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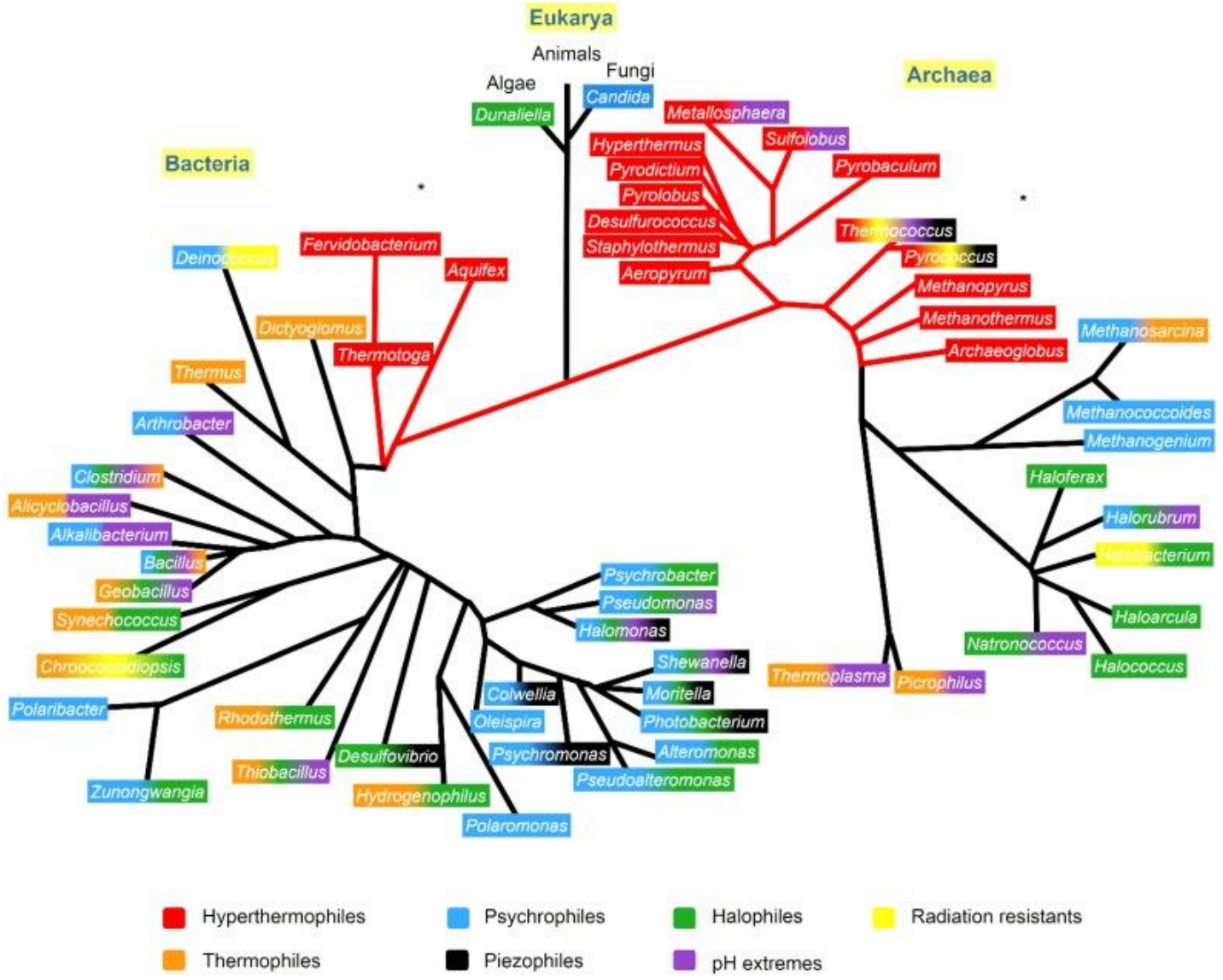
Linking Genomics and Metabolomics to Discover Bioactive Natural Products in Marine Extremophiles

Extremophiles are classified as living organisms able to survive and proliferate in environments with extreme physical (temperature, pressure, radiation, pollution) and geochemical parameters (salinity, pH, redox potential).

The vast majority of extremophile organisms belong to the prokaryotes (Archaea and Bacteria).



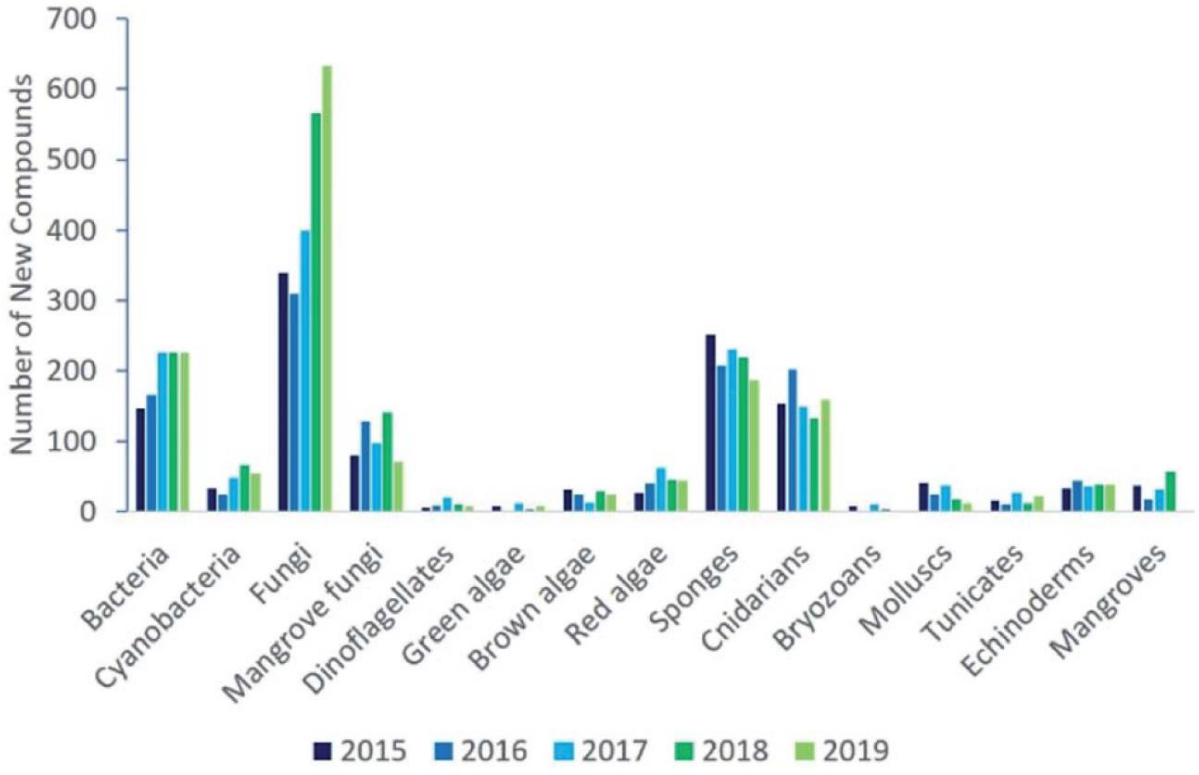
Mar Drugs, 2015, 13(4):1925-65. doi: 10.3390/md13041925.

