Marine Organic Geochemistry

Marine Natural Products Lecture

Tracy Mincer
May 3, 2011

Marine Natural Products, General Outline:

- 2º metabolism (specialized metabolism)
  - Types of bioactivity
- Toxins, bioaccumulation, chemical ecology
  - Plant metabolites
  - Invertebrate metabolites (soft, exposed, sessile-chemically defended
- Classifications and biosynthetic pathways
  - Acetogenins/polyketides
  - Terpenes
  - Peptides
  - Polyethers
- Microbial metabolism
  - Some molecules
  - Biosynthesis
  - Biodiversity
  - Siderophores- marine biogeochemistry relevance

Some types of bioactivities:

Partial classification of secondary metabolite effects on browsing animals

Agent	Nature of chemical	Effect	Suggested bio-assay
Attractant	volatile or soluble	smells/tastes good from afar	Y-tube
Repellent	volatile or soluble	smells/tastes nasty from afar	Y-tube
Revulsant	insoluble but readily released	tastes nasty when bitten into	spits out
Anorectic	insoluble until eaten	loss of appetite	stops eating
Emetic		induces vomiting	vomit
Indigestant	? tans proteins	impairs digestion	
Constipant	(cf spicules of sponges, SiO_2 skeletons of diatoms)	constipates	evident in faeces
Narcotic		temporary narcosis	keels over
Toxic		sickness	
Lethal		death	R.I.P.
Contraceptive			less/no progeny
Abortifacient			offspring dead
etc.			

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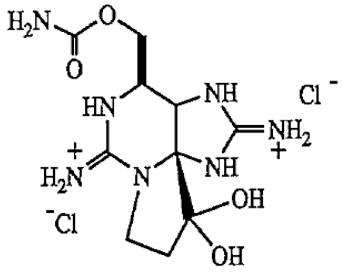
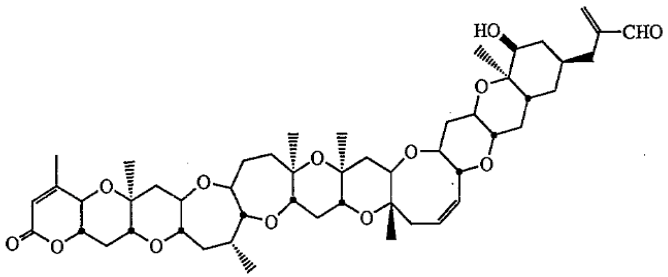
Major microalgal toxins:



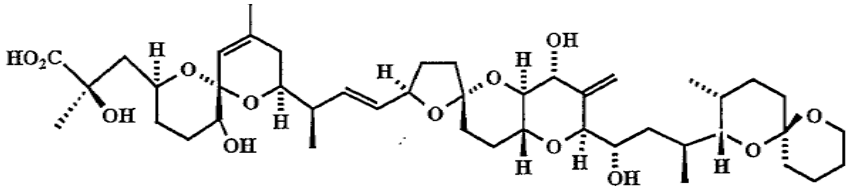
Saxitoxin (STX) - approximately 15 derivatives are now known to be found in the marine environment

Saxitoxin binds to receptor sites near sodium channels thus inhibiting ion influx, action potential and hence nerve impulses. Cause of death is respiratory failure.

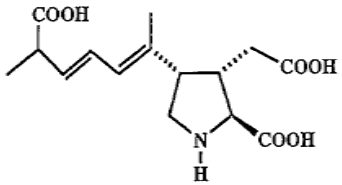


Brevetoxin -B (BTX-B) the major polyether toxin produced by the red tide dinoflagellate *Ptycodiscus brevis* (*Gymnodinium breve*)

Brevetoxins act to increase sodium ion influx through channels thus resulting in overall depolarization and reduction of acetylcholine levels at the synapses.



Okadaic Acid - a member of a family of severe diarrhea-producing metabolites found in fish and later isolated from dinoflagellates of the genus *Prorocentrum* and *Dinophysis*. ~~Diahretic~~ Diarrhetic Shellfish Poisoning, "DSP" now recognized.



Domoic

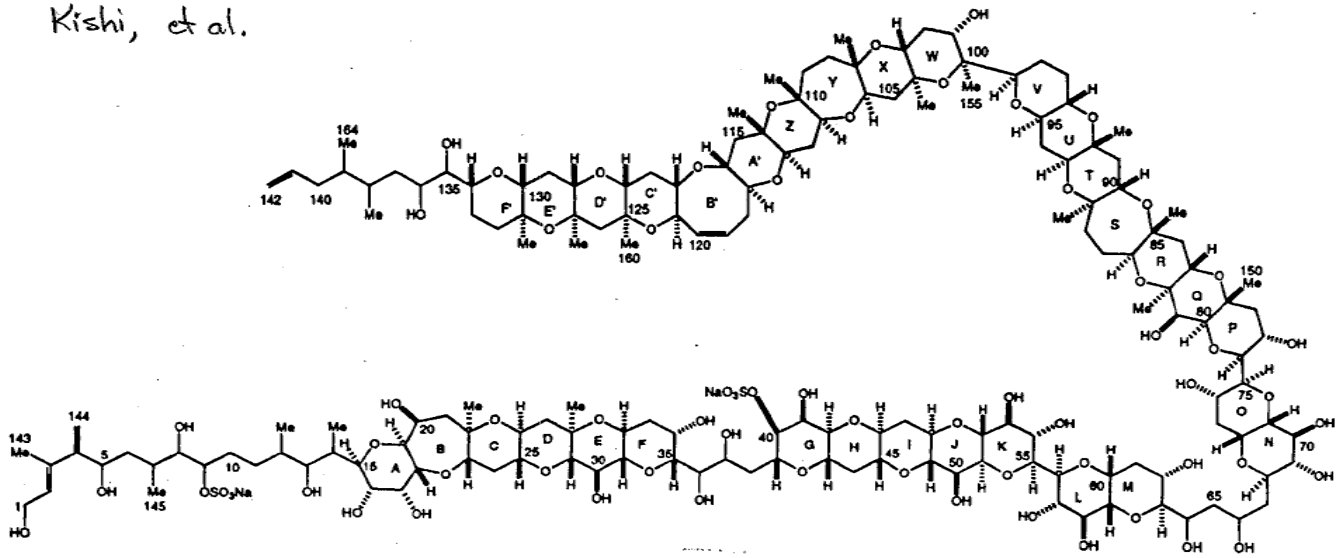
~~Kainic~~ Acid - a metabolite recently found responsible for cases of severe loss of memory, i.e. Amnesic Shellfish Poisoning, "ASP" now recognized as a disease associatedwith consumption of shellfish in Canada and US. Later found to have killed many fish and dolphins and to originate in a marine diatom.

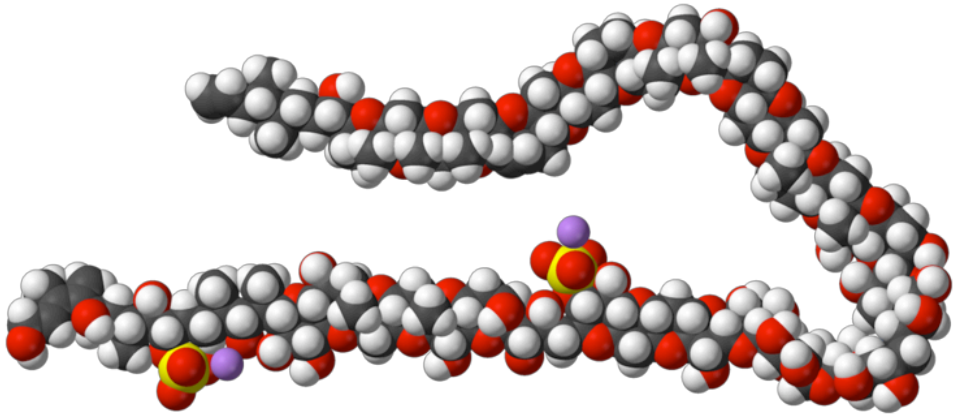
Major toxins, Maitotoxin from *Gambierdiscus toxicus*,:

Relative Stereochemistry of Maitotoxin

J. Am. Chem. Soc., Vol. 118, No. 34, 1996 7947

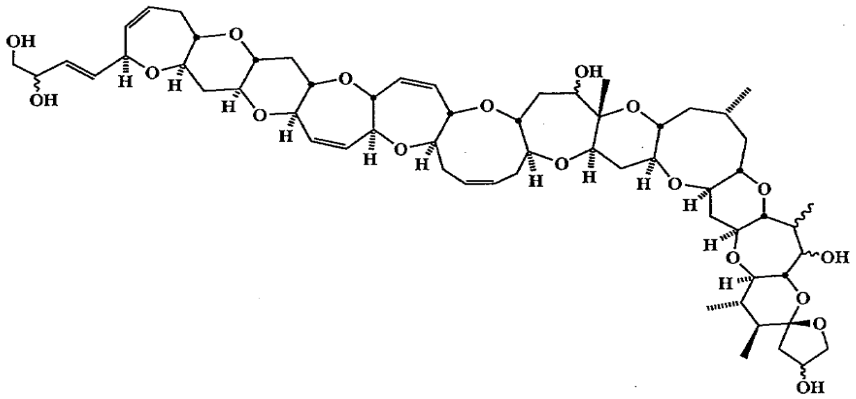
Kishi, et al.



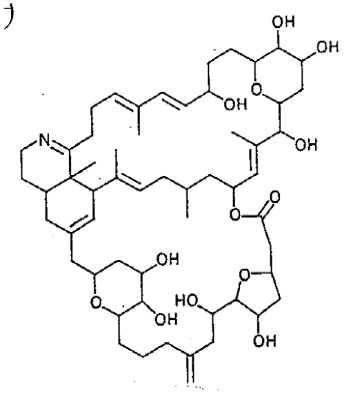


Benthic dinoflagelate produces this as well as ciguatoxin,

Major toxins:

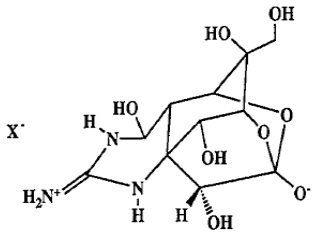


Ciguatoxin (CTX) a world-wide cause of fish intoxication, was recently shown to be produced by the dinoflagellate *Gambierdiscus toxicus*. Formally CTX is rarely toxic, but results in severe illness. As little as 0.1 μg will cause deleterious effects in man.

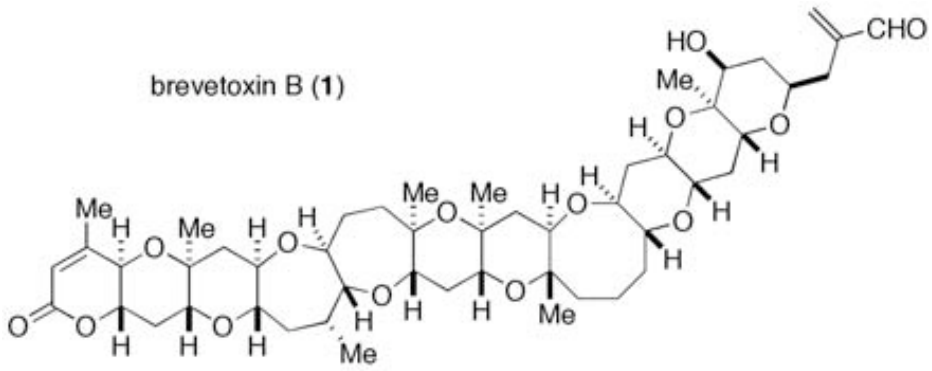


prorocentrolide

Prorocentrum lima



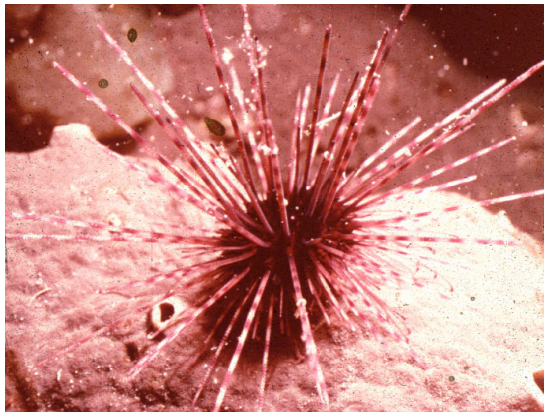
Tetrodotoxin (TTX) the major toxin from the puffer fish (Tetrodontidea) Isolated from *Fugu* species which are very highly regarded for food in Japan. Recently, this toxin has been shown to be produced by numerous marine bacteria!

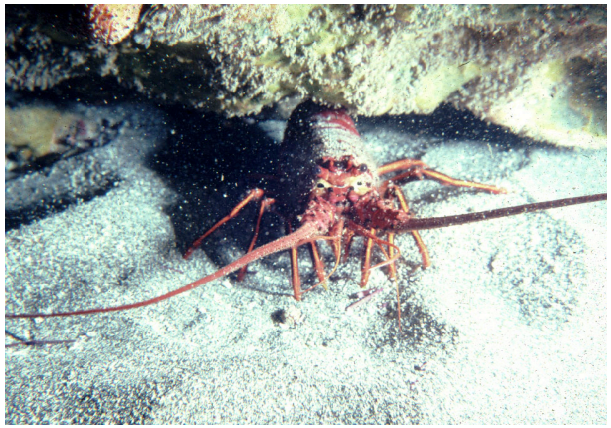


Brevetoxin, a red tide neurotoxin produced by Karenia brevis. Binds to voltage-gated sodium channels.

Chemical Ecology: Marine Invertebrates

Physical Defenses

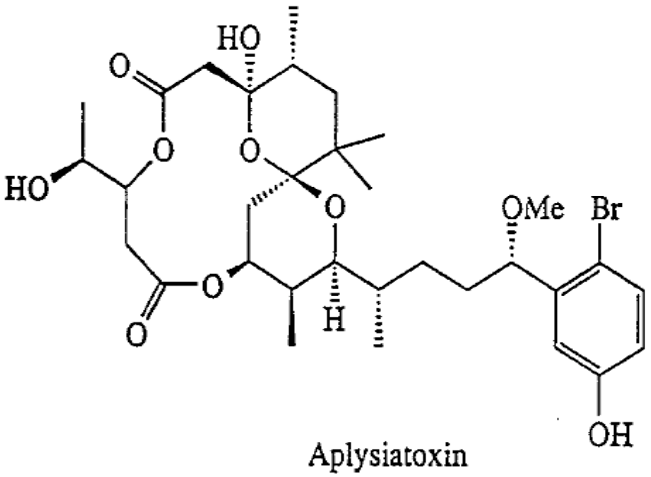




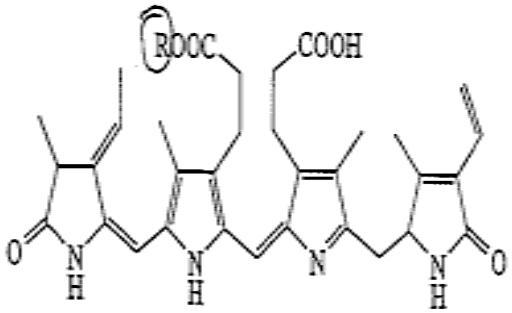
Soft-bodied animals with Chemical Defenses

Chemical defenses are also important for microbes!

Molecule sequestration for defense:



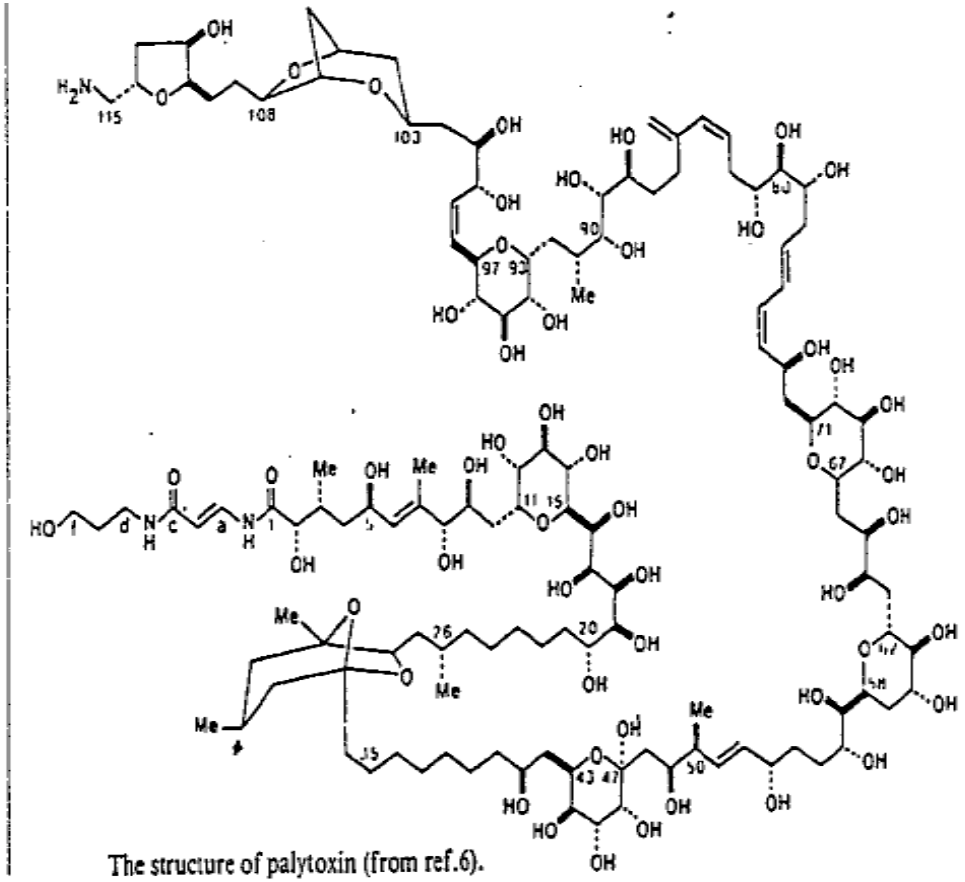
Aplysiatoxin



R = Me, aplysioviolin — phycoerythrobilin

Aplysia californica studied for its simple and large ganglia. Sequesters toxins from red *Laurencia* sp. alga- feed lettuce it eventually sheds toxin and turns green.

Palytoxin, used by Hawaiian natives to tip spears for mortal combat:



The structure of palytoxin (from ref.6).

Absolute stereochem solved: *JACS*, 104, p. 7369, 1982

Palythoa toxica a soft coral. Reefcorner.com

Ostreopsis siamensis, and other members of this genus of benthic dinoflagelate are also known or implicated in palytoxin production-bioaccumulation in bivalves and parrotfish

LD_{50} of 0.15µg pr kg in mice, one of most potent small molecules known *Nature*, Vol. 302:17 p212, 1983

Other Toxins: note extraordinary toxicity of protein type toxins

Table 1. Relative toxicities of a selected group of toxic substances.

