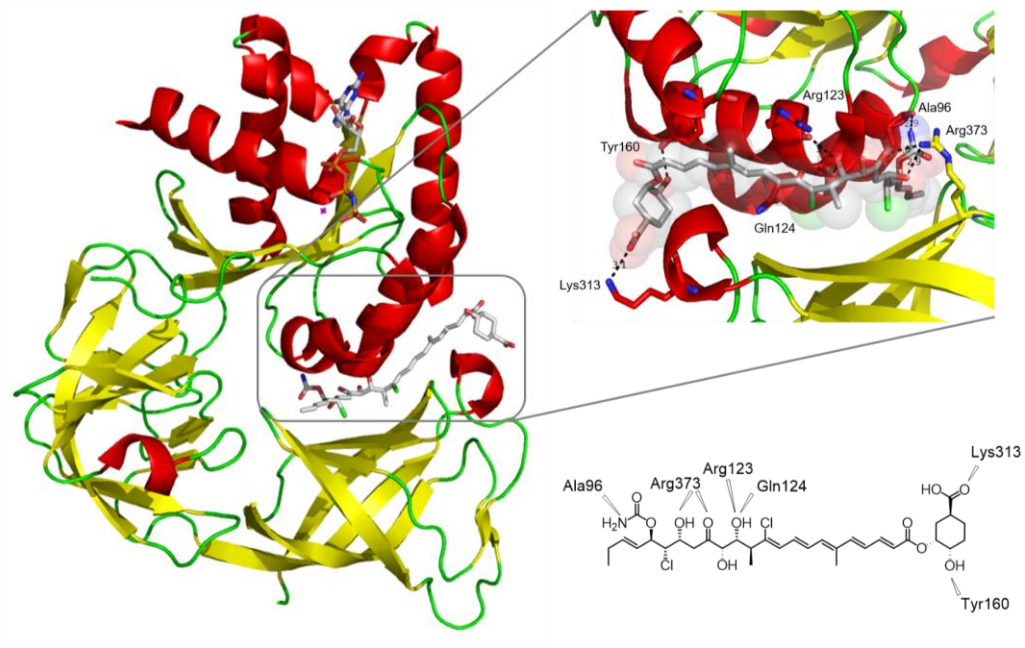

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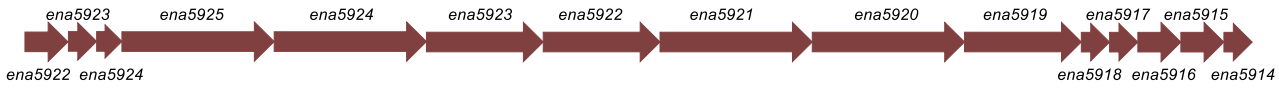
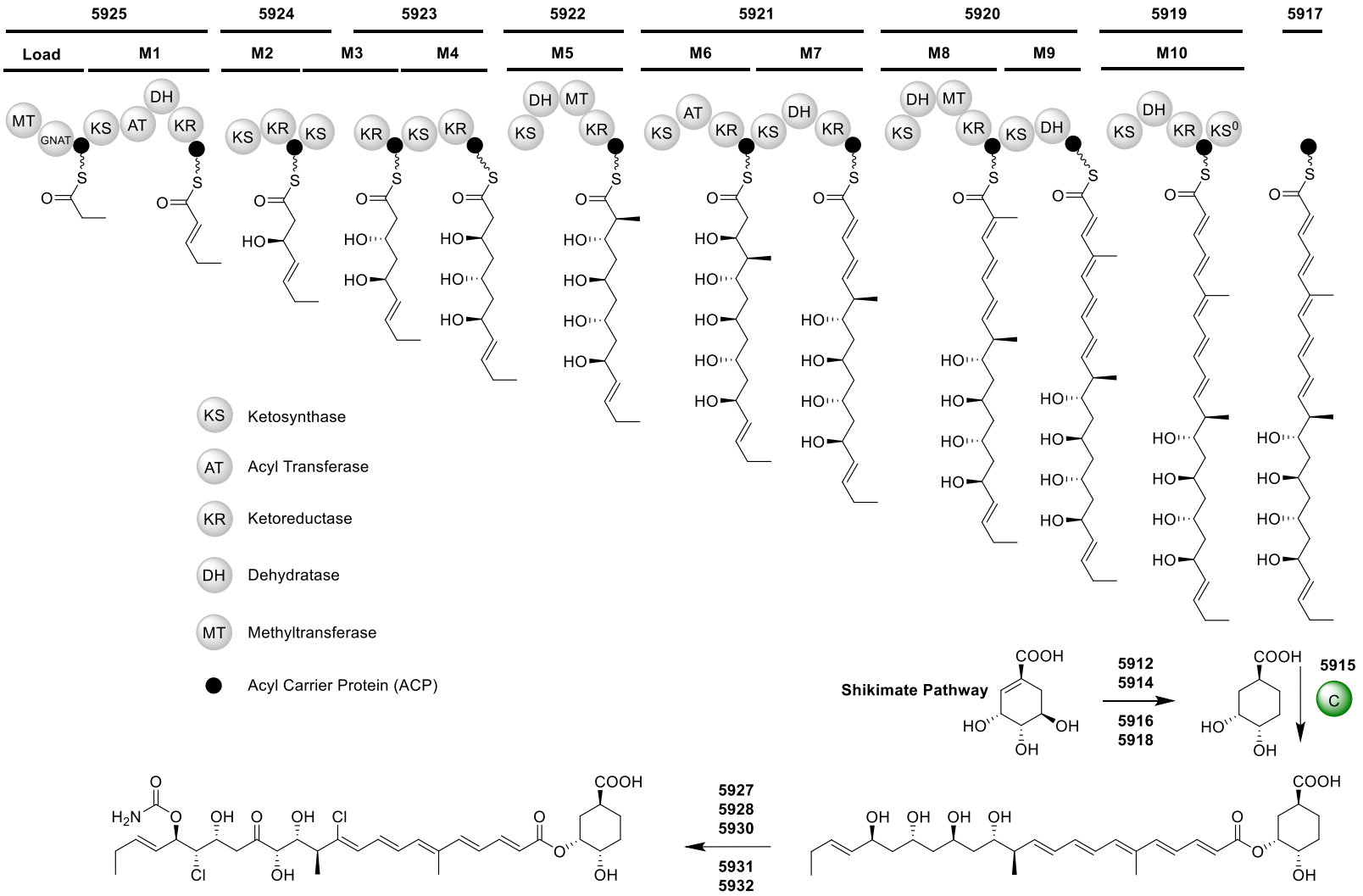
Enacyloxin Biosynthetic Gene Cluster



Chem. Biol. **2011**, *18*, 665.

Biosynthesis of Enacyloxin IIa

Hybrid *cis*/*trans*-AT Modular Polyketide Synthase

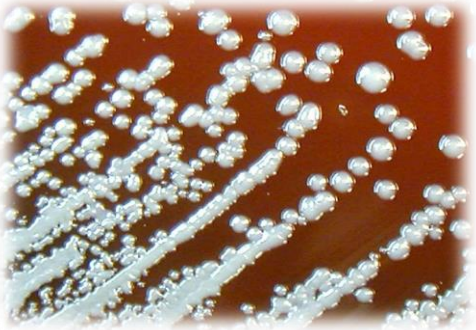


Chem. Biol. **2011**, *18*, 665.

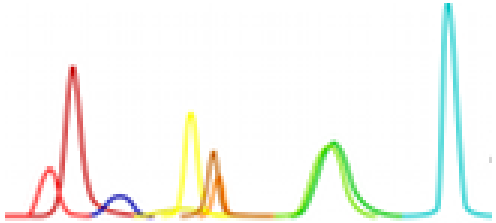
Screening Metabolites from *Burkholderia gladioli* BCC0238