Marine Natural Products

Many bioactive compounds from marine organisms are produced by **associated microorganisms** rather than the original source.

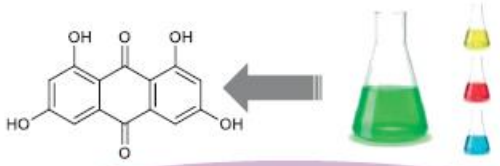
This observation has moved attention towards exploration of the **symbiotic microbiome**, thereby leading to identification of microbial producers of bioactive molecules.

In parallel, researchers focused on the chemical profiling of **free-living marine bacteria and fungi**.



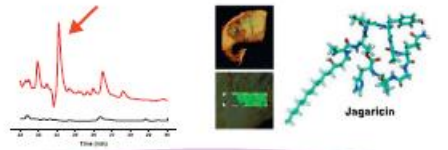
Nat. Prod. Rep., 2021,38, 362-413. doi.org/10.1039/D0NP00089B.

Strategies in Natural Product Discovery



Culture condition screening

Diverse sampling



Comparative metabolomic profiling

Top-down approaches begin with the sample collection for extraction or lab cultivation.
Extracts are screened for a desired bioactivity, with 'hits' isolated for structural characterization.

Top-Down Approaches

Natural Product Discovery

Bottom-Up Approaches

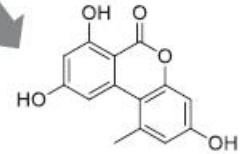
Bottom-up approaches allow discovery of NPs undetected under standard fermentation conditions, using functional genomics, bioinformatics, and genetic manipulation tools.

Bioinformatics tools

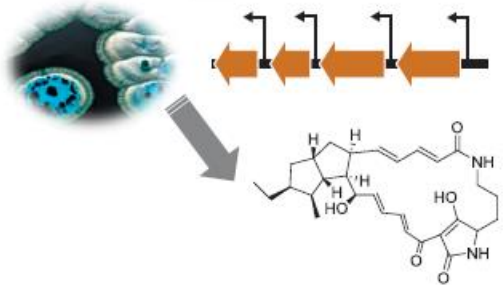
SMURF

anti SMASH

Native host



Heterologous host



Curr. Opin. Biotechnol., 2014,30, 230-237 . doi: 10.1016/j.copbio.2014.09.002.

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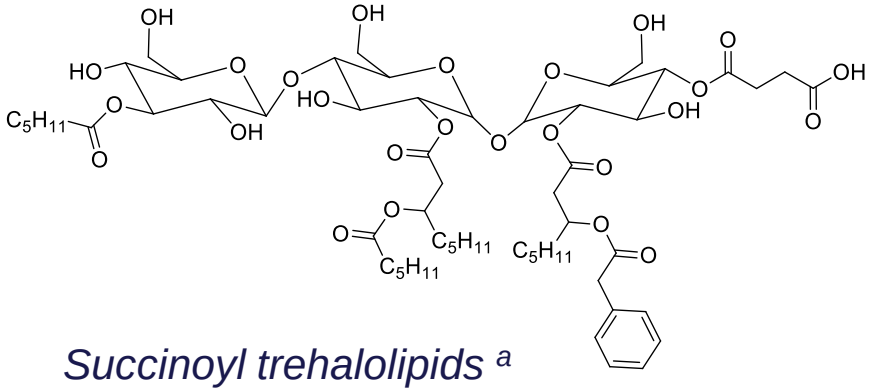
Lipopeptides from a Deep-Sea *Rhodococcus*

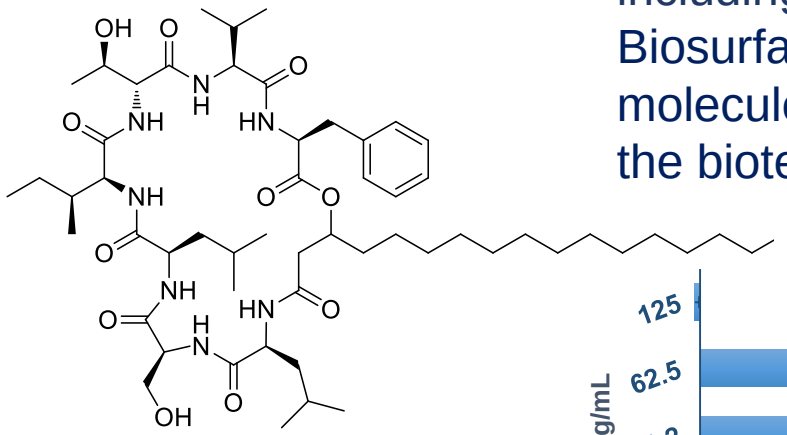
The *Rhodococcus* sp. I2R was isolated on agar plates containing Mineral Salt Medium (MSM) supplemented with phenanthrene.

Biosurfactant activity of the lipopeptide enriched fraction from *Rhodococcus* sp. I2R in the oil displacement assay

The bacterium was found to produce two distinct classes of biosurfactants, including glycolipids and lipopeptides. Biosurfactants are amphiphilic molecules, which play a crucial role in the biotech and biomedical industry.

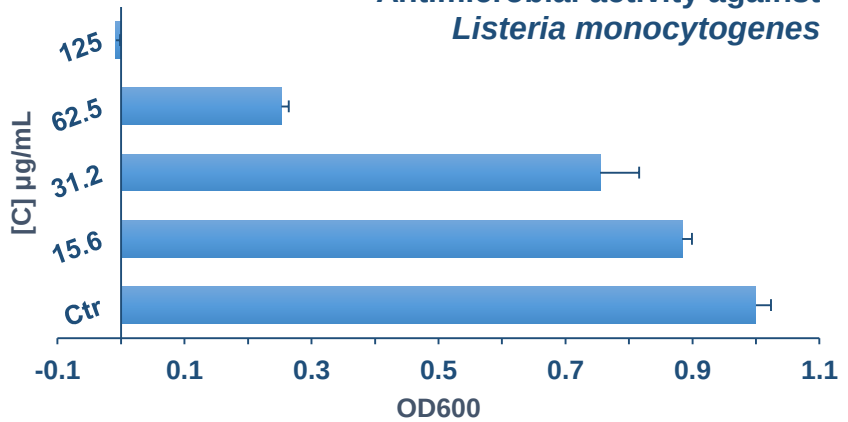


Succinoyl trehalolipids [a]