Toxin	Minimum lethal dose (μg/kg)*	Source	Form and/or structure	Molecular weight	Reference
		Protein			
Botulinus toxin A	0.00003	Bacterium: *Clostridium botulinum*	Crystalline, A	900,000	(*61*)
Tetanus toxin	.0001	Bacterium: *Clostridium tetani*	Crystalline	100,000	(*61*)
Ricin	.02	Plant: castor bean, *Ricinus communis*			(*62*)
Diphtheria toxin	.3	Bacterium: *Corynebacterium diphtheriae*		72,000	
Cobra neurotoxin	.3	Snake: *Naja naja*			(*62*)
Crotalus toxin	.2	Snake: rattlesnake, *Crotalus atrox*			(*62*)
		Nonprotein			
Kokoi venom	2.7†	Frog: *Phyllobates bicolor*		~400	(*11*)
Tarichatoxin	8	Newt: *Taricha torosa*	$(C_{11}H_{17}N_3O_9)$	319	(12)
Tetrodotoxin	8–20	Fish: *Sphoeroides rubripes*	$(C_{11}H_{17}N_3O_9)$	319	(*9*, *10*)
Saxitoxin	9	Shellfish. Produced by dinoflagellate *Gonyaulax catenella* ingested by shellfish	$(C_{10}H_{17}N_7O_4 \cdot 2HCl)$	372	(*9*)
Bufotoxin	390	Toad: *Bufo vulgaris*	Vulgarobufotoxin $(C_{40}H_{60}N_4O_{10})$	757	(*62*)
Curare	500	Plant: *Chondodendron tomentosum*	*d*-Tubocurarine $(C_{38}H_{44}N_2O_6Cl_2)$	696	(*62*)
Strychnine	500	Plant: *Strychnos nux-vomica*	$(C_{21}H_{22}N_2O_2)$	334	(*62*)
Muscarin	1,100	Mushroom: *Amanita muscaria*	$(C_9H_{20}O_2NCl)$	210	
Samandarin	1,500	Salamander: *Salamandra maculosa*	$(C_{19}H_{23}O_2N)$	397	(*33*)
Diisopropyl-fluorophosphate	3,000		Synthetic nerve gas $[(C_3H_7)_2PO_2F]$	184	
Sodium cyanide	10,000		Synthetic NaCN	49	(*62*)

* Minimum lethal dose refers to mouse except in the case of ricin, where it refers to guinea pig (see *61*), and of bufotoxin and muscarin, where it refers to cat. In cat, administration was intravenous; in all other cases it was intraperitoneal. Since the survival times are not always specified and the experiments are not direct comparisons, these values are of necessity approximate and indicative only of relative toxicity by the indicated route of administration. † LD_{50} in mouse, administered intravenously.

Toxins:

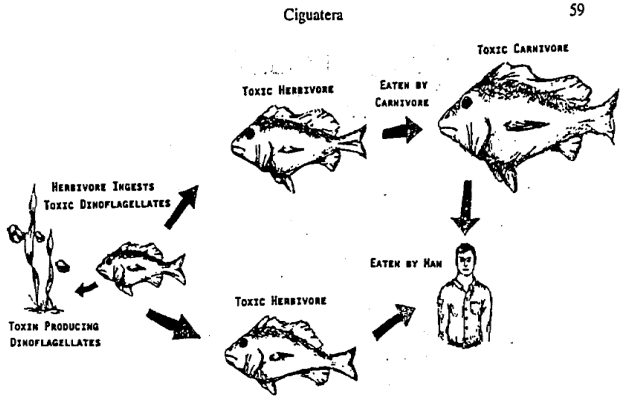


Fig. 2. Food chain hypothesis for the transmission of ciguatera toxins.

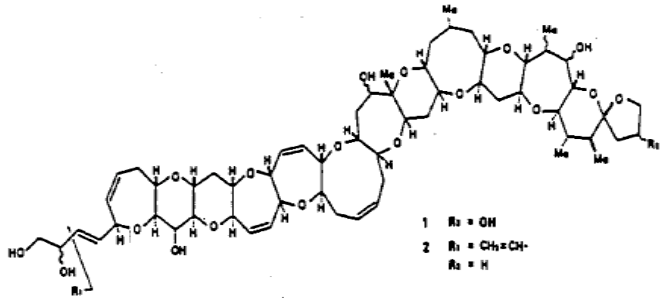


Fig. 5. Ciguatoxin structure and its analog.

Gambierdiscus toxicus, a dino, metabolites bioaccumulate as above

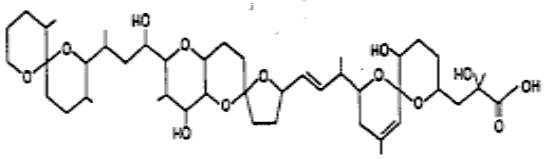


Fig. 6. Okadaic acid structure.

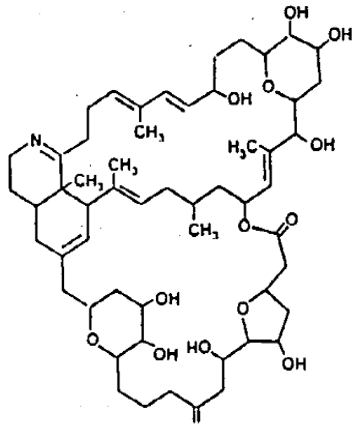


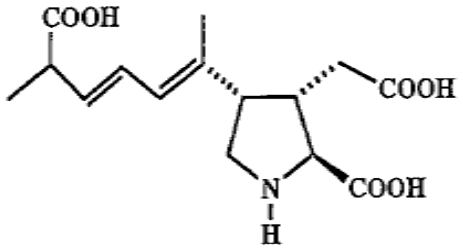
Fig. 7. Prorocentrolide structure.

Prorocentrum lima, dinoflagelate metabolites known to bioaccumulate

Basically, never eat and old, territorial, tropical fish...

From: Foodborne Toxins of Marine Origin. Rev. Environ. Contam. Ecol. Vol. 117 p. 51, 1991

Domoic Acid: toxicity to marine mammals



Frances Gulland, The Marine Mammal Center, San Francisco, California

Toxin causes Amnesic Shellfish Poisoning in humans- lesions to mammalian brain, odd behavior in california sea lions, such as above, and swimming up rivers as far as 50 miles- never before reported in California. Strange bird behavior as well- dive bombing cars.

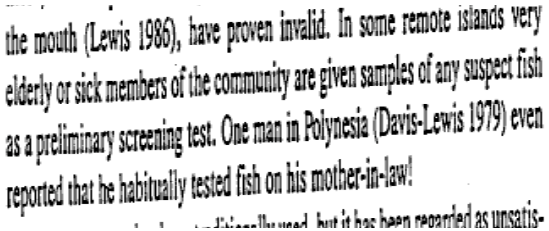
Monitoring for toxins: a major public health issue

•Direct testing using chemical method such as LC/MS, time-consuming

•Animal testing, mouse LD_{50} (response time not fast enough)

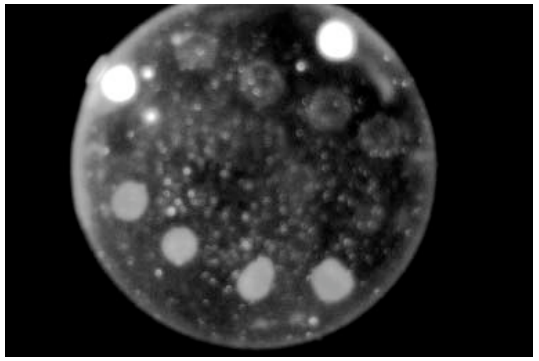
•Hapten formation with small-molecule enables immunogenic response to antigenic epitope. Hybridoma cell lines for large-scale monoclonal antibody production- big investment...

•Measure known producers using nucleic acid based methods, PCR, Southern Hybridization. ect.


the mouth (Lewis 1986), have proven invalid. In some remote islands very elderly or sick members of the community are given samples of any suspect fish as a preliminary screening test. One man in Polynesia (Davis-Lewis 1979) even reported that he habitually tested fish on his mother-in-law!

From: Foodborne Toxins of Marine Origin. Rev. Environ. Contam. Ecol. Vol. 117 p. 51, 1991

Measuring Domoic Acid directly in environment

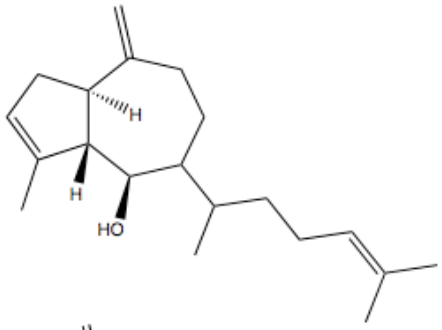


DNA hybridization/PCR detection (10 cells per mL) and monoclonal antibody ELISA assay (sensitive to 0.5 ng/mL) enable measurement of producer and molecule directly and process data within hours

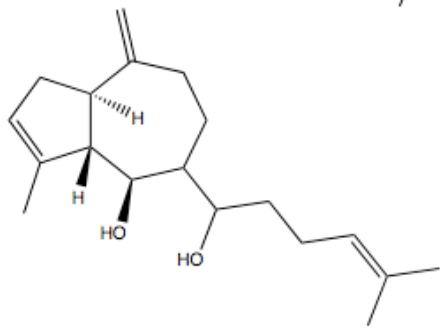
Environmental Sample Processor, Dr. Chris Scholin, MBARI

Pachydictyol:

Pachydictyol A



Pachydictyol E



JACS, 1973, 95 (12), pp 4049–4050

- PachA One of the most potent grazing deterrents known, working at 100 fold lower concentrations than natural abundance in *Dictyota coriaceum,* a brown alga (CA coast from Santa Barbara to Baja).
- Originally discovered as a weak antibiotic
- The amphipod *Pseudoampithoides incurvus* builds domiciles from leaves of alga, but does not sequester toxin
- PachE very weak antigrazing with fish, but high activity against sea urchins

Oecologia, v. 75, Number 2, 1988, 246-252

Conotoxins and Other Peptides:

14562–14568 | PNAS | November 25, 2003 | vol. 100 | suppl. 2

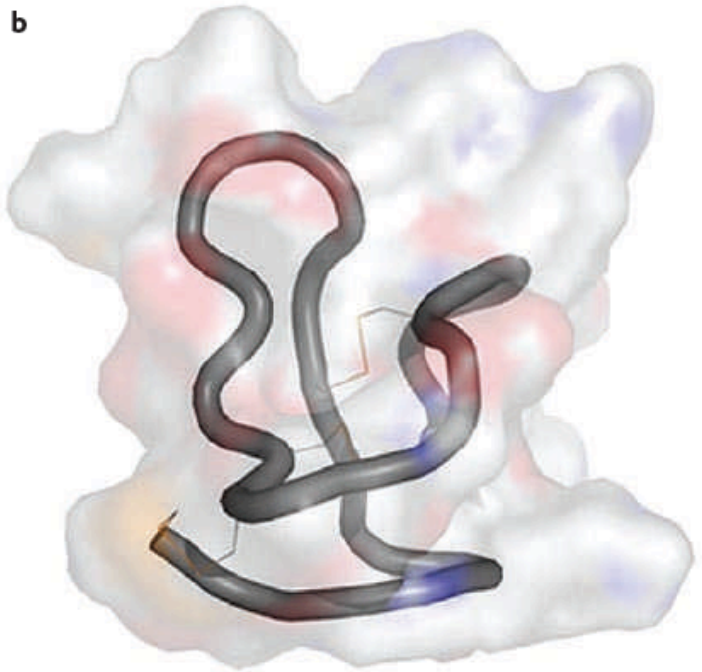


Figure 1 | **ω-Conotoxin VIIA. a** | Amino-acid sequence of the peptide ω-conotoxin MVIIA (ziconotide/Prialt; Elan Pharmaceuticals). **b** | Three-dimensional structure of the synthetic ω-conotoxin VIIA polypeptide. The cylinder represents the amide backbone of ω-conotoxin VIIA overlayed against an electrostatic potential surface. For a three-dimenational representation of ω-conotoxin MVIIA see the entry Ziconotide @ 3Dchem.com (see Further information).

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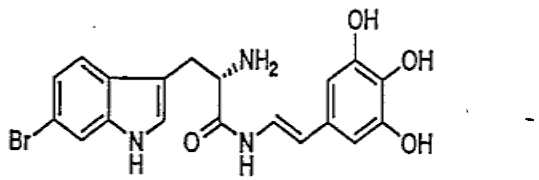
VOLUME 8 | JANUARY 2009 | 69

Ziconotide, the most potent pain reliever known. Just released on the market.

Boring Sponge metabolite: possible mechanism for calcium sequestering and degradation of hard substrate.

Cliona celata



clionamide

Andersen, R.J.; Stonard, R.J. *Can. J. Chem.* 1979, *57*, 2325.

Description: This is the largest and sponge, occurs in the British Isles. Bores into limestone and other calcareous substrata, shells, coral. The boring stage is seen as clear sulphur lemon lobes, which are the rounded tips of papillae in the rock. The raphyrus stage becomes massive lobose with raised, rounded ridges. Large specimens may be up to 100 cm across and 50 cm high. The colour is bright yellow in life. The surface of this form is evenly covered by tuberculate inhalant papillae. Large oscules with raised rims are found along the tops of the ridges.
www.habitas.org.uk/marinelife

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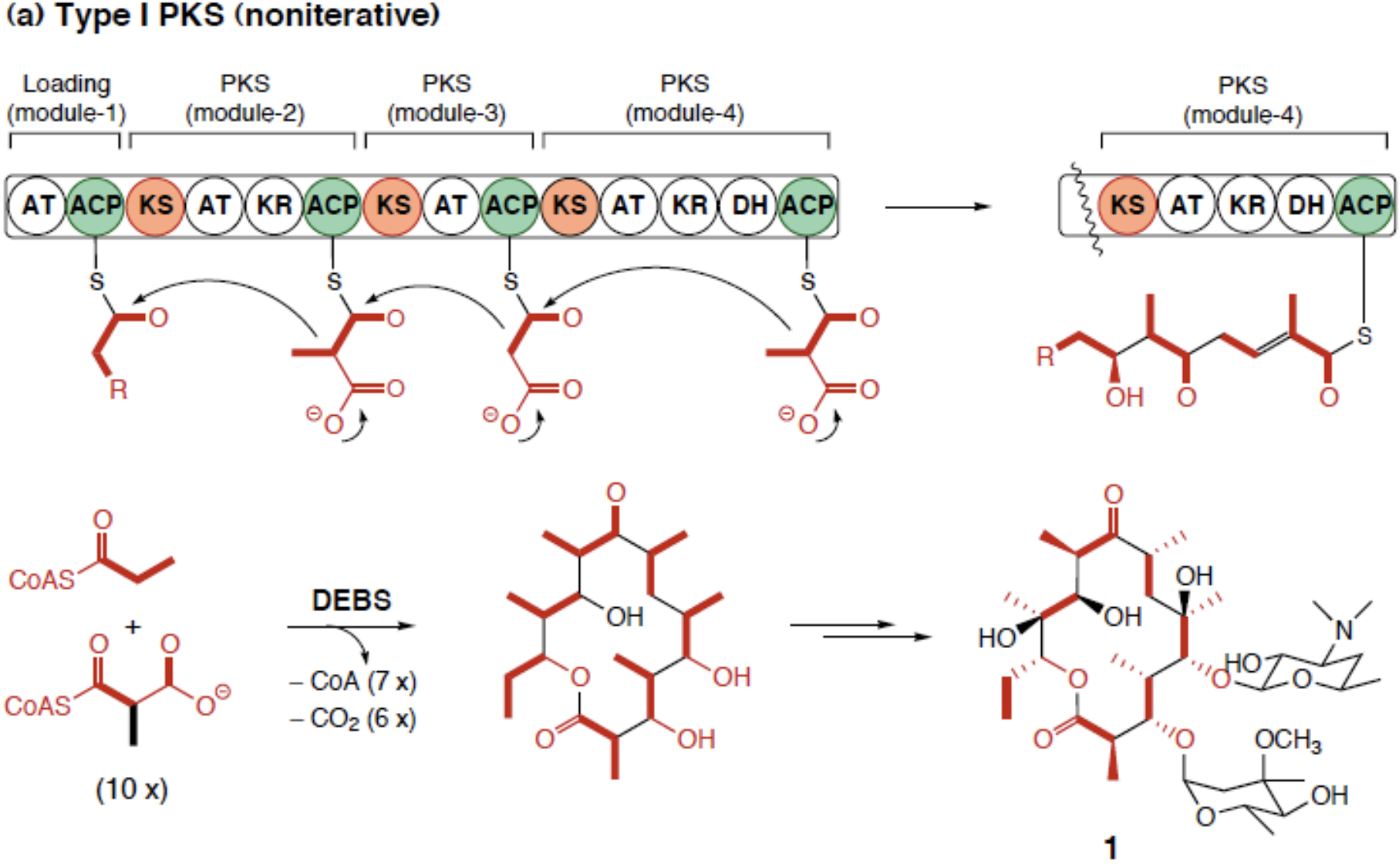
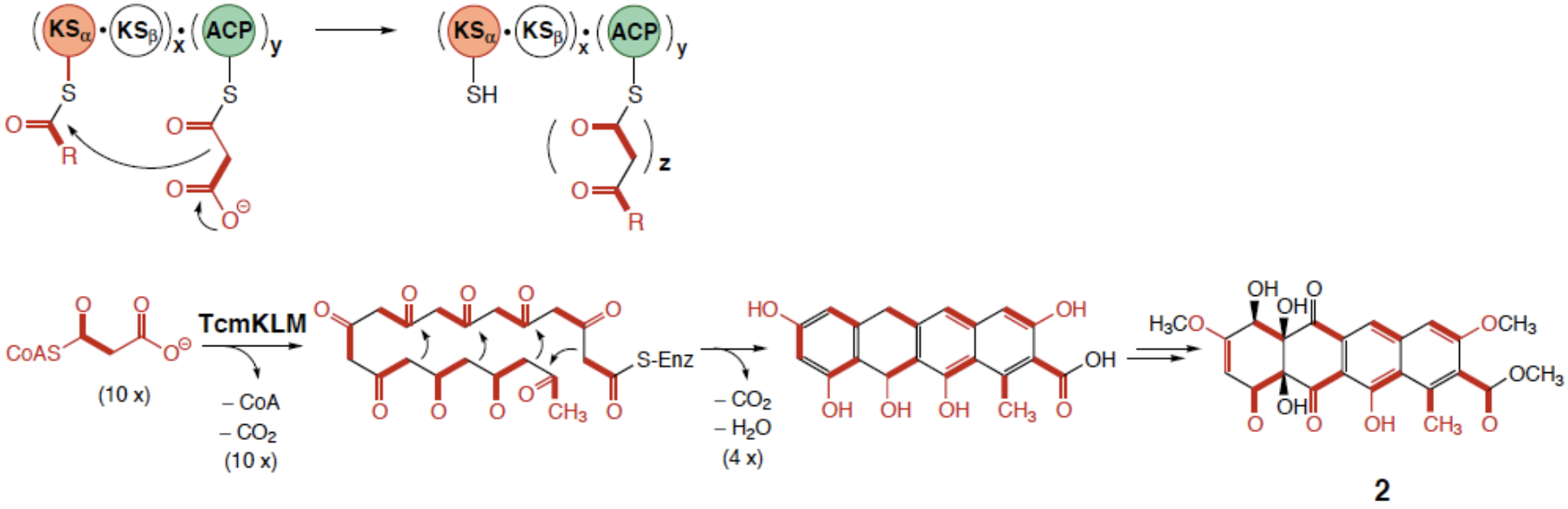

(a) Type I PKS (noniterative)
Loading (module-1)
PKS (module-2)
PKS (module-3)
PKS (module-4)
PKS (module-4)
AT ACP KS AT KR ACP KS AT ACP KS AT KR DH ACP
KS AT KR DH ACP
CoAS
CoAS
(10 x)
DEBS
– CoA (7 x)
– CO2 (6 x)
1

:

(b) Type II PKS (iterative)



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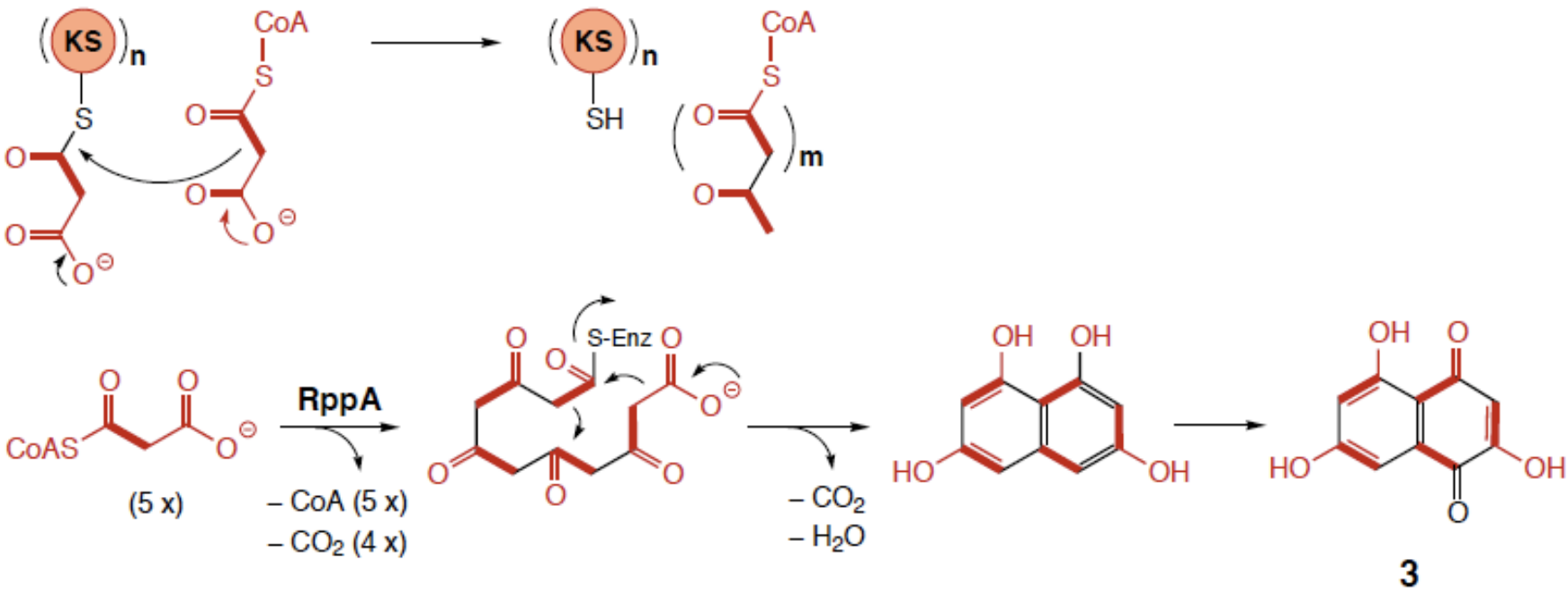
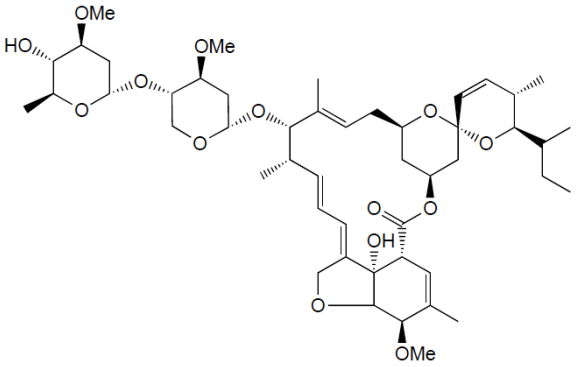
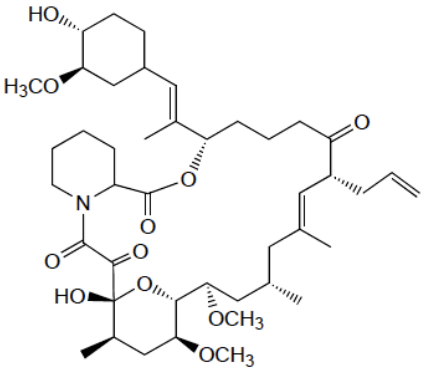
(c) Type III PKS (ACP-independent & iterative)



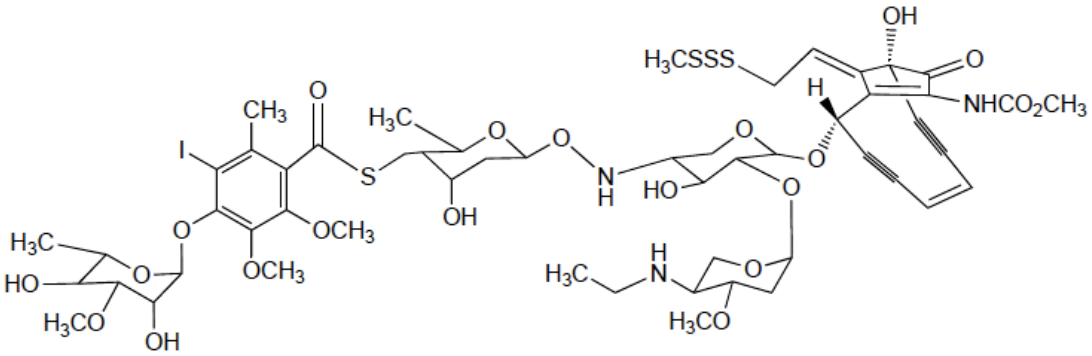
Polyketides, some extraordinary examples:



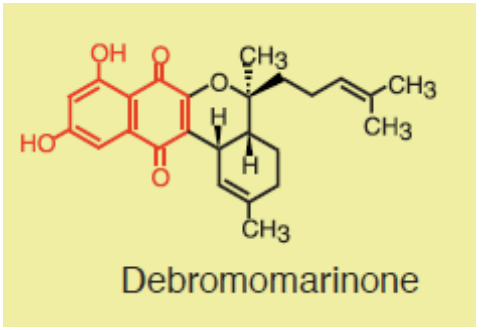
Avermectin A1a



FK-506



Calicheamicin γ1I



Biosynthesis/Terpenes:

Pachydictyol A

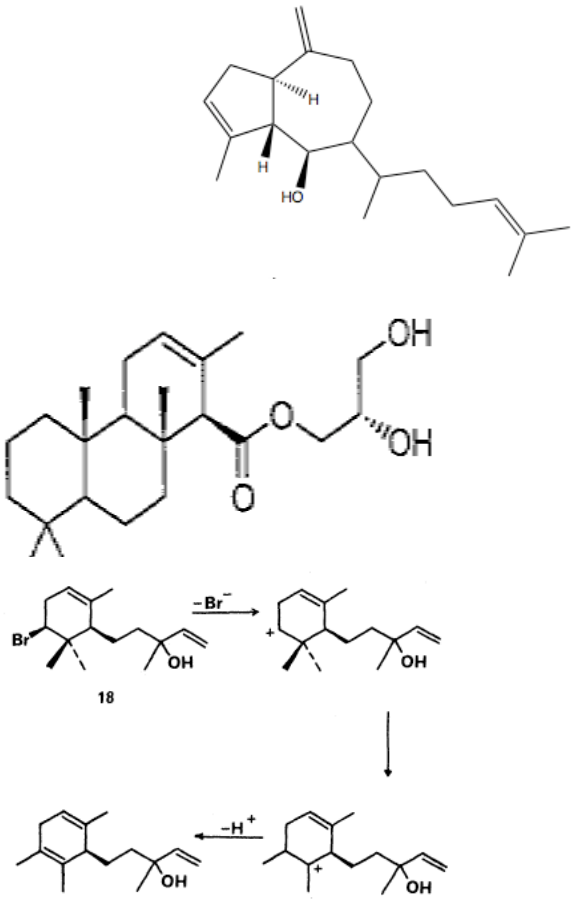
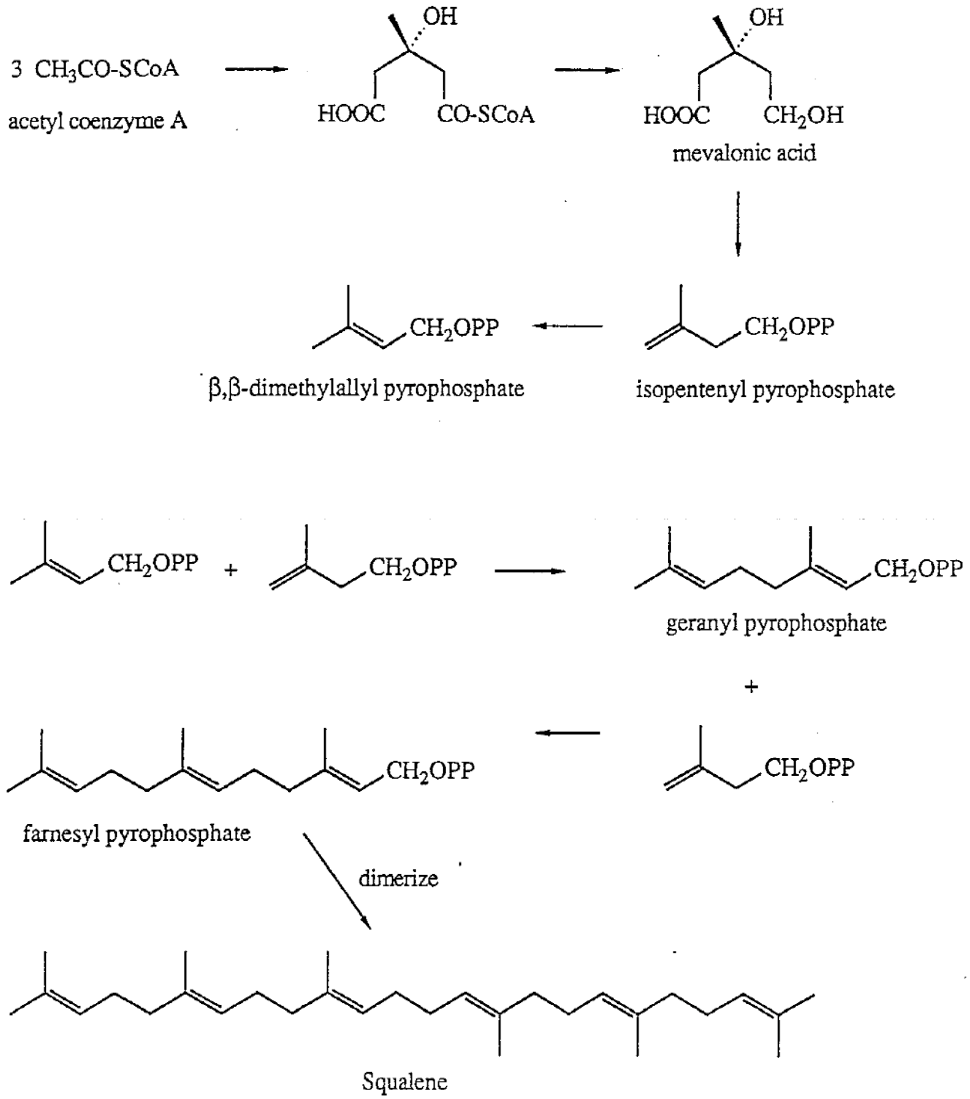


Fig. 7. A biosynthetic proposal involving the solvolysis of haloterpenoids which explains the production of the ''rearranged terpenoids'' found in marine organisms.

Outline of Terpene Biosynthesis

1. Terpenes and sterols are synthesized in nature from "isoprene" units.
2. The "isoprene" unit, isopentenyl pyrophosphate, is created from acetic acid.



Non Ribosomal Peptide biosynthesis:

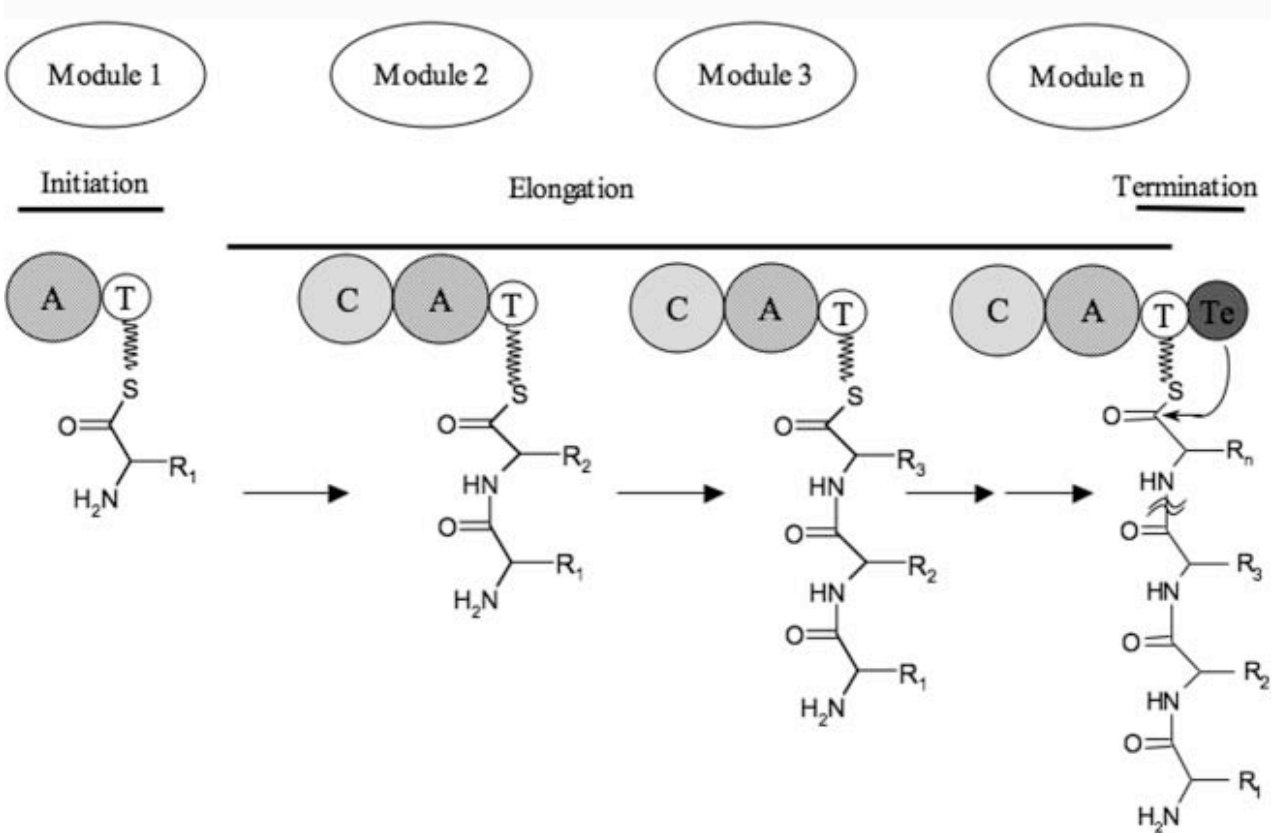
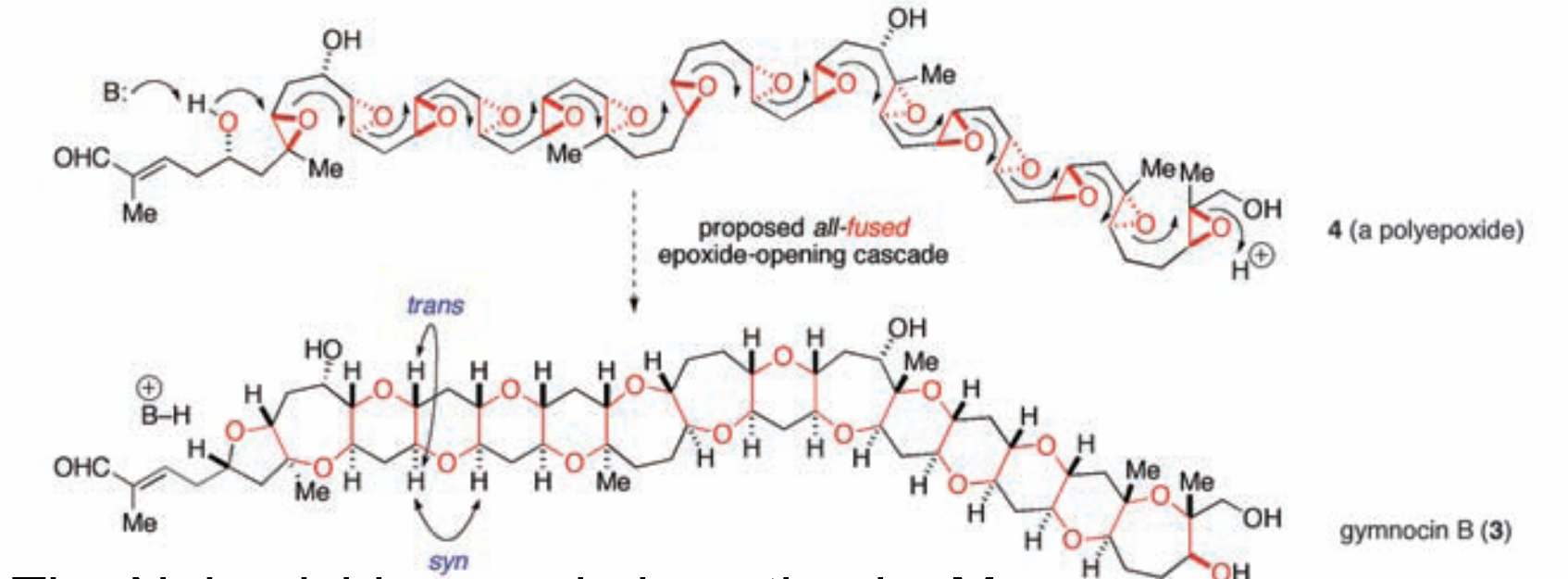


Fig. 1, each NRPS module consists of three core domains: an adenylation (A) domain, which selects the cognate amino acid, activates it as an amino acyl adenylate and transfers it to the T domain (also known as peptidyl carrier protein, or PCP) where a thioester bond is formed, a condensation (C) domain

Nat. Prod. Rep., 2007, ***24***, 1073–1109 | 1073

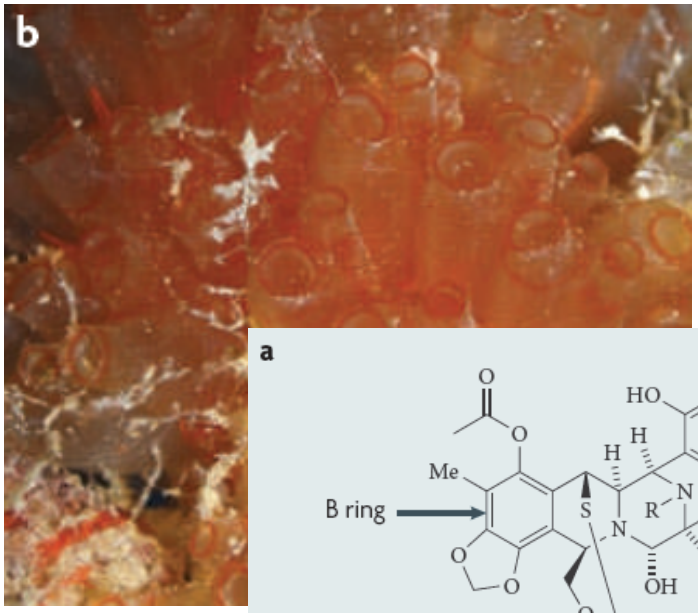
Polycyclic ether toxins, an aqueous synthetic route possibly occurring in nature:

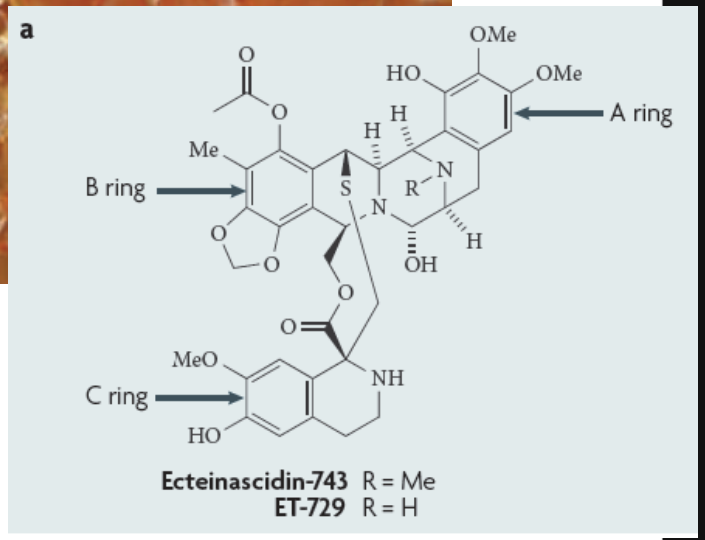


The Nakanishi cascade hypothesis. More than 20 years ago, Nakanishi put forth a hypothesis that accounts for these structural and stereochemical similarities—the transformation of a polyepoxide (e.g., 4) into a ladder polyether via a series or “cascade” of epoxide-opening

SCIENCE VOL 317 31 AUGUST 2007

Applications- Drugs from the Sea:





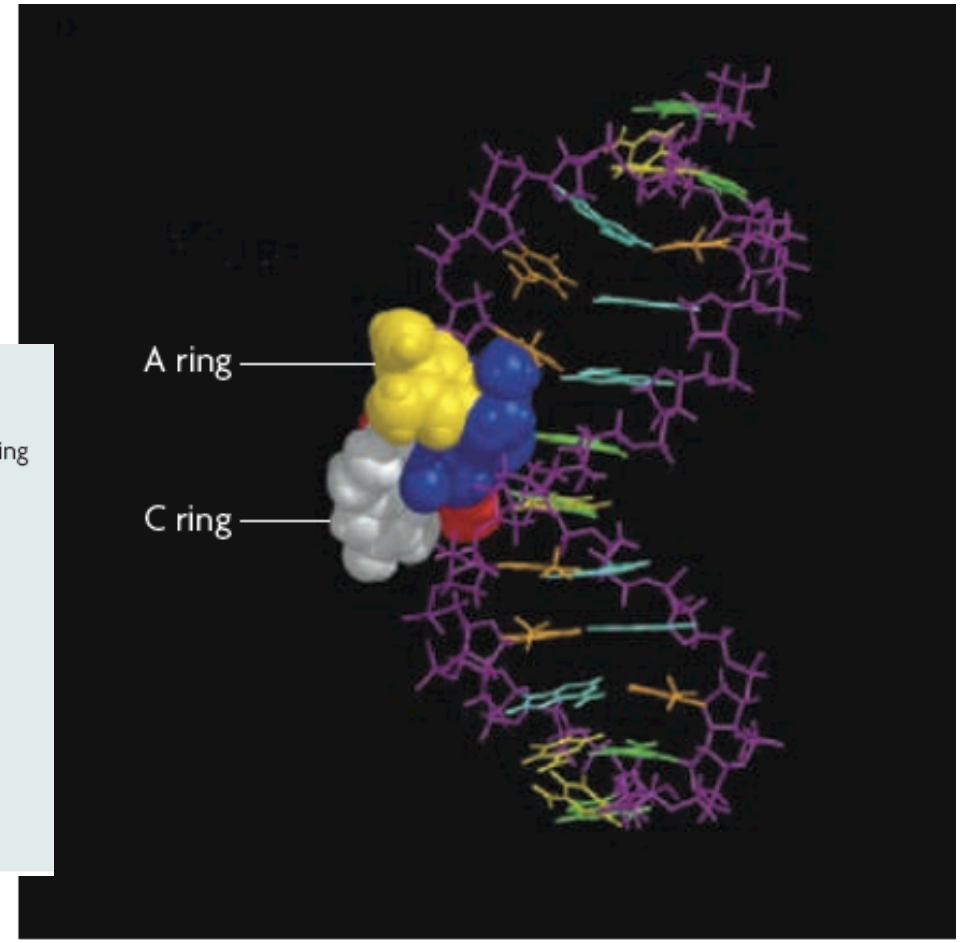


Figure 4 | **Molecular-dynamics model showing the alkylation of DNA by ET-743 at N2 of guanine in the minor groove.** The A Ring and C Ring represent the tetrahydroisoquinoline A and C rings of ET-743 (FIG. 3).

Potent activity against solid tumors, available in clinic now

•Semi-synthetic production by PharmaMar

Halogenation: Input of VHOC's by macroalgae

Table 1. Mean (and range) rates of release of the three major VHOC to seawater by temperate macroalgae (in nanograms per gram of dry algae per day); ND, not detected.

Algal species (Number of samples)	$CHBr_3$	$CHBr_2Cl$	CH_2Br_2
	Brown algae		
Ascophyllum nodosum (8)	4,500 (150–12,500)	1,100 (ND–3,000)	680 (ND–2,100)
Fucus vesiculosis (7)	2,200 (140–4,700)	150 (ND–820)	84 (ND–590)
	Green algae		
Enteromorpha linza (2)	(ND; 850)	(ND; ND)	(ND; 300)
Ulva lacta (2)	(1,700; 14,000)	(590; 4,300)	(ND; 250)
	Red algae		
Chondrus crispus (2)	ND	ND	ND
Gigartina stellata (3)	(ND; 320; 2,100)	(ND; ND; 3,000)	(ND; ND; ND)

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SCIENCE, VOL. 227 1 MARCH 1985

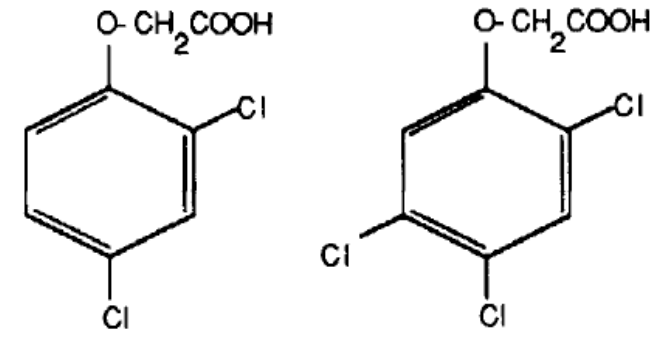
Halogenation: Input of VHOC's by macroalgae

Table 3. Estimated annual global inputs of organobromides and organoiodides to the atmosphere.

Sources	Bromine or iodine (g/year)
Organobromides	
Macroalgae*	
(10^{13} g algae) (1 to 10 μg Br per gram per day) (365 days per year)	$\sim 10^{10}$
Industrial products†	
CH_3Br (fumigant)	$\sim 5 \times 10^{10}$
BrCH=CHBr (fumigant and gasoline additive)	$\leq 10^{11}$
CF_3Br (flame retardant)	$\leq 10^{9}$
Chlorination‡	
Seawater (6×10^{10} g Cl per year) (1 percent organobromides)	6×10^{8}
Freshwater (4×10^{12} g Cl per year) (0.1 percent organobromides)	4×10^{9}
Organoiodides	
Macroalgae*	
(10^{13} g algae) (10 to 100 ng I per gram per day) (365 days per year)	$\sim 10^{8}$
Phytoplankton-derived CH_3I§	$\sim 10^{12}$

*Global algal biomass estimate from (*19*), on the assumption that 100 percent of the algal release is lost to the atmosphere. †Data from (*18*). ‡Assuming that the global chlorine usage is twice that of the United States (*21*); formation efficiency of volatile bromine species after (*22*). §Estimates from (*23*).

Halogenation: making a molecule resistant to biodegradation and/or a better ligand



2,4-D — BIODEGRADABLE

2,4,5-T — RECALCITRANT

FIGURE 1. Effect of halogen substituents on biodegradability.

Hardman. David J.(1991)'Critical Reviews in Biotechnology

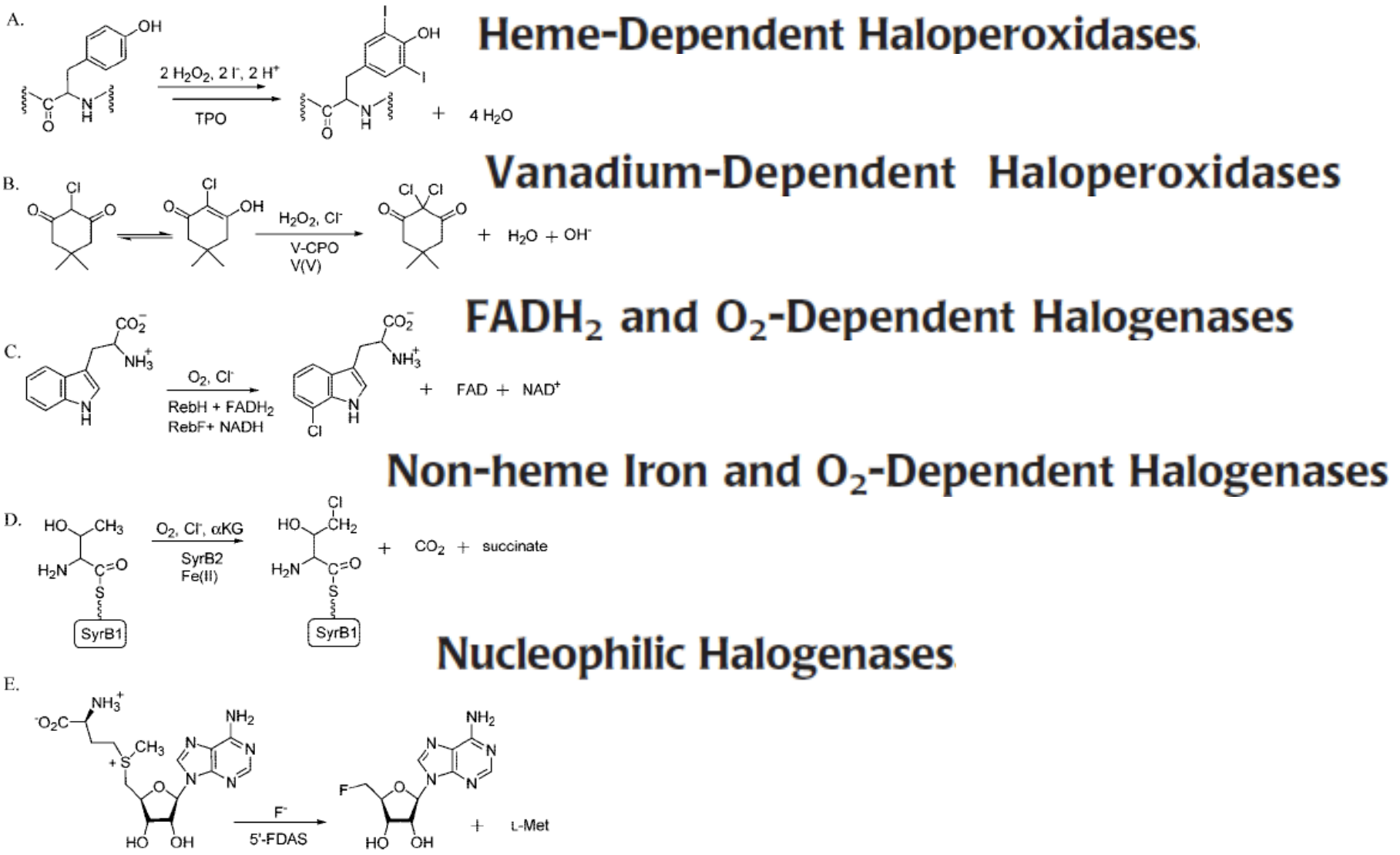
TABLE 2. Trends in the Halides

ionic radius	$F^- < Cl^- < Br^- < I^-$
electronegativity	$I^- < Br^- < Cl^- < F^-$
basicity	$I^- < Br^- < Cl^- < F^-$
nucleophilicity (protic solvent)	$F^- < Cl^- < Br^- < I^-$
nucleophilicity (gas phase)	$I^- < Br^- < Cl^- < F^-$
good leaving group	$F^- < Cl^- < Br^- < I^-$
standard redox potential of HOX/X^- couple	$I^- < Br^- < Cl^- < F^-$

Accounts of chemical research (2009) vol:42 iss:1 pg:147

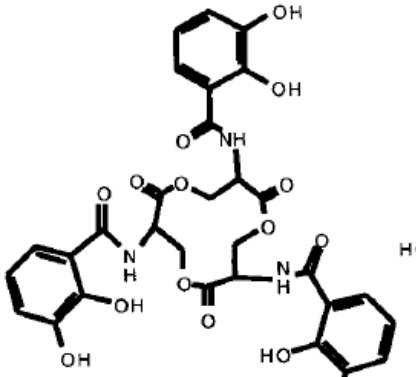
Halogenation: 5 basic types of enzymes known

SCHEME 1. Examples of Enzymatic Halogenation Reactions



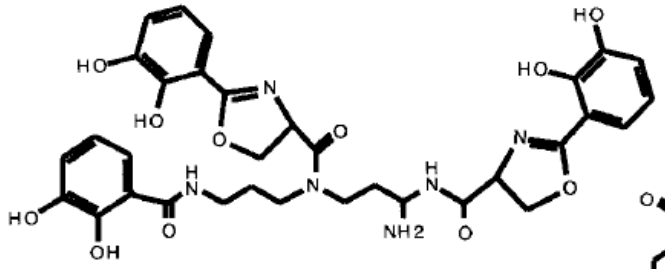
Siderophores: important in biomedicine

MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, June 2002, p. 223–249



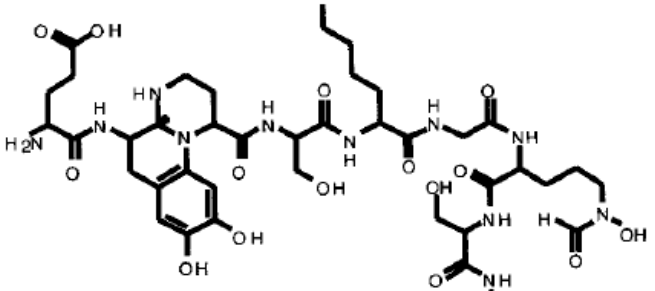
enterobactin

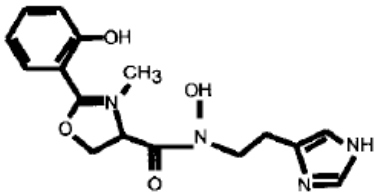
E. coli



vibriobactin

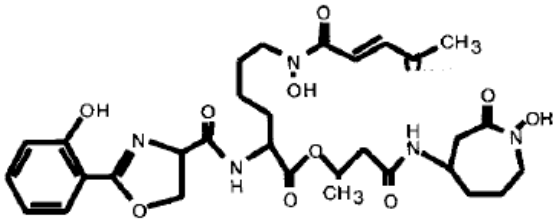
V. cholerae





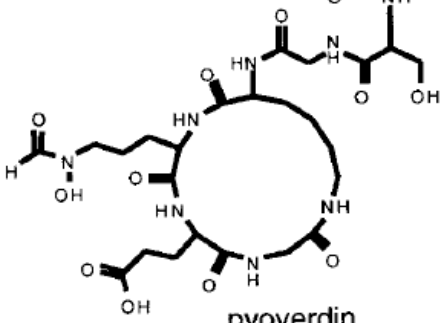
acinetobactin

Acinetobacter calcoaceticus



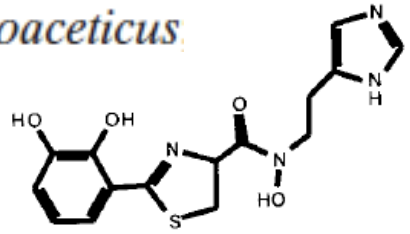
mycobactin T

M. tuberculosis



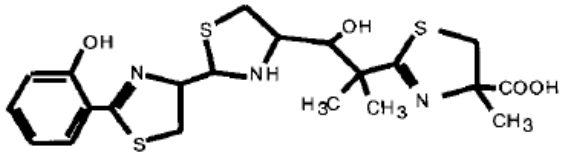
pyoverdin

P. aeruginosa



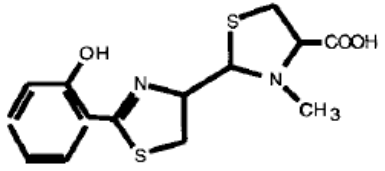
anguibactin

V. anguillarum



yersiniabactin

Y. pestis.

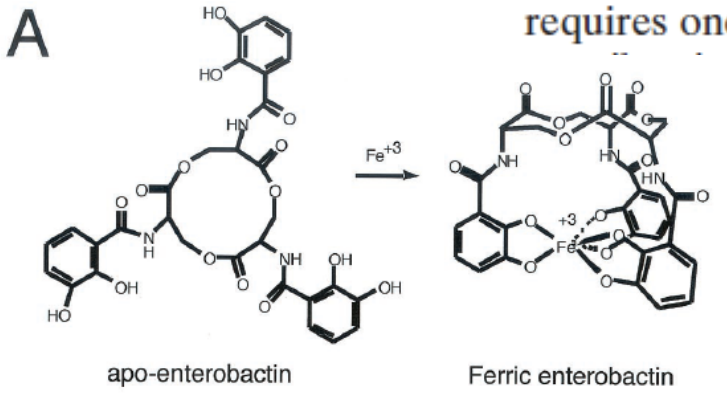


pyochelin

P. aeruginosa

Siderophores:

FIG. 2. Spatial structures of enterobactin and anguibactin. (A) Enterobactin without and with Fe^{3+}. Notice that the groups coordinating the iron are six oxygens from the three diphenolic groups. The complex requires one molecule of enterobactin. (B) X-ray structure of gallium

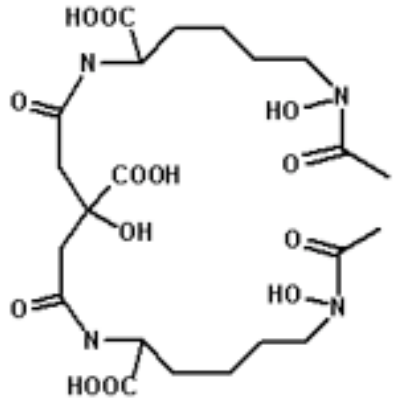


Enterobactin

constant of association for iron (about 10^{52} 1/M)

Aerobactin

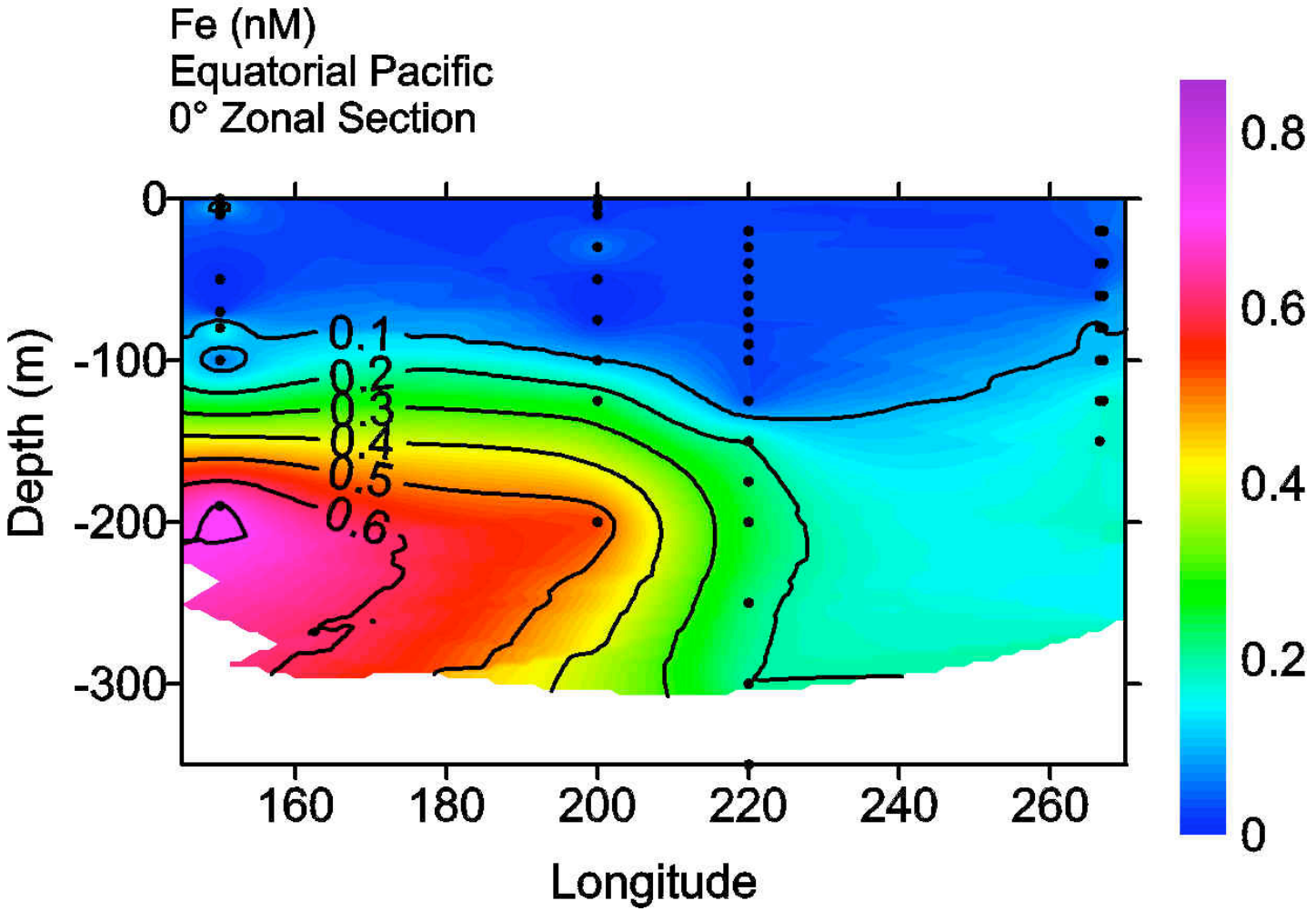
constant of association for iron (about 10^{23} 1/M



Biomedical Question: why is aerobactin favored by pathogenic serovars of E.coli?

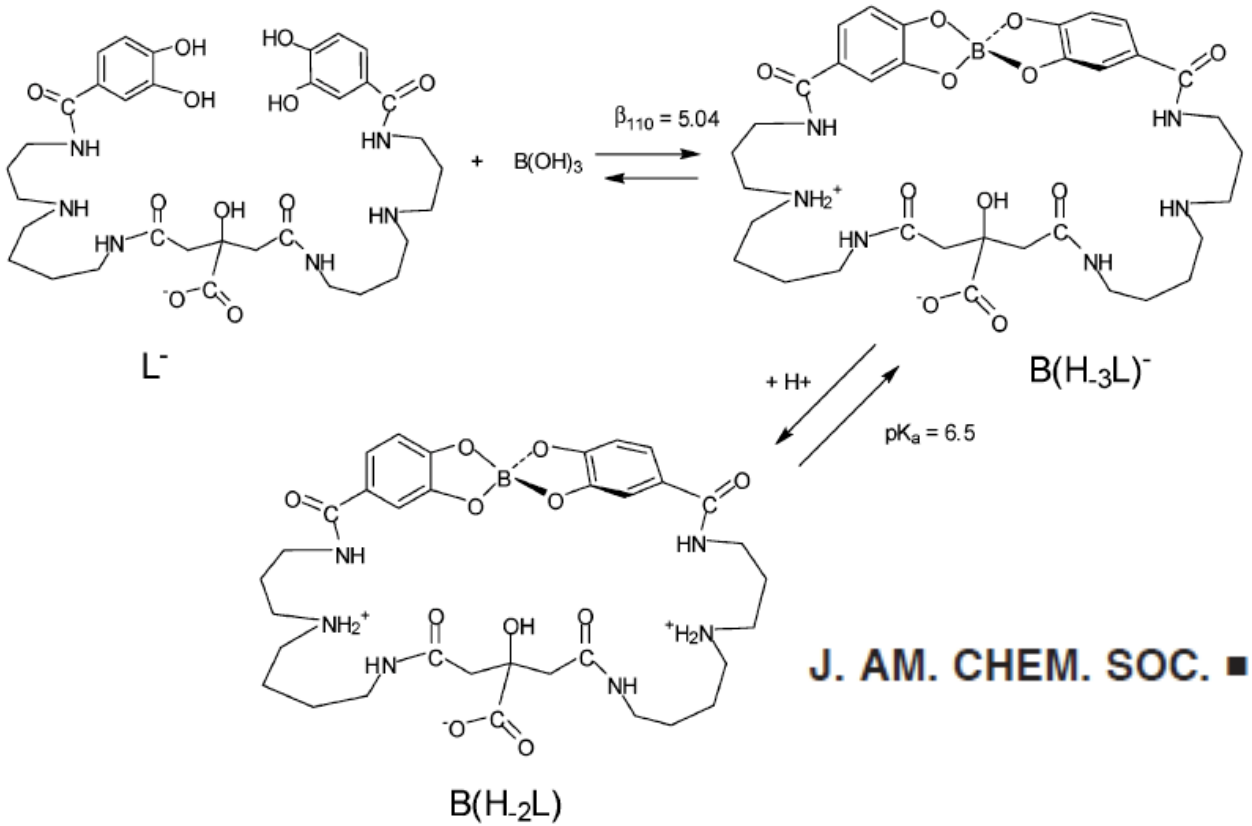
- Ferric enterobactin esterified or destroyed upon uptake
- Ferric aerobactin readily releases iron, stays intact
- Enterobactin has low serum solubility-studies show aerobactin more efficient at iron delivery in equimolar competition studies
- Enterobactin elicits host immune response
- Clustering of biosynth genes could aid in rapid evolution siderophores

Iron limitation in the marine environment:



Special requirements and selective pressures in marine environments?

Siderophores: Transporting Boron?

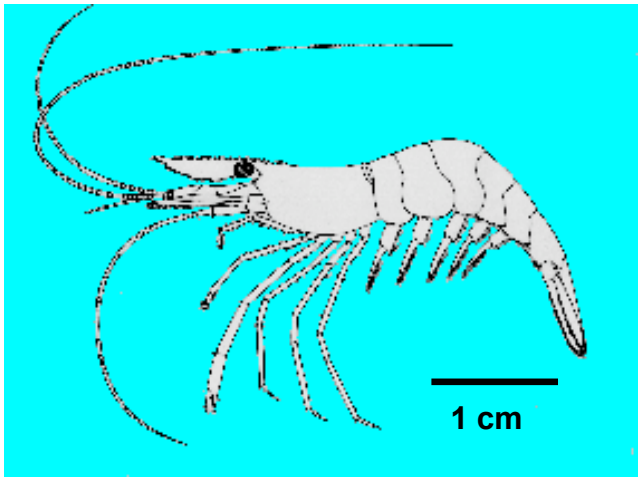


J. AM. CHEM. SOC. ■ VOL. 129, NO. 40, 2007

Petrobactin and vibrioferrin recently found to have unexpected binding affinity to Boron (a cofactor that is limiting in terrestrial env)- not all siderophores tested showed this. Cobalt and Zinc limiting in marine env, cognate chelators for these?

Symbiotic Marine Bacteria Chemically Defend Crustacean Embryos from a Pathogenic Fungus

M. Sofia Gil-Turnes, Mark E. Hay, William Fenical



"Embryos of the shrimp *Palaemon macrodactylus* are remarkably resistant to infection by the fungus *Lageniditlm callinectes*, a recognized pathogen of many crustaceans..."

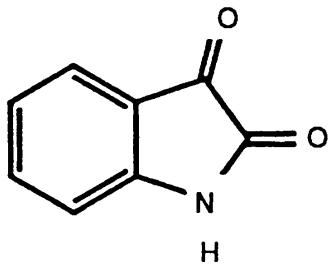


Fig. 1. The molecular structure of 2,3-indolinedione.

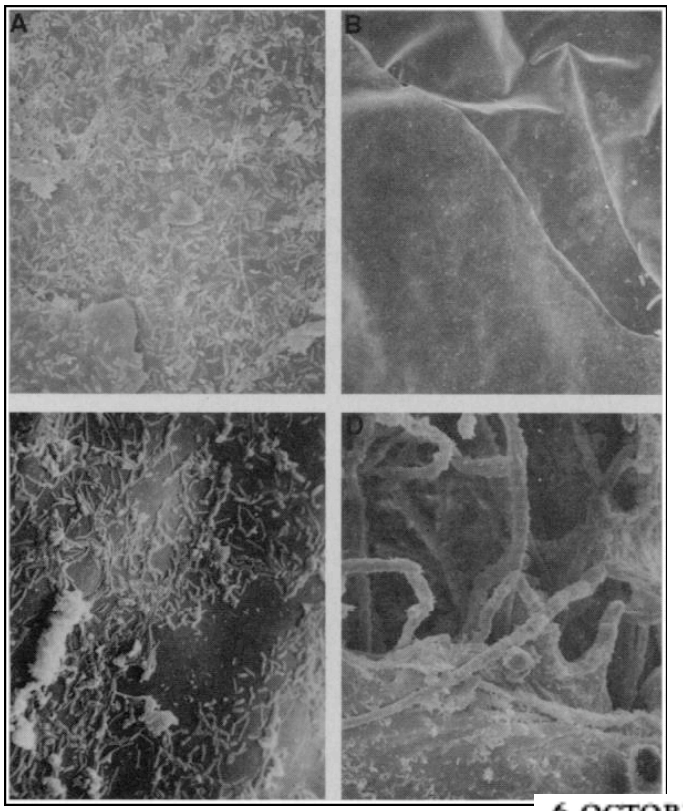
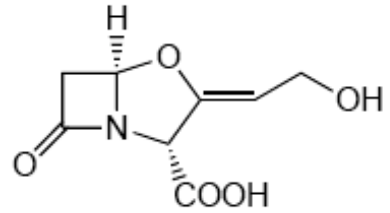


Fig. 2. Scanning electron micrographs of representative embryos during the experiment. (**A**) Bacteria on surface of healthy embryo (×2000). (**B**) Surface of embryo after treatment with penicillin-G (×1000). Although in some instances a few large bacteria could be seen on the embryos, most of the bacterial population was eliminated. (**C**) Embryos after recolonization with pure cultures of the protective bacterium (×2000). (**D**) Hyphae of the fungus *L. callinectes* penetrating the embryonal coat (×1000).

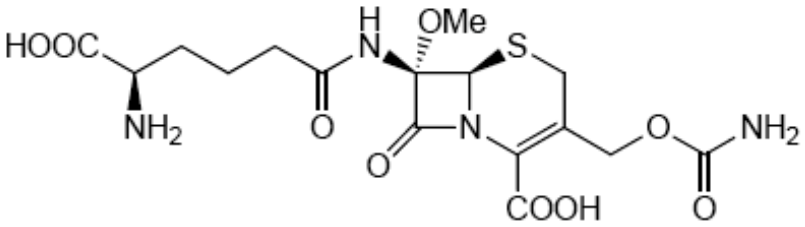
6 OCTOBER 1989 SCIENCE, VOL. 246

Do antibiotics function in natural setting?
Clavulanic acid and the story of Augmentin®

Potent inhibitor of
β-lactamase



Clavulanic acid



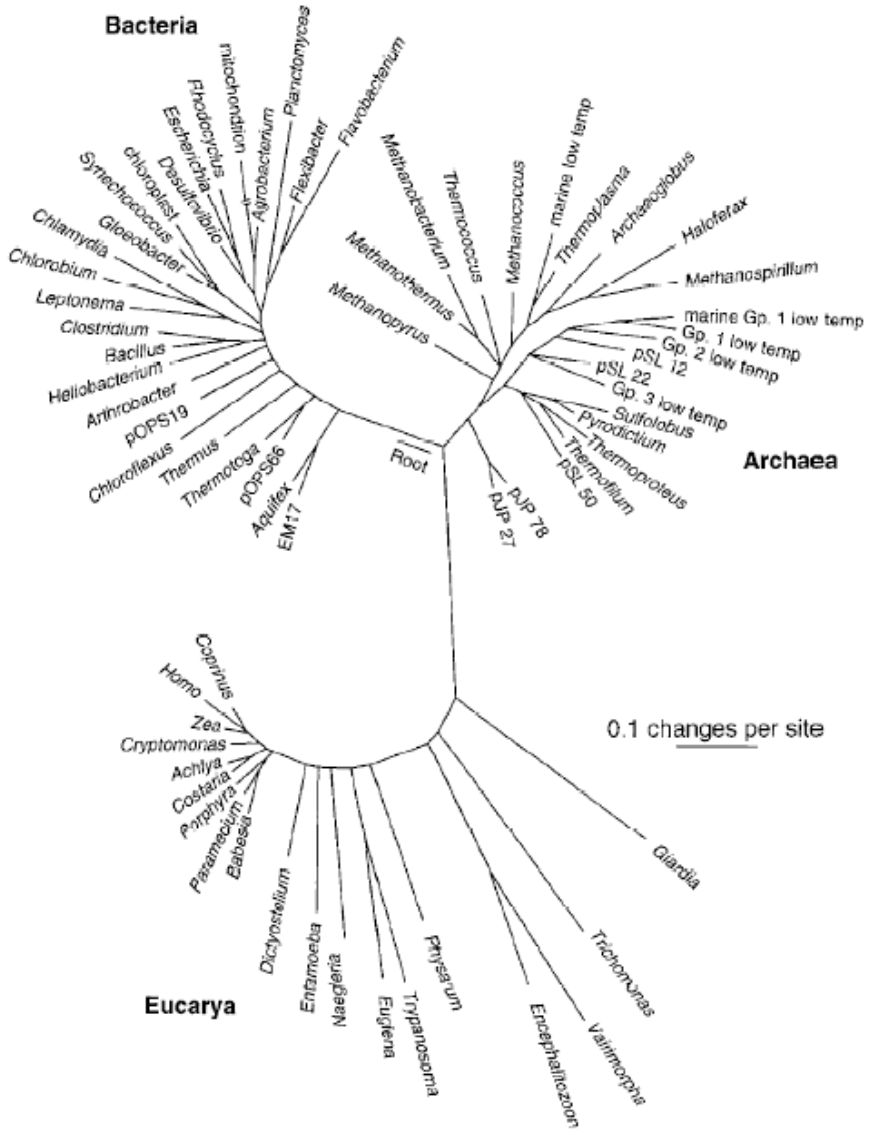
Cephamycin

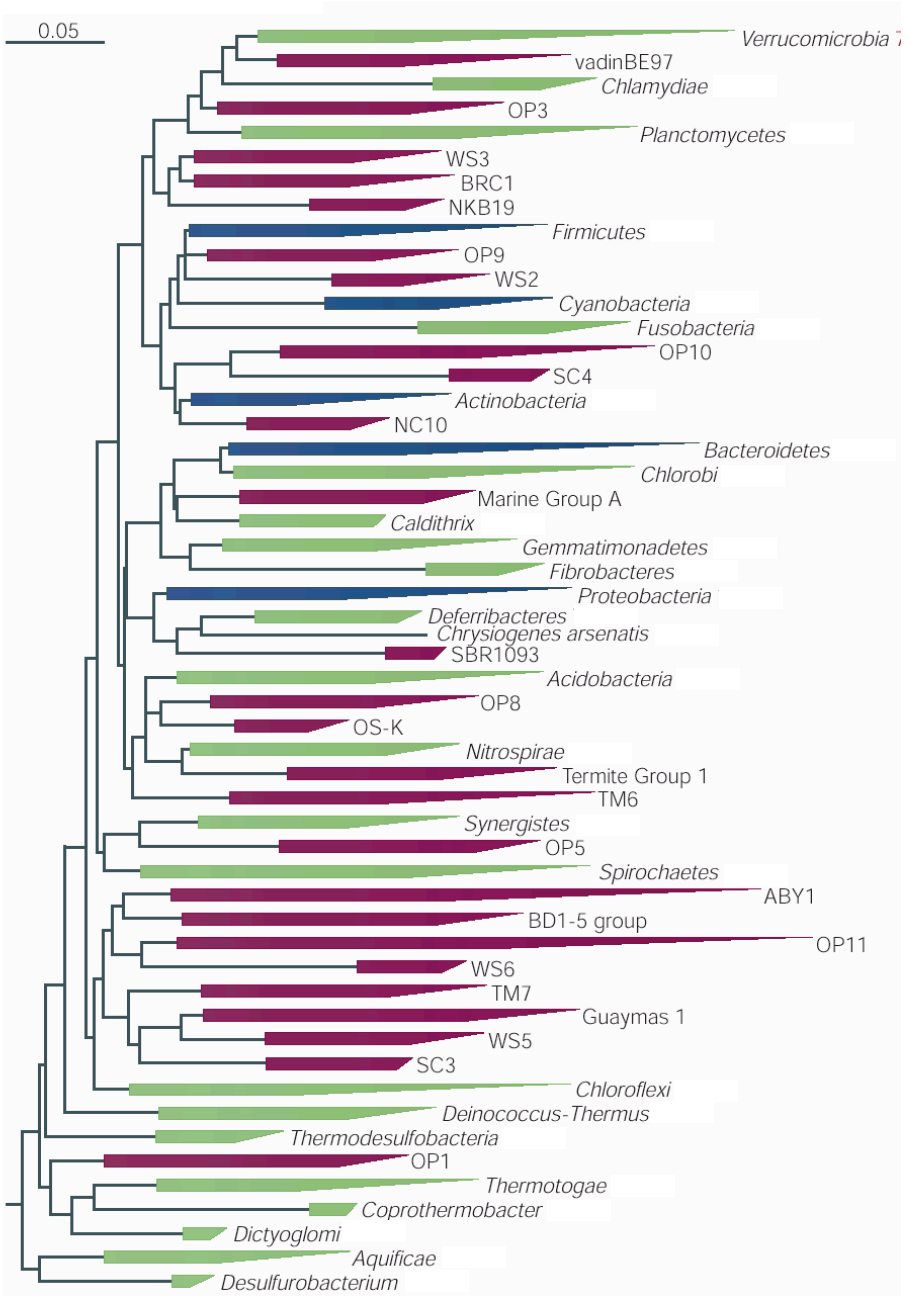
Potent inhibitior of cell-wall synthesis

Biodiversity: chemical diversity

Taken from N. R. Pace, Science, vol. 276. p 734-740, 1997.

- Vast majority of diversity is within the Archaea and Bacteria.
- Find-em-and-grind-em surveys shows certain hotspots-Why? Is this representative?
- What pressures are microbes experiencing and how do specialized metabolites aid adatpation?





Phylogenetic tree of the domain Bacteria based on 16S rRNA gene sequences

(from: Keller and Zengler 2004 Nature Rev. Microbiol. 2:141).

53 bacterial phyla with cultured representatives are shown in blue and green.

Only 5/24 cultivated phyla produce anti-infectives (in blue).

Actinomycetes represent the major source of fermentation-derived secondary metabolites.

Genomic survey of Thiotemplate Modular Systems (PKS and NRPS type clusters)

Table 1 Analyzed genomes by phylum

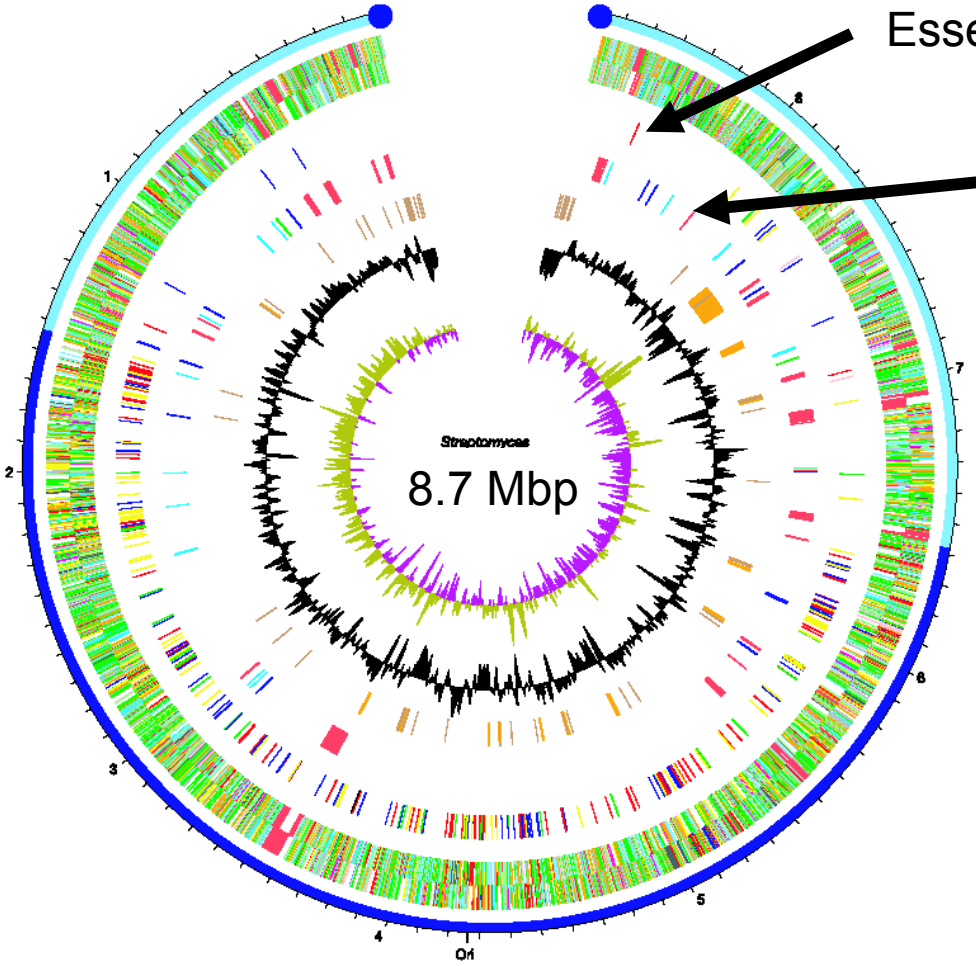
Phylum	Genomes[a]	Size[b]	TMS genes[c]	Density[d]
Actinobacteria	18	70 044 365	452 991	1.940
Aquificae	1	1 590 791	0	0.000
Bacteroidetes	5	22 776 893	2628	0.035
Chlamydiae	9	11 601 785	0	0.000
Chlorobi	1	2 154 946	0	0.000
Cyanobacteria	8	26 666 055	51 826	0.583
Deinococci	2	5 411 638	0	0.000
Firmicutes	63	159 832 396	133 511	0.251
Fusobacteria	1	21 74 500	0	0.000
Planctomycetes	1	7 145 576	11 197	0.470
α-Proteobacteria	25	77 768 614	39 940	0.154
β-Proteobacteria	13	59 473 882	236 438	1.193
γ-Proteobacteria	59	247 393 752	540 029	0.655
δ-Proteobacteria	4	15 226 925	0	0.000
ε-Proteobacteria	6	10 640 511	0	0.000
Spirochaetes	6	15 806 532	0	0.000
Thermotogae	1	1 860 725	0	0.000
Total	223	737 569 886	1 468 560	0.597

[a] Different strains with published sequenced genomes. [b] Cumulative genome size, in bp. [c] Cumulative size of TMS polypeptides, in aa. [d] Percentage of TMS genes, obtained dividing cumulative size of TMS genes by cumulative genome size.

Nat. Prod. Rep., 2007, **24**, 1073–1109

Streptomyces coelicolor: Knowledge Gained from Genome Analysis

Chromosome arms

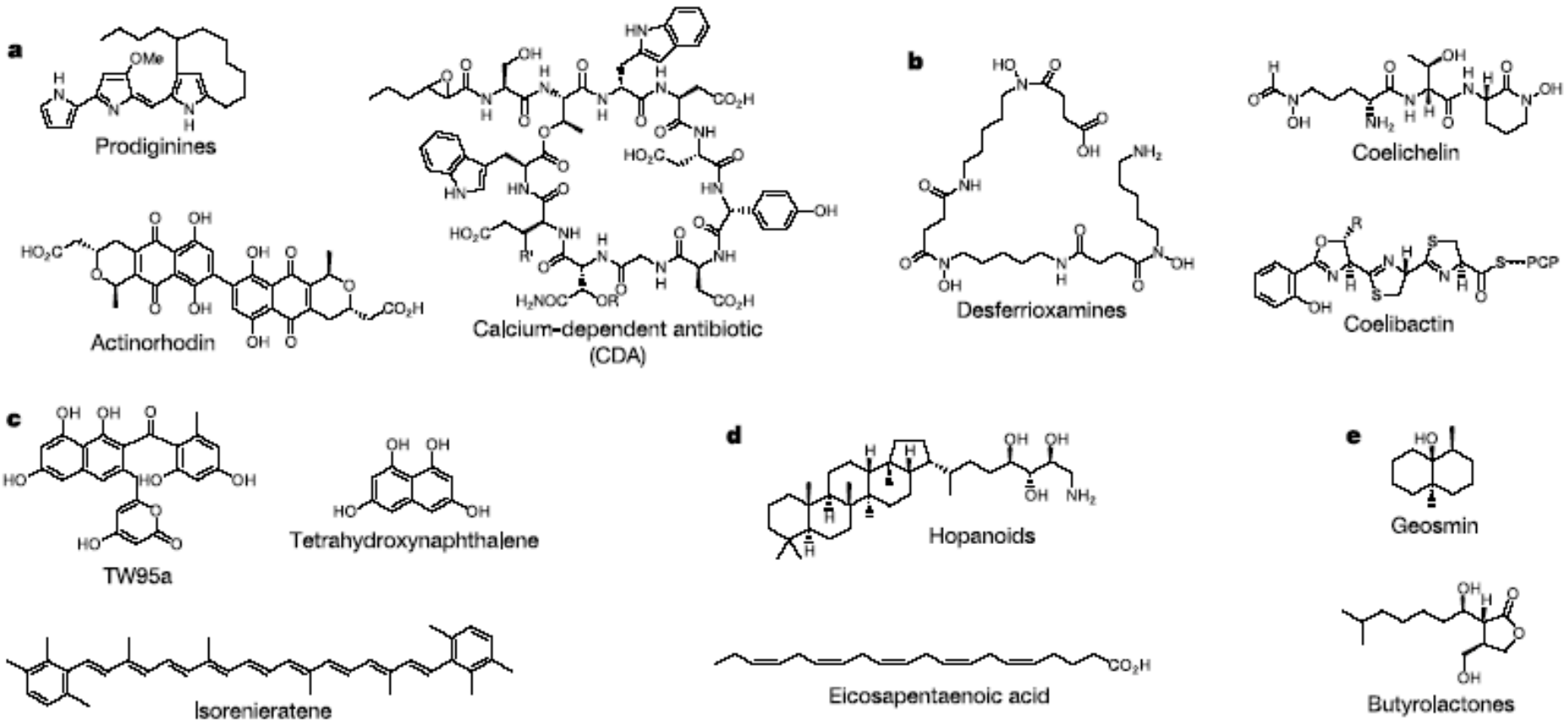


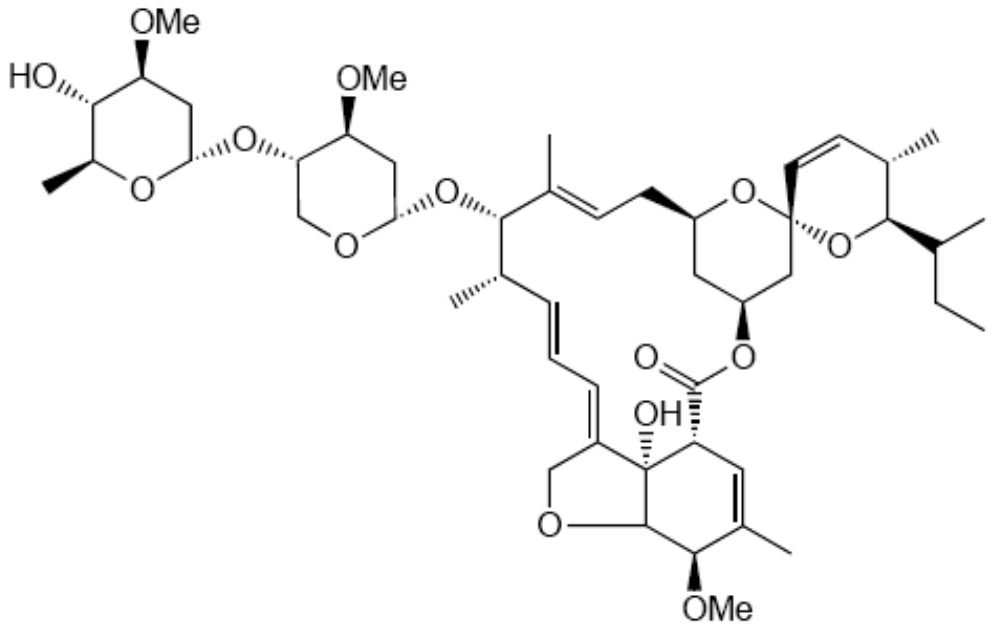
Essential metabolites

Secondary metabolites
Degradative enzymes

- 60 proteases/peptidases
- 13 chitinases/chitosanases
- 5X more secreted hydrolases than *B. subtilus*
- 13 different classes of secondary metabolites predicted

Bentley, et al, *Nature* (2002) 417, 141-147


a
Prodiginines
Actinorhodin
Calcium-dependent antibiotic
(CDA)
b
Desferrioxamines
Coelichelin
Coelibactin
c
TW95a
Tetrahydroxynaphthalene
Isorenieratene
d
Hopanoids
Eicosapentaenoic acid
e
Geosmin
Butyrolactones

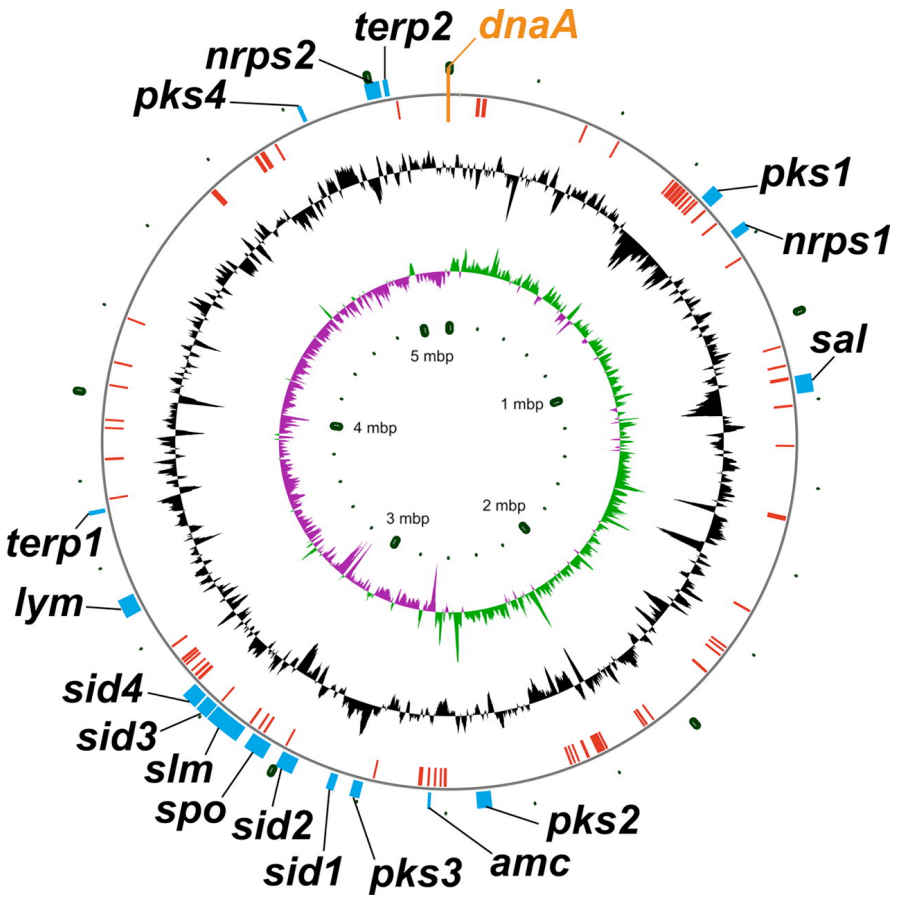


Avermectin A1a

-Potent anti-parasite compound
-Used in agriculture and medicine
-Kills mites which normally feed on hyphae

Genome sequencing reveals complex secondary metabolome in the marine actinomycete *Salinispora tropica*

Daniel W. Udwary*, Lisa Zeigler*, Ratnakar N. Asolkar*, Vasanth Singan†, Alla Lapidus†, William Fenical*, Paul R. Jensen*, and Bradley S. Moore*‡§



Nearly 10% of genome devoted to natural product synthesis

Highest proportion to date of any genome!

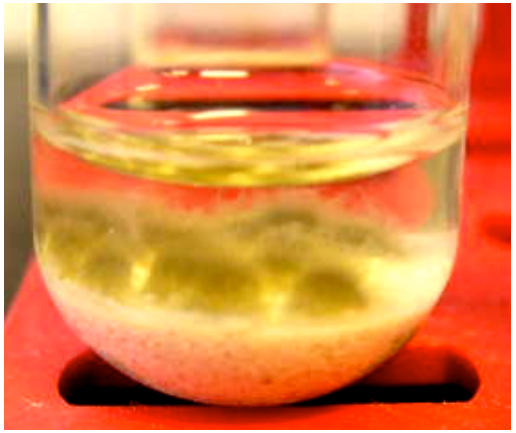


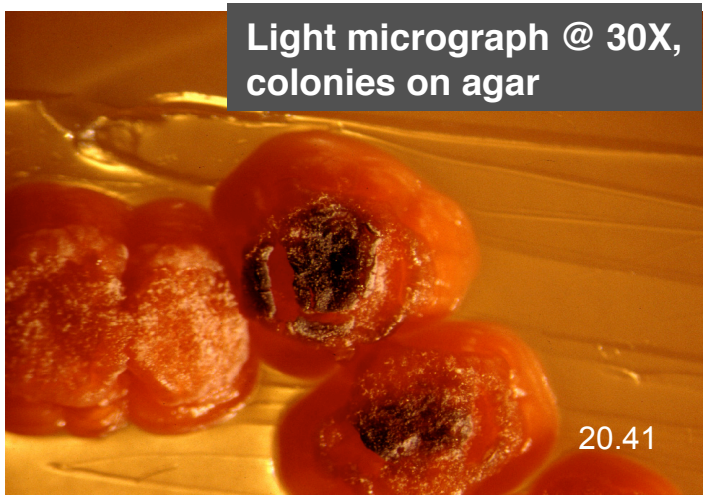
Fig. 1. Circular chromosome of S. tropica CNB-440, oriented to the dnaA gene

10376–10381 | PNAS | June 19, 2007 | vol. 104 | no. 25

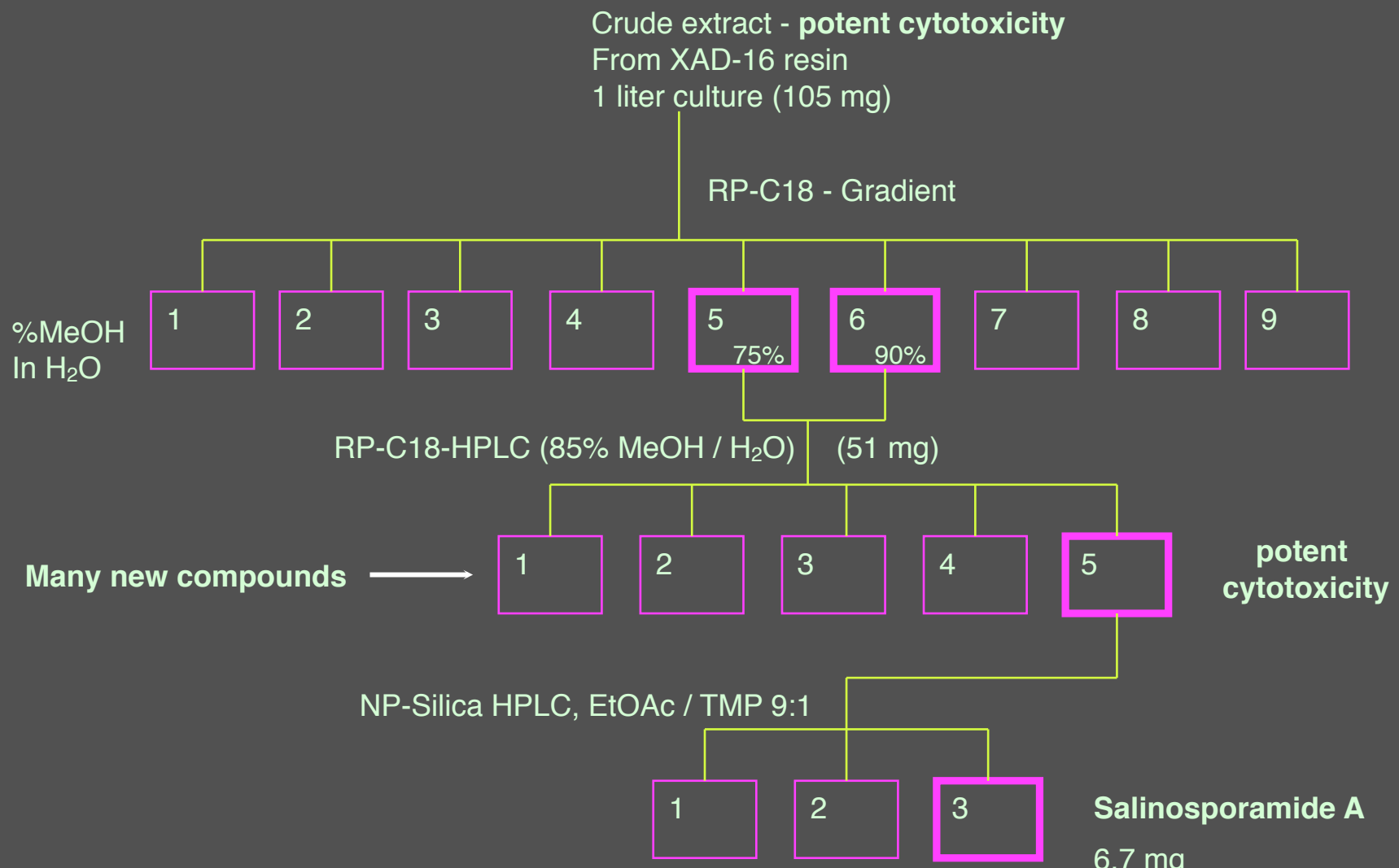
Investigation of *Salinospora* strain # CNB-392 - Bahamas

- Cultured in seawater medium
- Marine-derived nutrients

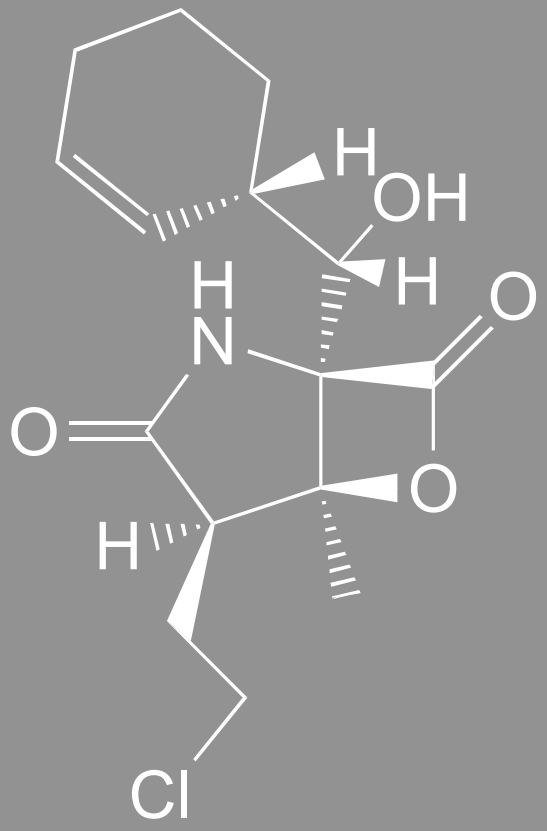
- Crude culture extract showed potent cytotoxicity against HCT-116 colon carcinoma.
- Cultured in shake flasks for 15 days with added XAD-16 absorbent resin.
- Resin filtered and eluted with methanol



Cytotoxicity-Guided Isolation of Salinosporamide A

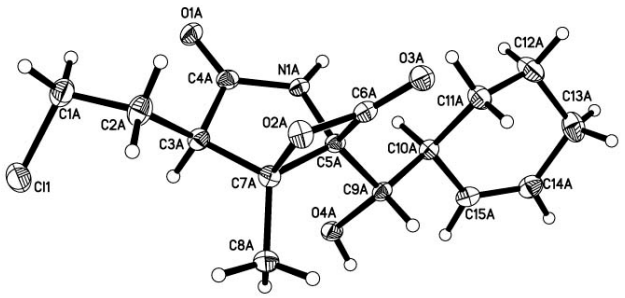


Anti-tumor / anti-malaria agent Salinosporamide A



Salinosporamide A

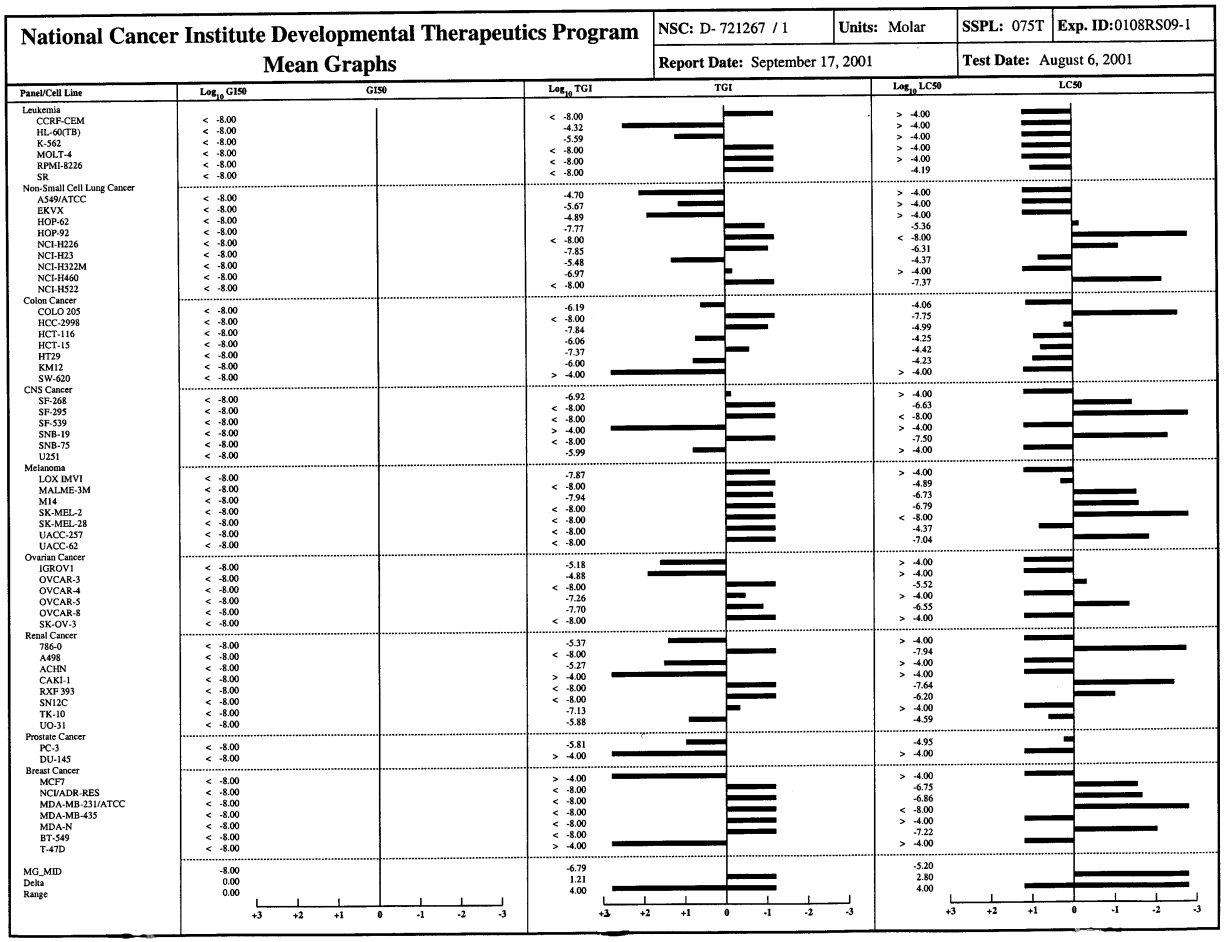
- Currently in Phase II clinical trials "Fast-Track" for Multiple Myeloma at Dana Farber and other clinics
- Potent inhibitor of the 20S proteasome
- Als… nti-mala…
- "Co… …" Sole… Phar…



Feling *et al.* Angew. Chem. 2003.

"Salinosporamides and methods for use thereof" R.T. Feling, T.J. Mincer, P.R. Jensen, W.F. Fenical. (U.S. Patent #7,176,233) 2007.

Cytotoxicity (NCI) of Salinosporamide A



National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC: D- 721267 / 1 | Units: Molar | SSPL: 075T | Exp. ID:0108RS09-1
Report Date: September 17, 2001 | Test Date: August 6, 2001

Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	< -8.00	< -8.00	> -4.00
HL-60(TB)	< -8.00	-4.32	> -4.00
K-562	< -8.00	-5.59	> -4.00
MOLT-4	< -8.00	< -8.00	> -4.00
RPMI-8226	< -8.00	< -8.00	> -4.00
SR	< -8.00	< -8.00	-4.19
Non-Small Cell Lung Cancer			
A549/ATCC	< -8.00	-4.70	> -4.00
EKVX	< -8.00	-5.67	> -4.00
HOP-62	< -8.00	-4.89	> -4.00
HOP-92	< -8.00	-7.77	-5.36
NCI-H226	< -8.00	< -8.00	< -8.00
NCI-H23	< -8.00	-7.85	-6.31
NCI-H322M	< -8.00	-5.48	-4.37
NCI-H460	< -8.00	-6.97	> -4.00
NCI-H522	< -8.00	< -8.00	-7.37
Colon Cancer			
COLO 205	< -8.00	-6.19	-4.06
HCC-2998	< -8.00	< -8.00	-7.75
HCT-116	< -8.00	-7.84	-4.99
HCT-15	< -8.00	-6.06	-4.25
HT29	< -8.00	-7.37	-4.42
KM12	< -8.00	-6.00	-4.23
SW-620	< -8.00	> -4.00	> -4.00
CNS Cancer			
SF-268	< -8.00	-6.92	> -4.00
SF-295	< -8.00	< -8.00	-6.63
SF-539	< -8.00	< -8.00	< -8.00
SNB-19	< -8.00	> -4.00	> -4.00
SNB-75	< -8.00	< -8.00	-7.50
U251	< -8.00	-5.99	> -4.00
Melanoma			
LOX IMVI	< -8.00	-7.87	> -4.00
MALME-3M	< -8.00	< -8.00	-4.89
M14	< -8.00	-7.94	-6.73
SK-MEL-2	< -8.00	< -8.00	-6.79
SK-MEL-28	< -8.00	< -8.00	< -8.00
UACC-257	< -8.00	< -8.00	-4.37
UACC-62	< -8.00	< -8.00	-7.04
Ovarian Cancer			
IGROV1	< -8.00	-5.18	> -4.00
OVCAR-3	< -8.00	-4.88	> -4.00
OVCAR-4	< -8.00	< -8.00	-5.52
OVCAR-5	< -8.00	-7.26	> -4.00
OVCAR-8	< -8.00	-7.70	-6.55
SK-OV-3	< -8.00	< -8.00	> -4.00
Renal Cancer			
786-0	< -8.00	-5.37	> -4.00
A498	< -8.00	< -8.00	-7.94
ACHN	< -8.00	-5.27	> -4.00
CAKI-1	< -8.00	> -4.00	> -4.00
RXF 393	< -8.00	< -8.00	-7.64
SN12C	< -8.00	< -8.00	-6.20
TK-10	< -8.00	-7.13	> -4.00
UO-31	< -8.00	-5.88	-4.59
Prostate Cancer			
PC-3	< -8.00	-5.81	-4.95
DU-145	< -8.00	> -4.00	> -4.00
Breast Cancer			
MCF7	< -8.00	> -4.00	> -4.00
NCI/ADR-RES	< -8.00	< -8.00	-6.75
MDA-MB-231/ATCC	< -8.00	< -8.00	-6.86
MDA-MB-435	< -8.00	< -8.00	< -8.00
MDA-N	< -8.00	< -8.00	> -4.00
BT-549	< -8.00	< -8.00	-7.22
T-47D	< -8.00	> -4.00	> -4.00
MG_MID	-8.00	-6.79	-5.20
Delta	0.00	1.21	2.80
Range	0.00	4.00	4.00

+3 +2 +1 0 -1 -2 -3

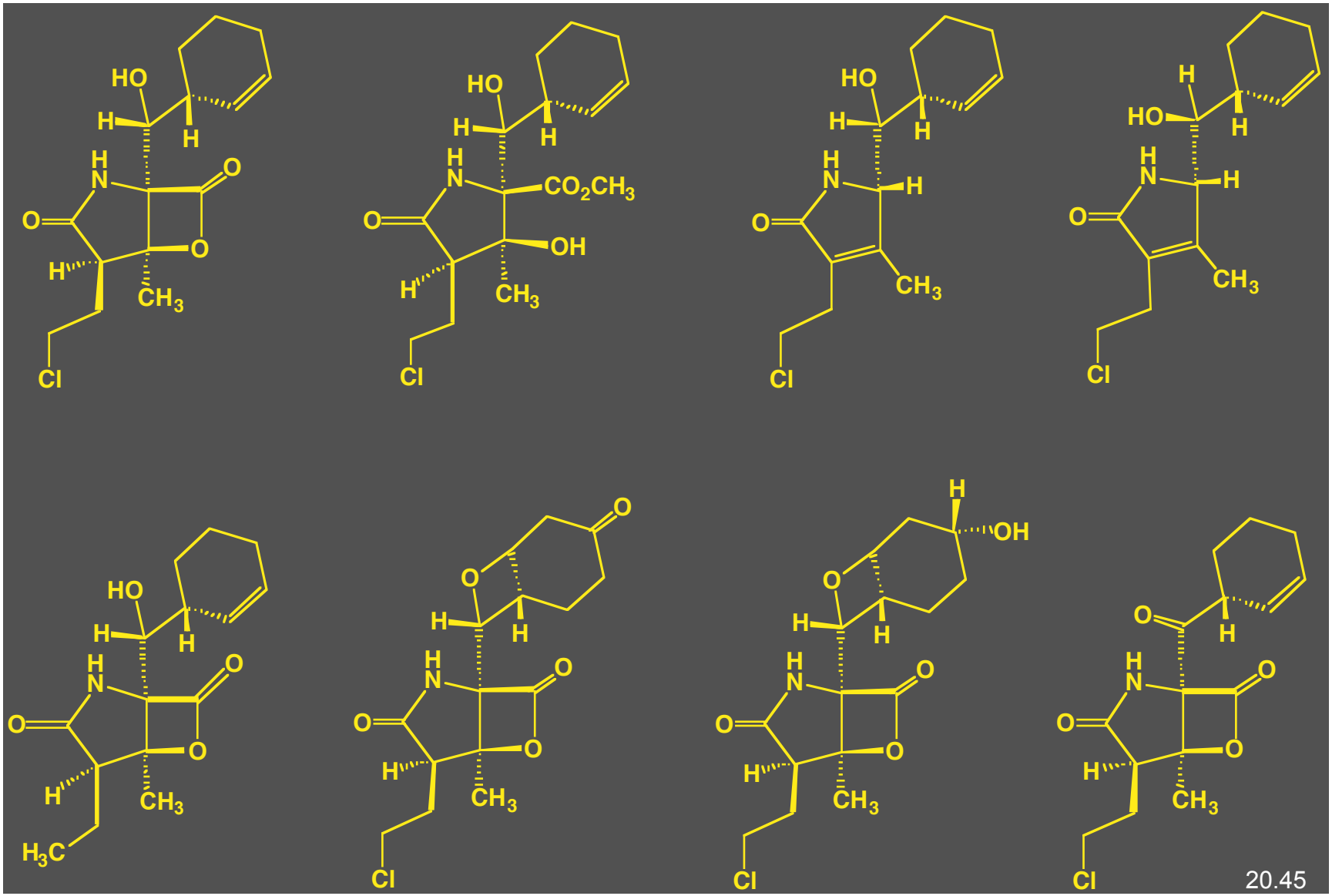
GI_{50} < 10 nM

TGI 0.2 μM

LC_{50} 6.3 μM

- **At 10 nM, never reached a GI_{50} for any cell line.**
- **Over a 4 log range between resistant and sensitive cell types.**
- **Several diverse cancer types show excellent sensitivities.**

Other Salinosporamides Isolated



Mycolactone: A Polyketide Toxin from *Mycobacterium ulcerans* Required for Virulence

Kathleen M. George,[1] Delphi Chatterjee,[2] Geewananda Gunawardana,[3] Diane Welty,[1] John Hayman,[4] Richard Lee,[5] P. L. C. Small[1]*

5 FEBRUARY 1999 VOL 283 SCIENCE

Fig. 3. Pathology in guinea pig skin after intradermal injection of mycolactone and *M. ulcerans* 8 days after infection. (**A**) Injection of mycobacterial medium or tox$^-$ mutant *M. ulcerans* 1615A did not produce a lesion, whereas injection of either viable *M. ulcerans* or 100 µg of mycolactone resulted in formation of a necrotic, noninflammatory lesion. Histopathology was evaluated from paraffin-embedded sections stained with hematoxylin and eosin. WT, wild type.

"We speculate that mycolactone protects *M.ulcerans* from predatory eukaryotes in its natural habitat(22, 23)."

Mycolactone: a metabolite that induces necrosis

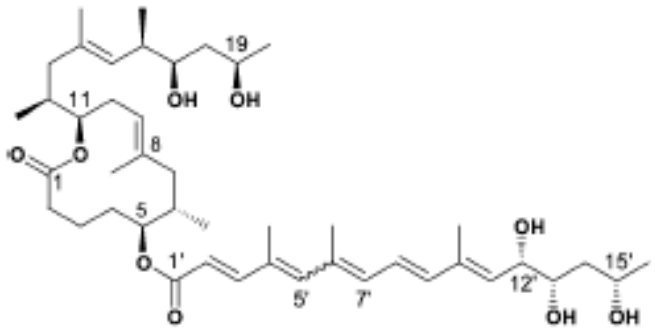


Fig. 1 The structures of mycolactone A (Z-$\Delta^{4',5'}$) and B (E-$\Delta^{4',5'}$).

Hui Hong,[a] Paul J. Gates,[a] James Staunton,[a] Tim Stinear,[b] Stewart T. Cole,[b] Peter F. Leadlay[ab] and Jonathan B. Spencer*[a]

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[b] *Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, Paris 75015, France*

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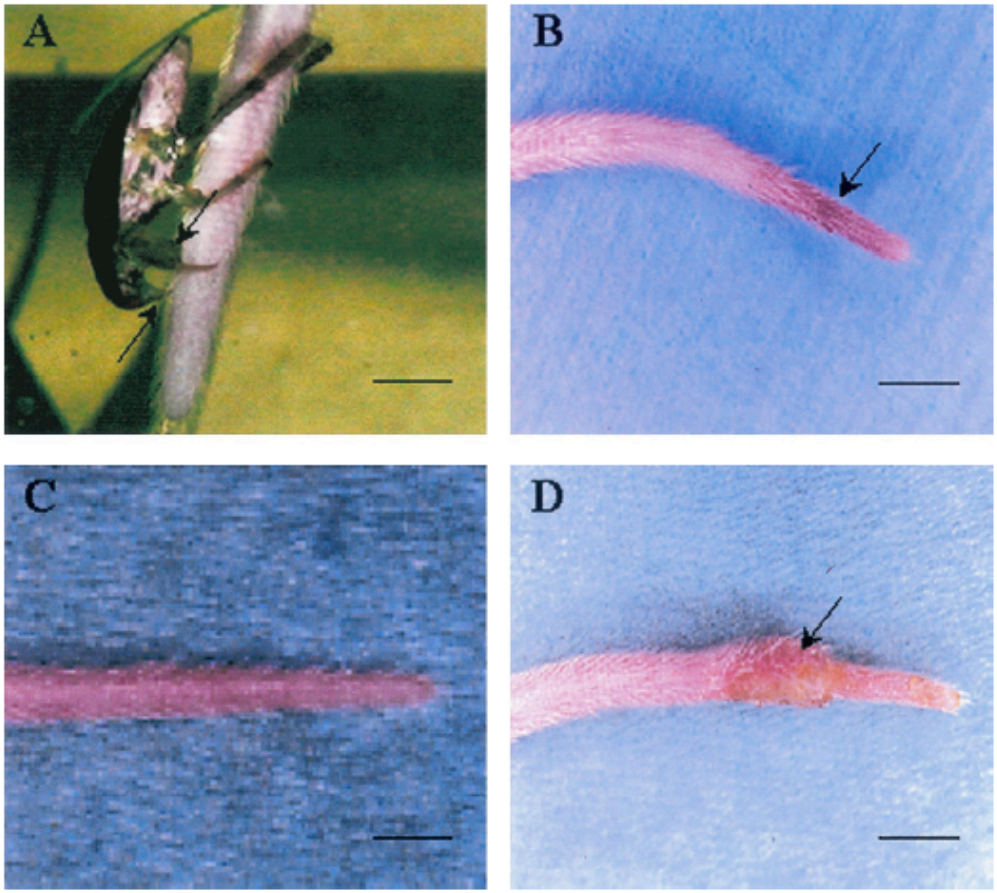
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.9.4623–4628.2002

Aquatic Insects as a Vector for *Mycobacterium ulcerans*

Laurent Marsollier,[1] Raymond Robert,[2] Jacques Aubry,[3] Jean-Paul Saint André,[4] Henri Kouakou,[5] Pierre Legras,[1] Anne-Lise Manceau,[1] Chetaou Mahaza,[1] and Bernard Carbonnelle[1]*



Rethinking 'secondary' metabolism: physiological roles for phenazine antibiotics

Alexa Price-Whelan[1], Lars E P Dietrich[2] & Dianne K Newman[2,3]

NATURE CHEMICAL BIOLOGY VOLUME 2 NUMBER 2 FEBRUARY 2006

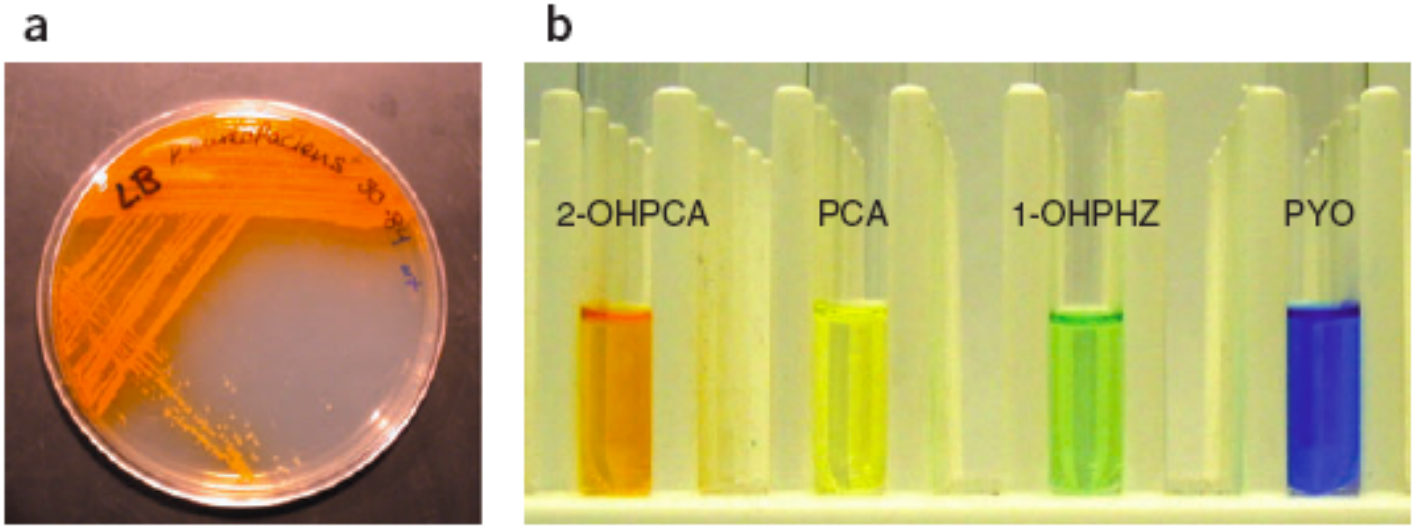
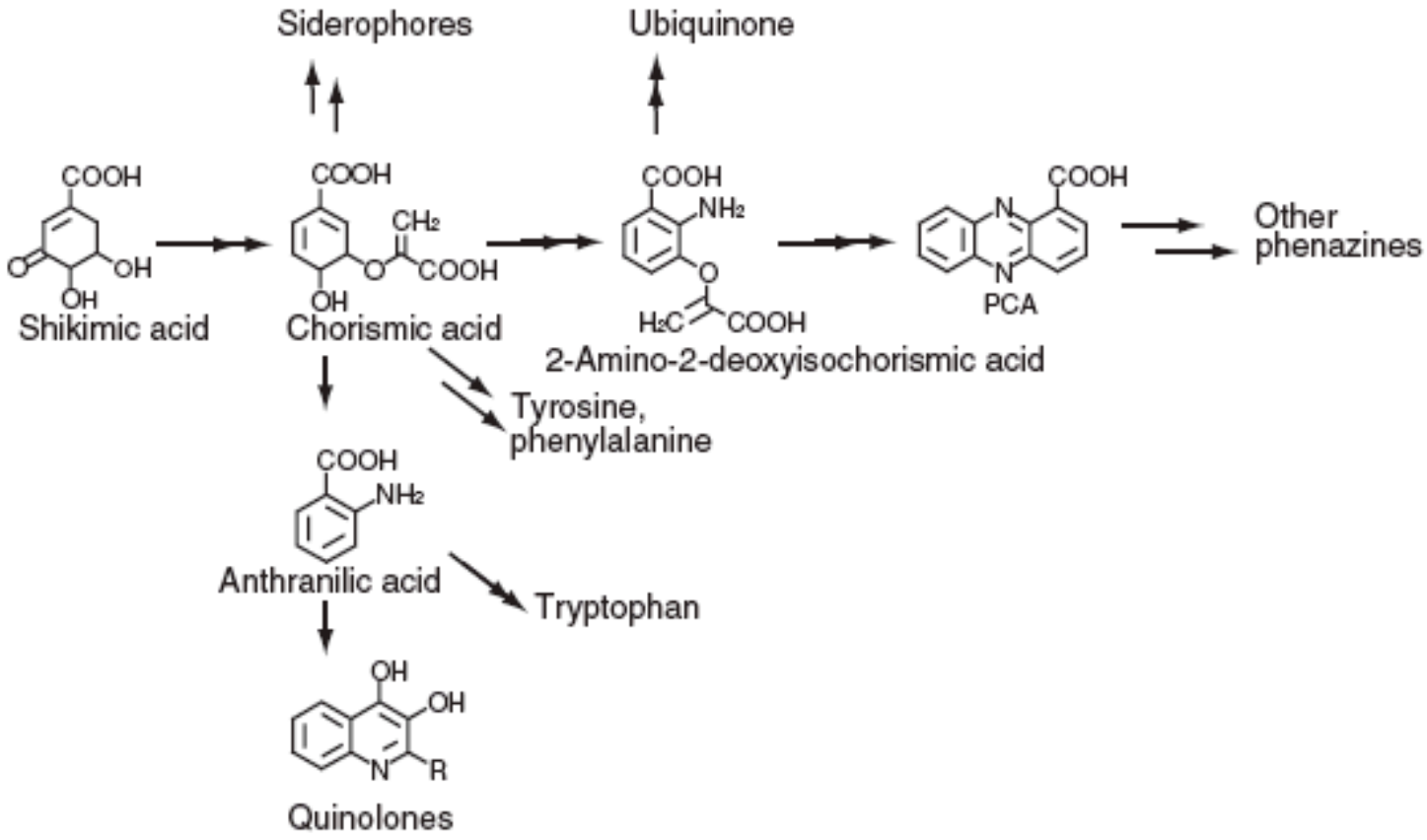
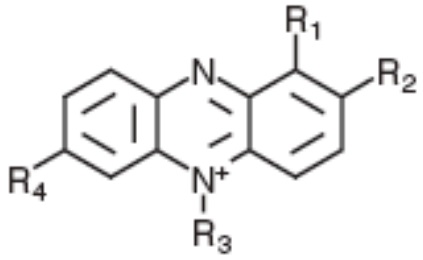


Figure 1 Phenazines are colorful, diffusible bacterial metabolites. (**a**) Streak plate of the biocontrol strain *P. aureofaciens* 30-84. The phenazine 2-OHPCA turns the agar bright orange. (**b**) Aqueous solutions of some of the phenazines produced by various *Pseudomonas* strains.



Scheme 1 Phenazine biosynthesis and its relation to the shikimic acid pathway in pseudomonads[24,45,46,93].



No	Name	R_1	R_2	R_3	R_4	K_{OW} ox	K_{OW} red	$E^{0\prime}$ (mV)
1	Aeruginosin A	COOH		CH_3	NH_2	–0.71	0.46	NA
2	Phenazine-1-carboxylic acid (PCA)	COOH				2.17	3.72	–177
3	Pyocyanin (PYO)	OH		CH_3		1.60	2.89	–34
4	2-Hydroxyphenazine-1-carboxylic acid (2-OHPCA)	COOH	OH			2.54	3.32	NA
5	Phenazine-1-carboxamide (PCN)	$CONH_2$				1.04	2.19	–115
6	1-Hydroxyphenazine (1-OHPHZ)	OH				1.81	2.35	–172

Table 1 Characteristics of some phenazines excreted by pseudomonads[90–92]. Octanol-water partition coefficients (K_{ow}) were calculated using the KOWWIN demo program available at http://www.syrres.com/esc/est_kowdemo.htm. $E^{o\prime}$, standard redox potential, pH 7; NA, not available.

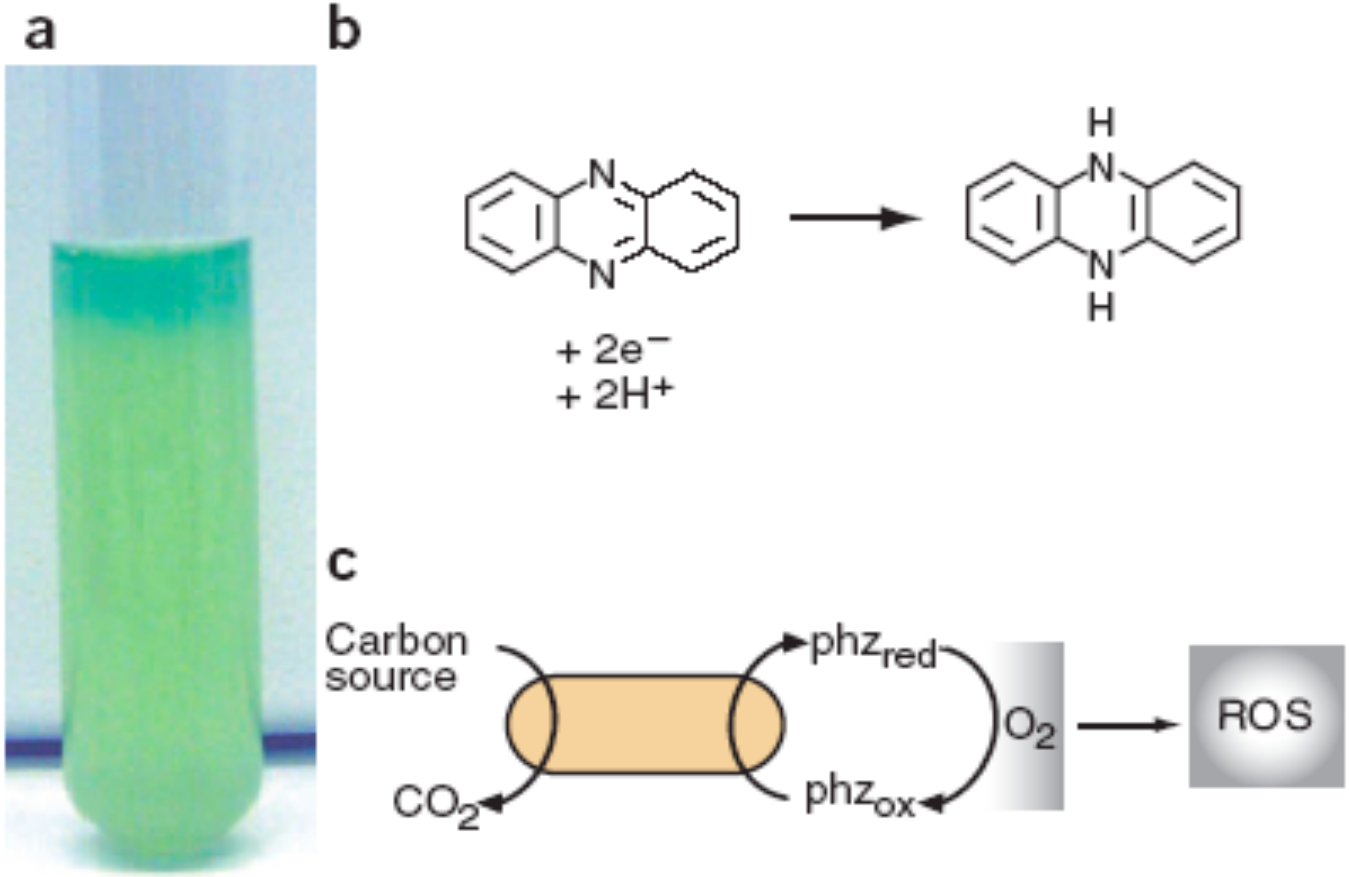


Figure 2 Pseudomonads stimulate phenazine reduction. (**a**) Characteristic gradient formed by standing cultures of *P. aeruginosa*. Bacterial respiration renders most of the culture anoxic. Phenazines (phz) are reduced and, in the case of PYO, become colorless. The darker blue at the top represents oxidized PYO. (**b**) Half-reaction representing generic two-electron phenazine reduction. (**c**) Schematic of phenazine reduction and auto-oxidation responsible for gradient formation in standing cultures. Reduced phenazines are oxidized abiotically by oxygen, generating reactive oxygen species (ROS).