A Highly Bioactive Strain

Cystic fibrosis patient
Minneapolis, USA.
1996

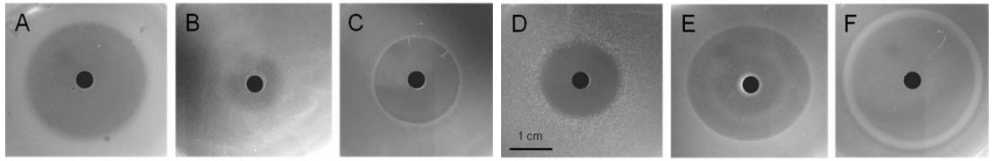


Burkholderia gladioli
BCC0238



Metabolite Screening

B. gladioli BCC0238 overlays



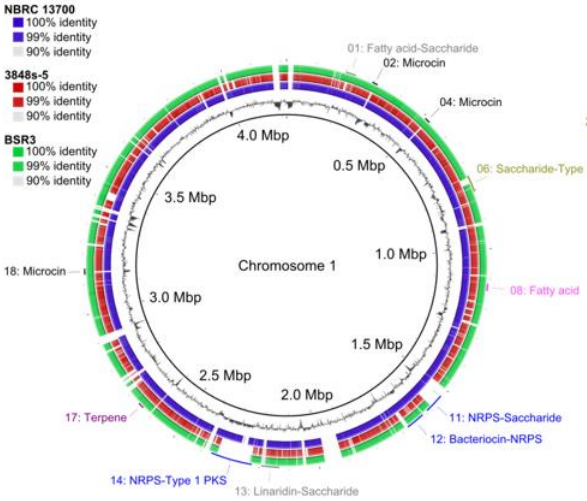
MRSA | *B. multivorans* | *B. subtilis* | *R. mannitolilytica* | *E. faecium* | *C. albicans*

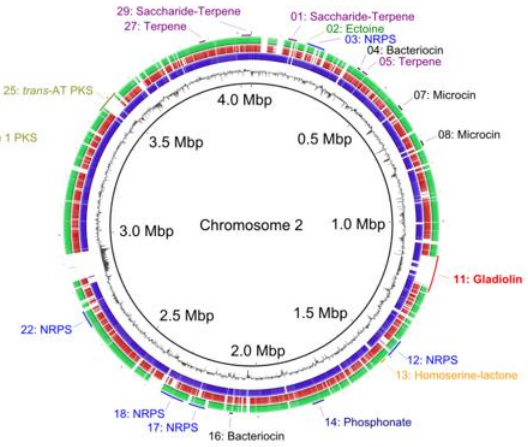
J. Am. Chem. Soc. **2017**, *139*, 7974.

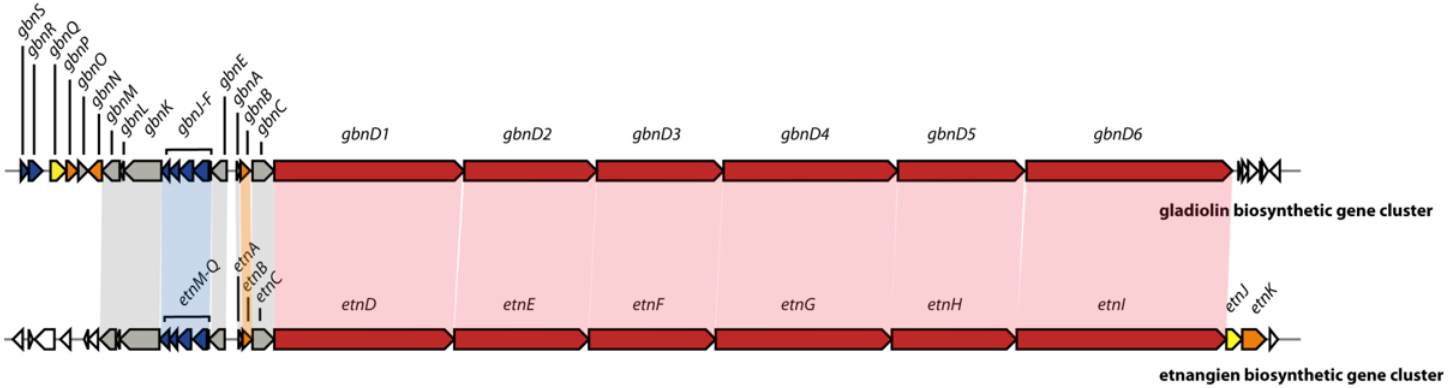
Genome Sequencing of *Burkholderia gladioli* BCC0238

