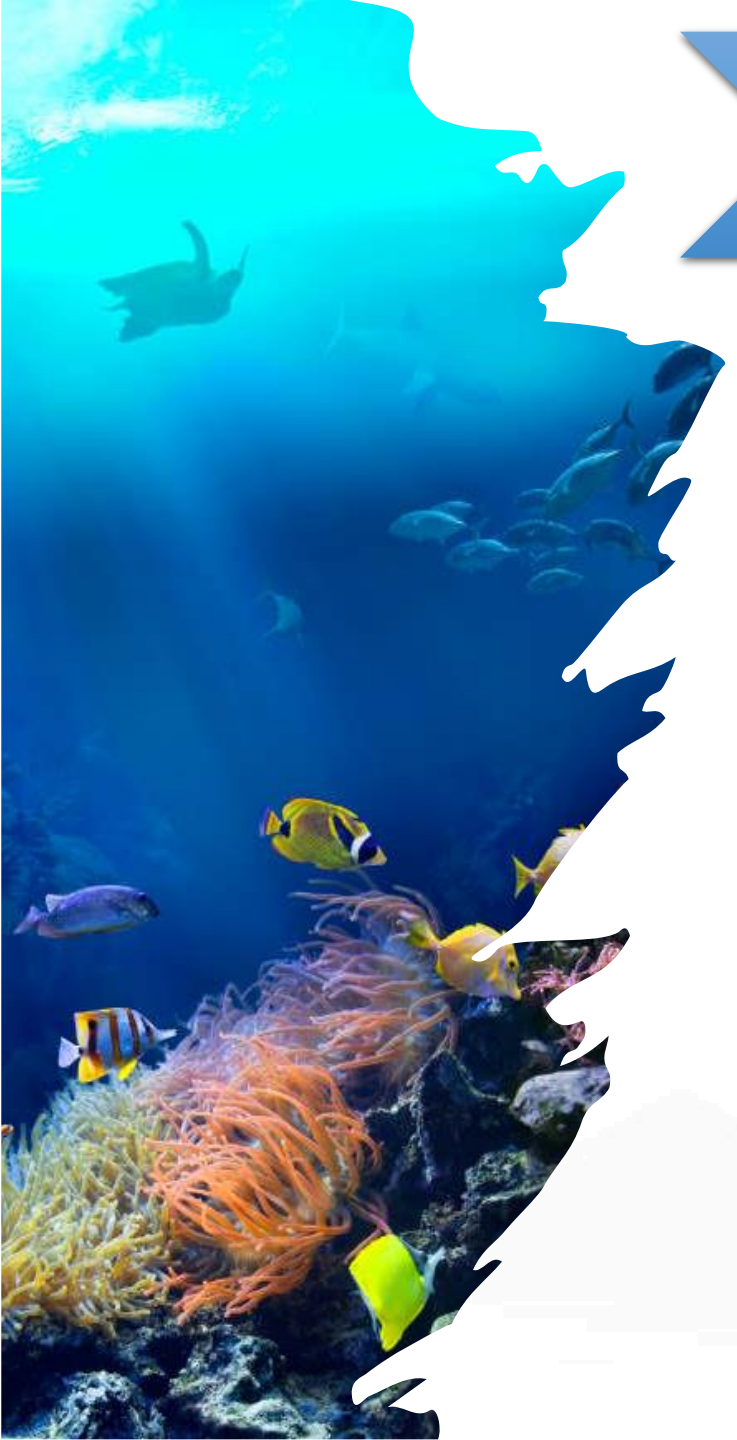
Combining genomic and metabolomic approaches to discover novel biosurfactants from a marine *Rhodococcus* sp. I2R

Costanza Ragozzino
PhD in Applied Biology and Experimental Medicine
Università degli Studi di Messina
Eco-Sustainable Marine Biotechnology Department
Stazione Zoologica Anton Dohrn, Napoli

Malaga, 22th-24th November

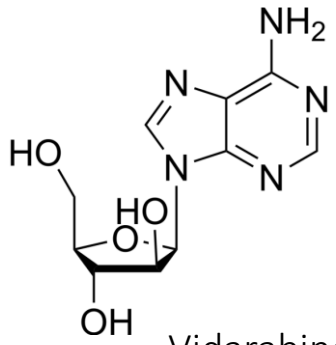
The SECRETed project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement No. 101000794



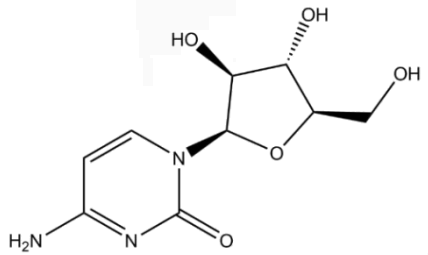
Marine Natural Products

METABOLITES

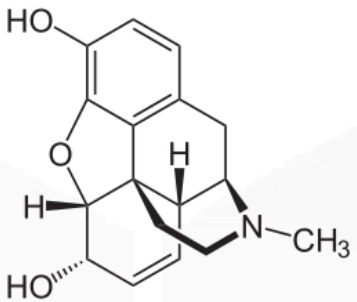
- Defense from predator
- Survival in extreme conditions
- Intra and inter species comunication



