Symbiosis Based Antimicrobial Discovery and Development Platform – and Lead Progress

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University of Wisconsin

Outline

- Antimicrobial discovery history
- Symbiotic discovery platform
- Lead progress

Wisconsin Antimicrobial Discovery Team

David Andes
Pharmacology
Fungal Biology

Lindsay Kalan
Skin Microbiome

Tim Bugni
Natural Product Chemist

Cameron Currie
Evolutionary Biology

Nasia Safdar
Resistance Microbiome

Bruce Klein
Fungal Biology

Rod Welch
Bacterial Biology

Jon Clardy
Natural Product Chemist

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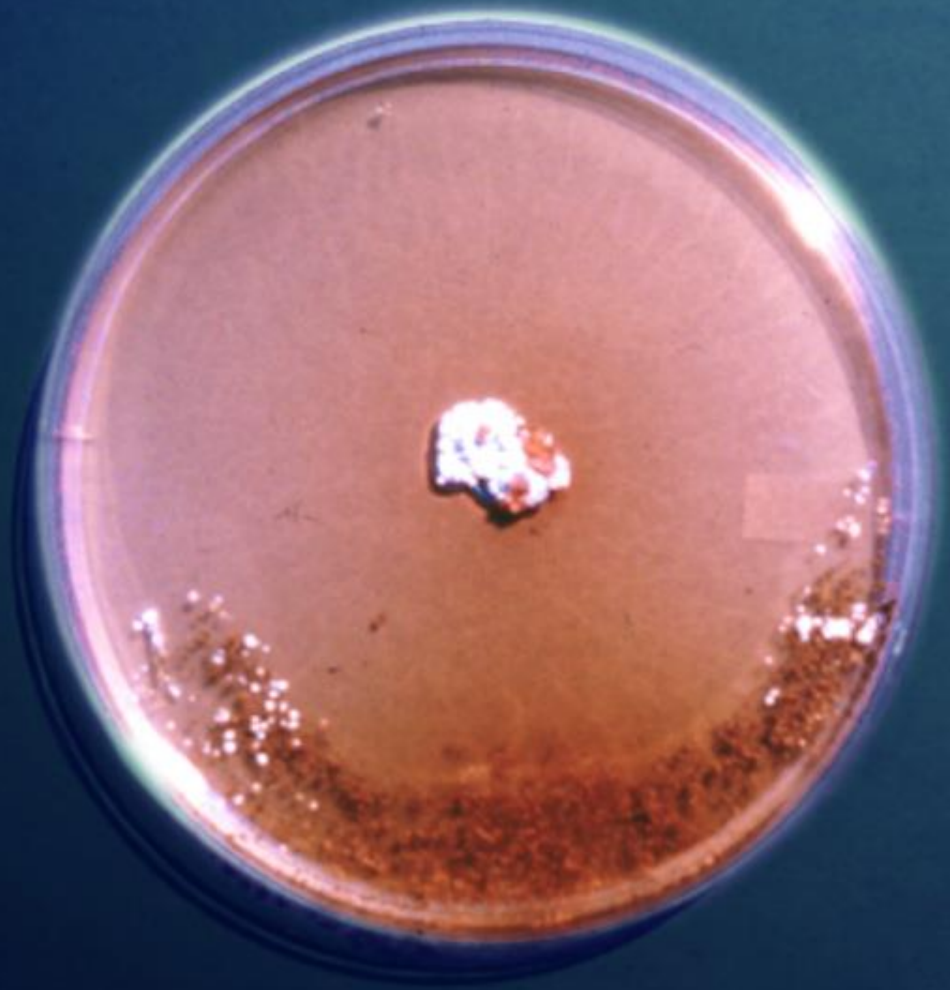
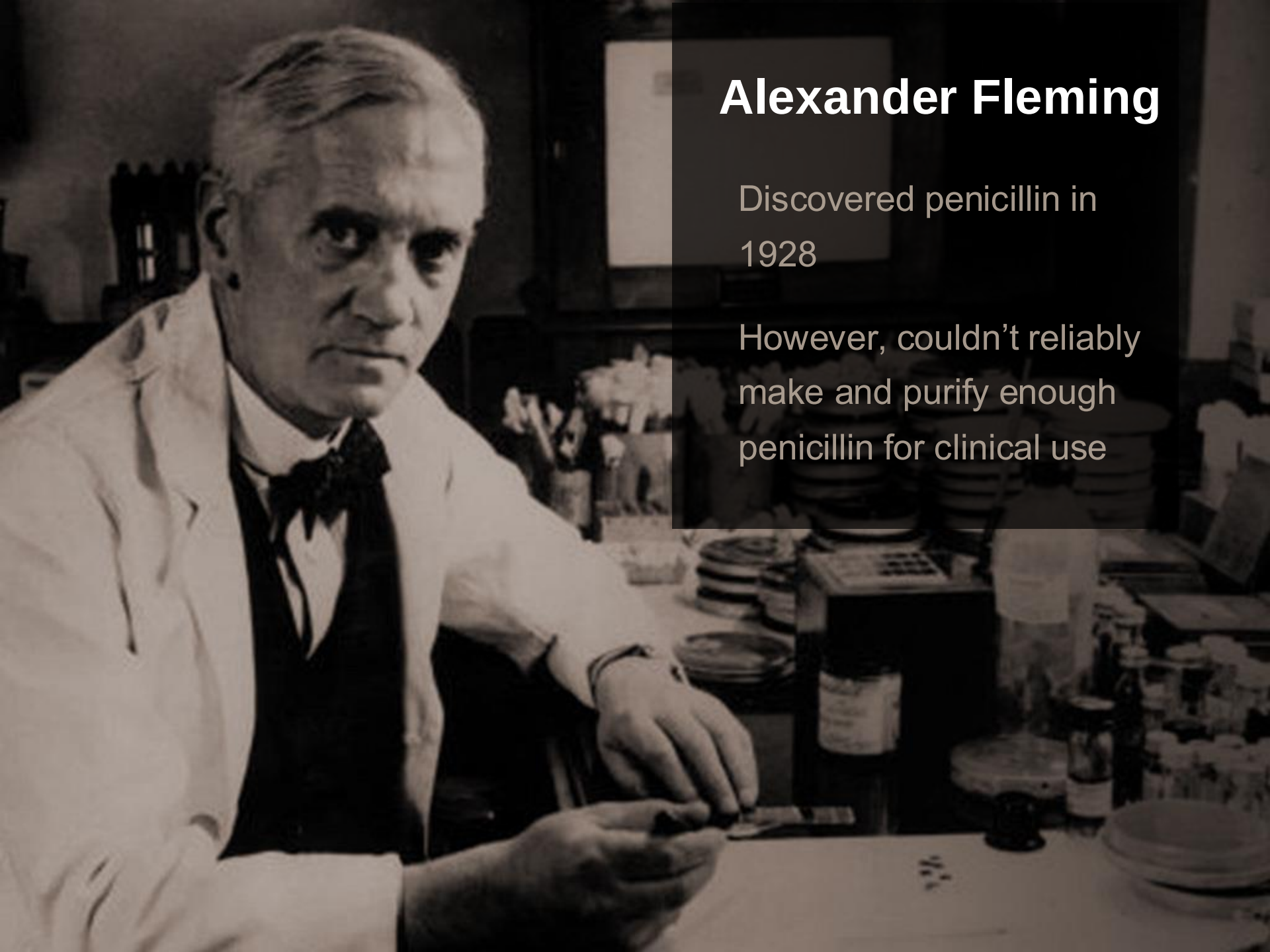
First Inklings of Success

- In 1910, Paul Ehrlich and Sahachiro Hata found arsphenamine (Salvarsan, #606) for syphilis.
- More than 100 FDA approved antimicrobials since



Antibiotic Sources

- 75% of antimicrobials are natural products
- Natural products are chemicals made by plant, animal or microbe
- Different from synthetic molecules by having an evolutionary history

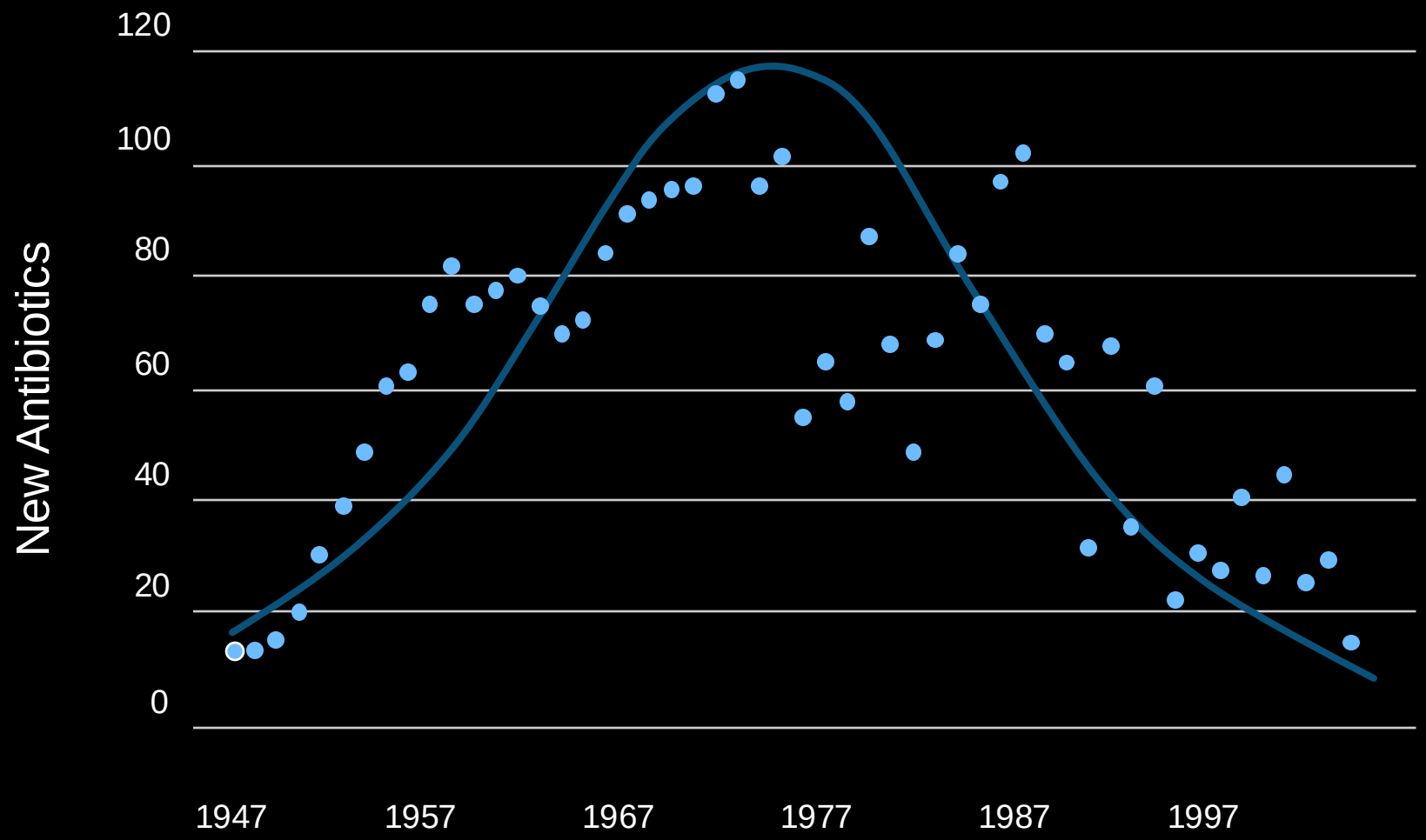


Alexander Fleming

Discovered penicillin in 1928

However, couldn’t reliably make and purify enough penicillin for clinical use

Natural Product Antibiotic Discovery Pipeline



Watve et al, *Arch Microbiol* 2001;176, 386

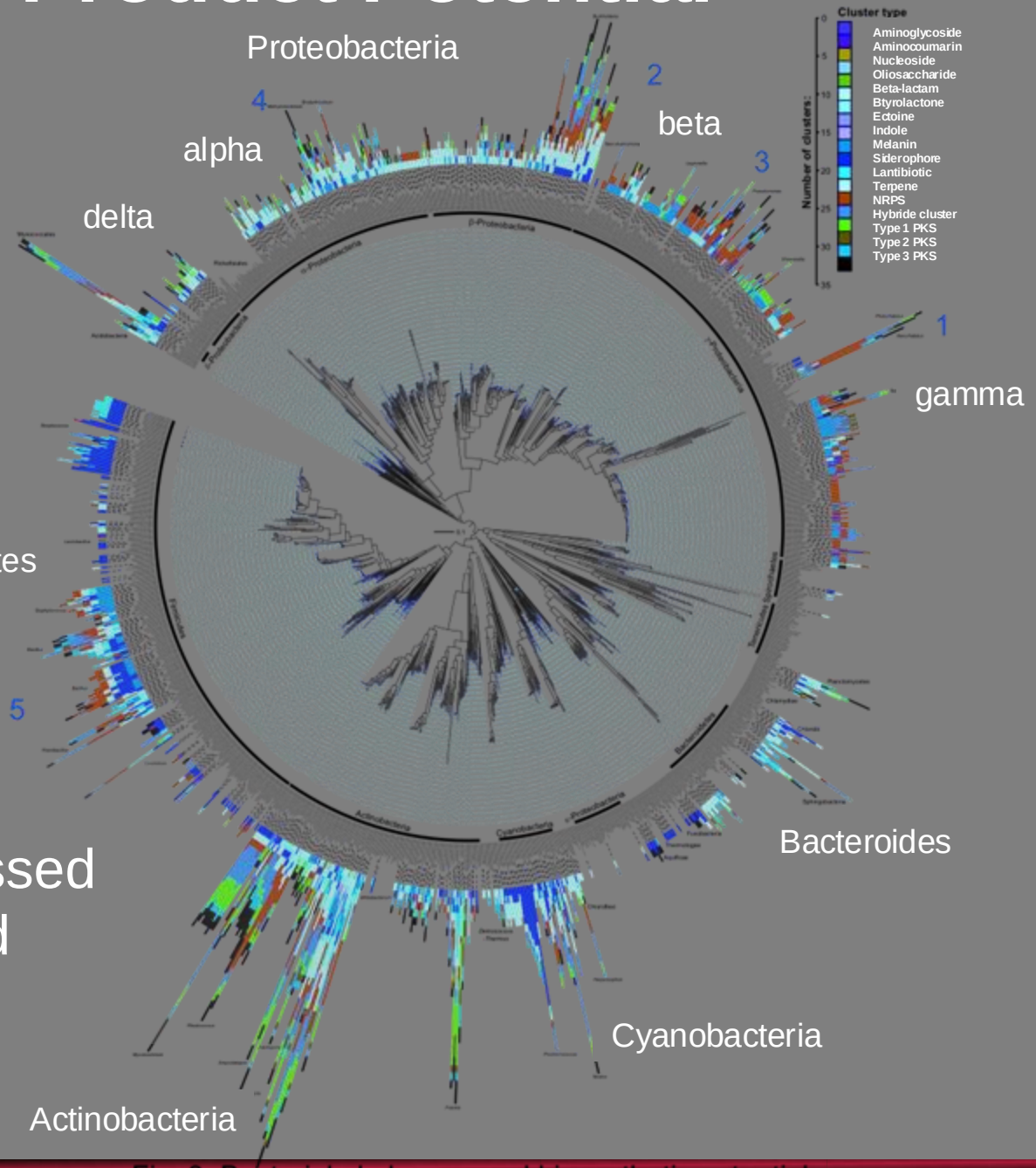
Traditional Product Discovery

Non-biased bioprospecting of soil bacteria now results in 99.5% rediscovery rate

Daptomycin from 1M strain screen


The well is dry!