Biosynthetic Gene Cluster Analysis

Burkholderia gladioli
BCC0238







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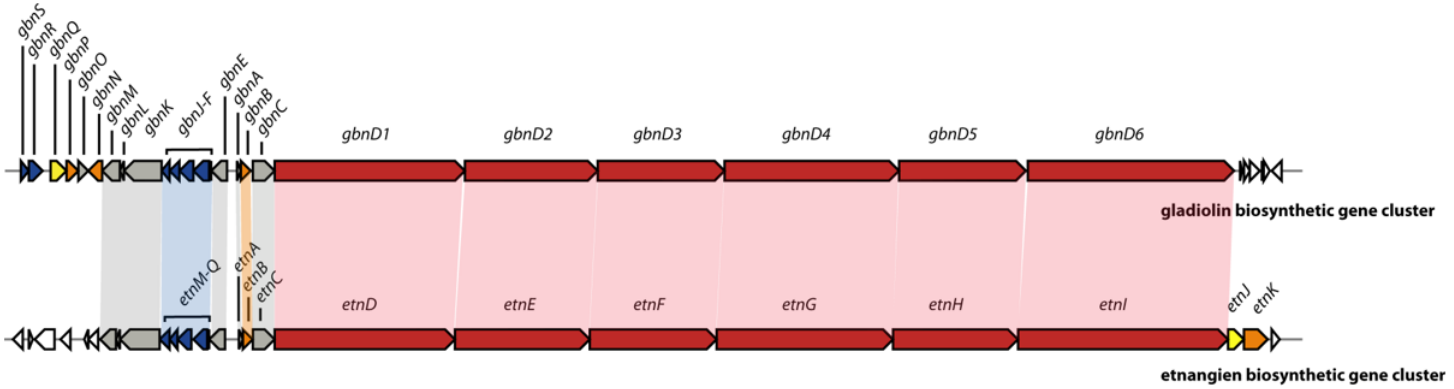
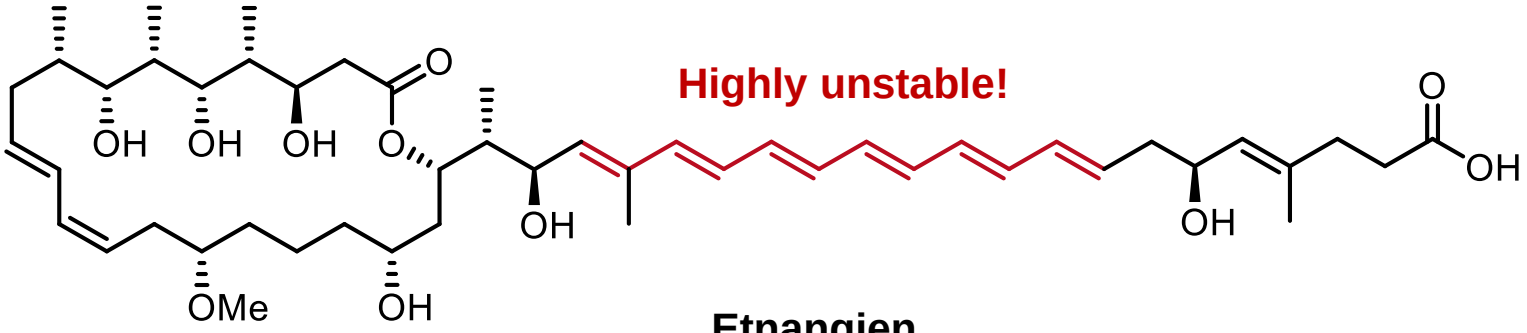
Discovery of Gladiolin: A Novel Macrolide Antibiotic

Structure Elucidation and Comparison to Etnangien





Highly unstable!

Etnangien

Sorangium cellulosum

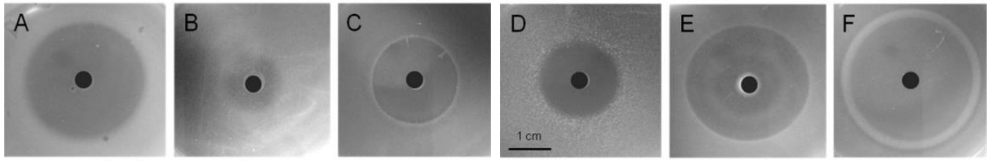
Mycobacterium smegmatis (MIC 1 µg/mL)