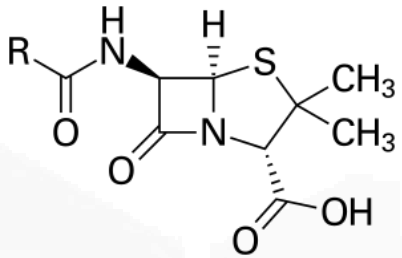
Vidarabine



Cytarabine

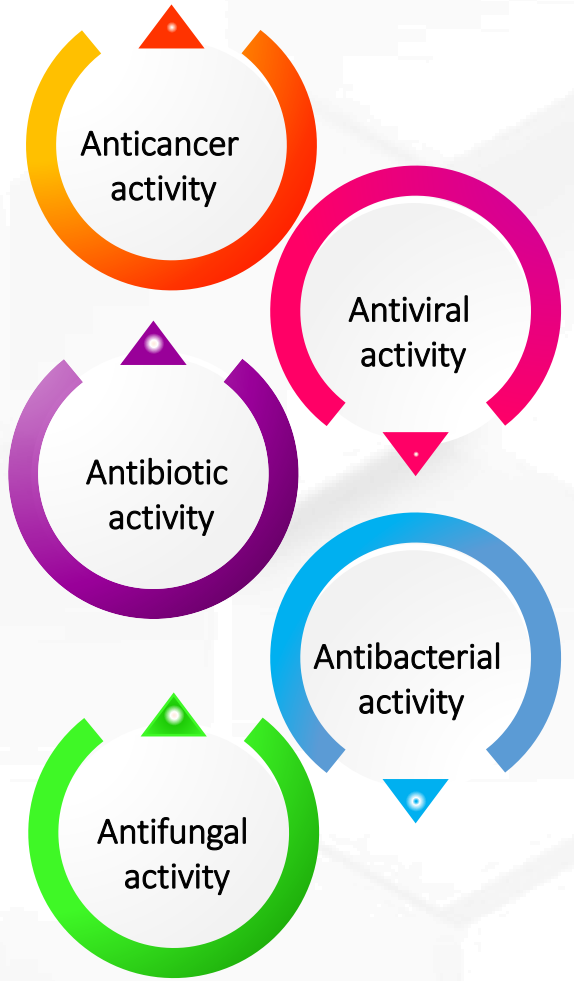


Morphine



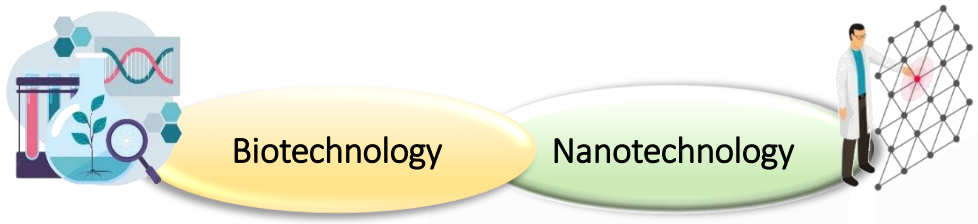
Penicillina

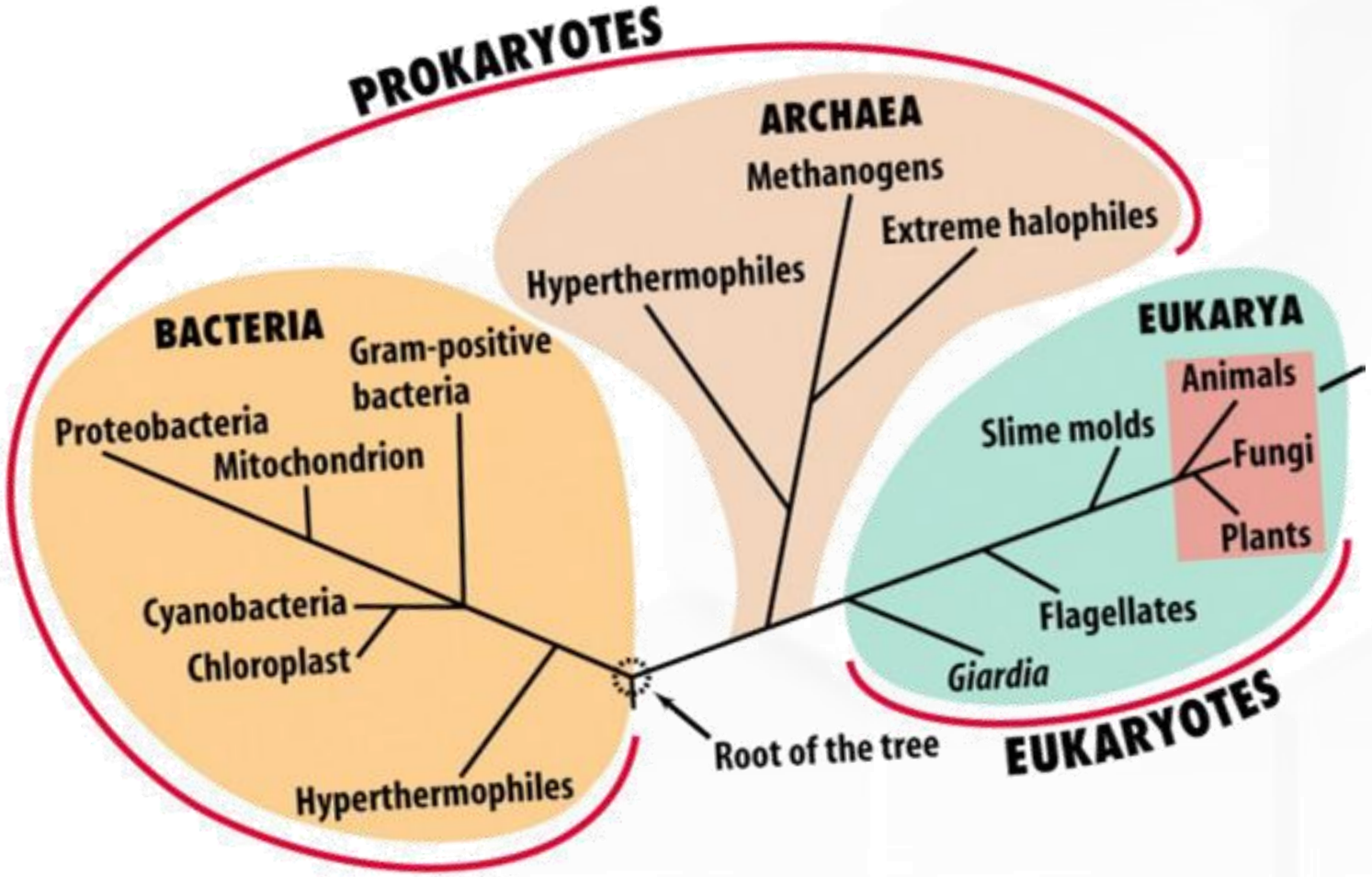
Pharmacological Activity



Extremophilic Microorganisms

Extremophiles are microorganisms that survive and proliferate in extreme conditions in environmental that have extremely high or low values of temperature, pressure, pH, salinity and radiation.



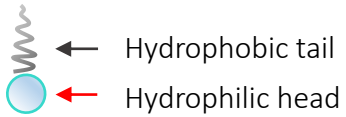


The majority of extremophile organisms belong to the prokaryotes (Archaea and Bacteria).

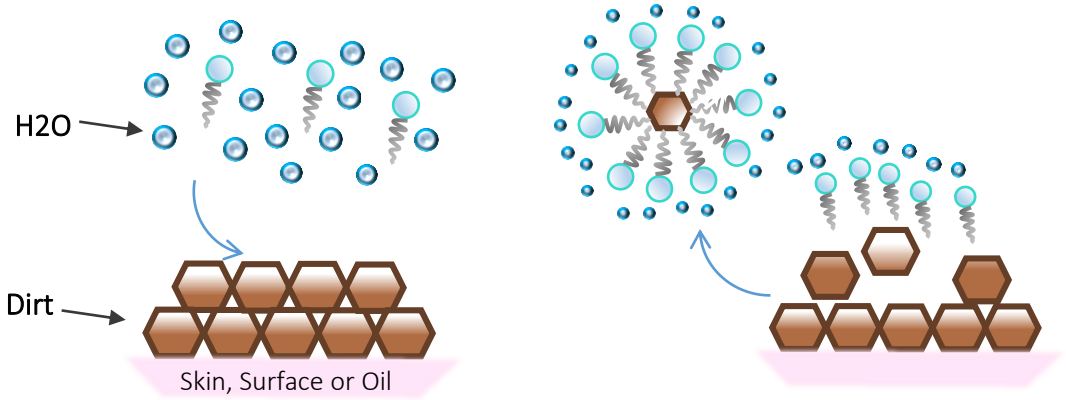
Brock Biology of Microorganisms 11/e2006 Pearson PrenticeHall, Inc

Biosurfactants

Biosurfactants are amphiphilic molecules, with natural tendency to form self-aggregates and lower surface tension.