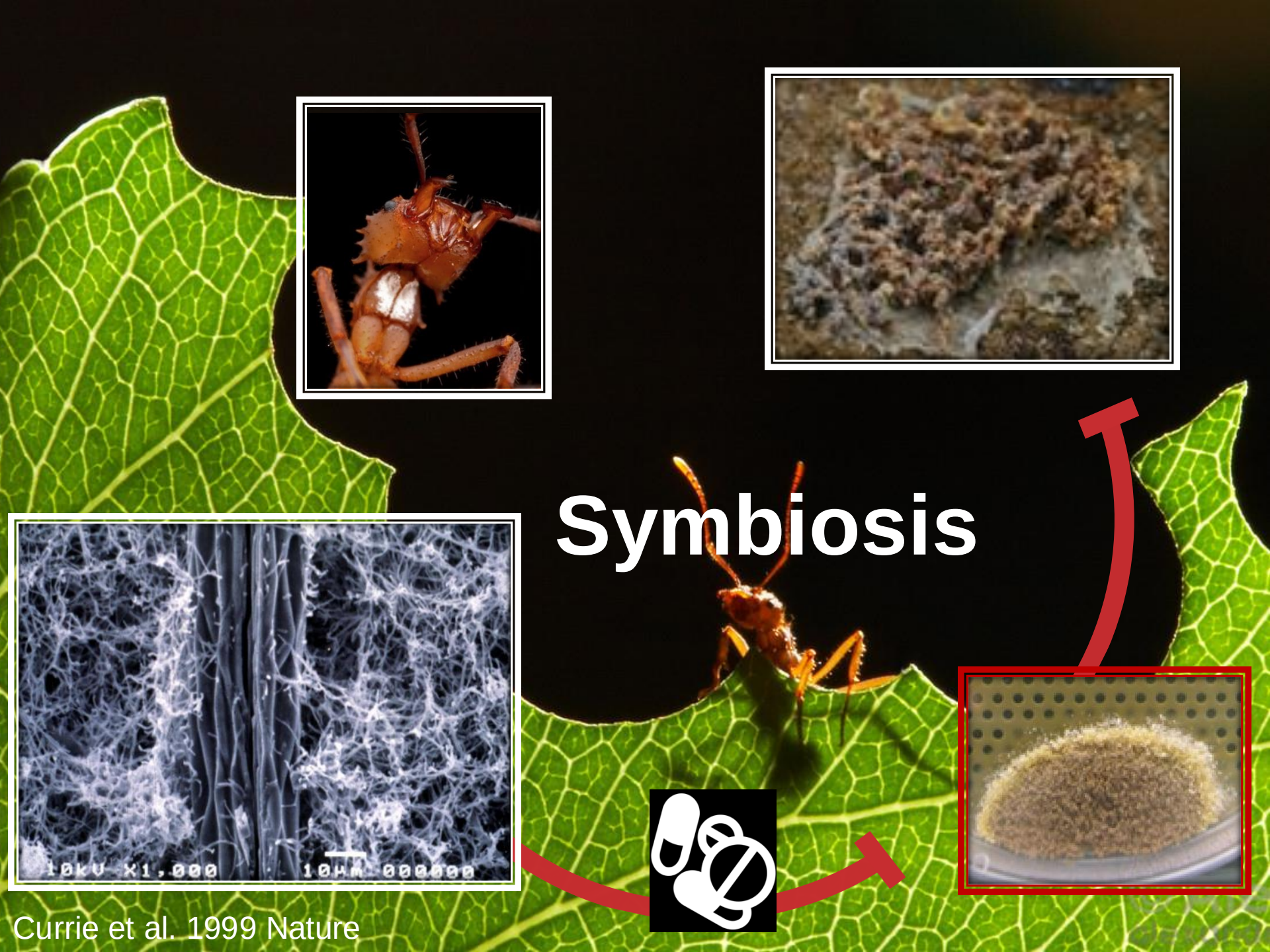
Nature Product Potential



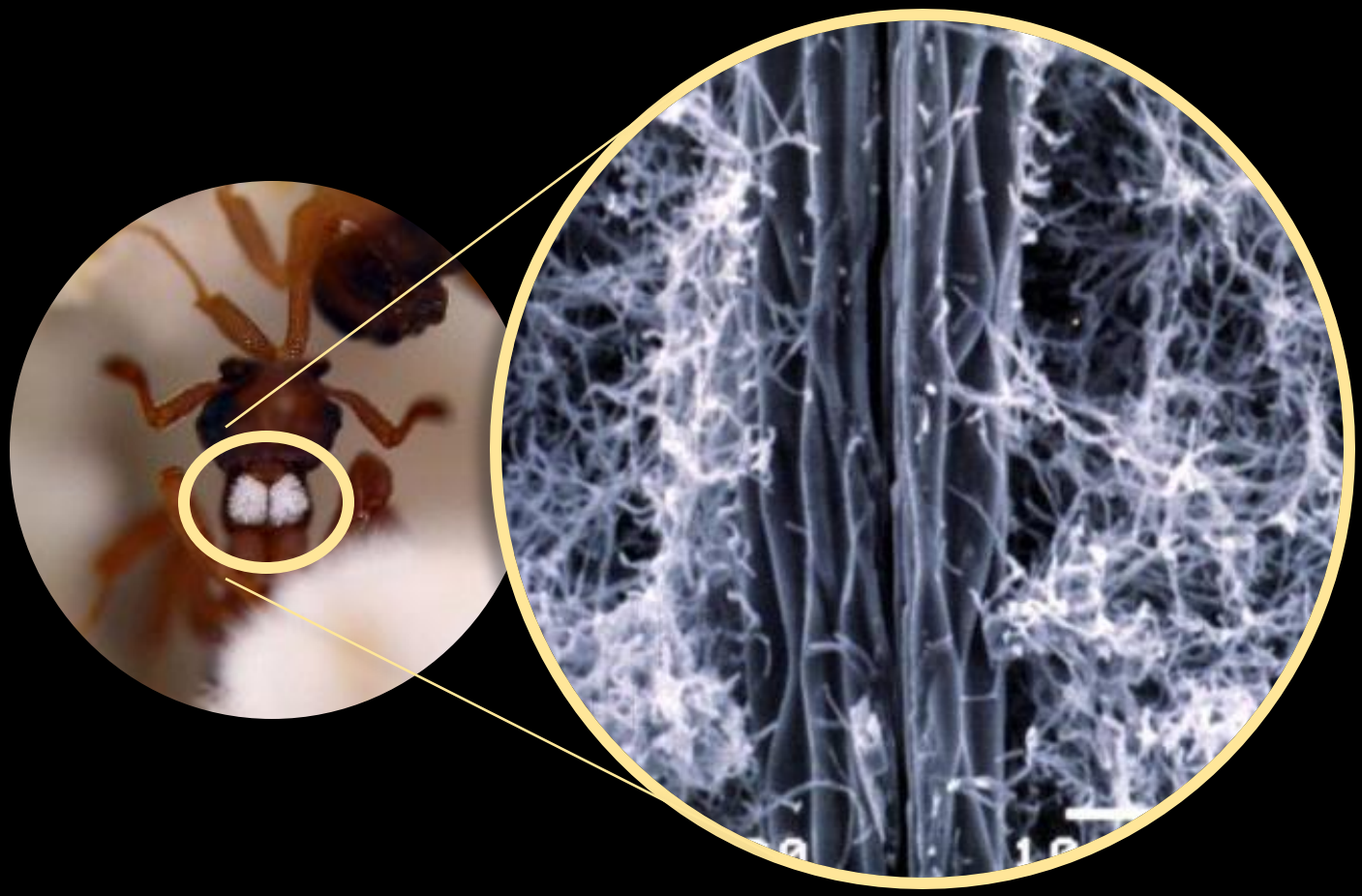
Genetic Potential

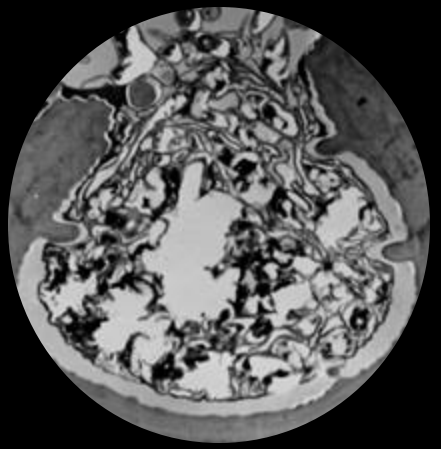
- <1% microbes assessed
- Genetically predicted antibiotics

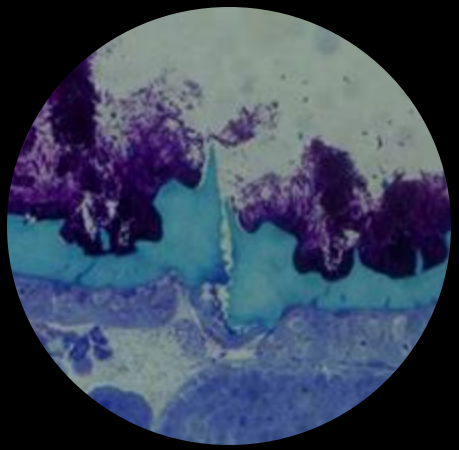
Unexpected Source

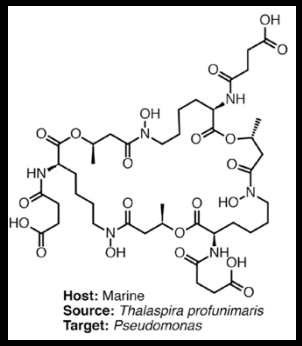

Symbiosis
10kV X1,000 10µm 000000
Currie et al. 1999 Nature

Evidence for Ancient Association



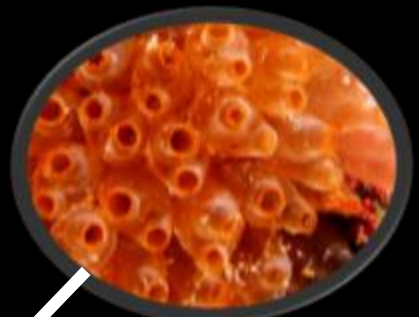






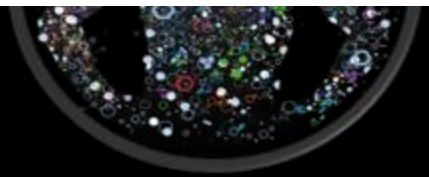
Host: Marine
Source: *Thalaspira profunimaris*
Target: *Pseudomonas*





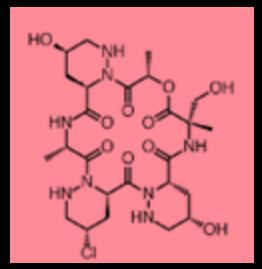
- Our studies have identified 100s novel antibiotics from 1000s symbiotic antibiotic producing strains
- 100- to 1000-fold greater hit rate than historical industry approach
- **>10 MILLION INSECT, MARINE SPECIES, and MAMMALIAN SKIN MICROBIOME CONSTITUENTS**





Symbiotic Discovery Hypothesis

Chemical Diversity

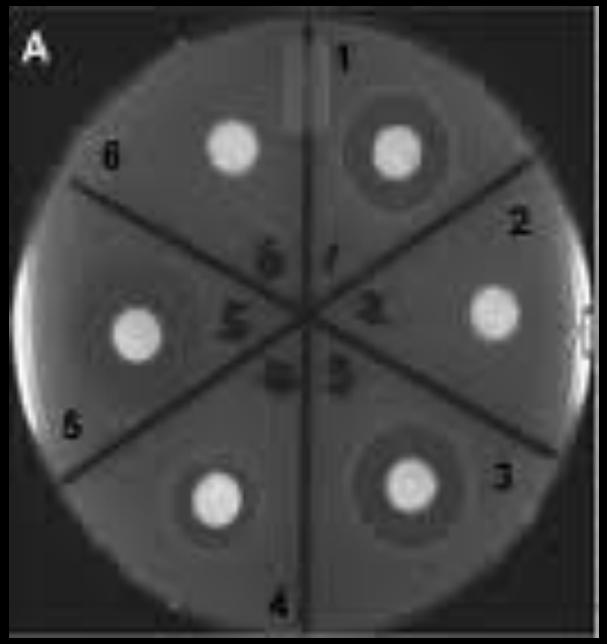


Biological Activity

Host Safety

History: How Has Industry Looked?

- Traditional natural product discovery = non-biased bioprospecting of soil bacteria
- Screening broth culture supernatant fractions for antimicrobial activity



Selman Waksman

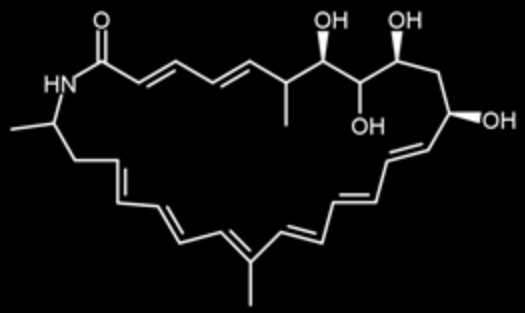

How Are We Looking?

Genome-based Natural Product Discovery

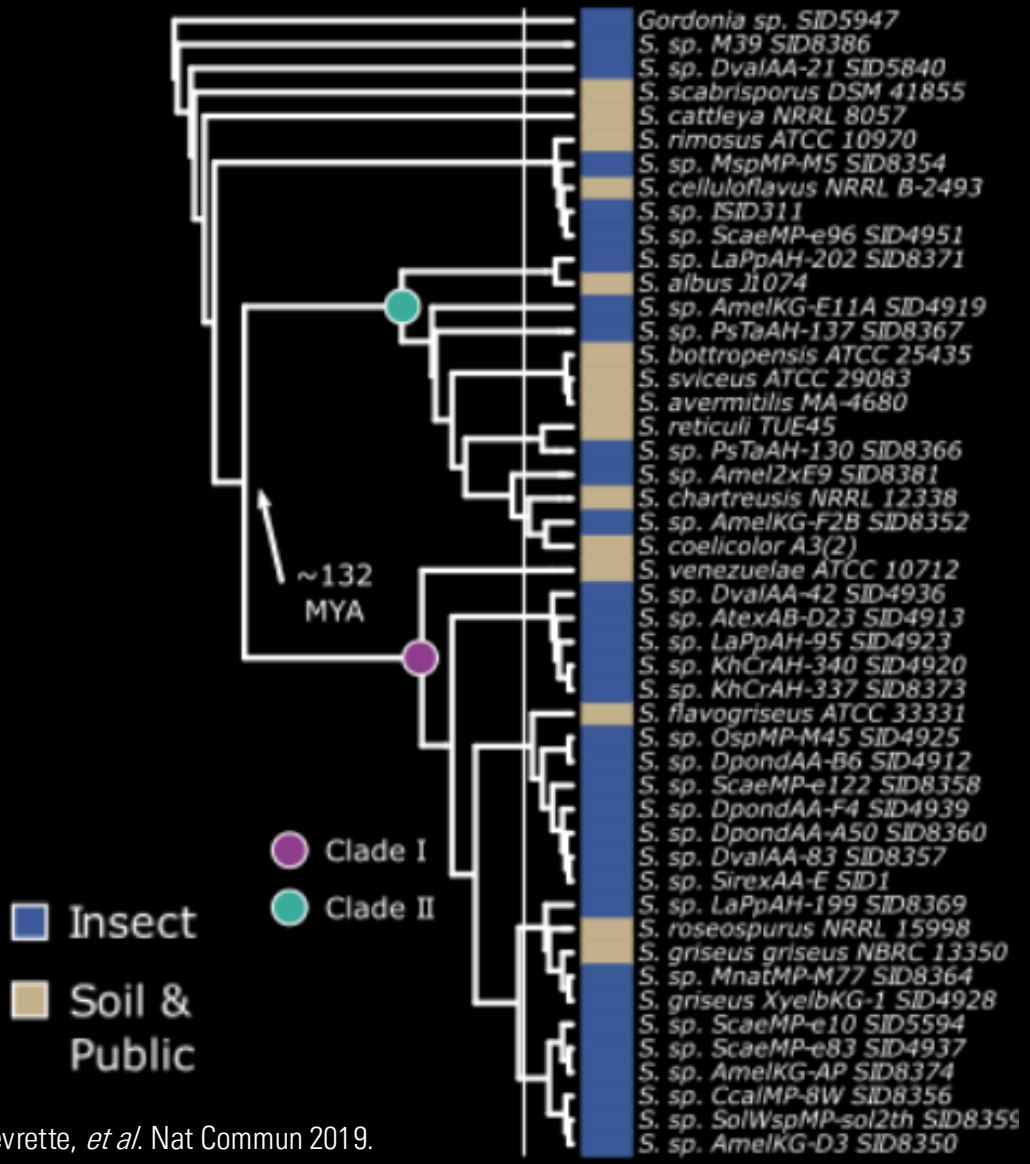
- Genes involved in biosynthesis of NPs are spatially clustered on bacterial genomes.
- Searching genomic space for enzyme signatures reveals the comprehensive biosynthetic potential of an organism.
- Novelty can be assessed from genomic predictions.

~80kb
70% GC

Salinilactam



Biosynthetic Potential of Insect *Streptomyces*



Chevrette, *et al*. Nat Commun 2019.