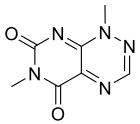
Inhibits *E. coli* **RNA Polymerase**

J. Am. Chem. Soc. **2017**, *139*, 7974.

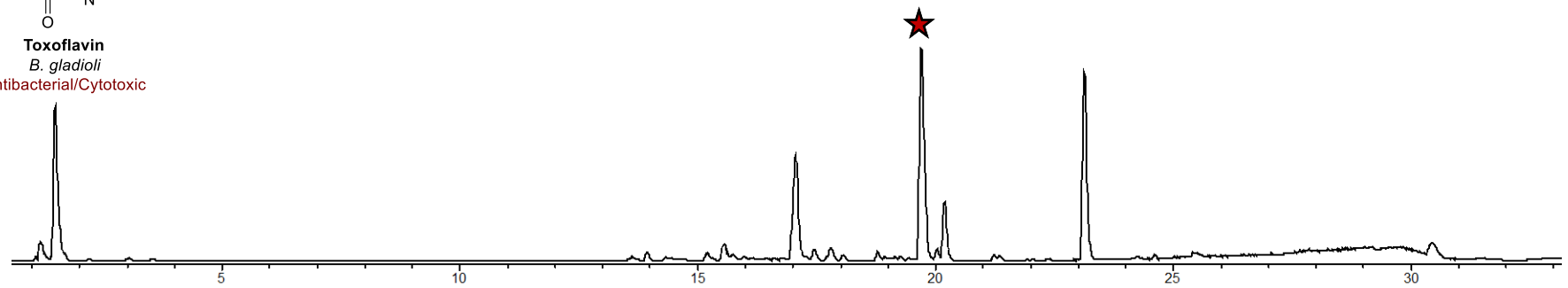
Screening Metabolites from *Burkholderia gladioli* BCC0238

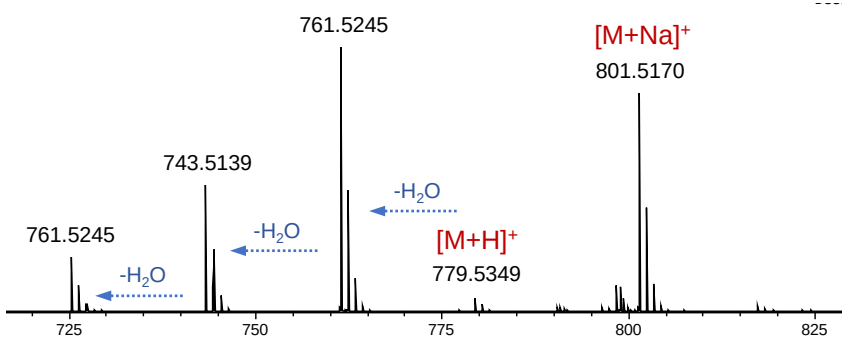
LC-MS Metabolite Screens of Crude Extracts