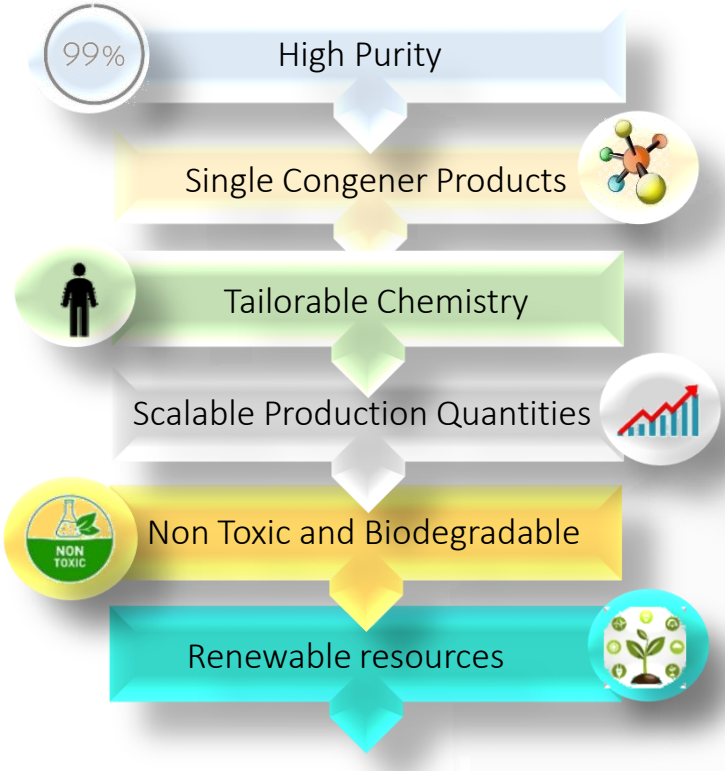


Biosurfactant hydrophobic ends around and lift the dirt while hydrophilic heads face the water and get caught in the flow, carrying the dirt away.