Lipopeptides

Antimicrobial activity against *Listeria monocytogenes*



[a] *Int. J. Mol. Sci.* 2021, 2(16):9055. doi: 10.3390/ijms22169055

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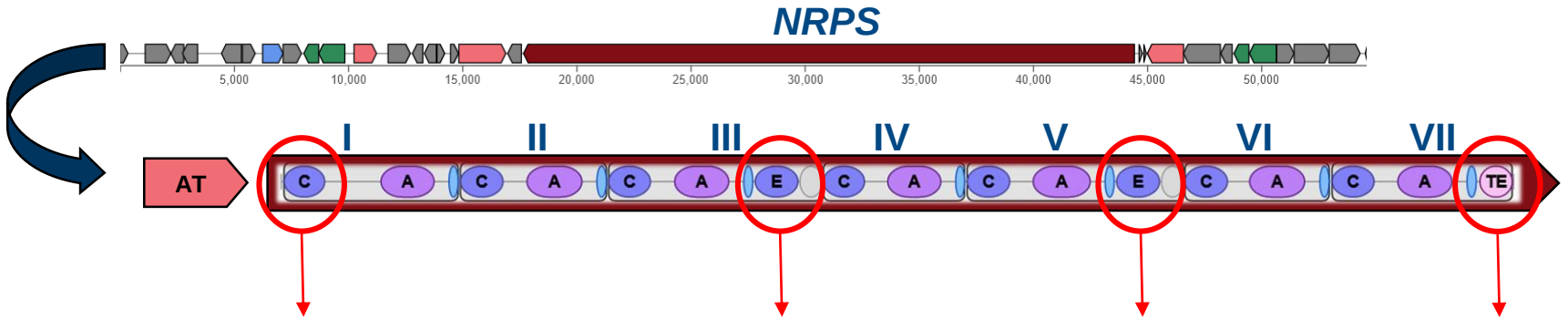
Structure and biosynthesis of novel lipopeptides from *Rhodococcus* sp. I2R

***Rhodococcus* sp. I2R**

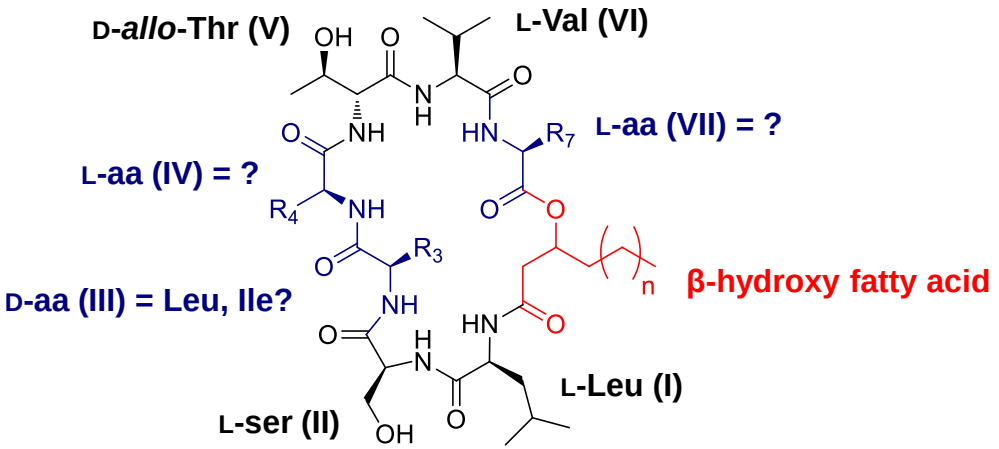
Genome mining



Structure prediction

Adenylation domain selectivity (Stachelhaus code)

	Prediction	Binding pocket signatures (position)								
		235	236	239	278	299	301	322	330	331
Ad_I	L-Leu	D	A	L	F	V	G	A	V	F
Ad_II	L-Ser	D	V	W	H	F	S	L	V	D
Ad_III	D-Leu or *allo*-Ile	D	A	L	F	A	G	A	I	F
Ad_IV	L-aa?	D	A	L	F	V	G	A	V	F
Ad_V	D-*allo*-Thr	D	F	W	N	I	G	M	V	H
Ad_VI	L-Val	D	A	L	F	V	G	G	I	M
Ad_VII	L-aa?	D	A	Y	F	A	G	G	I	Q



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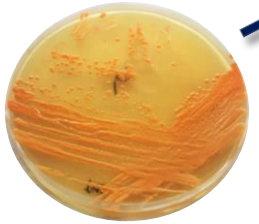
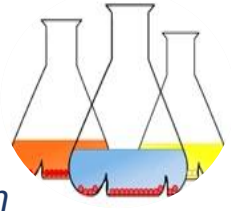
Structure and biosynthesis of novel lipopeptides from Rhodococcus sp. I2R

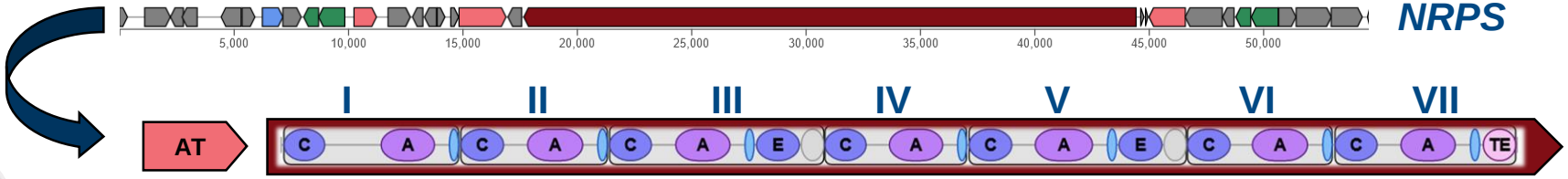
Genome mining



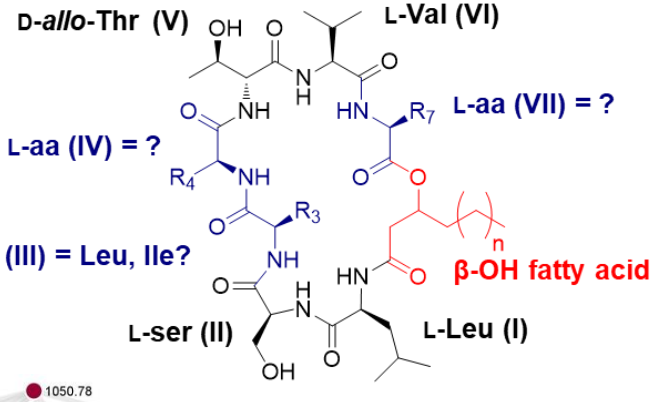
***Rhodococcus* sp. I2R**

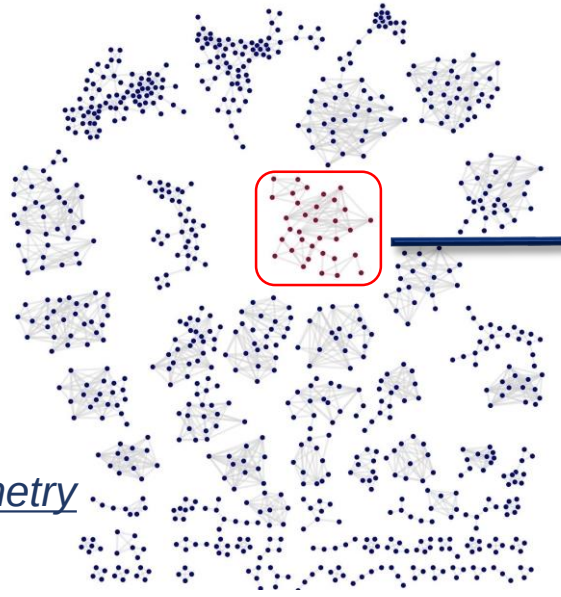


- *Cultivation in ASG medium with 1% hexadecane and 0.5 µM $FeCl_3$*
- *Chemical extraction*