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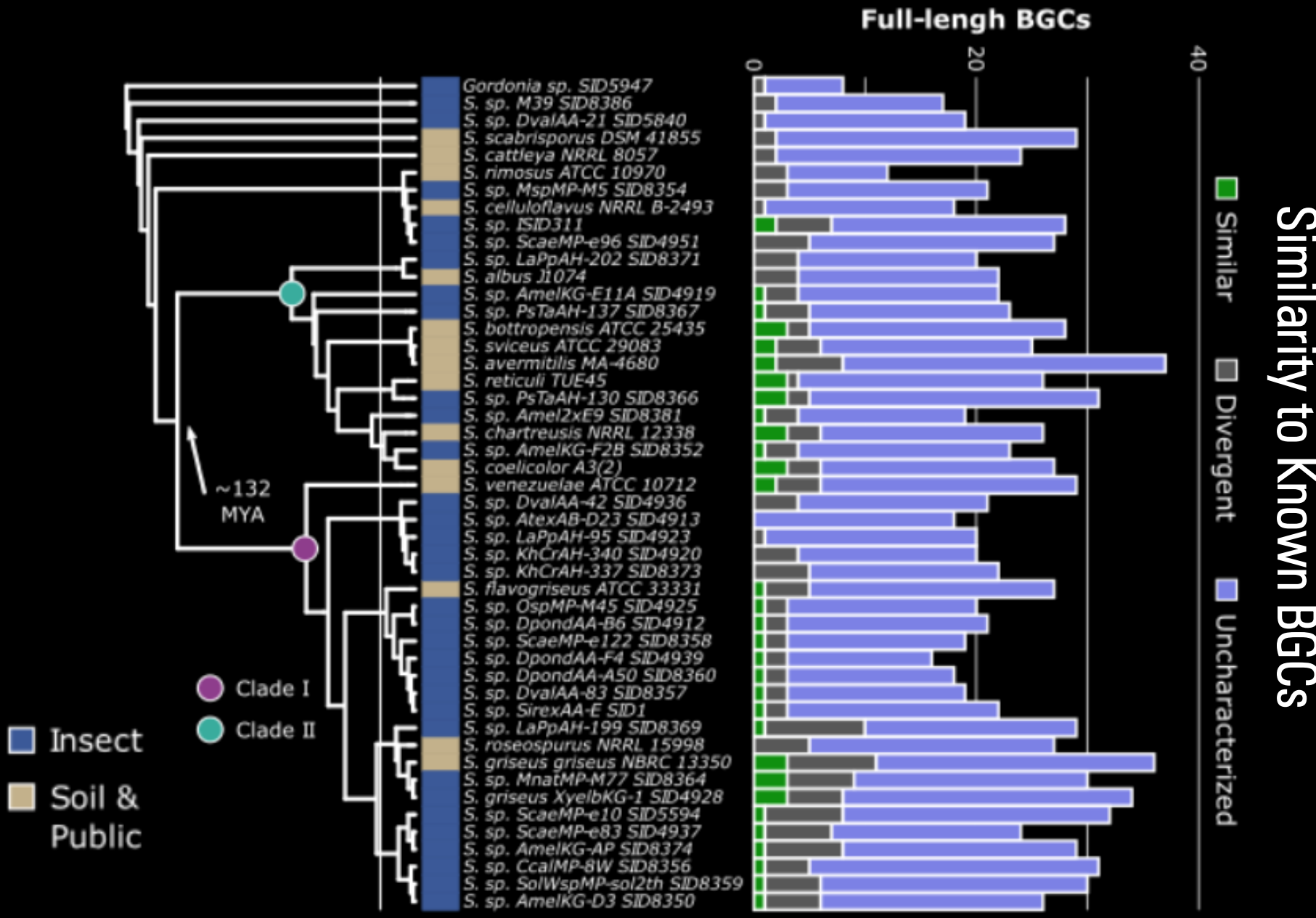
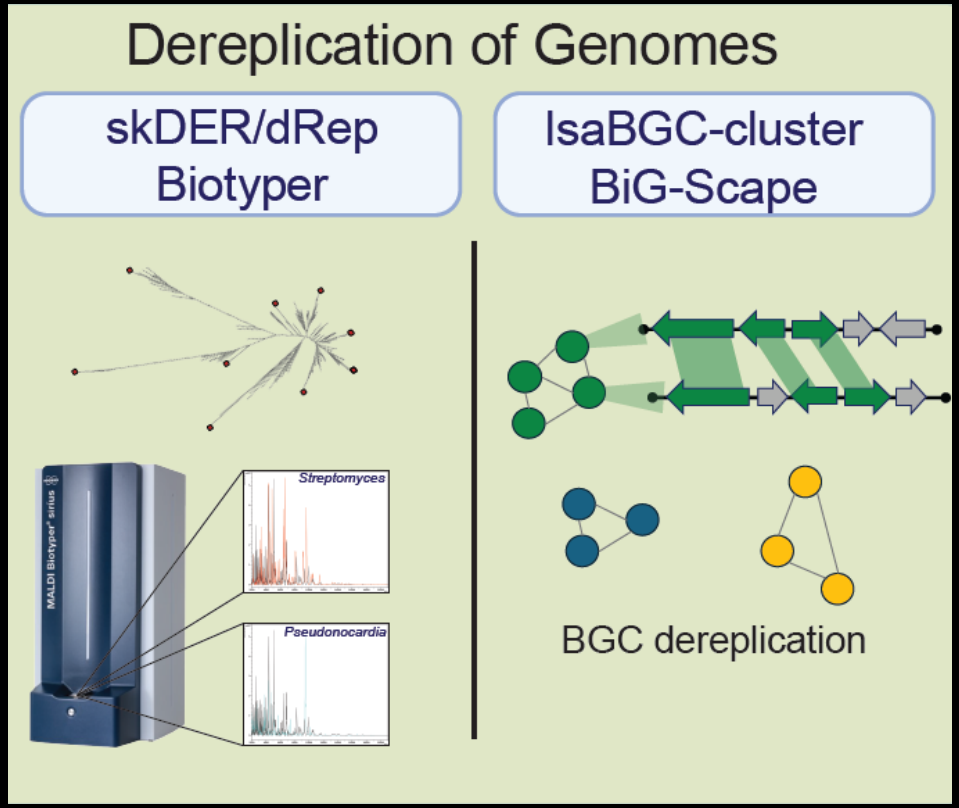


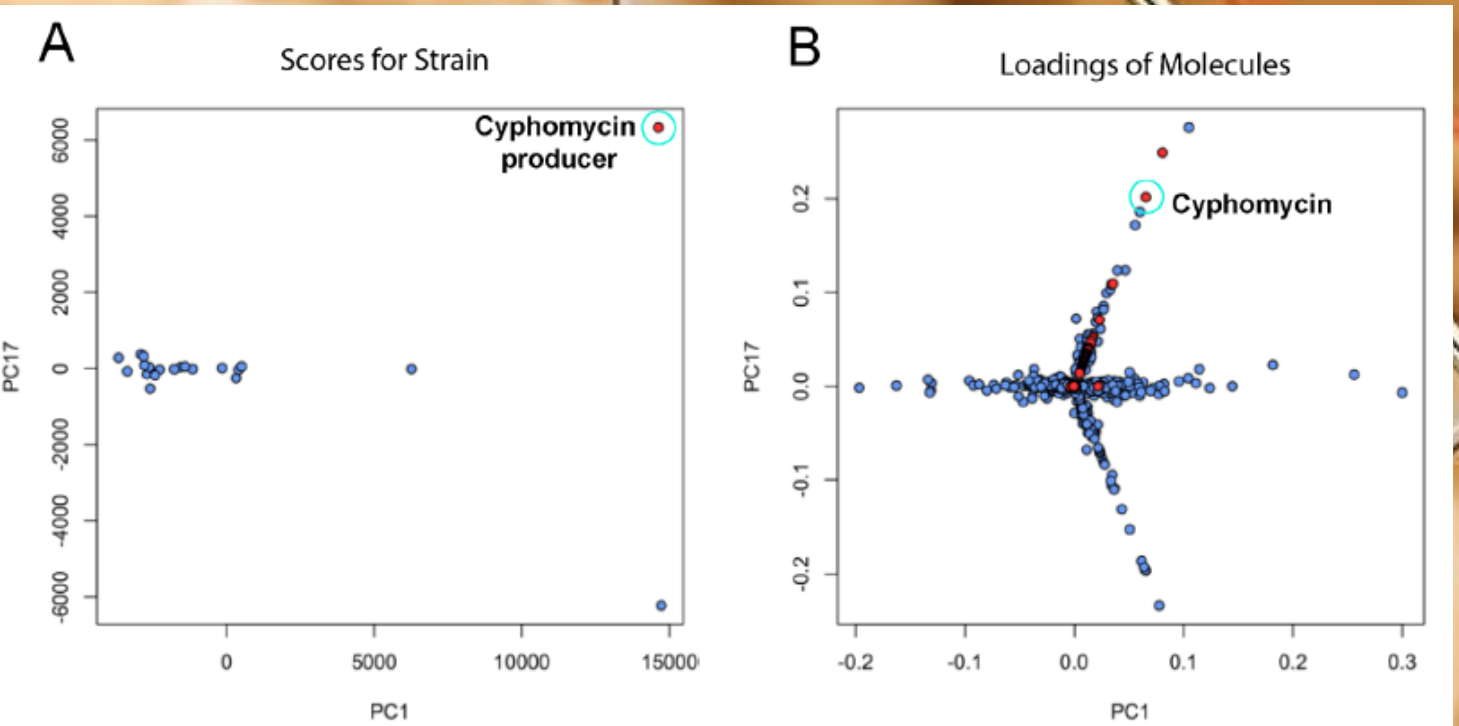
Machine Learning Enabled Genome Dereplication

- WGS of each strain is then dereplicated using a software program we developed called skDER76.
- Genomes are analyzed by antiSMASH to annotate biosynthetic gene clusters
- Structural prediction performed based on conserved protein domains or deepBGC, an AI-driven tool to detect additional noncanonical BGCs.

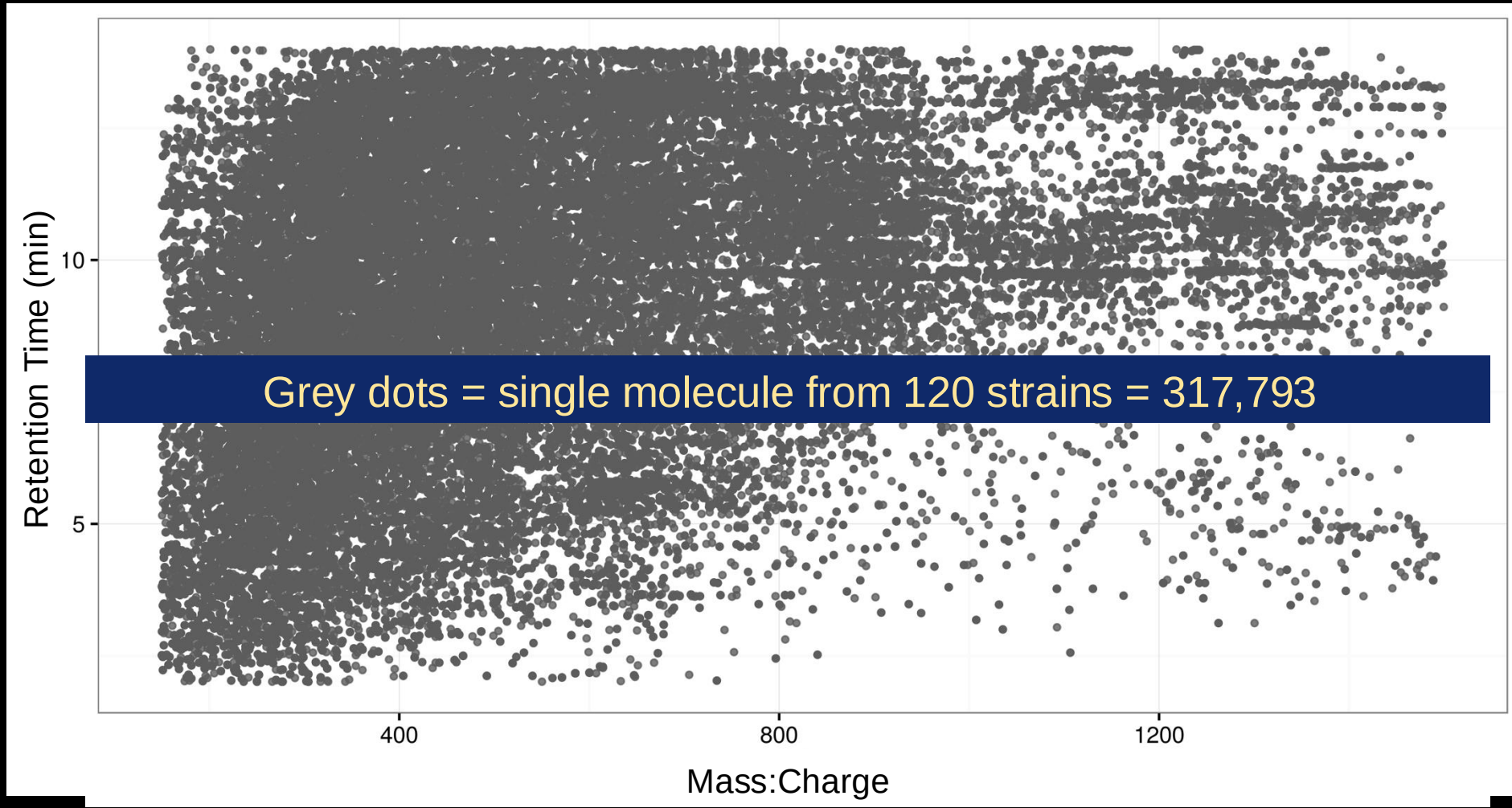


Analyzing Chemical Structure to Predict New Antibiotic Potential

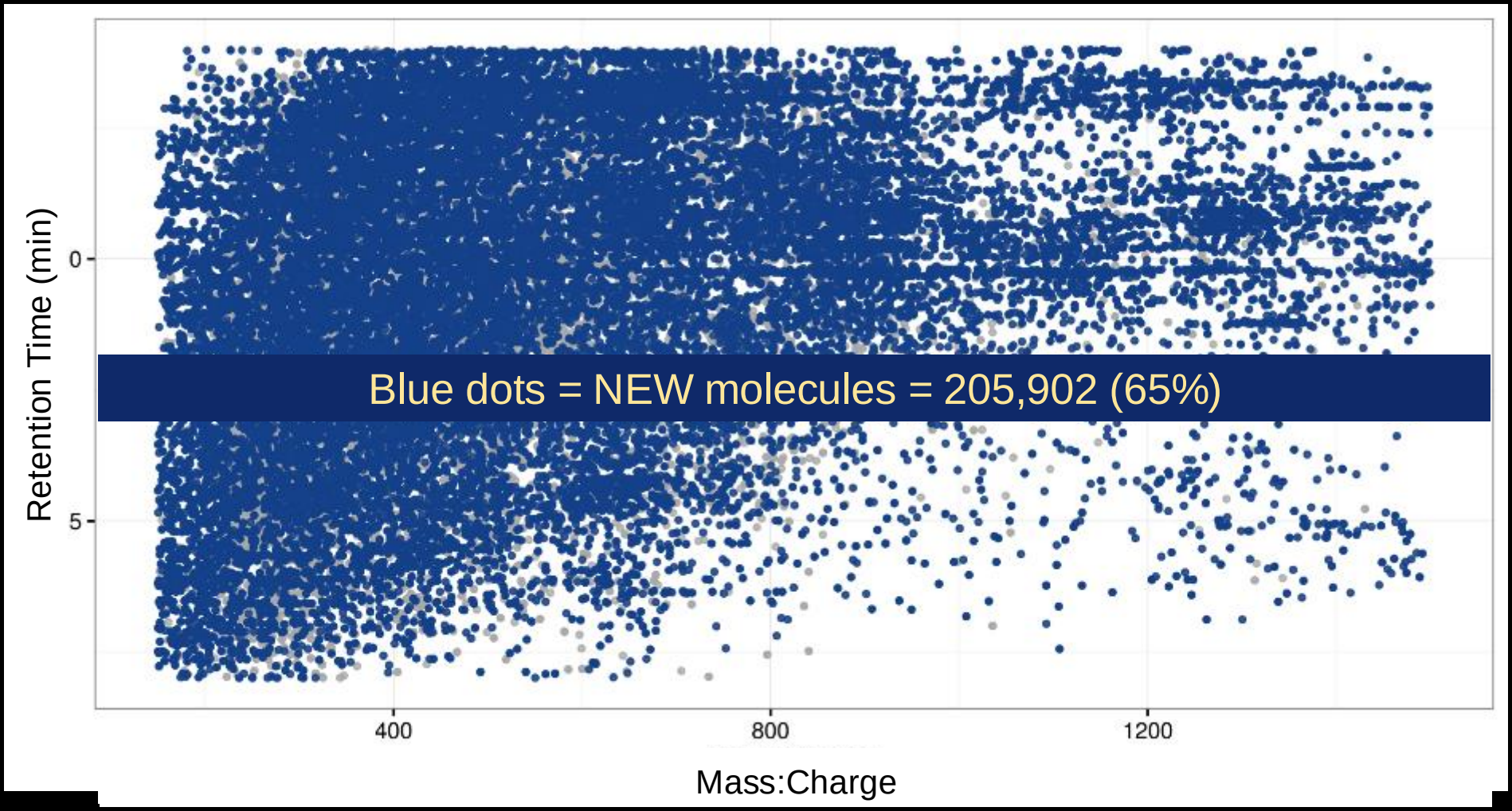
- Chemical assessment and novelty prediction (LC/MS/MS + PCA)
- Library comparison for new chemical structure (AntiBase + SIRIUS