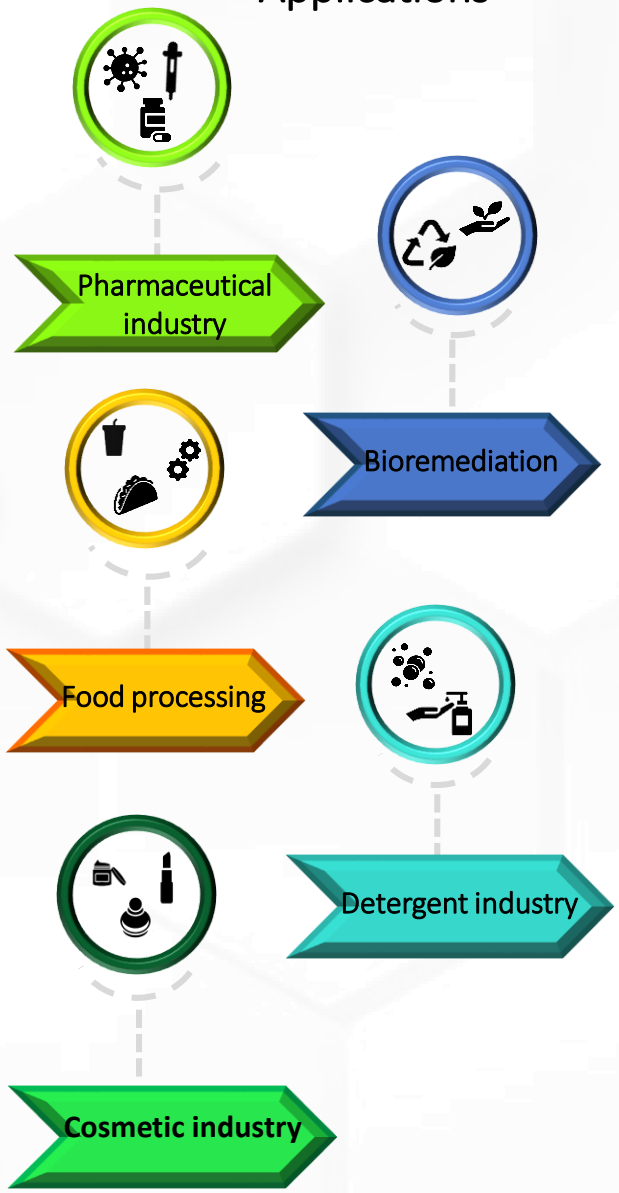


- LIPOPEPTIDES
- POLYMERS
- PHOSPHOLIPIDS
- GLYCOLIPIDS

Advantages

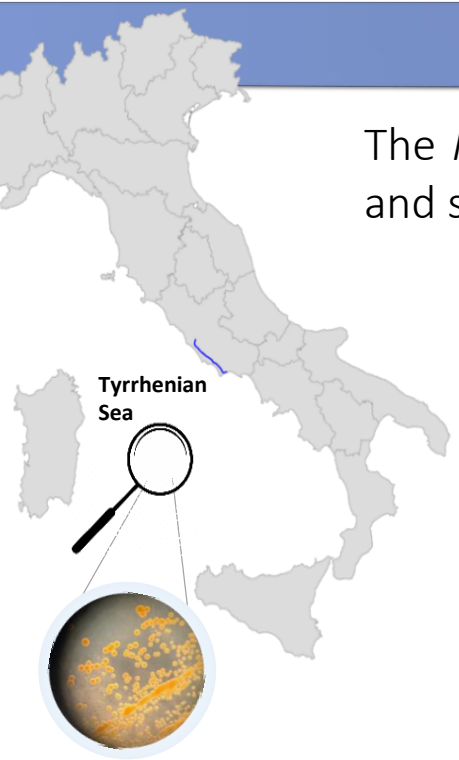


Applications

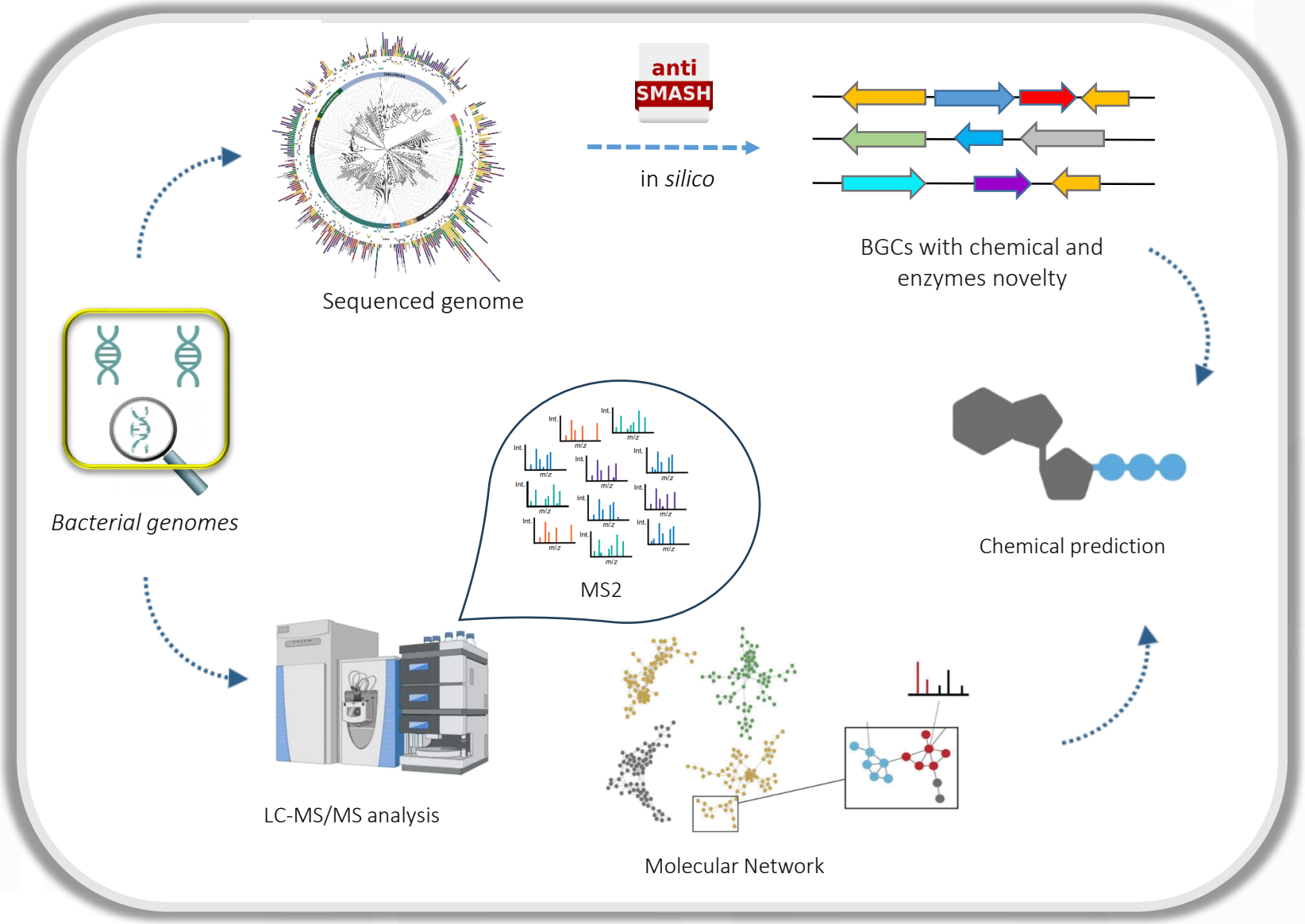


Front. Microbiol. 2020, 11:1341. doi: 10.3389/fmicb.2020.01341

Rhodococcus sp. I2R

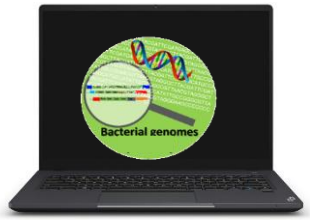


The *Rhodococcus* sp. I2R was isolated from deep-sea sediments collected in the Southern Tyrrhenian Sea and stored at 20 °C on agar plates (MSM).



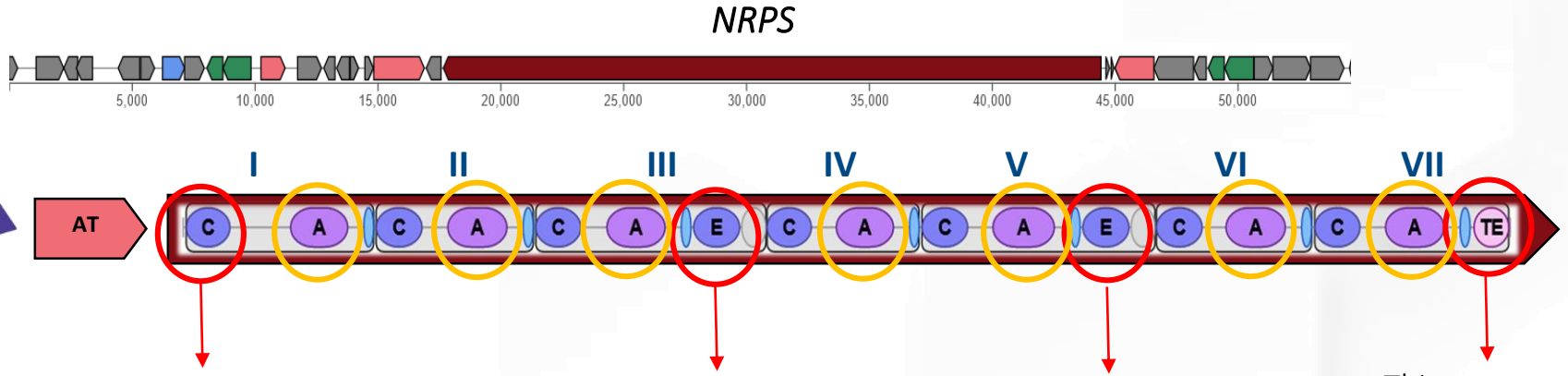
Bottom-up approaches allow discovery of NPs undetected under standard fermentation conditions, using functional genomics, bioinformatics, and genetic manipulation tools.

Genome Mining and Prediction



Genome mining

anti SMASH



Starter Condensation Domain

Epimerase

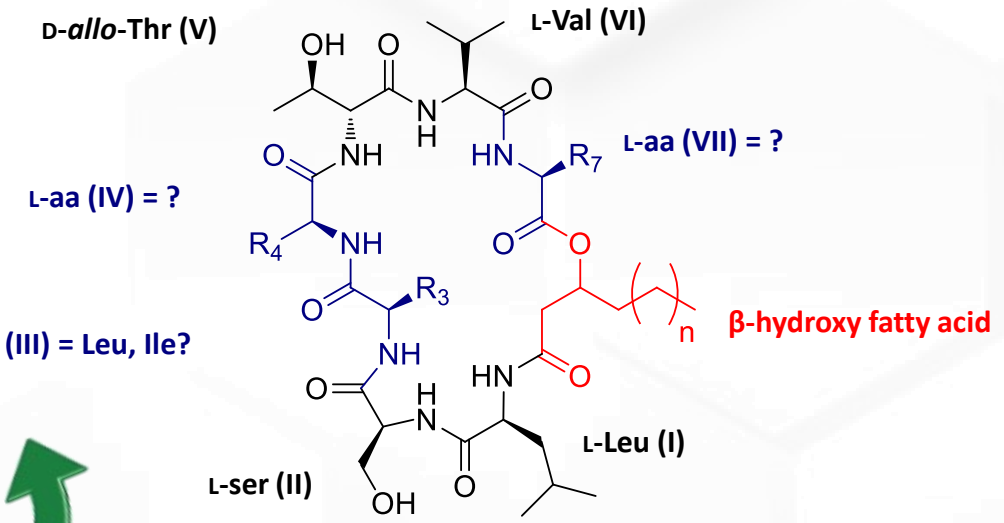
Epimerase

Thioesterase (cyclization)

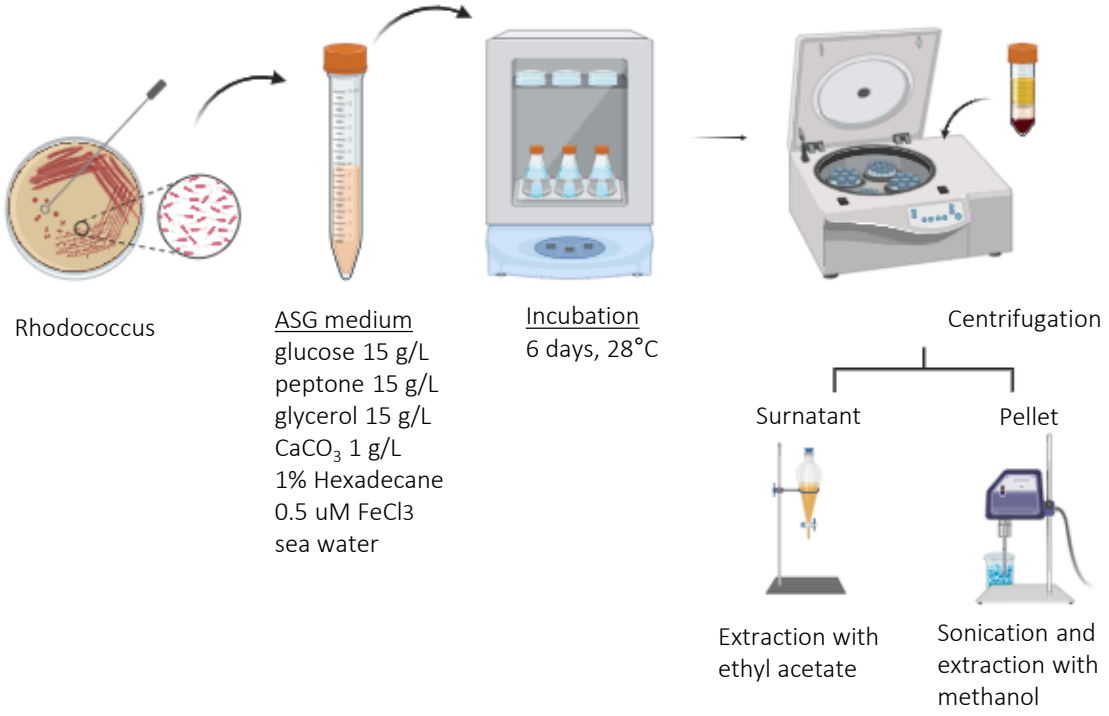
Structure prediction

Adenylation domain selectivity (Stachelhaus code)