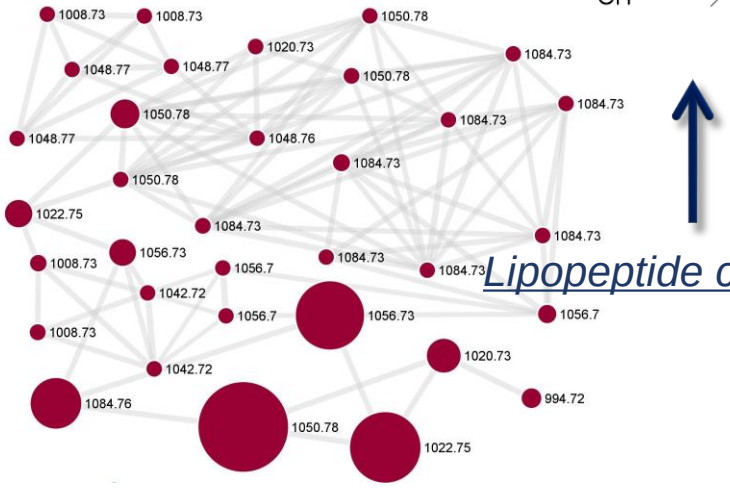


Structure prediction





Tandem Mass Spectrometry Molecular Networkig



Lipopeptide cluster

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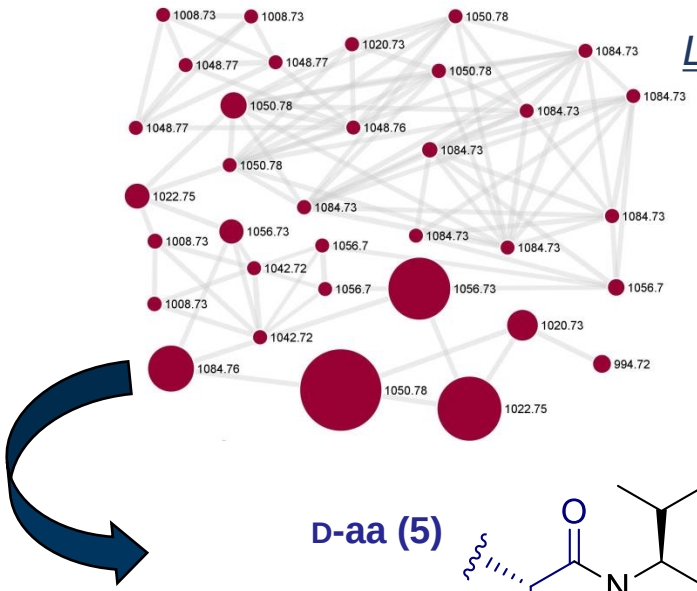
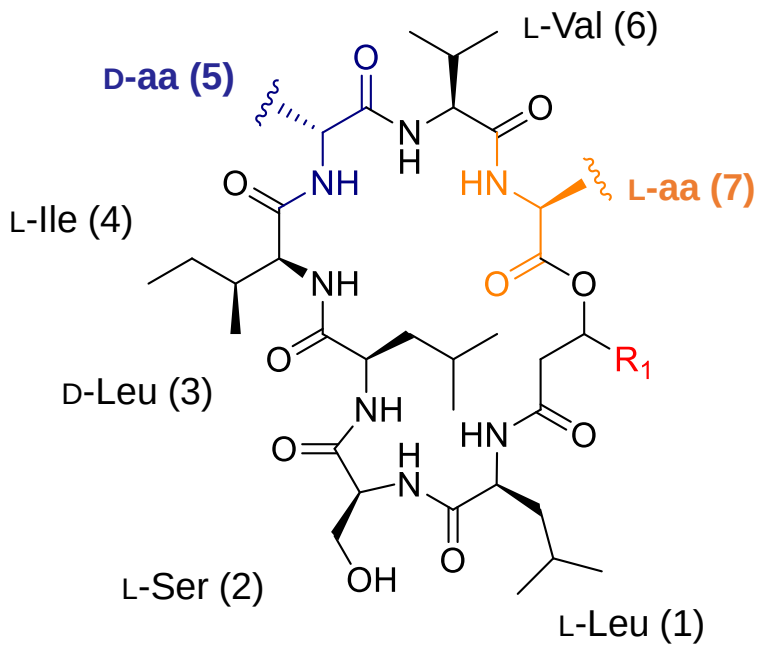
Structure and biosynthesis of novel lipopeptides from Rhodococcus sp. I2R