Rich and Untapped Source

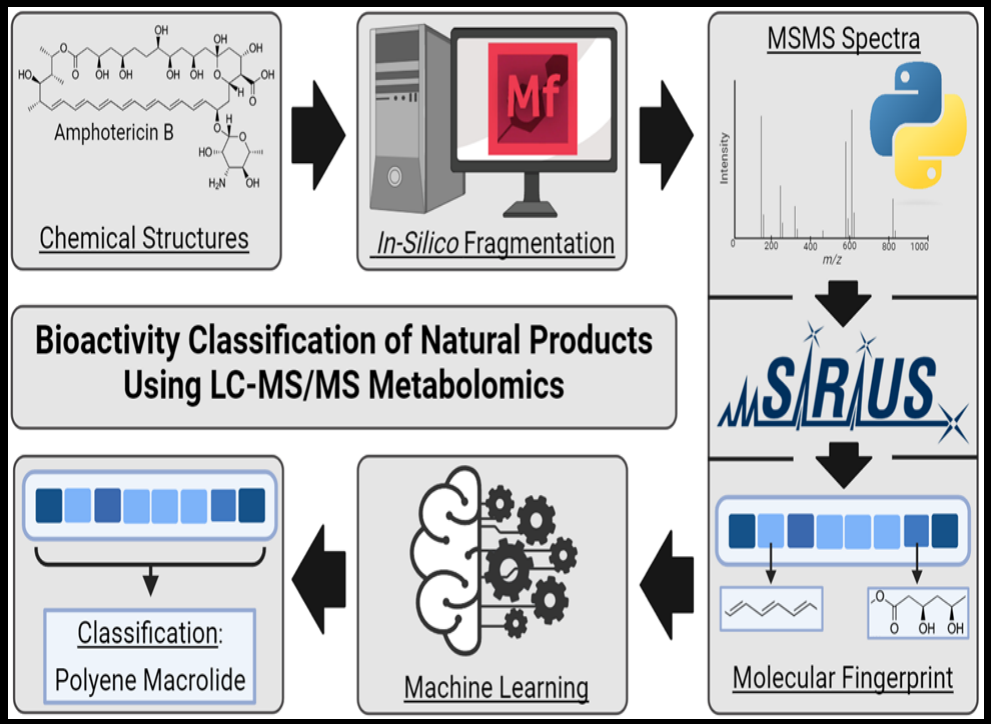


Rich and Novel Source



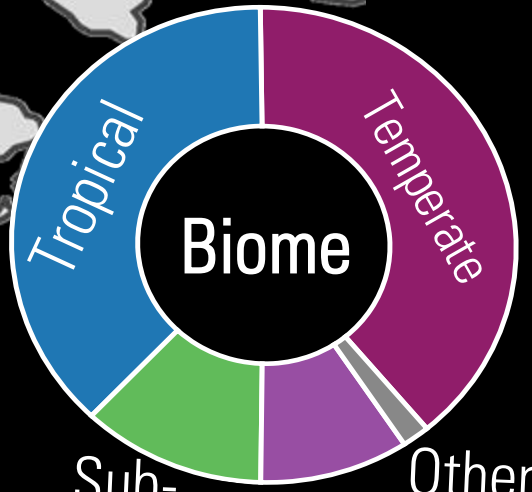
Machine Learning Enabled Structural Dereplication

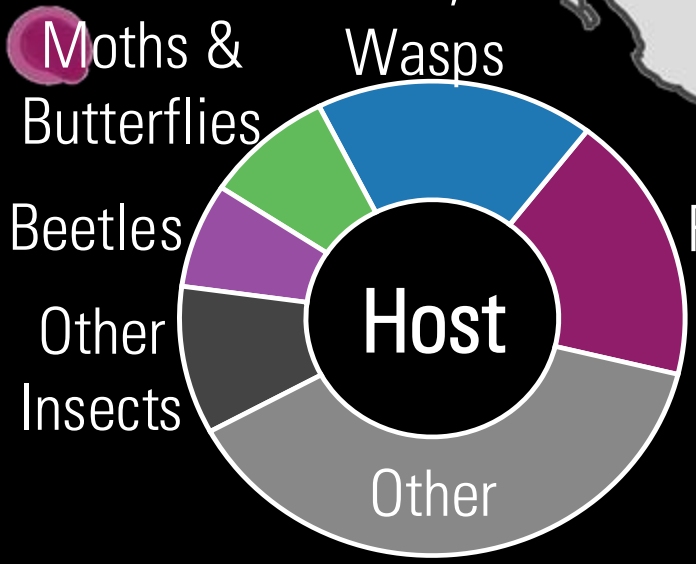
- Extract structural information from MS2 fragmentation into fingerprints
- Trained neural network model to learn the fingerprints of classes for enhanced dereplication
- Exhibited 98% accuracy on polyene antifungals

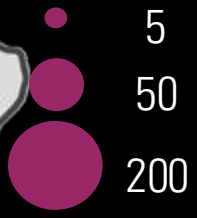


Where We Are Looking

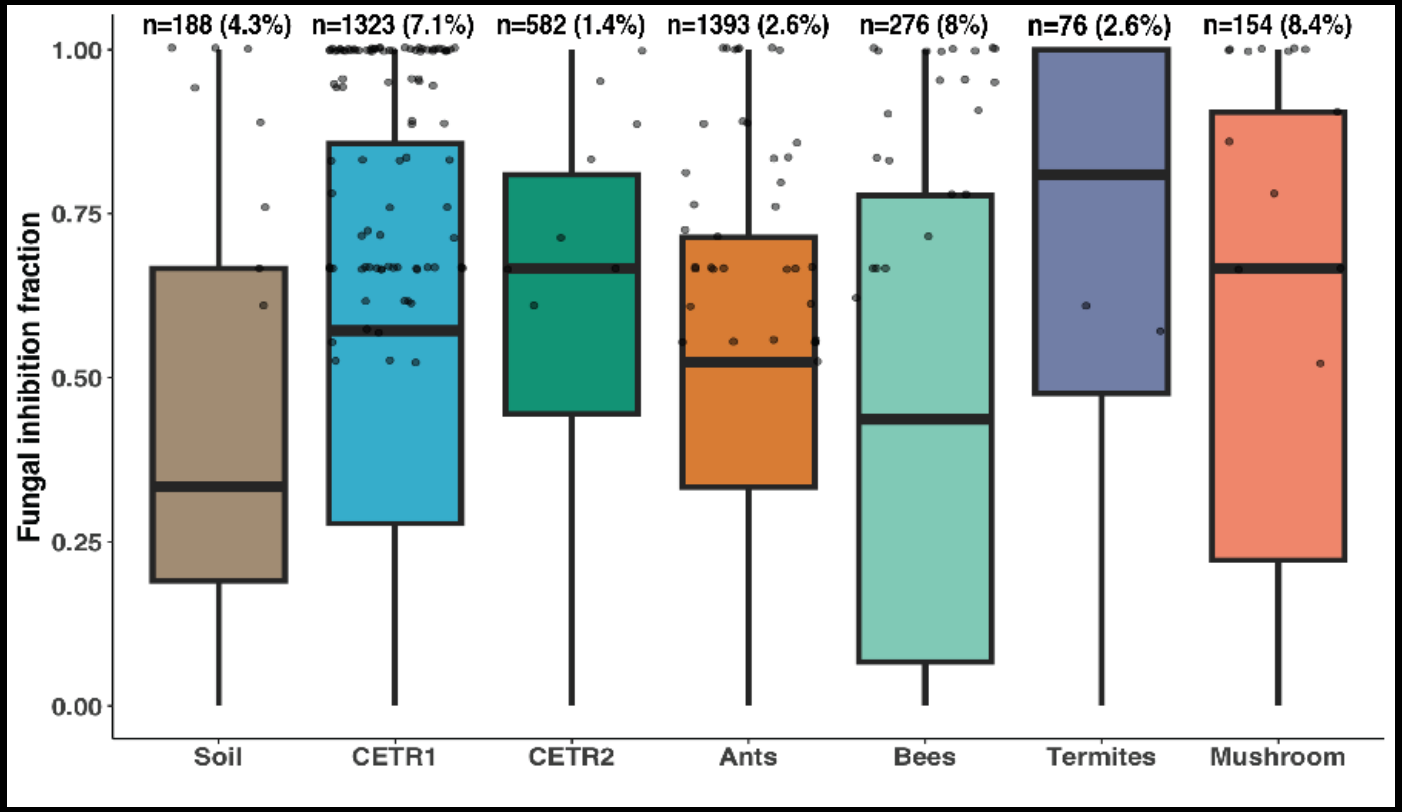
> 40,000 insects, marine animals, human microbiomes







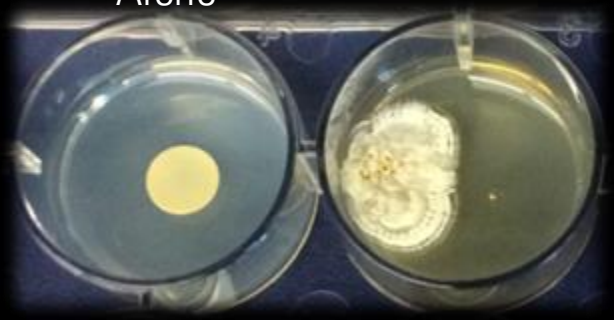
High Rate of Antifungal Activity



Candida albicans

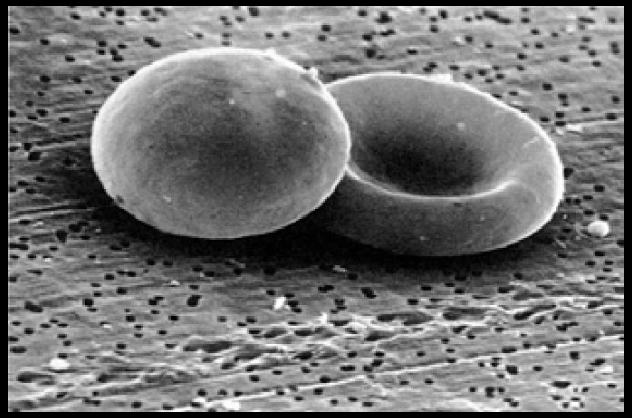
Pathogen Alone

Challenge



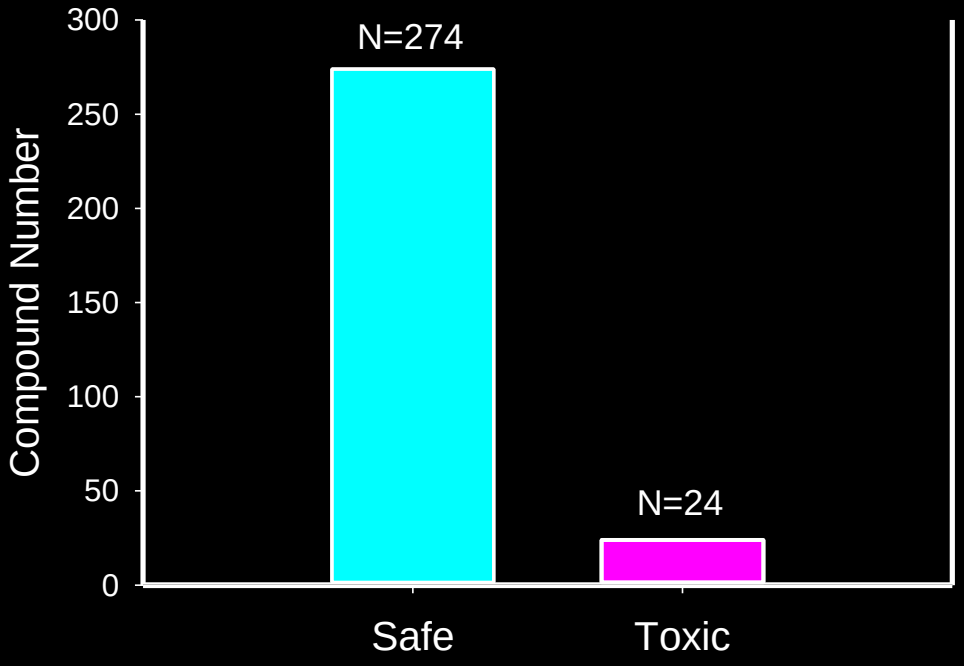
Chevrette, *et al.* Nat Commun 2019

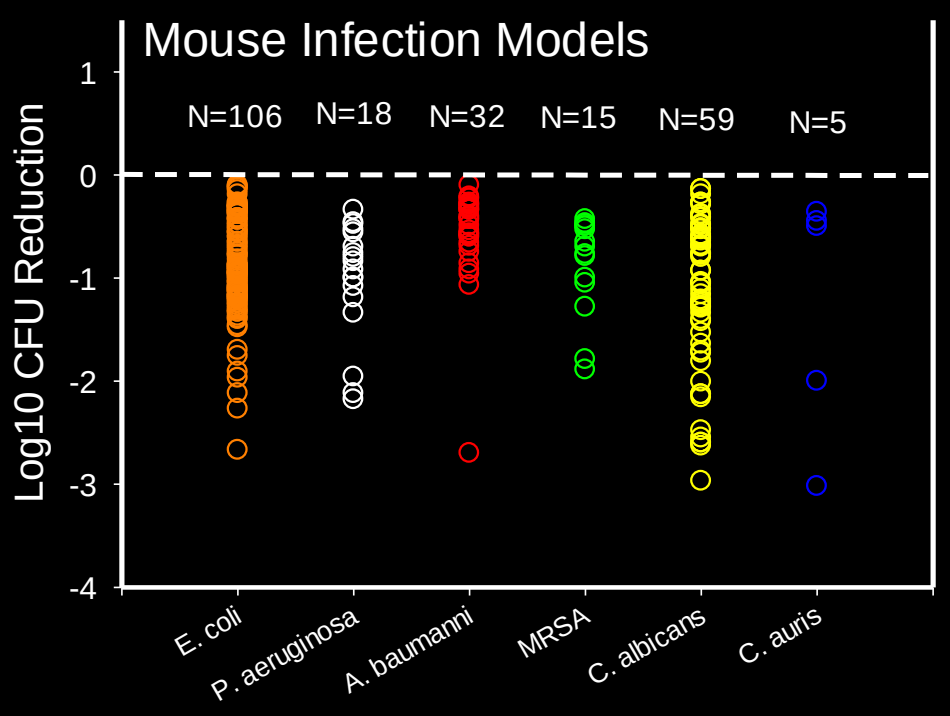
In vitro Activity Against Drug Resistant Pathogens and Human Cell Safety



Drug Resistant Microbial Targets	Compound Hit #
Candida glabrata 4720 (FKS2 HS1 mutation S645P)	718
Candida auris B11211 (azole, AmB, candin resistant)	

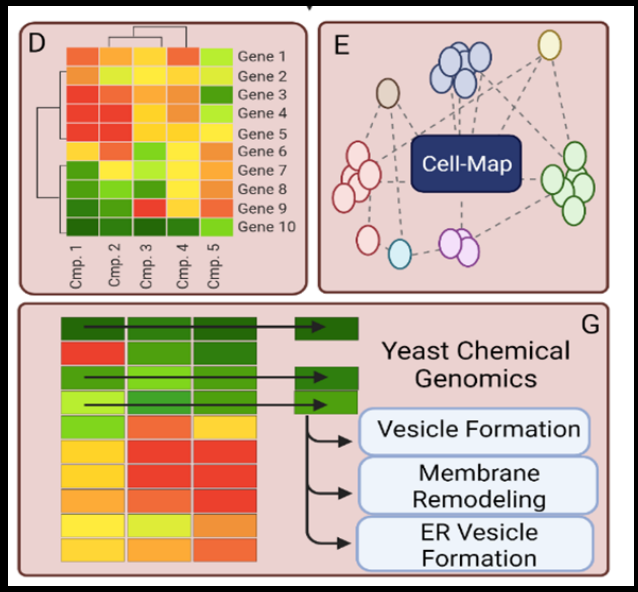
Antifungal Safety and Efficacy in In vivo

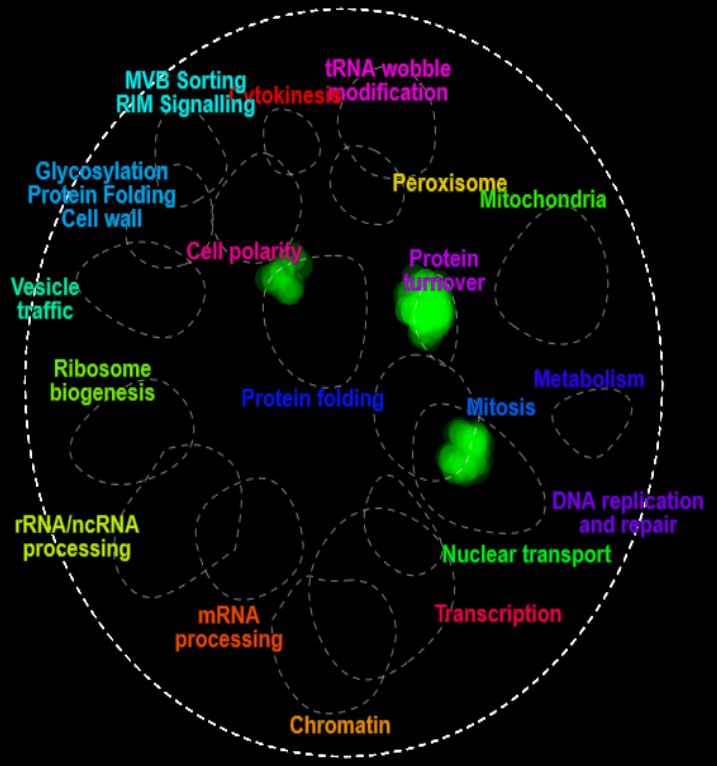




Chemical Genomic Dereplication

- Using 310 DNA-barcoded knockouts (Diagnostic set)
- Optimized for 384 well and semi-automated
- Also mapping sensitive knockouts using cell map (Right)
- Using CG-TARGET to identify likely cellular processes that are affected

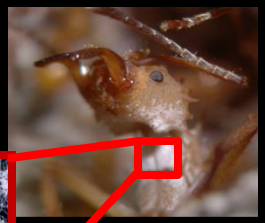


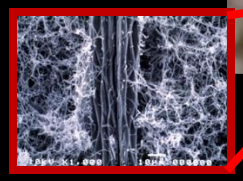


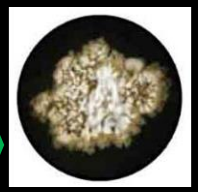
Cell Map: Constanzo, Boone, et al. (2016) Science, 353, 6306
BEAN-COUNTER: Simpkins, Meyers et al. (2019) Nat. Prot. 14, 415-440
CG-TARGET: Simpkins, Meyers et al. (2018) PLoS Comput. Biol. 14, e1006532.