Toxoflavin
B. gladioli
Antibacterial/Cytotoxic





Molecular Formula: $C_{44}H_{75}O_{11}$

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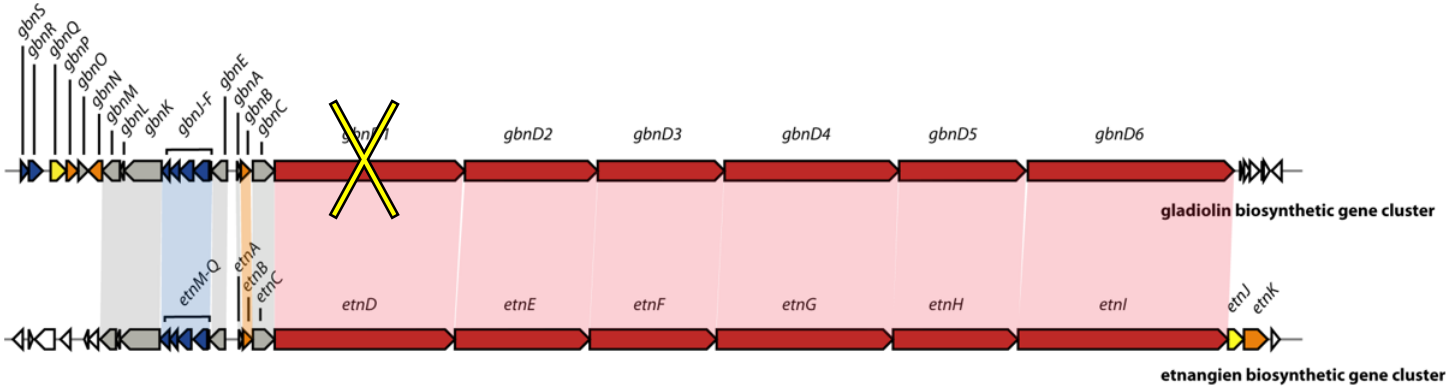
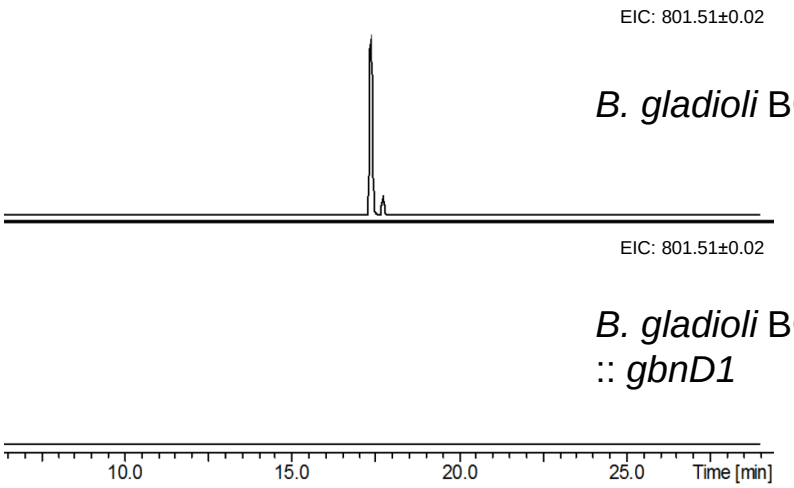
Genome Sequencing of *Burkholderia gladioli* BCC0238

Insertional Mutation Abolishes Gladiolin Production





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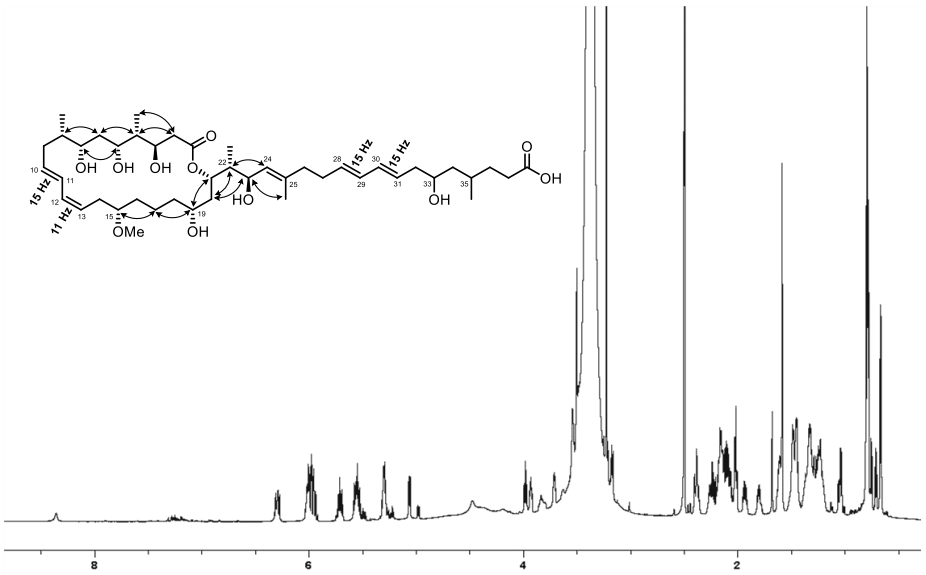
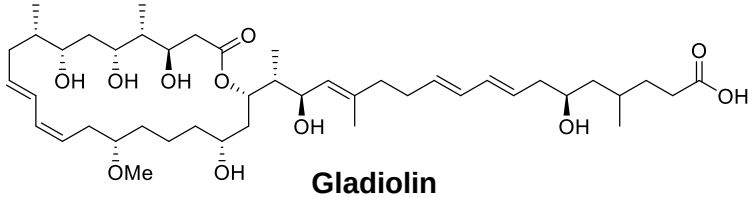
Discovery of Gladiolin: A Novel Macrolide Antibiotic

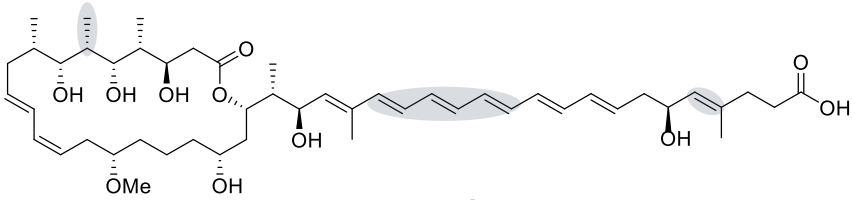
Structure Elucidation and Comparison to Etnangien





Gladiolin

Burkholderia gladioli



Etnangien

Sorangium cellulosum

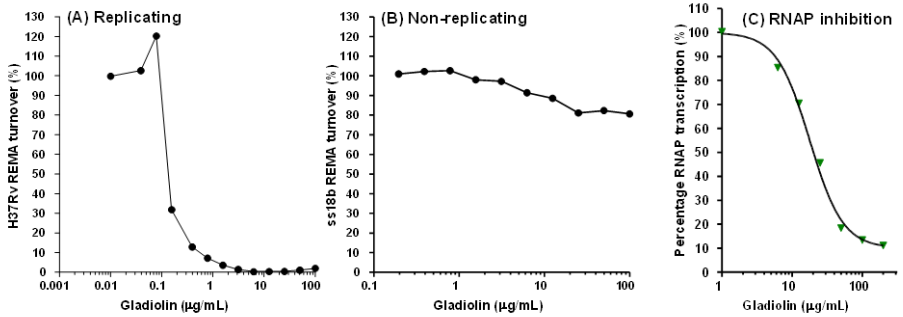
Mycobacterium smegmatis (MIC 1 µg/mL)

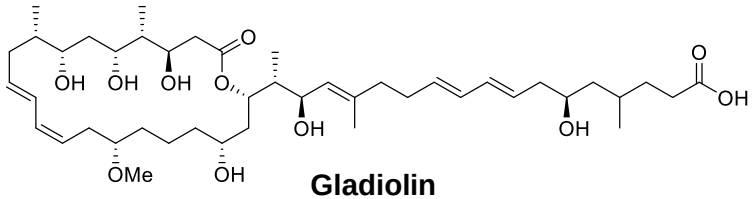
Inhibits *E. coli* **RNA Polymerase**

J. Am. Chem. Soc. **2017**, *139*, 7974.

Discovery of Gladiolin: A Novel Macrolide Antibiotic

Gladiolin Inhibition of RNA Polymerase and Cytotoxicity



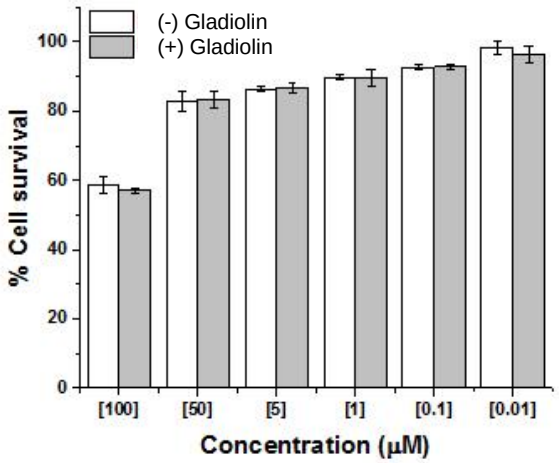


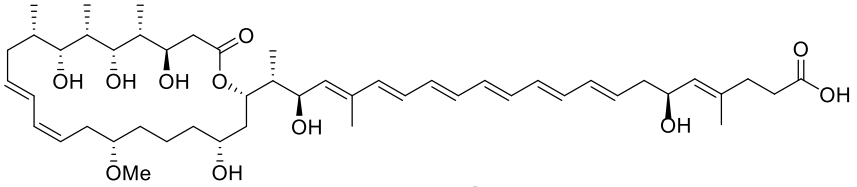
Gladiolin

Burkholderia gladioli

Mycobacterium tuberculosis (MIC 0.3 µg/mL)

Inhibits *Mycobacterium smegmatis* **RNA Polymerase** (23 µM)





Etnangien

Sorangium cellulosum

Mycobacterium smegmatis (MIC 1 µg/mL)