Lipopeptide cluster



Lipopeptides from *Rhodococcus* sp. I2R

[M+H]+	m/z	Δ (ppm)	R_t (min)	R_1	aa-5	aa-7
$C_{59}H_{102}N_7O_{11}$	1084.7633 [a]	0.1	15.3	$C_{17}H_{35}$	D-allo-Thr	L-Phe
$C_{54}H_{100}N_7O_{11}$	1022.7470	-0.5	30.1	$C_{15}H_{31}$	D-allo-Thr	L-Leu/Ile
$C_{57}H_{98}N_7O_{11}$	1056.7317	-0.1	28.8	$C_{15}H_{31}$	D-allo-Thr	L-Phe
$C_{56}H_{104}N_7O_{11}$	1050.7787	-0.1	19.1	$C_{17}H_{35}$	D-allo-Thr	L-Leu/Ile
$C_{56}H_{102}N_7O_{11}$	1048.7637	0.5	29.8	$C_{17}H_{33}$	D-allo-Thr	L-Leu/Ile
$C_{54}H_{98}N_7O_{11}$	1020.7308	-1.1	23.2	$C_{15}H_{29}$	D-allo-Thr	L-Leu/Ile
$C_{57}H_{96}N_7O_{11}$	1054.7154	-1.1	22.3	$C_{15}H_{29}$	D-allo-Thr	L-Phe
$C_{58}H_{106}N_7O_{11}$	1076.7948	0.3	14.7	$C_{19}H_{37}$	D-allo-Thr	L-Leu/Ile
$C_{58}H_{100}N_7O_{11}$	1070.7470	-0.5	30.4	$C_{15}H_{31}$	D-allo-Thr	L-Phe
$C_{59}H_{100}N_7O_{11}$	1082.7472	-0.3	28.6	$C_{17}H_{33}$	D-allo-Thr	L-Phe
$C_{63}H_{108}N_7O_{11}$	1138.8105	0.3	32.3	$C_{21}H_{41}$	D-allo-Thr	L-Phe
$C_{60}H_{110}N_7O_{11}$	1104.8262	0.4	34.8	$C_{21}H_{41}$	D-allo-Thr	L-Leu/Ile
$C_{57}H_{106}N_7O_{11}$	1064.7940	-0.4	22.1	$C_{18}H_{37}$	D-allo-Thr	L-Leu/Ile
$C_{56}H_{96}N_7O_{11}$	1042.7154	-0.8	26.6	$C_{15}H_{31}$	D-Ser	L-Phe
$C_{55}H_{94}N_7O_{11}$	1028.7001	-0.5	21.4	$C_{13}H_{27}$	D-allo-Thr	L-Phe
$C_{53}H_{98}N_7O_{11}$	1008.7305	-1.4	26.0	$C_{14}H_{29}$	D-allo-Thr	L-Leu/Ile
$C_{53}H_{98}N_7O_{11}$	1008.7311	-0.8	27.3	$C_{15}H_{31}$	D-Ser	L-Leu/Ile
$C_{55}H_{102}N_7O_{12}S$	1084.7290	-1.1	25.8	$C_{17}H_{35}$	D-allo-Thr	L-MetO
$C_{53}H_{98}N_7O_{12}S$	1056.6981	-0.7	21.8	$C_{15}H_{31}$	D-allo-Thr	L-MetO
$C_{52}H_{96}N_7O_{11}$	994.7152	-1.0	22.3	$C_{13}H_{27}$	D-allo-Thr	L-Leu/Ile
$C_{60}H_{104}N_7O_{11}$	1098.7781	-0.7	18.9	$C_{18}H_{37}$	D-allo-Thr	L-Phe

[a] *J Am Soc Mass Spectrom*, 2020, 31(3), 611–623. doi.org/10.1021/jasms.9b00059

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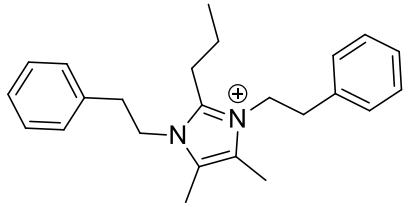
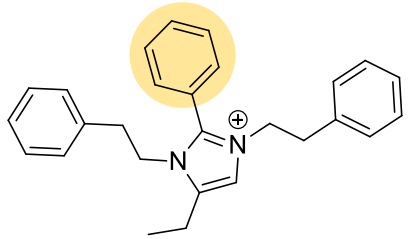
Imidazolium alkaloids from *Shewanella aquimarina*

Shewanella aquimarina was isolated from a seawater sample collected in the Yellow Sea (South Korea) and deposited in the Pasteur Institute Collection.

Combining OSMAC approach and tandem mass spectrometry molecular networking showed the bacterium to be a source of novel imidazolium alkaloids, namely shewazoles.

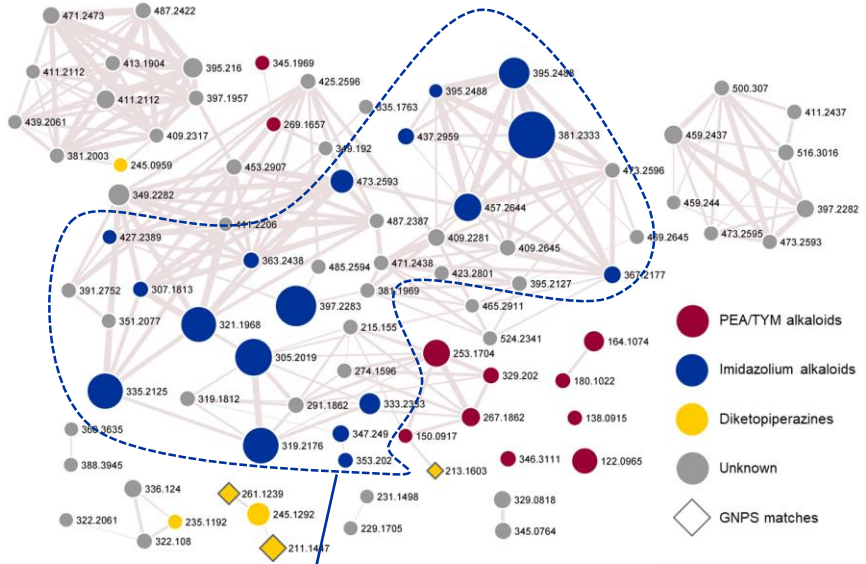


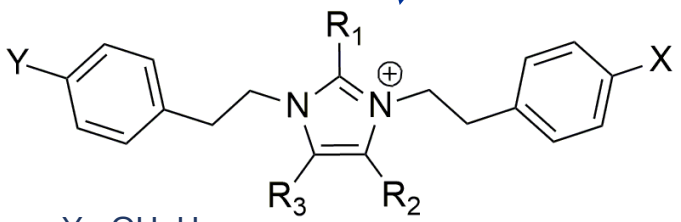
discolin A



shewazole G

These cationic biosurfactants share the central imidazolium core with discolins A,B, and F-H from *Tenacibaculum discolor* sv11 and stand out as featuring a different substitution pattern. Indeed, several congeners bear an unprecedented phenyl group.





Y= OH, H
X= OH, H
R_1= H, CH_3, C_3H_7, C_4H_9, Ph
R_2= H, CH_3, C_2H_5, C_3H_7, Ph
R_3= H, CH_3, C_2H_5

Biosurfactant activity of shewazoles in the oil displacement assay

Pharmaceutics, 2023, 15(8), 2139. doi.org/10.3390/pharmaceutics15082139.