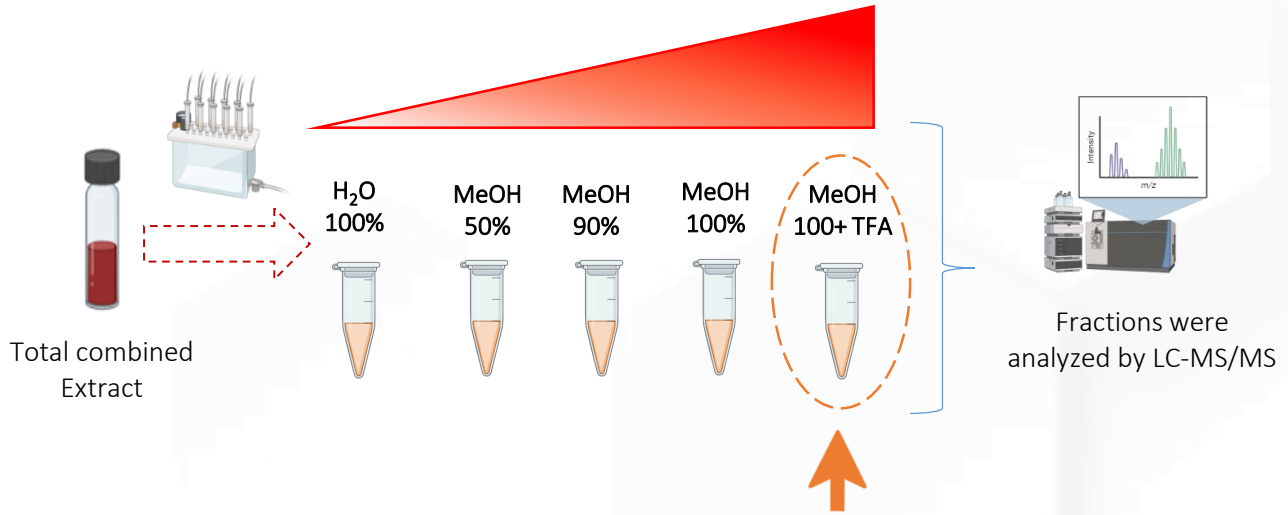
	Prediction	Binding pocket signatures (position)								
		235	236	239	278	299	301	322	330	331
Ad_I	L-Leu	D	A	L	F	V	G	A	V	F
Ad_II	L-Ser	D	V	W	H	F	S	L	V	D
Ad_III	D-Leu or allo-Ile	D	A	L	F	A	G	A	I	F
Ad_IV	L-aa?	D	A	L	F	V	G	A	V	F
Ad_V	D-allo-Thr	D	F	W	N	I	G	M	V	H
Ad_VI	L-Val	D	A	L	F	V	G	G	I	M
Ad_VII	L-aa?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