Inhibits *E. coli* **RNA Polymerase**

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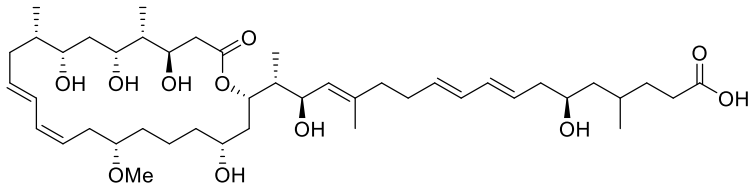
Discovery of Gladiolin: A Novel Macrolide Antibiotic

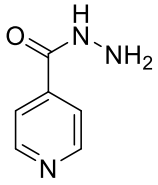
Cross-Resistance with Rifampicin and Isoniazid

			MIC (μg/mL)		
Strain	**Mutation**	**Resistance**	**Gladiolin**	**Isoniazid**	**Rifampicin**
H37Rv	None	None	0.3	0.04	0.001
HUG.MB.6726	*inhA*	Isoniazid	0.3	2.2	0.001
CHUV80037024	*inhA/katG/rpoB*	Isoniazid/Rifampicin	1.7	>10	>10



Gladiolin

Target: *M. tuberculosis* RNA Polymerase

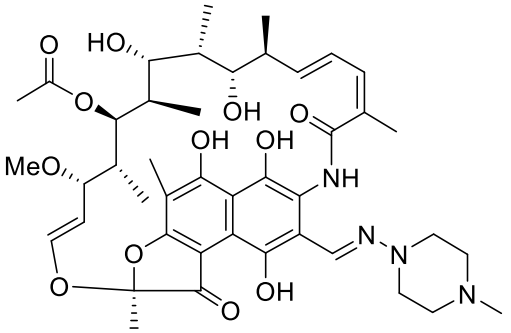


Isoniazid

Target: *M. tuberculosis* Enoyl-ACP Reductase (InhA)

&

M. tuberculosis Catalase peroxidase (KatG)



Rifampicin

Target: *M. tuberculosis* RNA Polymerase β-subunit (RpoB)

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Discovery of Gladiolin: A Novel Macrolide Antibiotic

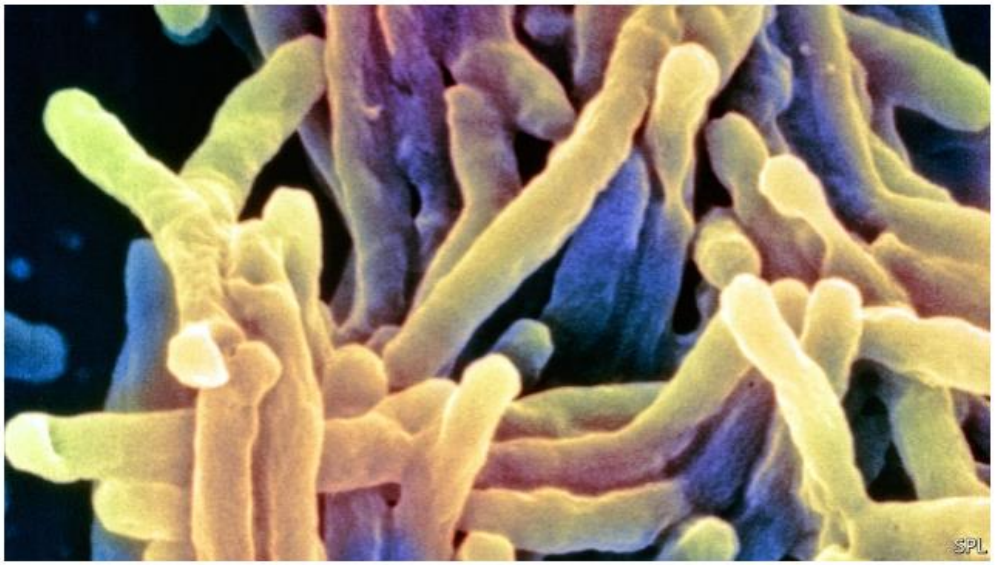
A Bit of Press Attention…

The Economist

The enemy of my enemy

A new antibiotic for drug-resistant tuberculosis

A bug that infects people with cystic fibrosis may yield a treatment for TB



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Nat. Prod. Rep. **2017**, *37*, 713.

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