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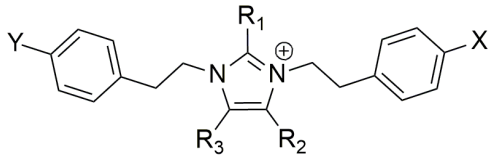
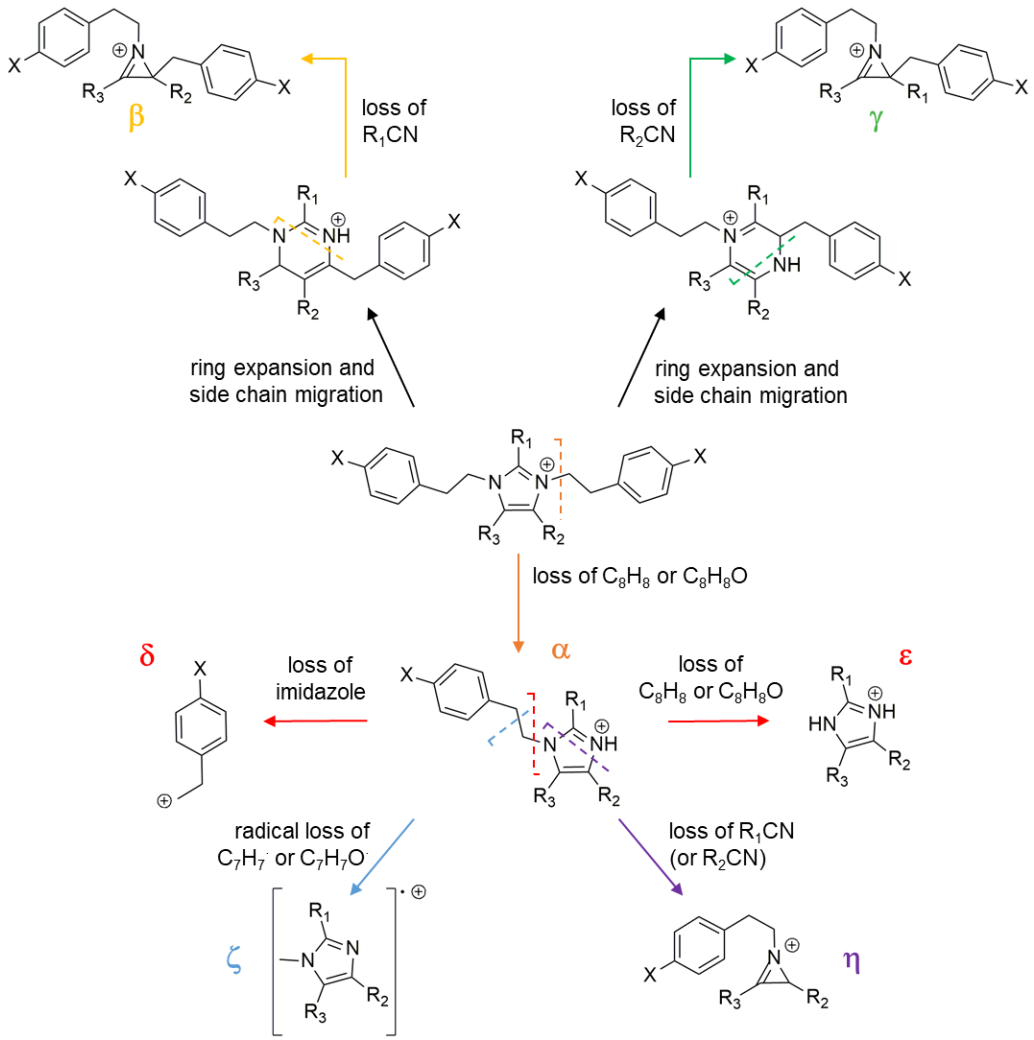
Imidazolium alkaloids from Shewanella aquimarina



Imidazolium PEA Alkaloids								
Compound	**R_t (min.)**	**$[M+H]^+$**	***m/z***	**R_1**	**R_2**	**R_3**	**X**	Y
shewazole A	15.8	$C_{20}H_{23}N_2$	291.1862	H	CH_3	H	H	H
bacillimidazole A	16.5	$C_{21}H_{25}N_2$	305.2017	H	CH_3	CH_3	H	H
shewazole B	17.1	$C_{22}H_{27}N_2$	319.2174	CH_3	H	C_2H_5	H	H
isoshewazole B	17.5	$C_{22}H_{27}N_2$	319.2174	H	C_2H_5	CH_3	H	H
shewazole C	17.9	$C_{23}H_{29}N_2$	333.2333	CH_3	C_2H_5	CH_3	H	H
isoshewazole C	18.5	$C_{23}H_{29}N_2$	333.2333	C_3H_7	CH_3	H	H	H
shewazole D	18.5	$C_{25}H_{25}N_2$	353.2018	H	Ph	H	H	H
shewazole E	18.8	$C_{26}H_{27}N_2$	367.2178	Ph	CH_3	H	H	H
shewazole F	18.9	$C_{24}H_{31}N_2$	347.2490	CH_3	C_3H_7	CH_3	H	H
isoshewazole F	19.3	$C_{24}H_{31}N_2$	347.2490	C_4H_9	CH_3	H	H	H
shewazole G	19.4	$C_{27}H_{29}N_2$	381.2331	Ph	H	C_2H_5	H	H
shewazole H	19.5	$C_{25}H_{33}N_2$	361.2645	C_4H_9	CH_3	CH_3	H	H
shewazole I	19.8	$C_{28}H_{31}N_2$	395.2488	Ph	C_2H_5	CH_3	H	H
isoshewazole I	20.0	$C_{28}H_{31}N_2$	395.2488	Ph	C_3H_7	H	H	H
shewazole J	21.6	$C_{33}H_{33}N_2$	457.2643	Ph	Ph	C_2H_5	H	H
shewazole K	21.8	$C_{31}H_{37}N_2$	437.2958	C_4H_9	Ph	C_2H_5	H	H

Imidazolium TYM Alkaloids								
Compound	**R_t (min.)**	**$[M+H]^+$**	***m/z***	**R_1**	**R_2**	**R_3**	**X**	**Y**
hydroxyshewazole A	13.5	$C_{20}H_{23}N_2O$	307.1809	H	CH_3	H	OH *	H
hydroxybacillimidazole A	14.3	$C_{21}H_{25}N_2O$	321.1967	H	CH_3	CH_3	OH	H
hydroxyshewazole B	14.8	$C_{22}H_{27}N_2O$	335.2122	CH_3	H	C_2H_5	OH	H
dihydroxyshewazole I	16.0	$C_{28}H_{31}N_2O_2$	427.2388	Ph	C_2H_5	CH_3	OH	OH
hydroxyshewazole F	16.8	$C_{24}H_{31}N_2O$	363.2438	CH_3	C_3H_7	CH_3	OH *	H
hydroxyshewazole H	17.3	$C_{25}H_{33}N_2O$	377.2596	C_4H_9	CH_3	CH_3	OH	H
hydroxyshewazole G	17.4	$C_{27}H_{29}N_2O$	397.2280	Ph	H	C_2H_5	OH *	H
hydroxyshewazole I	17.9	$C_{28}H_{31}N_2O$	411.2440	Ph	C_2H_5	CH_3	OH *	H

Proposed fragmentation pathway of shewazoles



Pharmaceutics, 2023, 15(8), 2139. doi.org/10.3390/pharmaceutics15082139.