Antifungal Lead Progress

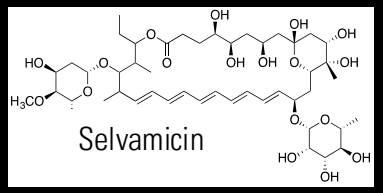
HOST
Atine Ant







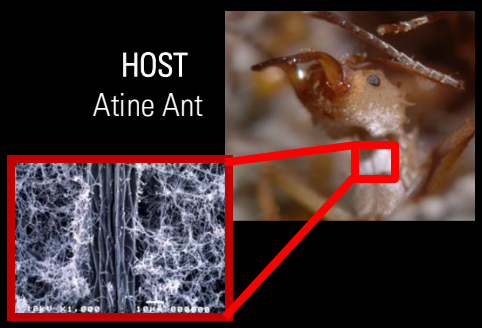
SYMBIONT MICROBE
Pseudonocardia

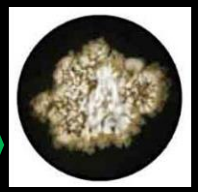


MDR Human
Fungal Pathogen

Van Arnam et al Proc Natl Acad Sci 2016

Selvamicin





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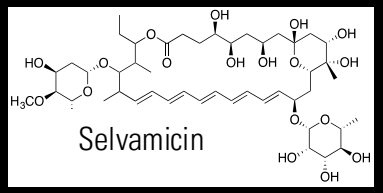

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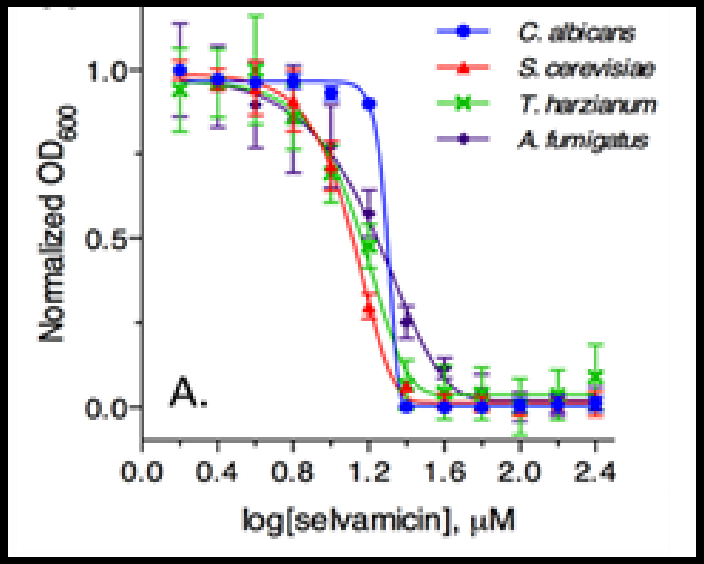
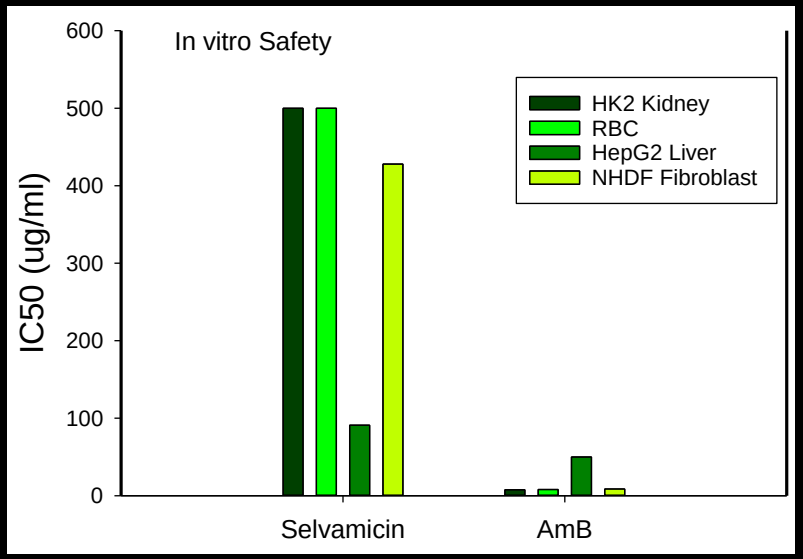
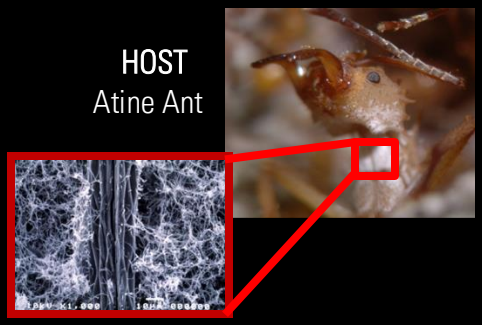
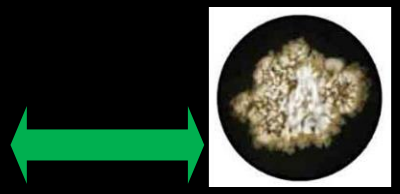
Fungal Pathogen



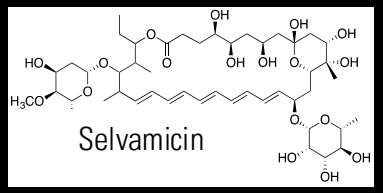


Selvamicin





SYMBIONT MICROBE
Pseudonocardia



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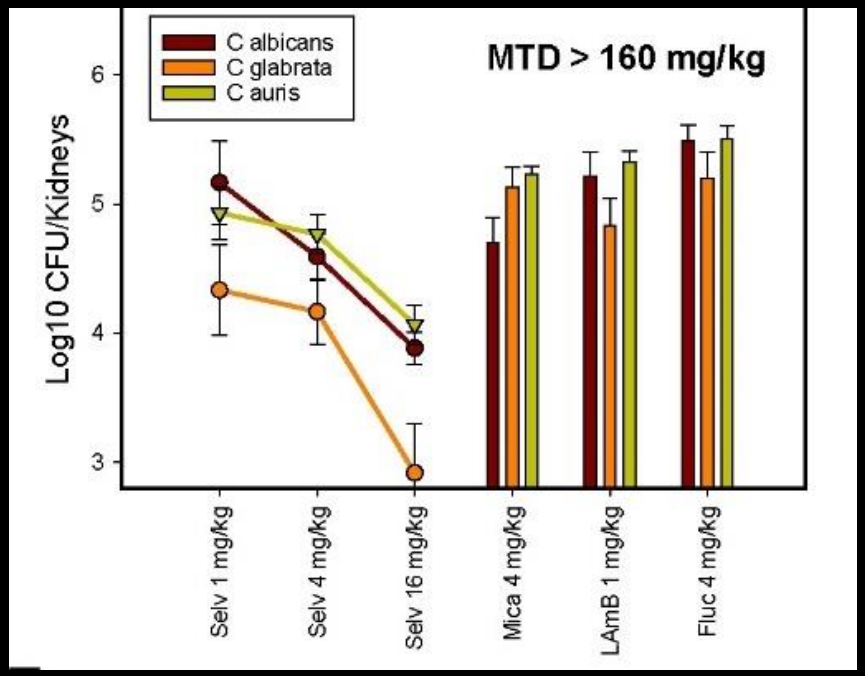
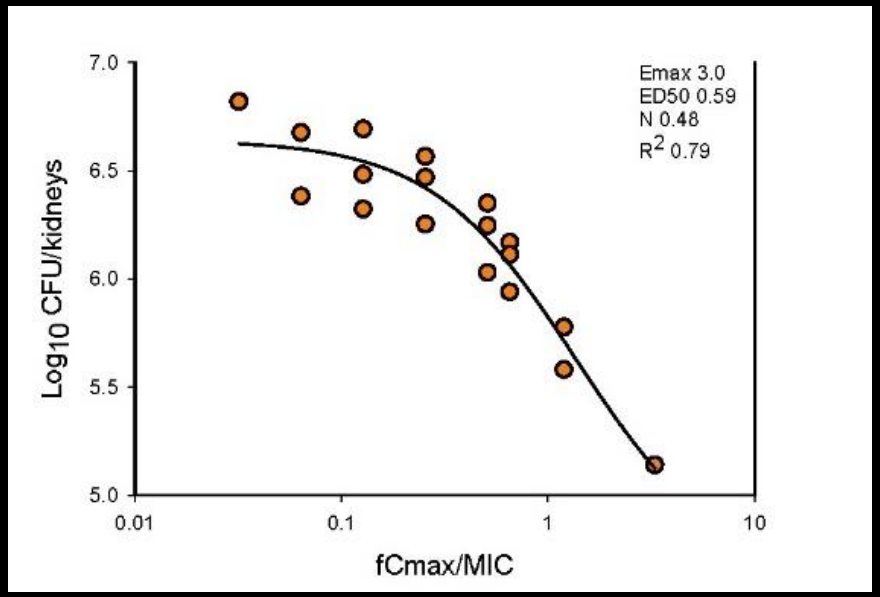
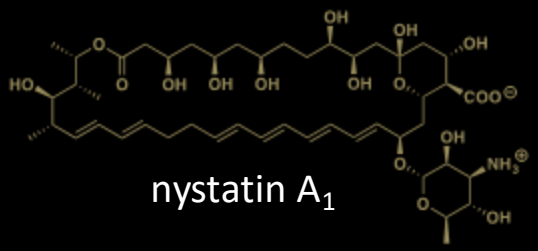
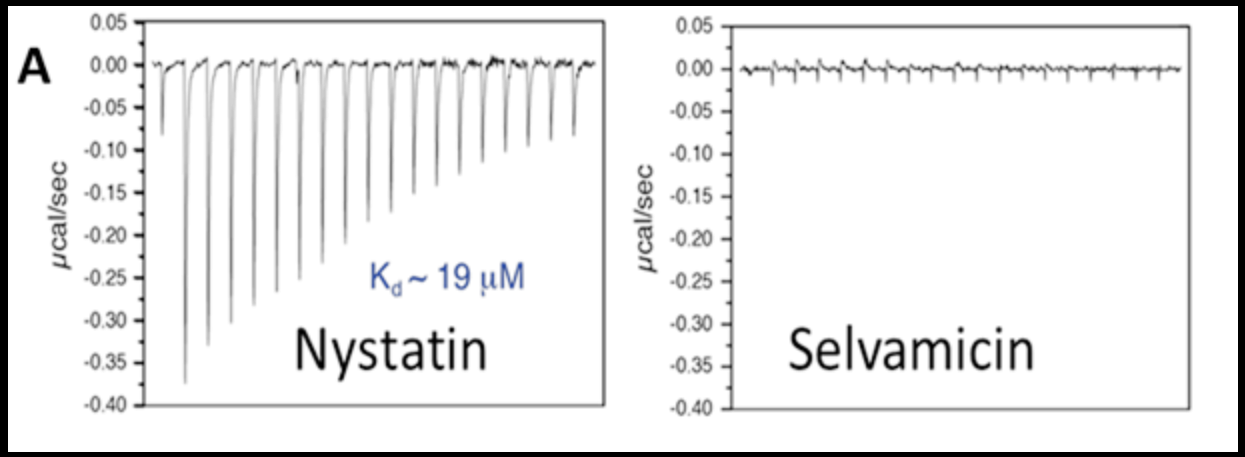




Selvamicin

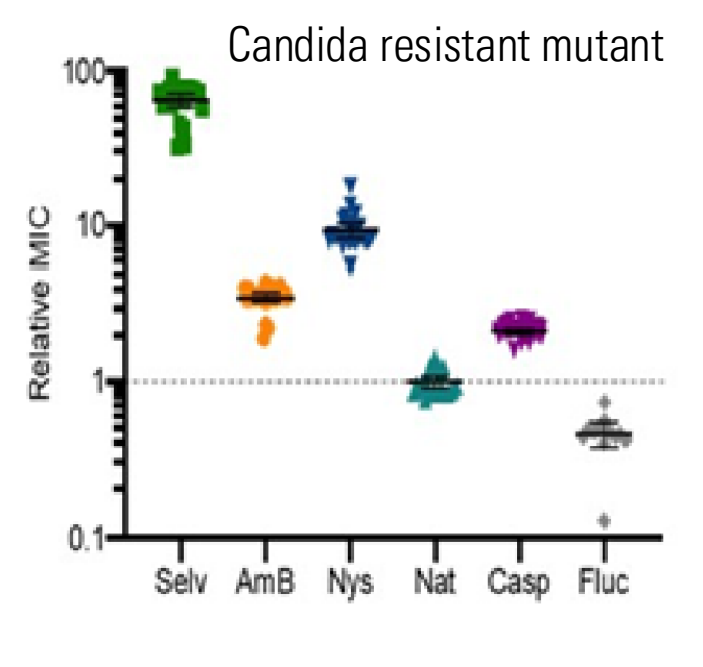
A Novel Mechanism of Action?

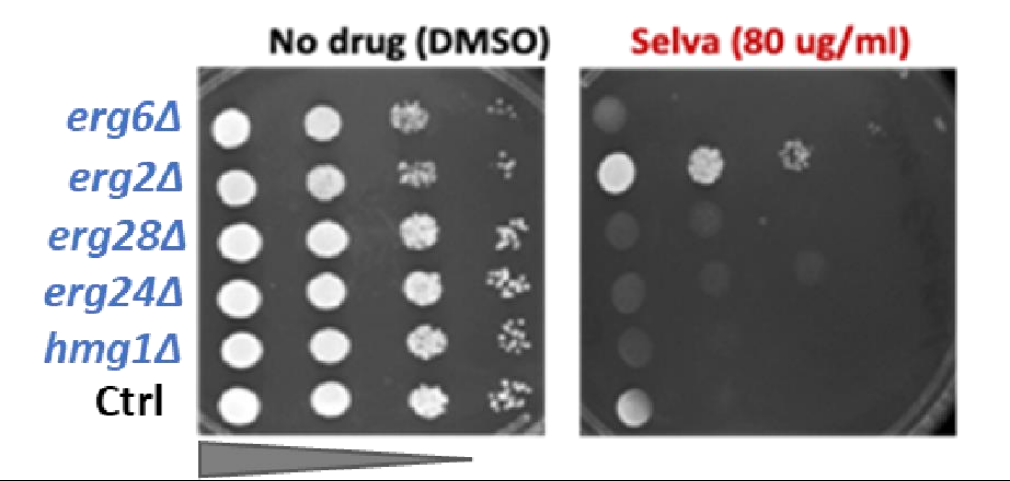


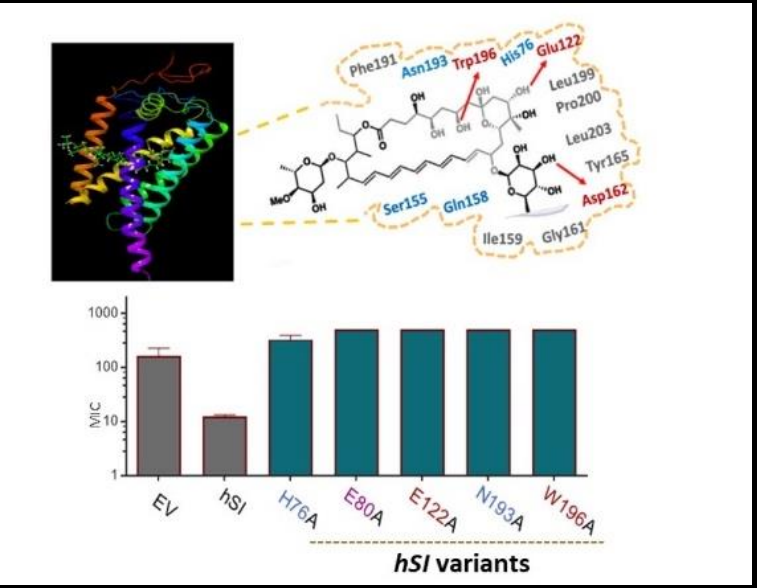


Isothermal Calorimetry Titration Assay

A Novel Mechanism of Action







Symbiotica

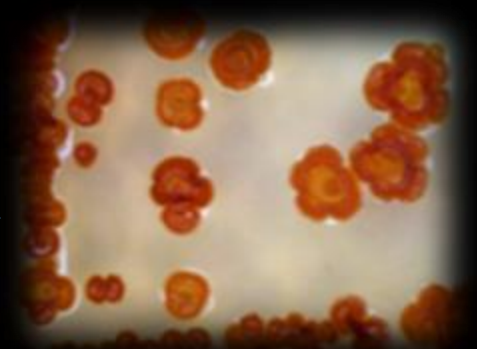
ANTIFUNGAL DISCOVERY

A marine microbiome antifungal targets urgent-threat drug-resistant fungi

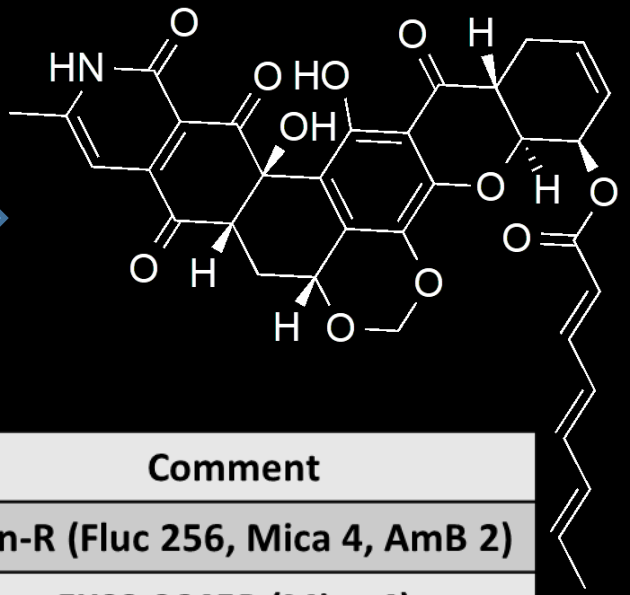
Zhang et al., Science 370, 974–978 (2020)