SMALL-SCALE PRODUCTION

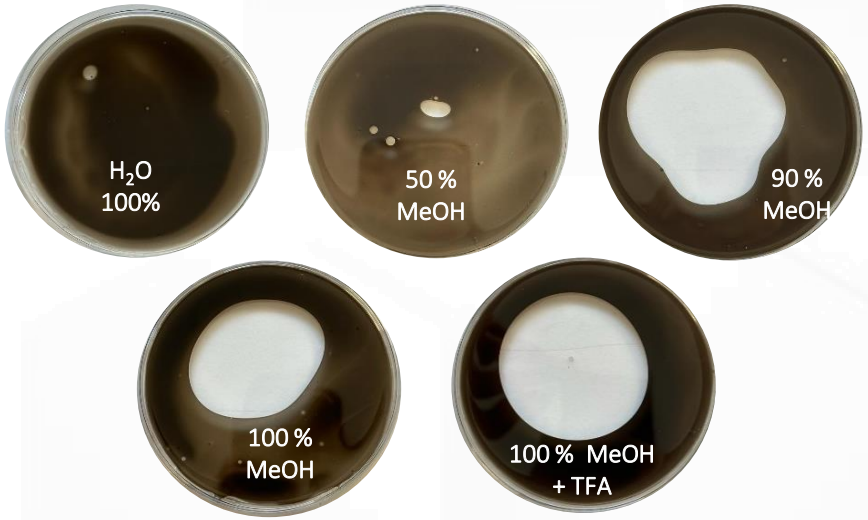


A) FRACTIONATION

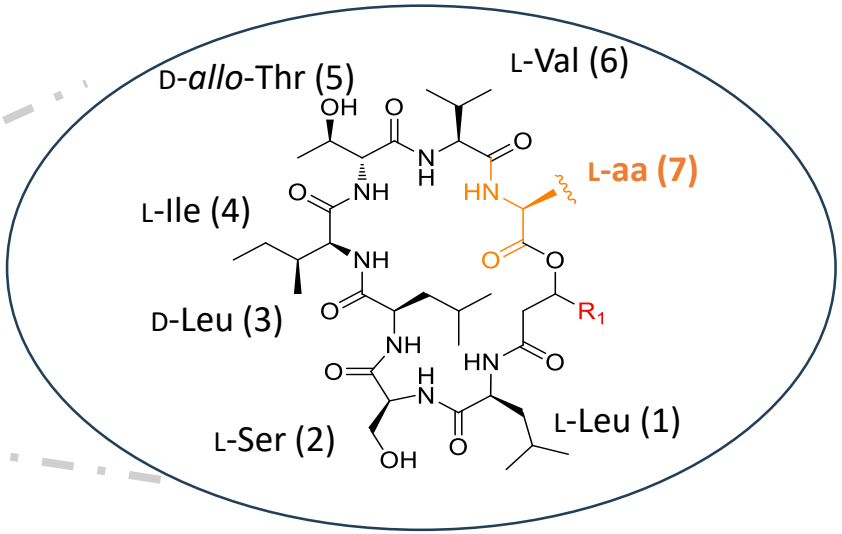


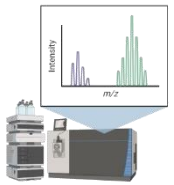
B) BIOSURFACTANT ACTIVITY

Oil displacement assay



Molecular Network

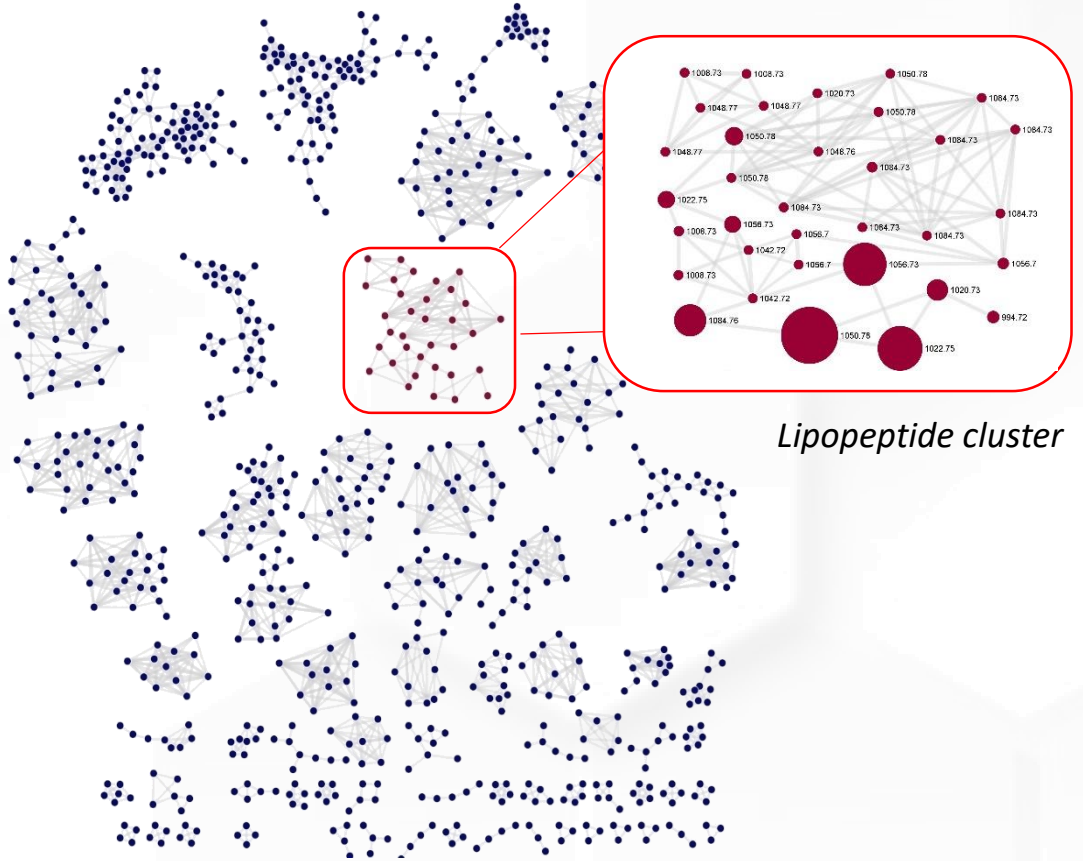




MZmine 2

GNPS

Cytoscape



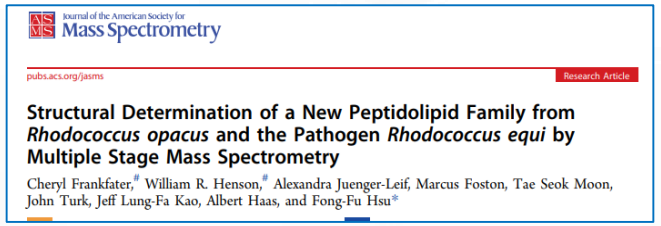
Lipopeptide cluster

Molecular Network

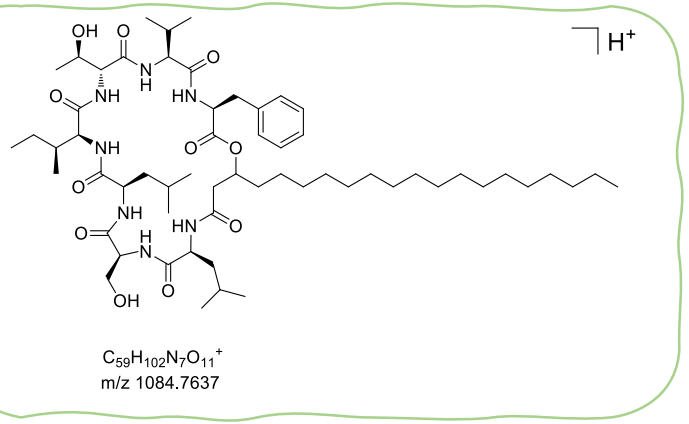
Lipopeptides from *Rhodococcus* sp. I2R

[M+H]$^+$	m/z	Δ (ppm)	R_t (min)	R_1	aa-7
$C_{59}H_{102}N_7O_{11}$	1084.7633 [a]	0.1	15.3	$C_{17}H_{35}$	L-Phe
$C_{54}H_{100}N_7O_{11}$	1022.7470	-0.5	30.1	$C_{15}H_{31}$	L-Leu/Ile
$C_{57}H_{98}N_7O_{11}$	1056.7317	-0.1	28.8	$C_{15}H_{31}$	L-Phe
$C_{56}H_{104}N_7O_{11}$	1050.7787	-0.1	19.1	$C_{17}H_{35}$	L-Leu/Ile
$C_{56}H_{102}N_7O_{11}$	1048.7637	0.5	29.8	$C_{17}H_{33}$	L-Leu/Ile
$C_{54}H_{98}N_7O_{11}$	1020.7308	-1.1	23.2	$C_{15}H_{29}$	L-Leu/Ile
$C_{57}H_{96}N_7O_{11}$	1054.7154	-1.1	22.3	$C_{15}H_{29}$	L-Phe
$C_{58}H_{106}N_7O_{11}$	1076.7948	0.3	14.7	$C_{19}H_{37}$	L-Leu/Ile

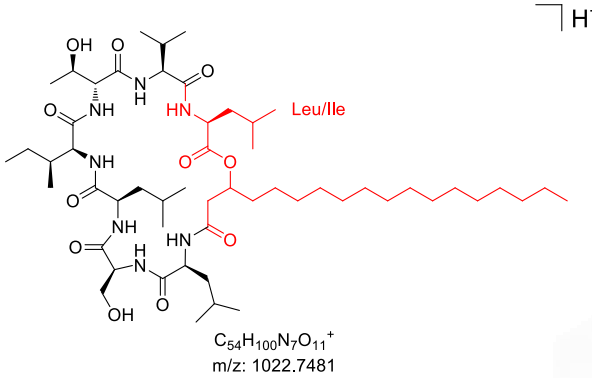
https://dx.doi.org/10.1021/jasms.9b00059



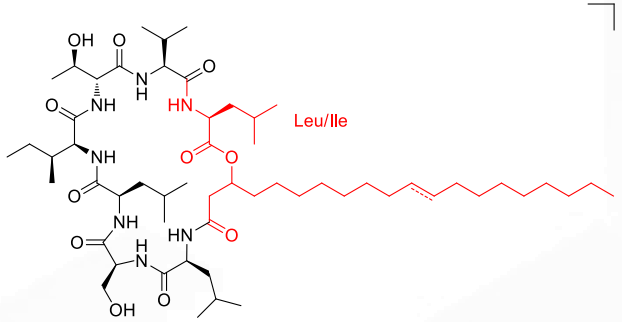
Lipopeptides structures from *Rhodococcus* sp. I2R



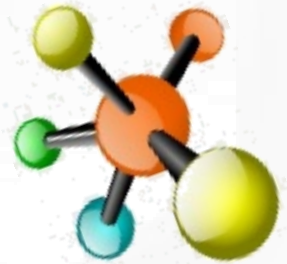
$C_{59}H_{102}N_7O_{11}^+$
m/z 1084.7637