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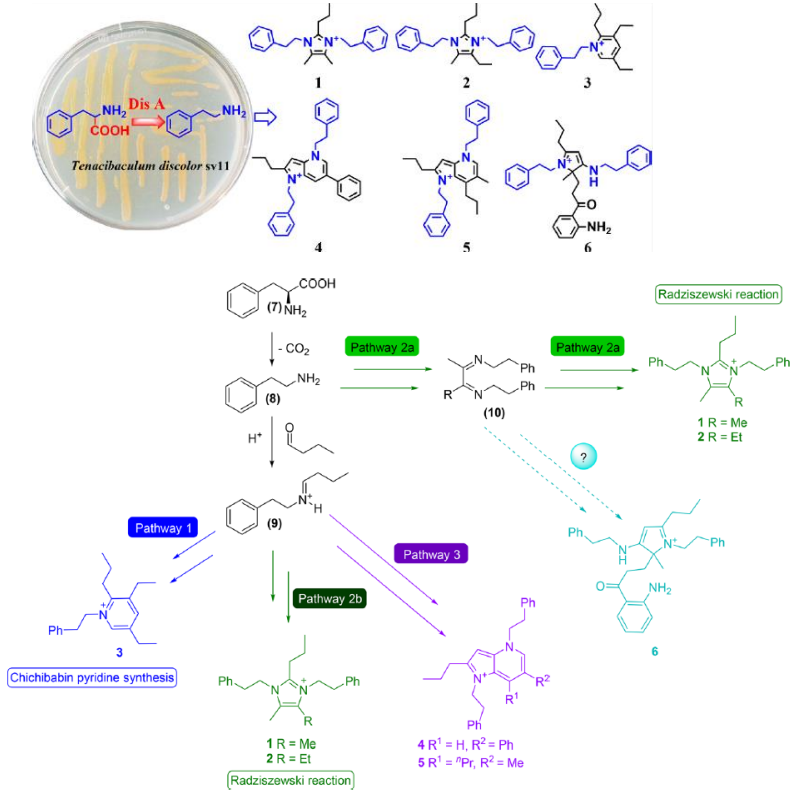
Imidazolium alkaloids from Shewanella aquimarina

Biosynthesis of shewazoles

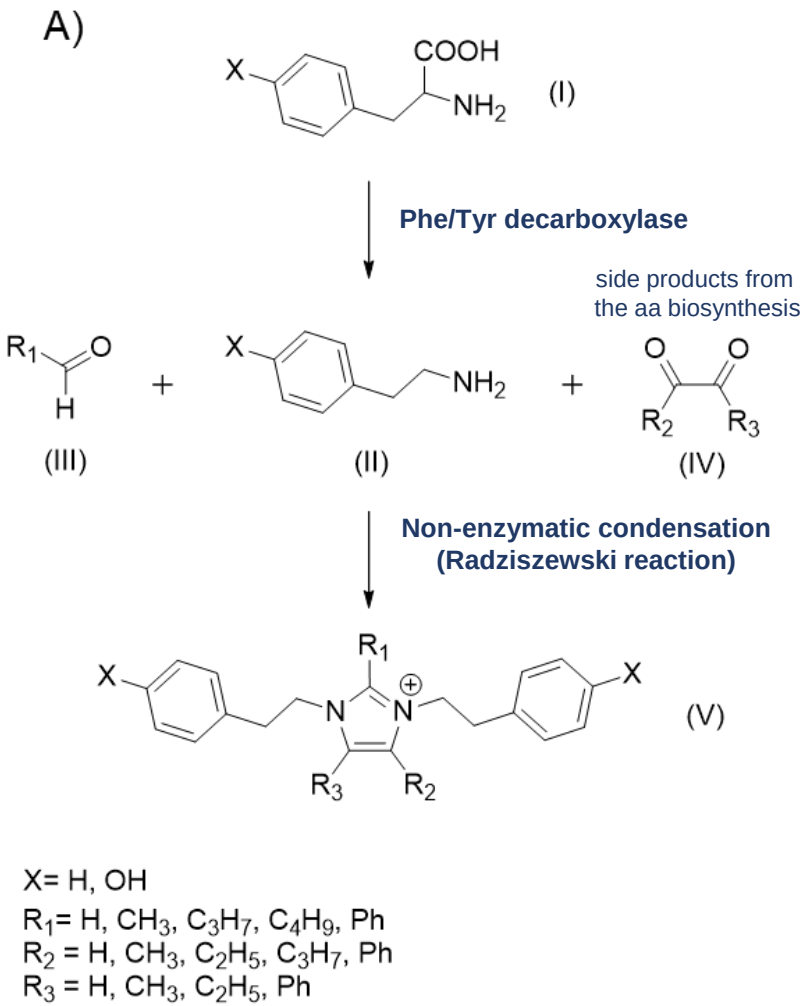
JOURNAL OF NATURAL PRODUCTS
pubs.acs.org/jnp Article

Discovery and Biosynthesis of Antimicrobial Phenethylamine Alkaloids from the Marine Flavobacterium *Tenacibaculum discolor* sv11

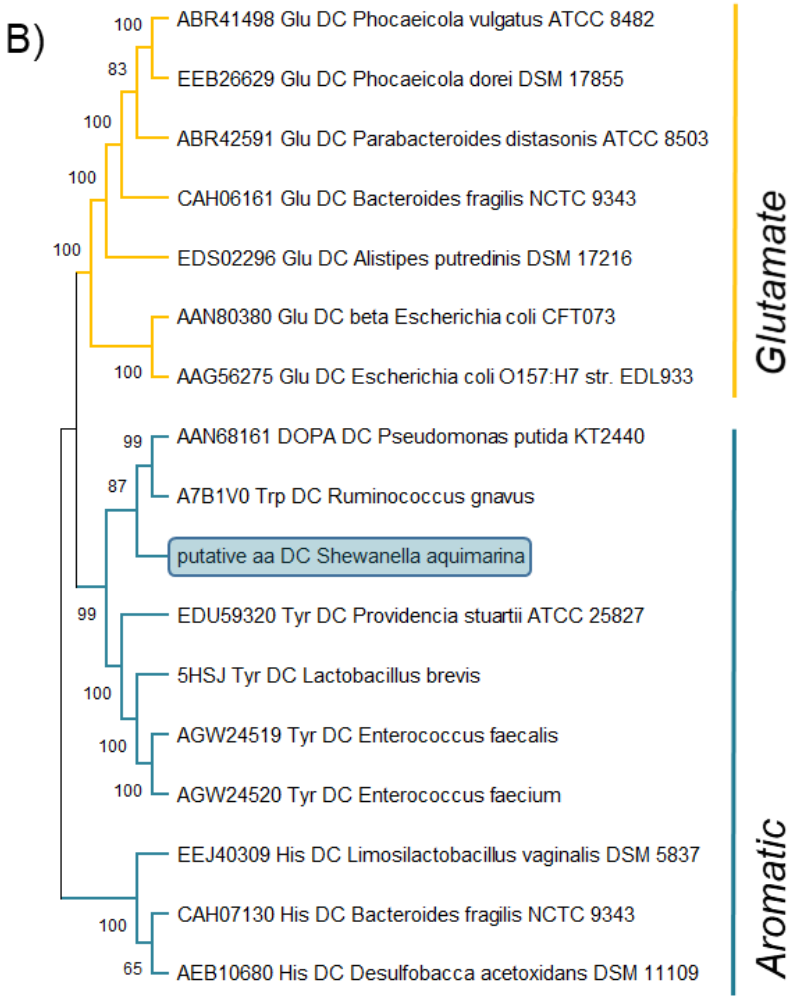
Lei Wang, Virginia Linares-Otoya, Yang Liu,* Ute Mettal, Michael Marner, Lizbeth Armas-Mantilla, Sabine Willbold, Tibor Kurtán, Luis Linares-Otoya,* and Till F. Schäberle*



Proposed biosynthetic pathway of shewazoles



Neighbor-joining tree of pyridoxal-dependent decarboxylases

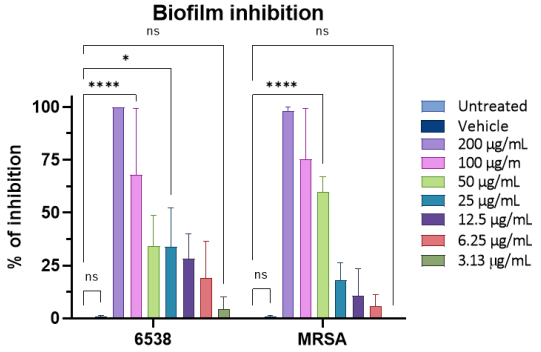


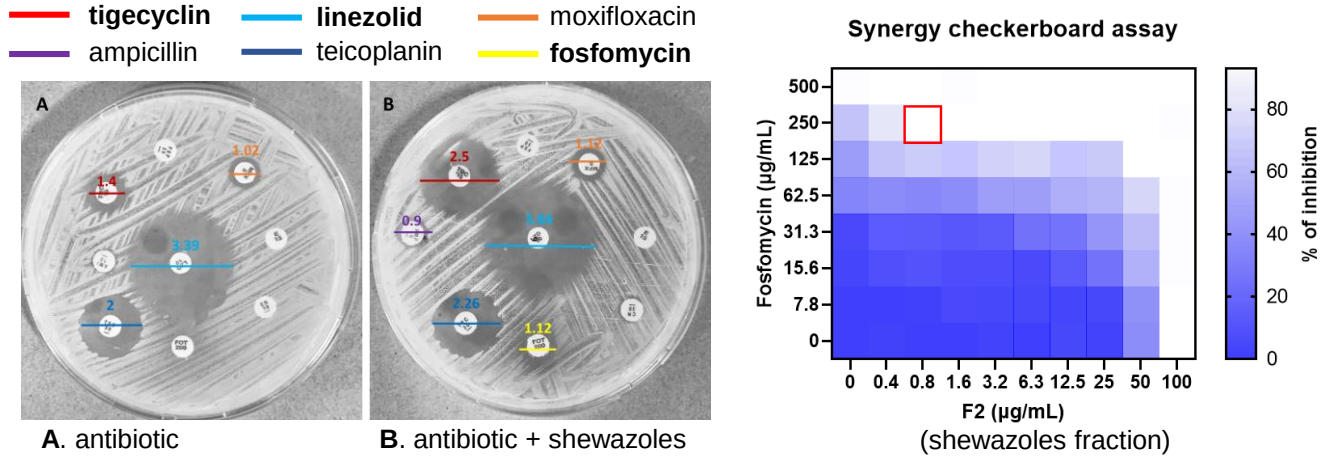
Gerardo Della Sala, PhD
Stazione Zoologica Anton Dohrn, Naples

31st INTERNATIONAL SYMPOSIUM ON THE CHEMISTRY OF NATURAL PRODUCTS
11th INTERNATIONAL CONGRESS ON BIODIVERSITY
15-19 October 2023, Naples

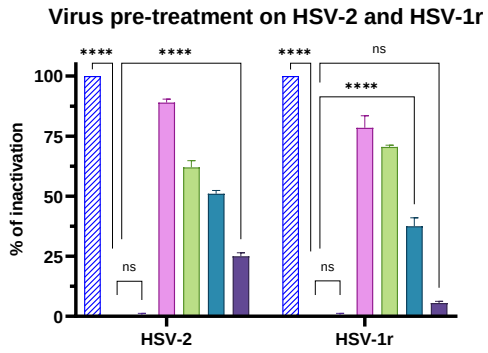
Broad-spectrum activity of shewazoles

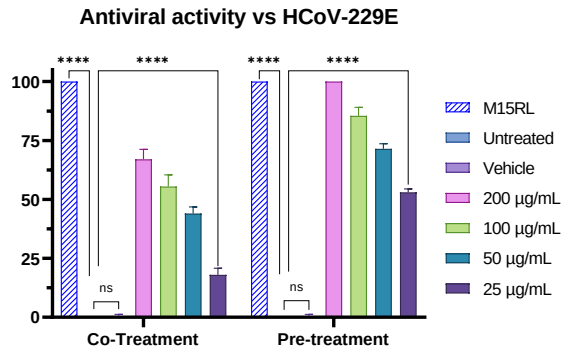
S. aureus Strain	MIC (µg/mL)	MBC (µg/mL)
ATCC 6538	100	100
MLSB	100	200
Quinolone res.	100	200
β-lactamase	100	200
MSSA	100	200
MRSA	100	100

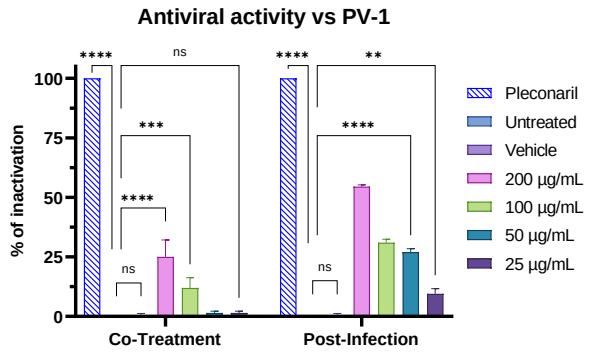


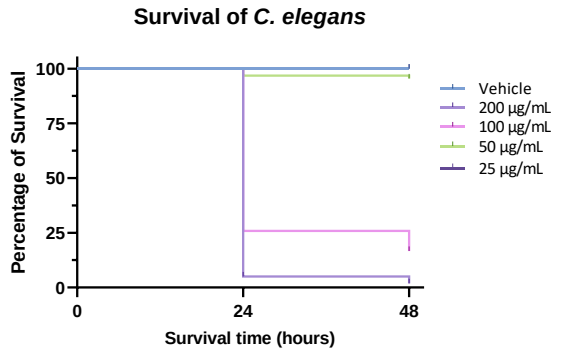


A. antibiotic **B**. antibiotic + shewazoles









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HORIZON 2020

SUSTAINABLE EXPLOITATION OF BIO-BASED COMPOUNDS REVEALED AND ENGINEERED FROM NATURAL SOURCES

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