Turbinmicin – A New Antifungal

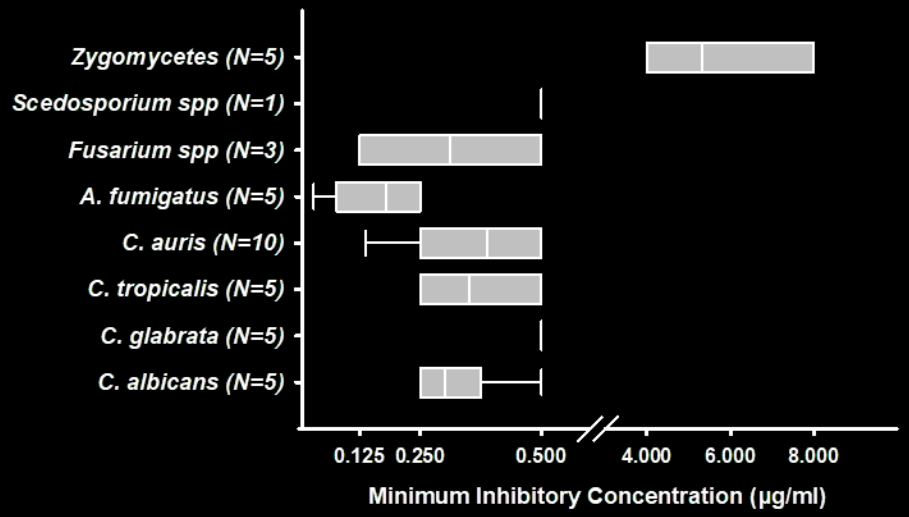
Ecteinascidia turbinata



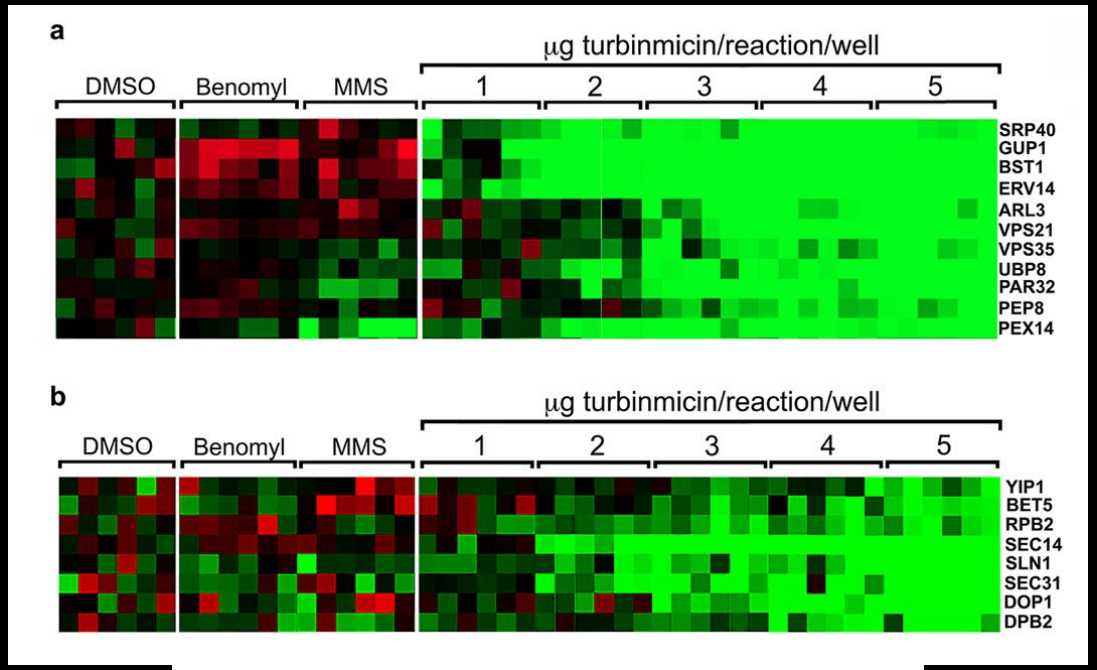
Micromonospora sp.

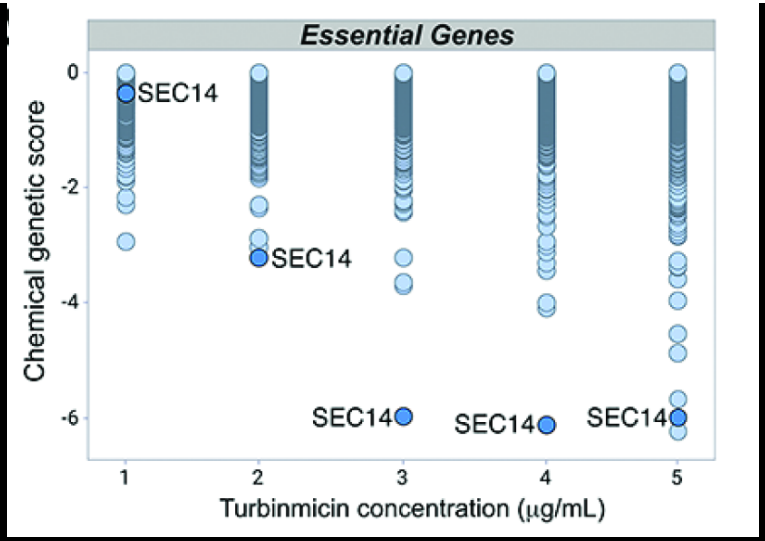


Target Pathogen	Turbinmicin MIC (ug/ml)	Comment
C auris B11211	0.25	Pan-R (Fluc 256, Mica 4, AmB 2)
C glabrata 4720	0.5	FKS2 S645P (Mica 4)
A fumigatus 11628	0.03	Cyp15 G54R (Posa 8)



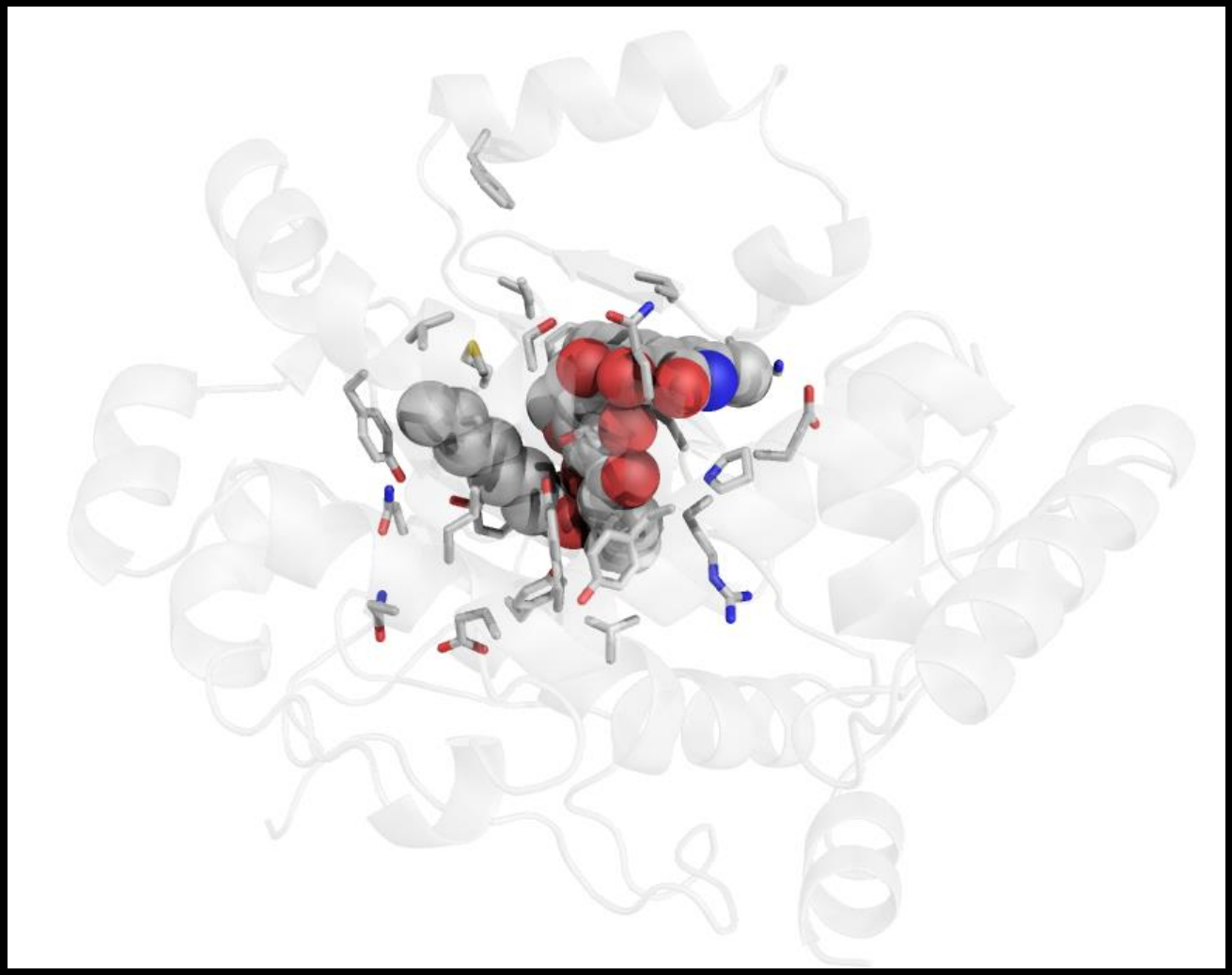
Turbinmicin MOA



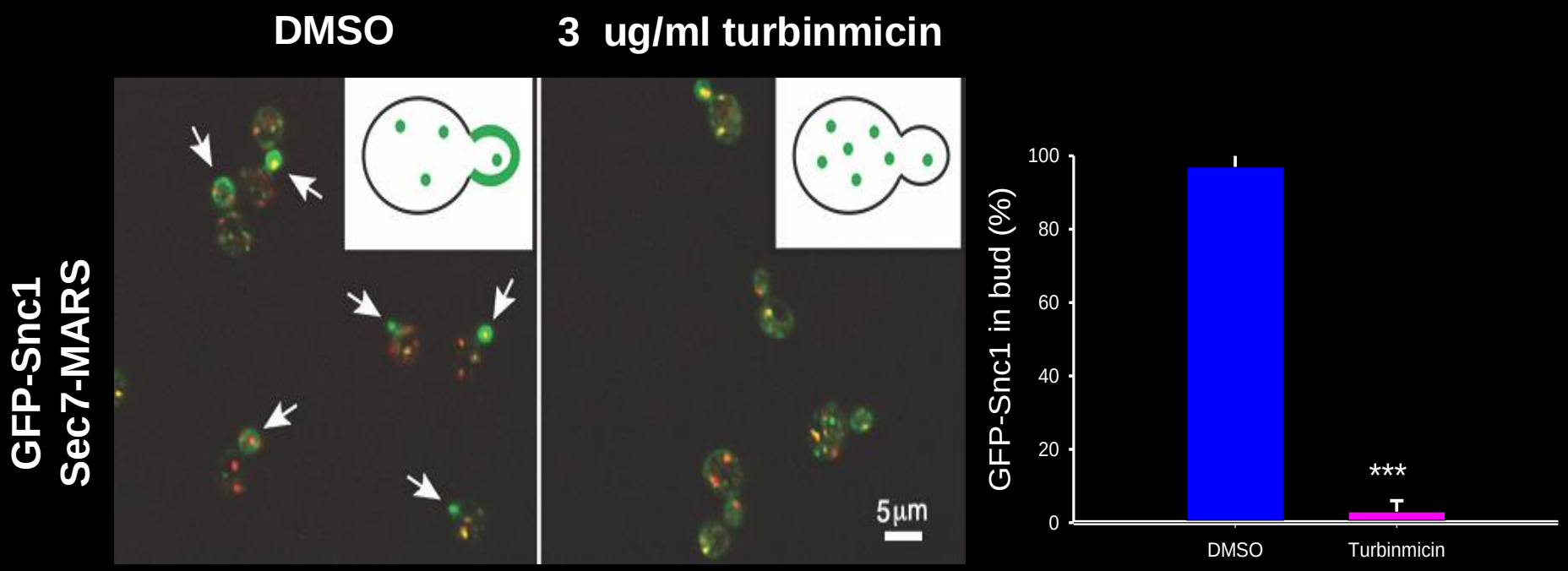


SC DAmP Library

Turbinmicin MOA – Insilico Docking

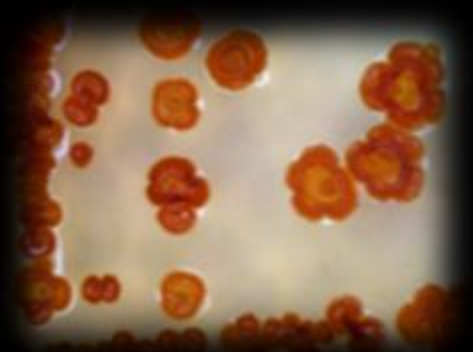


Turbinmicin MOA – Vesicle Trafficking Cell Biology

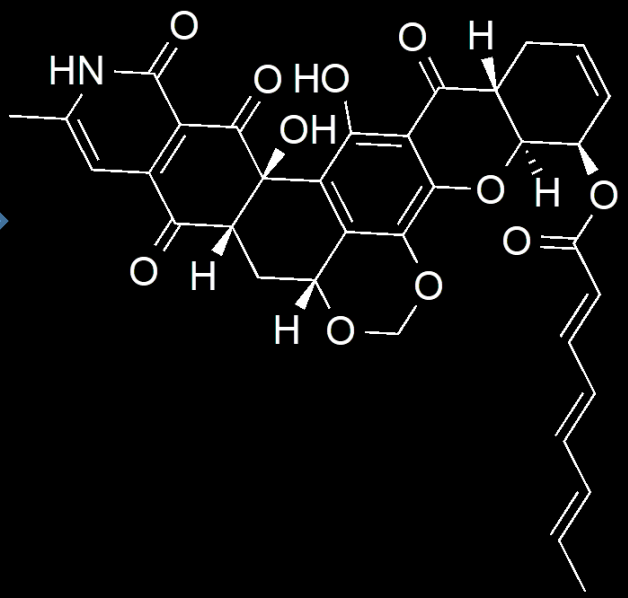


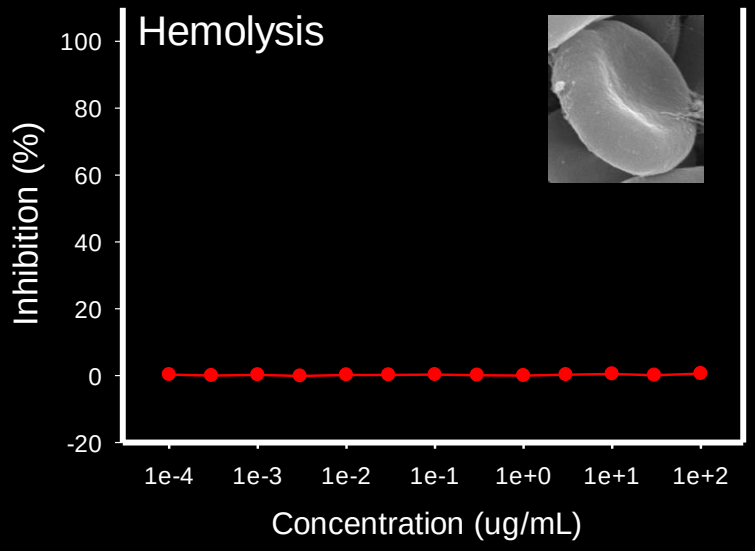
Turbinmicin – Safe In vivo

Ecteinascidia turbinata



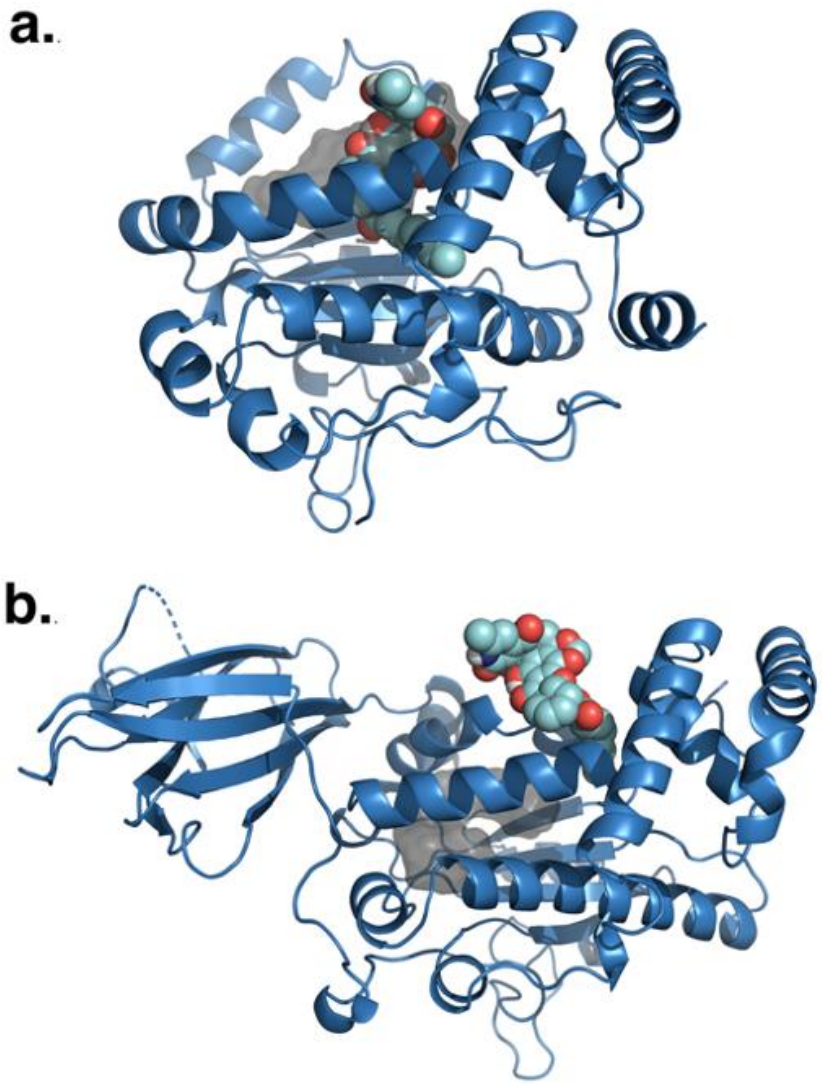
Micromonospora sp.

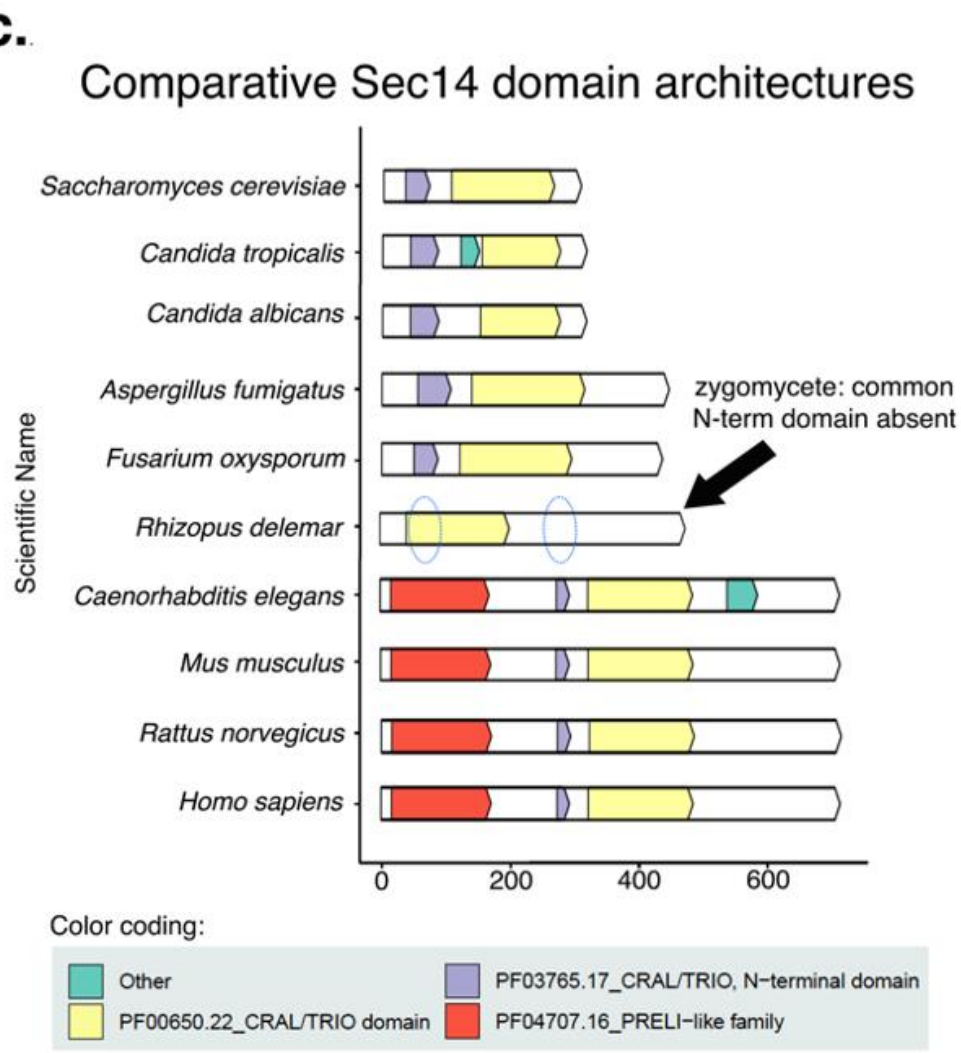




No C. elegans toxicity at highest concentration tested (>100 times MIC)

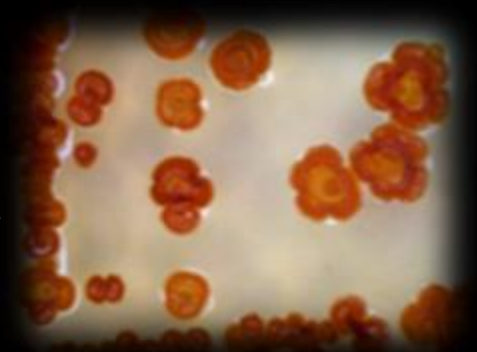
Maximal Tolerated Dose > 256 mg/kg


a.
b.

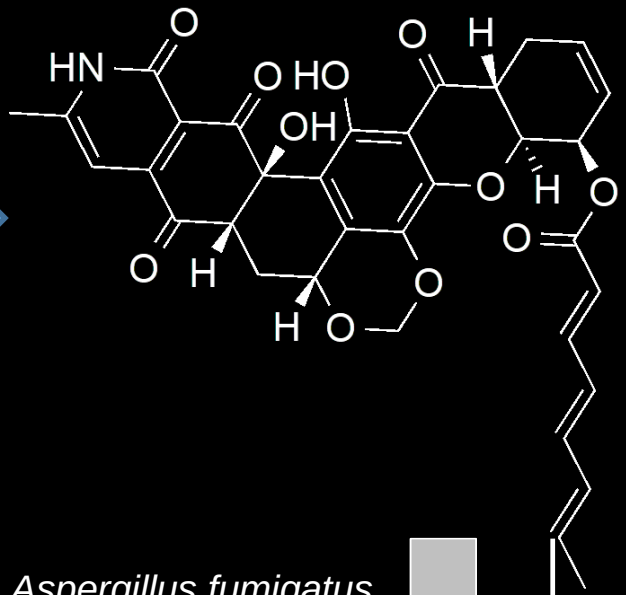

c.
Comparative Sec14 domain architectures
Saccharomyces cerevisiae
Candida tropicalis
Candida albicans
Aspergillus fumigatus
Fusarium oxysporum
Rhizopus delemar
Caenorhabditis elegans
Mus musculus
Rattus norvegicus
Homo sapiens
Scientific Name
zygomycete: common
N-term domain absent
0
200
400
600
Color coding:
Other
PF00650.22_CRAL/TRIO domain
PF03765.17_CRAL/TRIO, N-terminal domain
PF04707.16_PRELI-like family

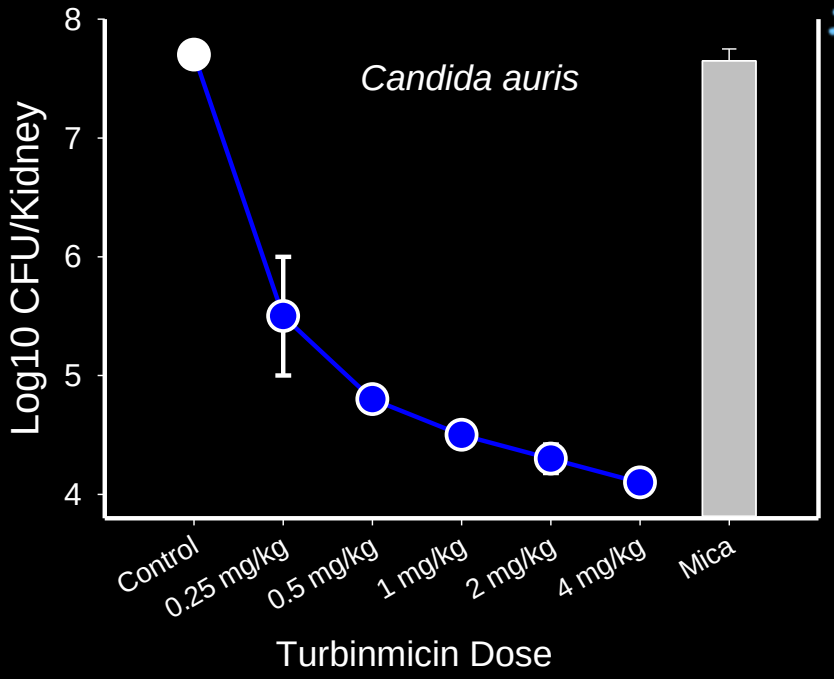
Turbinmicin – Safe and Potent In vivo

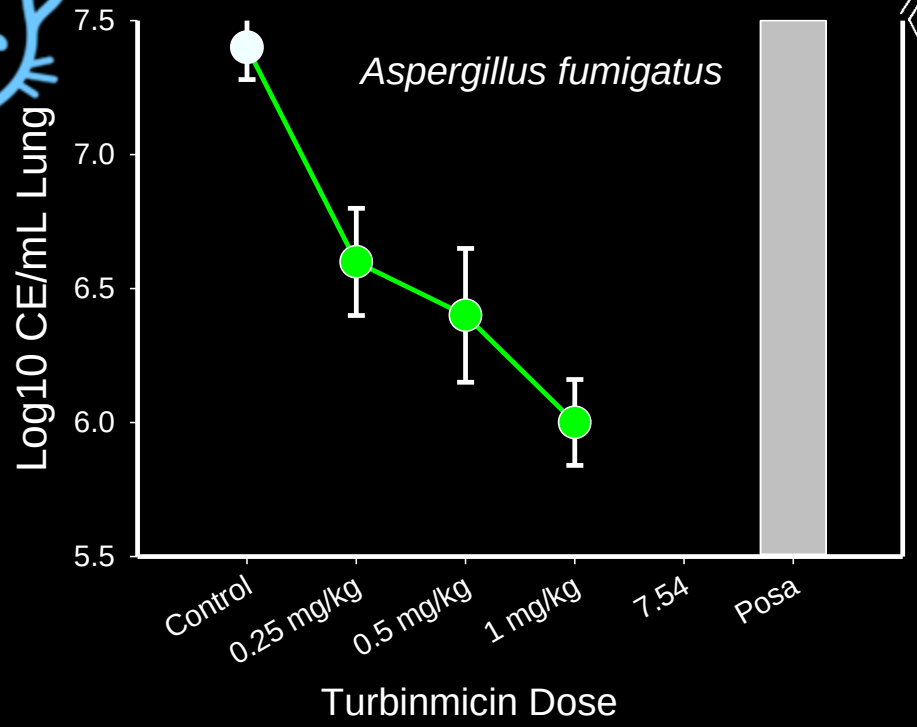
Ecteinascidia turbinata



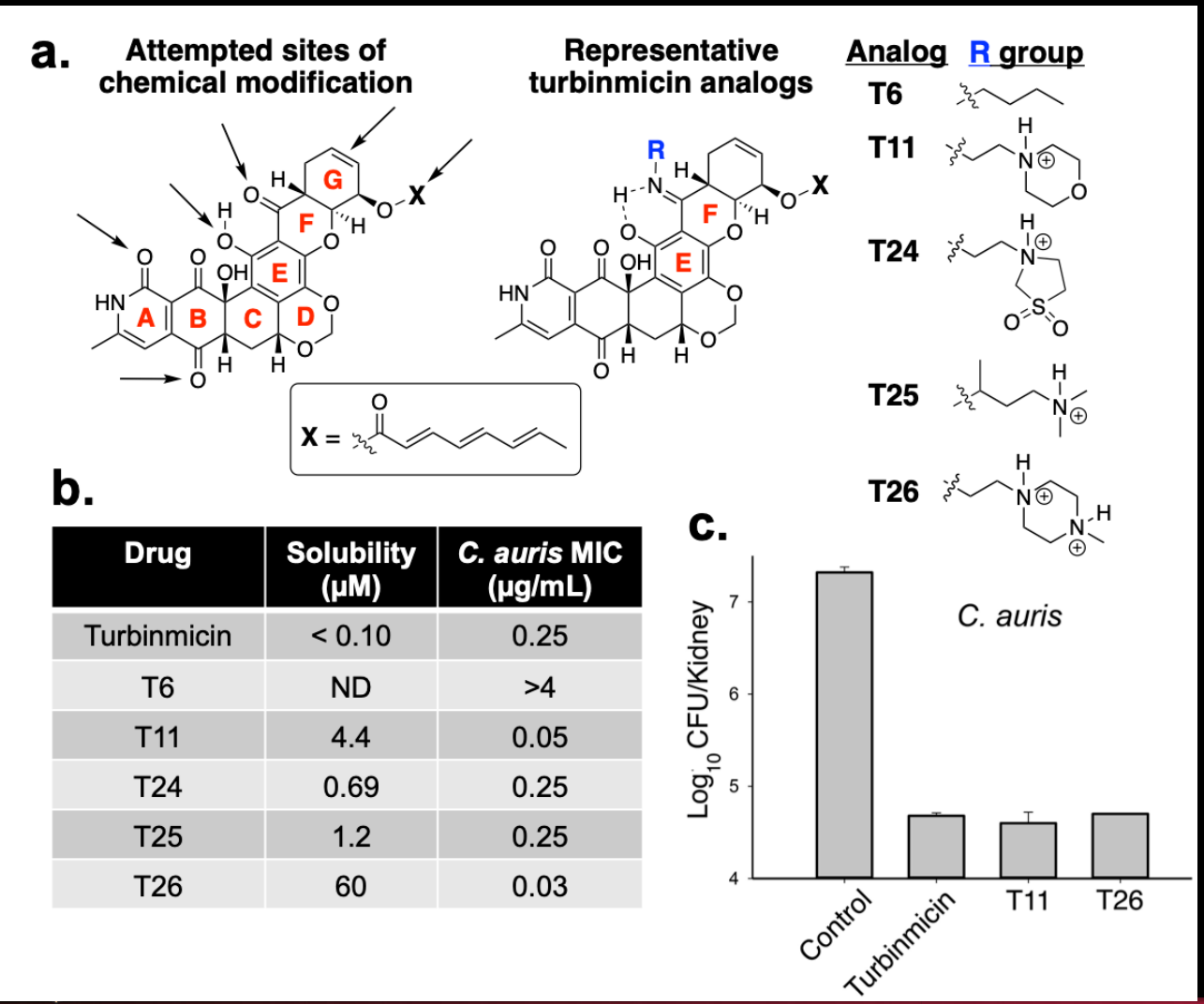
Micromonospora sp.







Turbinmicin Progress and Development



Drug	Solubility (μM)	*C. auris* MIC (μg/mL)
Turbinmicin	< 0.10	0.25
T6	ND	>4
T11	4.4	0.05
T24	0.69	0.25
T25	1.2	0.25
T26	60	0.03

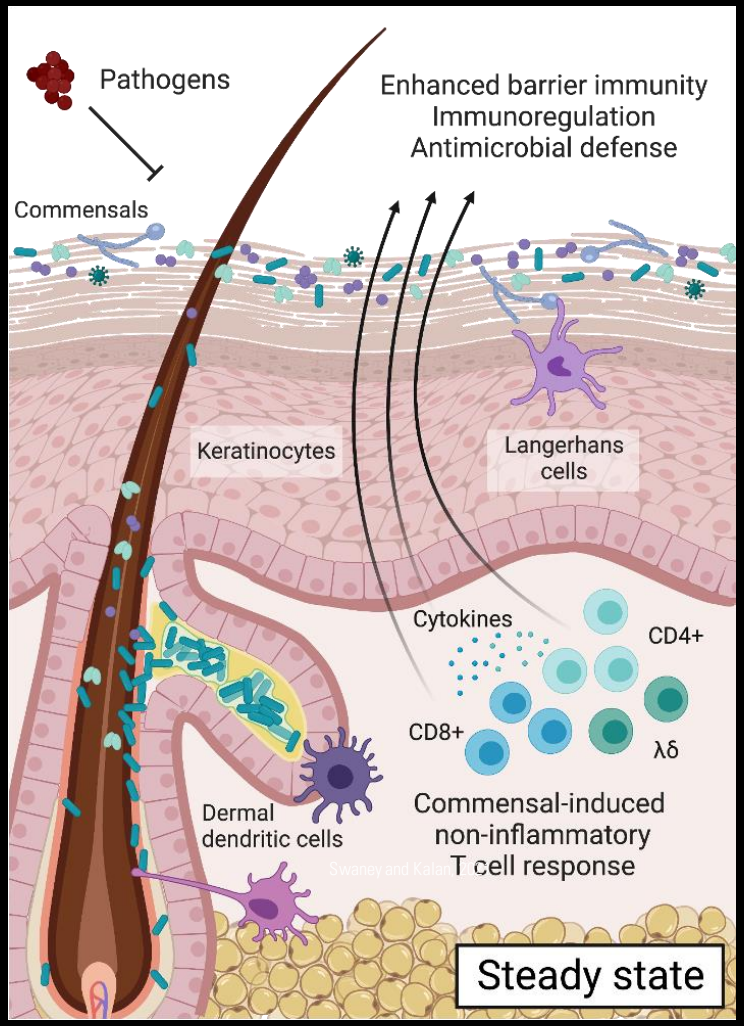
National Institute of Allergy and Infectious Diseases

R01AI167883

- Lead selection
- Preclinical PK/PD
- Preclinical Safety
- Large scale production

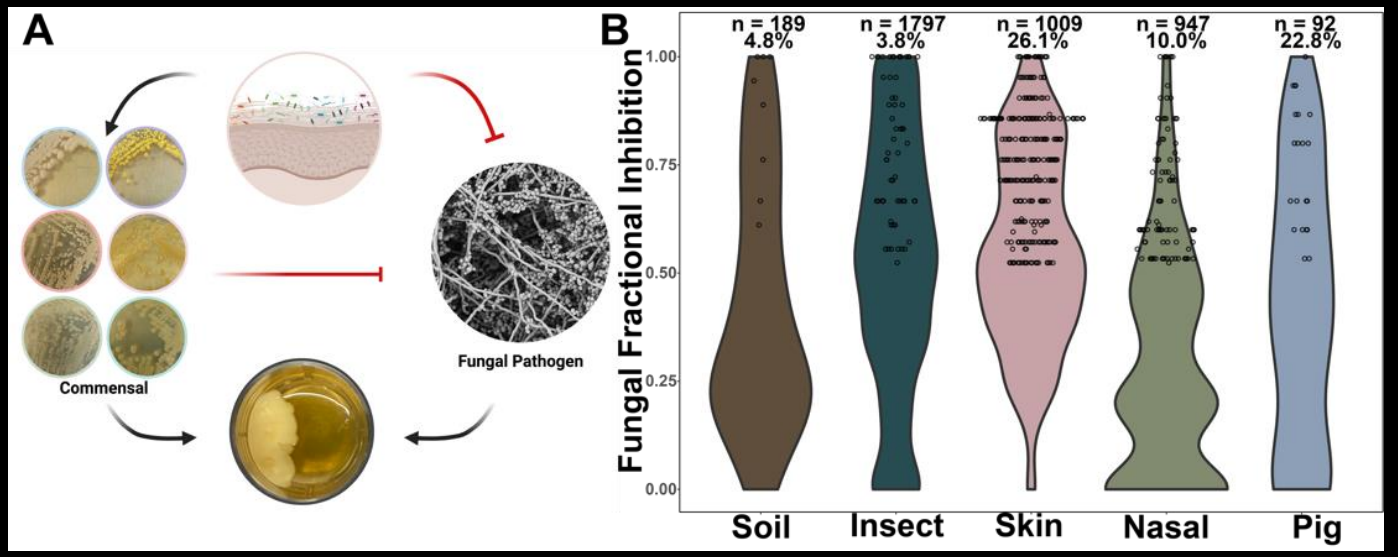
Skin & the microbiome

- Skin is the first line of defense
- Provides protection against disturbances, including pathogen invasion
- Ecosystem of microorganisms contributes to barrier maintenance
- Communication and competition within this niche maintains structural stability

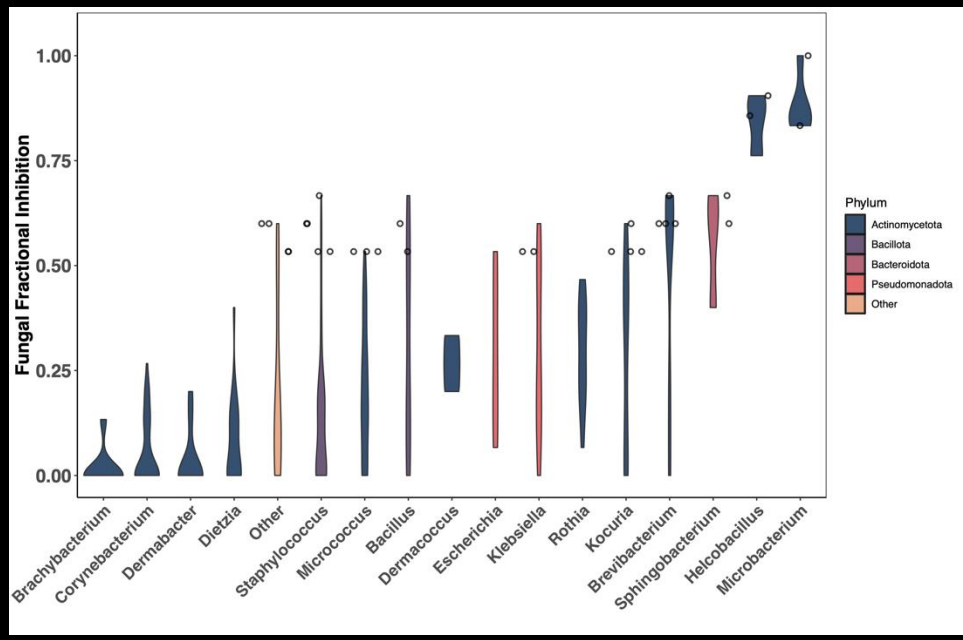


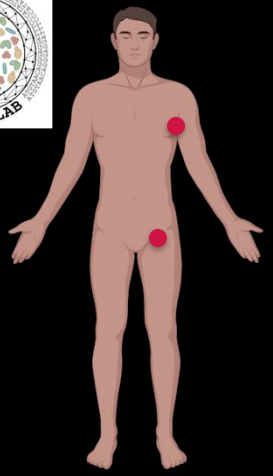
Skin Symbiont Antimicrobial Production

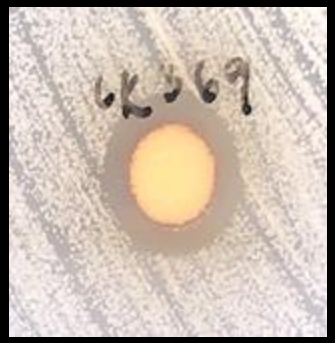
Skin symbionts as antifungal source



Antifungal source across skin genera

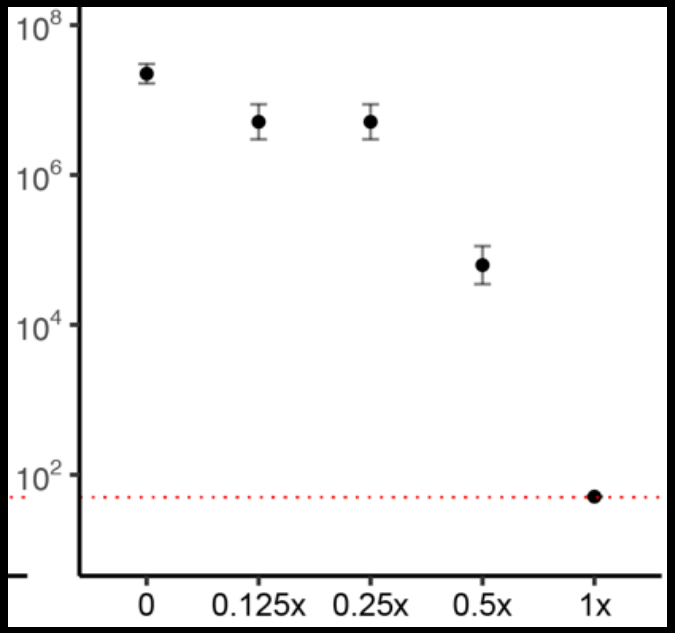






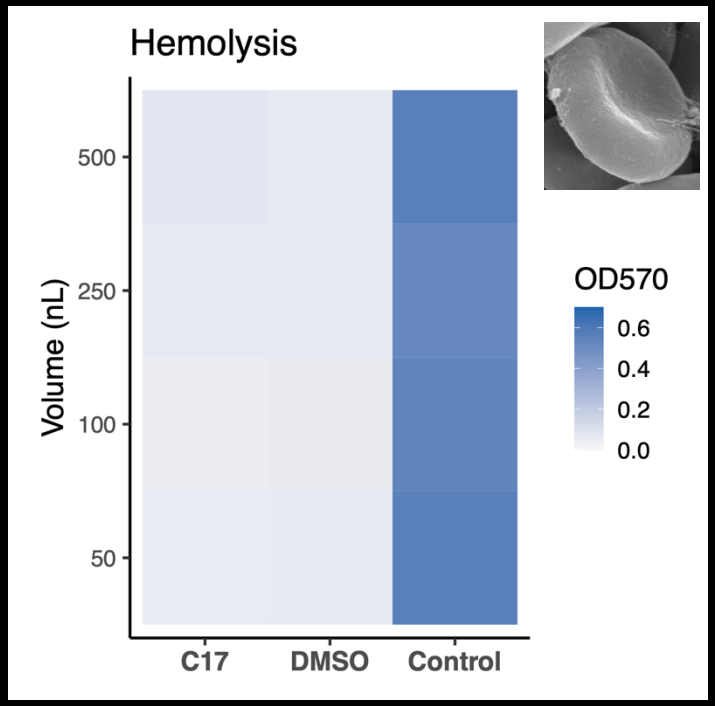
Helcobacillus massiliensis

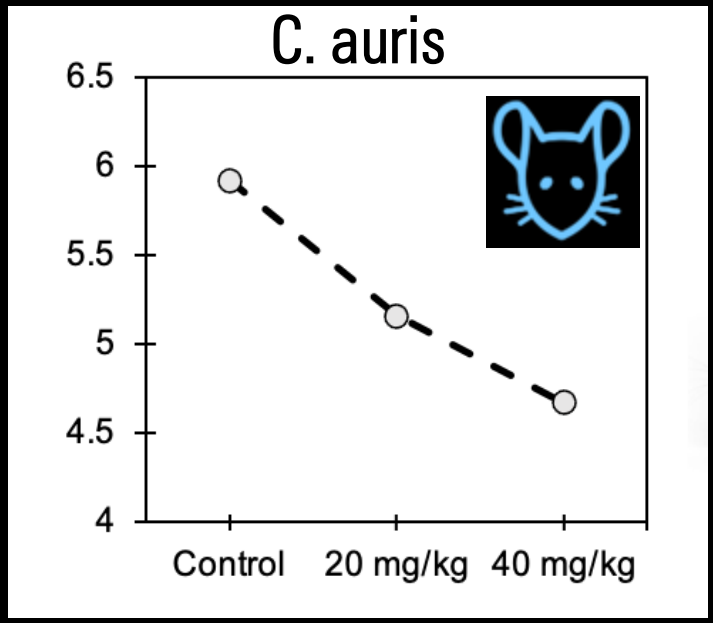
Aurimycin
m/z 256.135
$C_{16}H_{18}NO_2$



C. albicans, C. auris, C. glabrata, C. neoformans
MIC range 0.25-2 µg/ml

Aurimycin – Safe and Efficacious

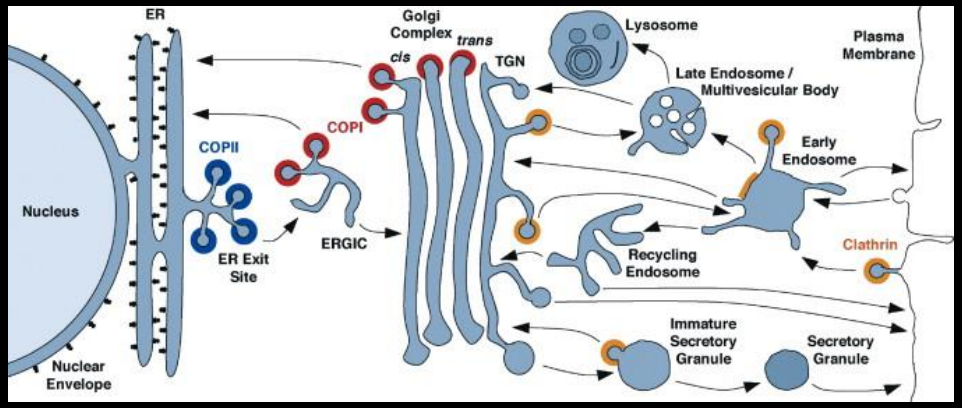


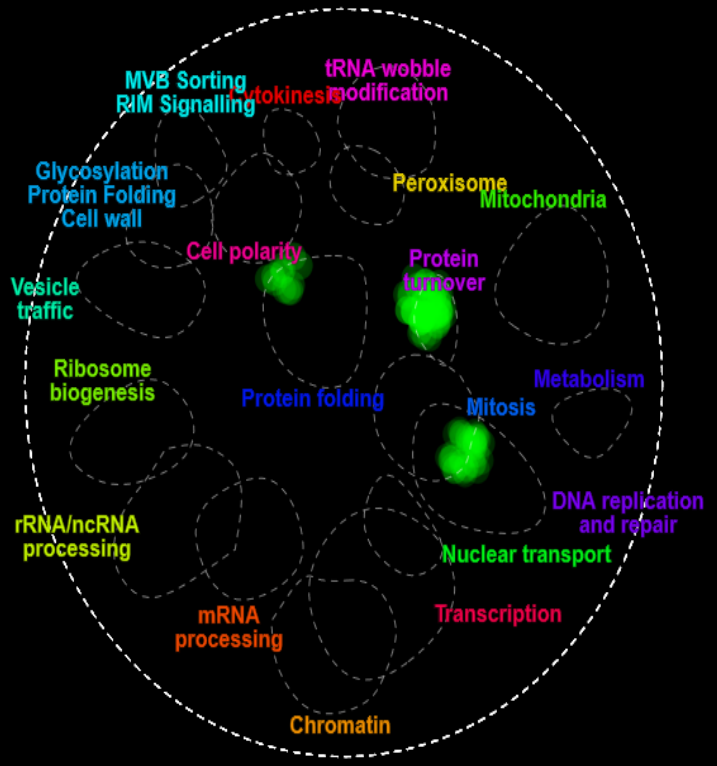


MTD > 640 mg/kg

Aurimycin – Vesicular Transport Signature

String DB pathway analysis: supports MOA targeting Golgi vesicle transport





“Fishing in a stocked pond”

Joe Heitman


THANK
YOU