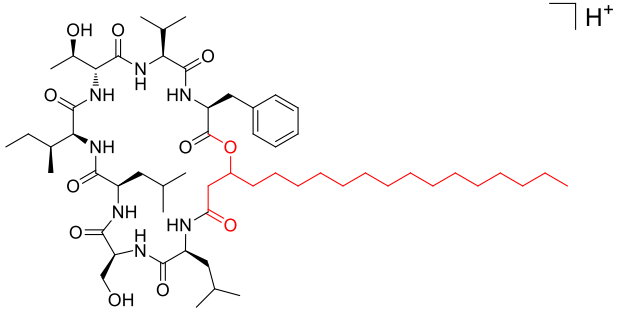


$C_{54}H_{100}N_7O_{11}^+$
m/z: 1022.7481

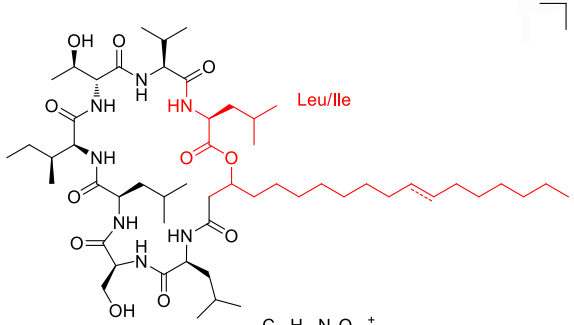


$C_{56}H_{102}N_7O_{11}^+$
m/z 1048.7637

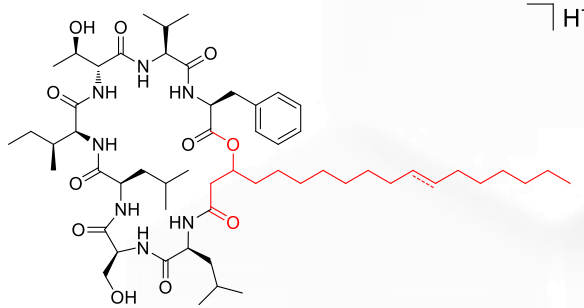




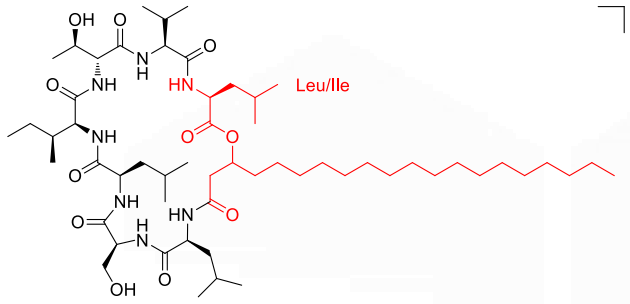
$C_{57}H_{98}N_7O_{11}^+$
m/z 1056.7324



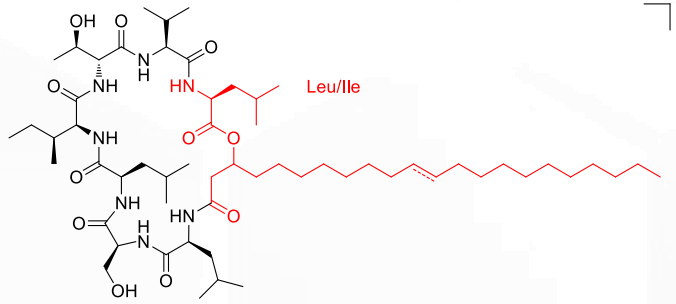
$C_{54}H_{98}N_7O_{11}^+$
m/z 1020.7324



$C_{57}H_{96}N_7O_{11}^+$
m/z 1054.7168



$C_{56}H_{104}N_7O_{11}^+$
m/z 1050.7794

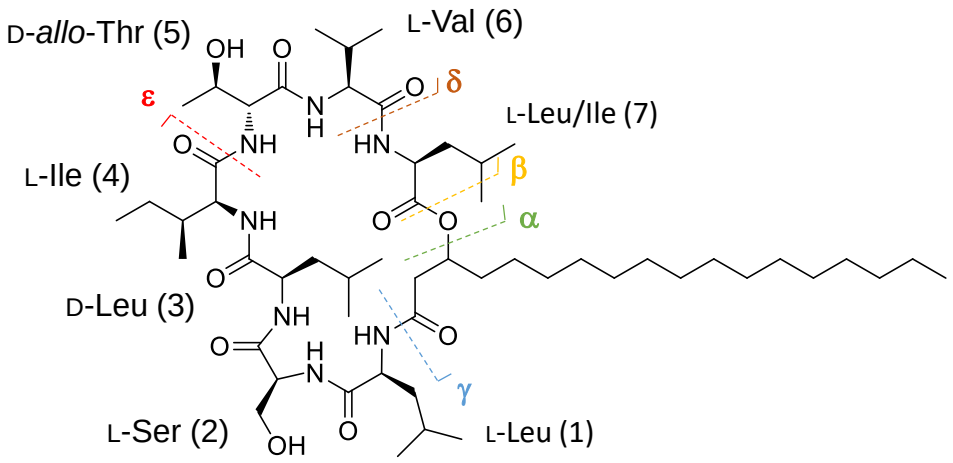


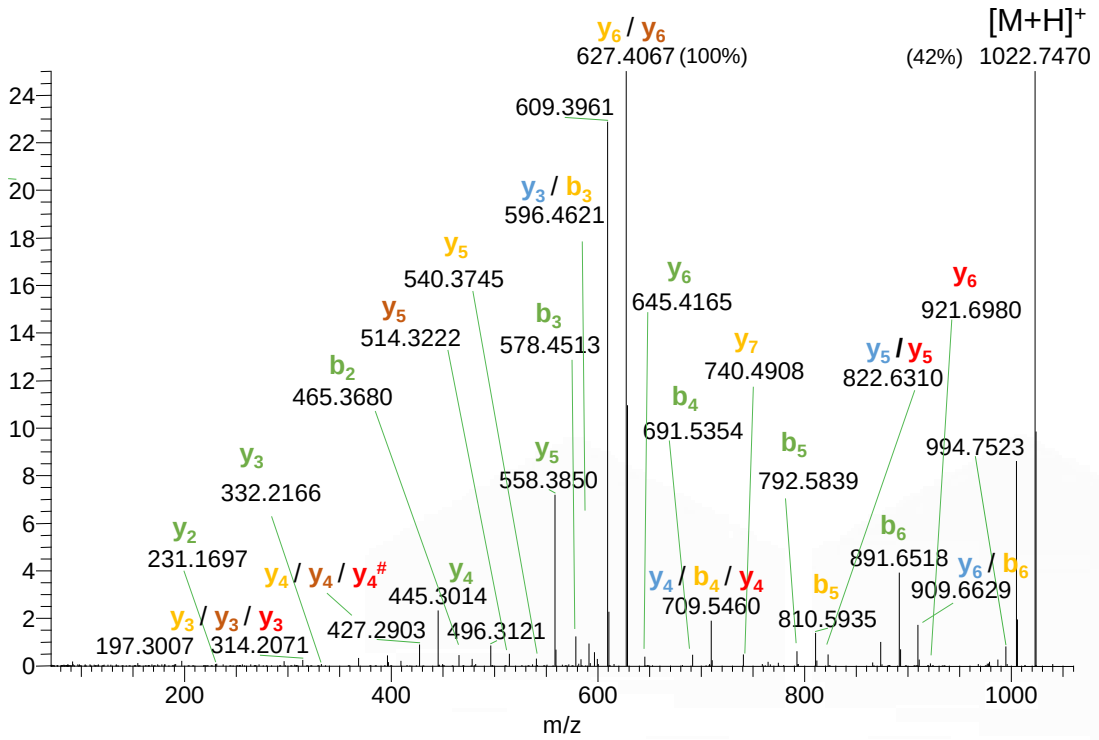
$C_{58}H_{106}N_7O_{11}^+$
m/z 1076.7950

Unpublished data

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

Lipopeptides structures from *Rhodococcus* sp. I2R





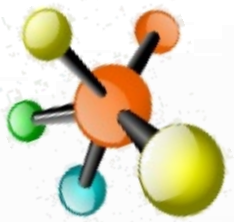
α) $C_{15}H_{31}$—Leu—Ser—Leu—Ile—Thr—Val—Leu/Ile—COOH

β) $C_{15}H_{31}$(OH)—Leu—Ser—Leu—Ile—Thr—Val—Leu/Ile—C≡O

γ) Leu—Ser—Leu—Ile—Thr—Val—Leu/Ile—O—$C_{15}H_{31}$

δ) Leu/Ile—O—$C_{15}H_{31}$—Leu'—Ser—Leu—Ile—Thr—Val—C≡O

ε) Thr—Val—Leu/Ile—O—$C_{15}H_{31}$—Leu—Ser—Leu—Ile—C≡O

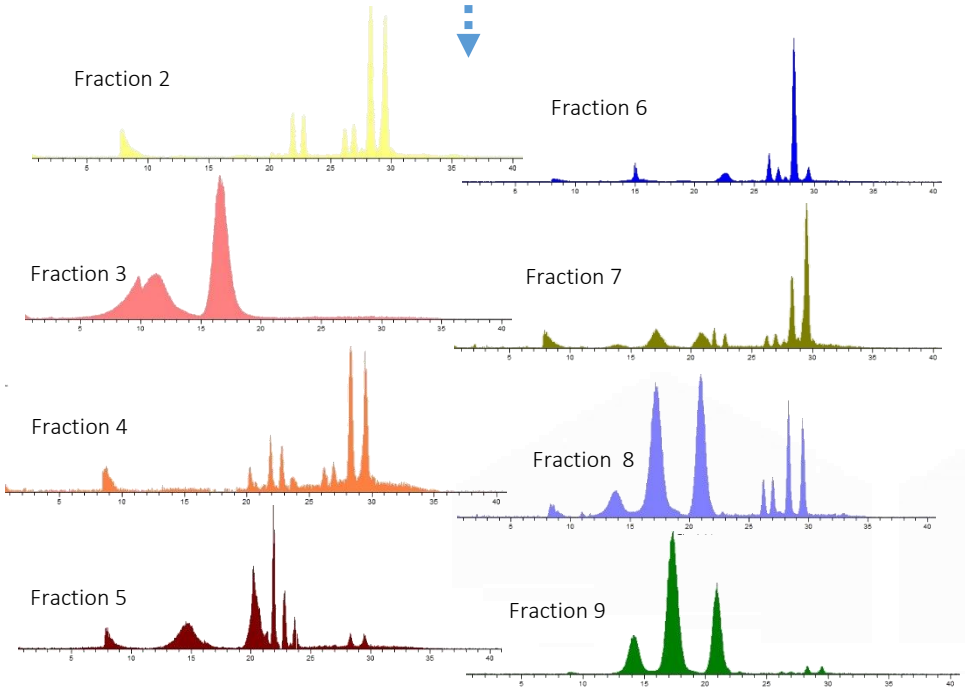


HPLC

Purification of the extract was done with semi-preparative column C18. The column was eluted with H_2O + 0.1% TFA and acetonitrile + 0.1% TFA.

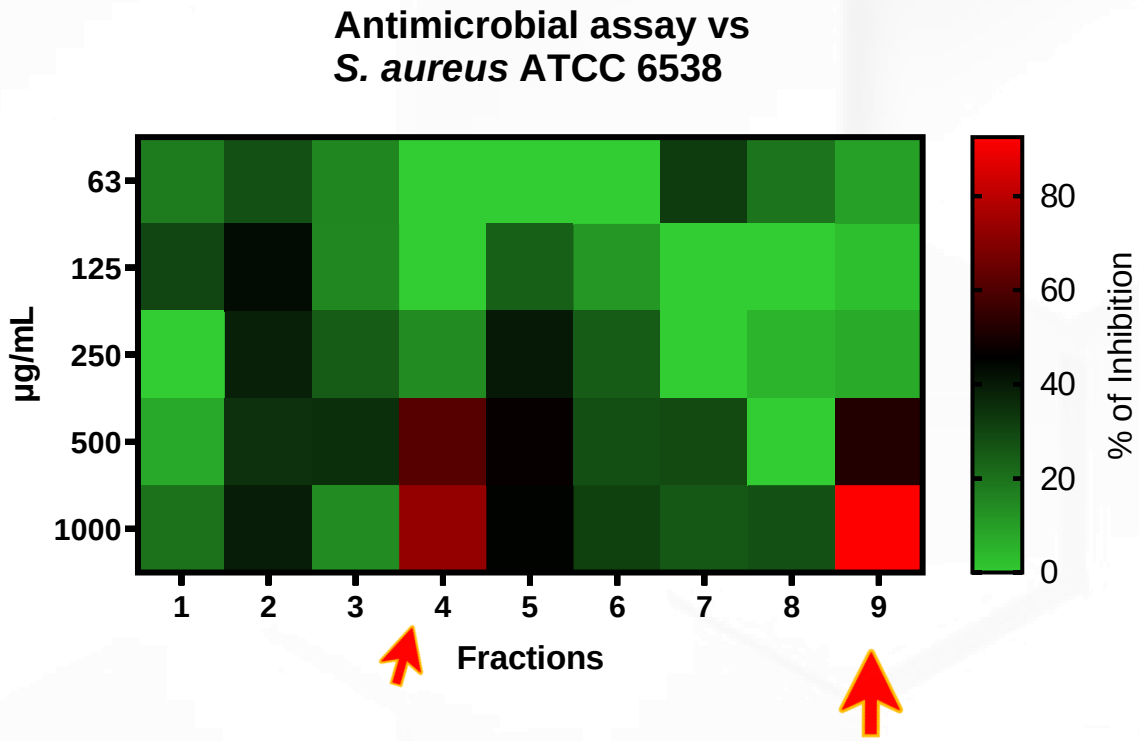
Fraction
MeOH 100 + TFA

9 fractions



Antimicrobial activity

Antimicrobial activity against *Staphilococcus aureus* ATCC 6538 (*)



*Preliminary test

Next step

(a)Purification of single molecules for NMR stereo-structural elucidation.
(b)Evaluation of putative antimicrobial, antiviral and anticancer activities of pure compounds.
(c)Identification of other lipopeptides included in the cluster.
(d)Heterologous expression to improve production yields.

Thank you for your kind attention!

- Donatella De Pascale
- Gerardo Della Sala
- Fortunato Palma Esposito
- Carmine Buonocore
- Pietro Tedesco
- Daniela Coppola
- Laura Vitale
- Vincenza Casella
- Alessandro Coppola
- Antonella Consiglio
- Silvia Scarpato
- Andrea Lopez-Mobilia

H2020 GA: 101000794

The SECRETed project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement No. 101000794

Project website: