



Università
degli Studi di
Messina

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ECMB 21th-24th November 23



*Eco-Sustainable Marine Biotechnology
Department*

Director: Dr. Donatella de Pascale

REVEALING THE BIOSYNTHETIC POTENTIAL OF DEEP-SEA BACILLUS sp. BCP32:

PRODUCTION OF NOVEL BIOSURFACTANTS AND ANTIBIOTICS



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



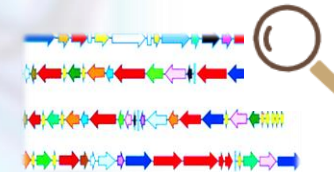
AIM of the PROJECT



The aim of the project is the search for novel natural compounds from marine bacteria



Identification of the biosynthetic pathway



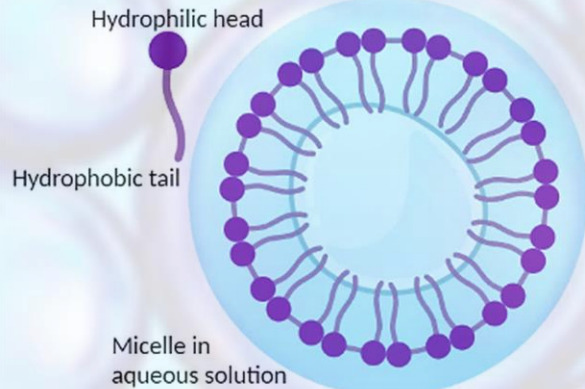
Potential application



COMPOUNDS from MARINE BACTERIA

BIOSURFACTANTS

BSs are amphiphilic compounds produced on the microbial cell surface or excreted extracellularly.



GLYCOLIPIDS

LIPOPEPTIDES

FATTY ACIDS

APPLICATIONS

BIOREMEDIATION

OIL
RECOVERY

EMULSIFIER

FOOD
&
AGRICULTURE

MEDICINE
USE

INDUSTRIAL
USE

ACCEPTABLE PRODUCTION
ECONOMICS

HIGH BIODEGRADABILITY

GENERAL LOW TOXICITY

GENTLE ON SKIN

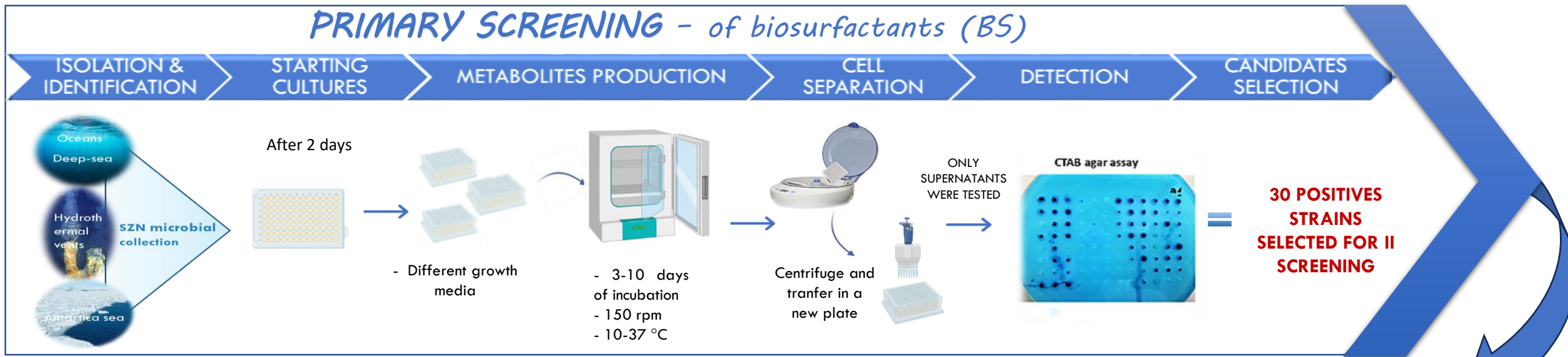
SPECIFICITY &
EFFECTIVENESS



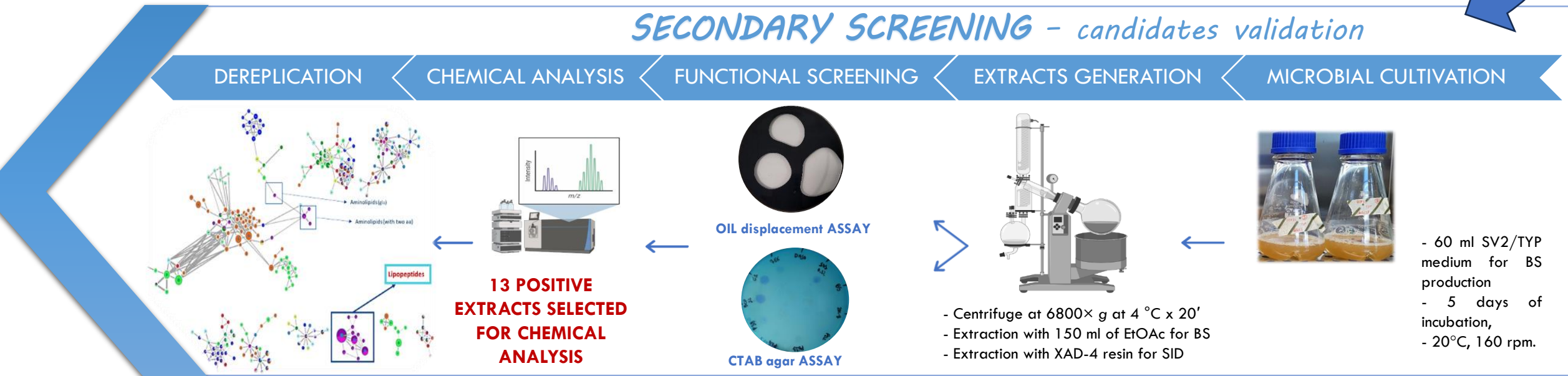
SCREENING & SELECTION of PROMISING STRAINS



PRIMARY SCREENING - of biosurfactants (BS)



SECONDARY SCREENING - candidates validation

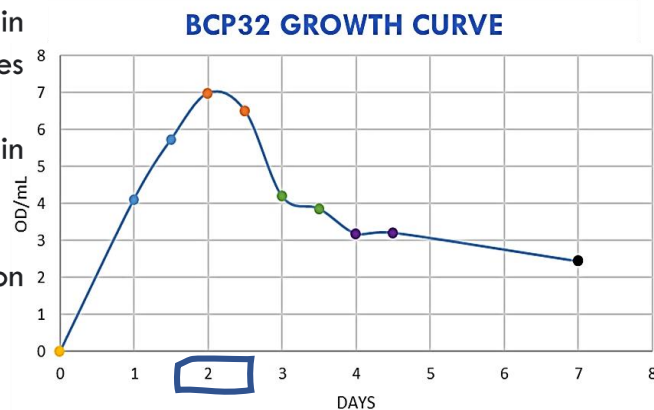


SELECTION of BACILLUS sp. BCP32 for BS PRODUCTION



TIME-COURSE of BIOACTIVITY

- ✓ Isolated from deep sea in the Gulf of Naples (Canyon Dohrn).
- ✓ 60 ml of TYP medium in 250 ml flask
- ✓ 28°C, 7 days, 150 rpm
- ✓ Daily Chemical extraction by EtOAc

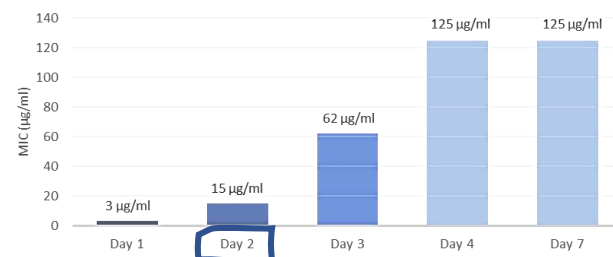


Each extract was tested for BS activity evaluated based on the size of the clear zone on the oil surface.



BS (Lipopeptides) often have antimicrobial activity against Gram+. The liquid inhibition assay was performed against *Staphylococcus aureus* and confirmed the bioactivity. The graph shows the minimum inhibitory concentration (MIC) of each extract vs *S. aureus*.

ANTIMICROBIAL ACTIVITY vs *S. aureus* 6538p



SCALE-UP & FRACTIONATION

MICROBIAL CULTURE



- 1L of TYP
- TYP MEDIUM
16 g/L triptone
16 g/L yeast extract
10 g/L NaCl



48 H



150 rpm



28 °C

SPE C18



H₂O MeOH 50% MeOH 90% MeOH 100% MeOH /TFA



MASS SPECTROMETRY



METABOLOMICS & IDENTIFICATION

FUNCTIONAL SCREENING

OIL DISPLACEMENT TEST



Fr. H₂O
MeOH 90%

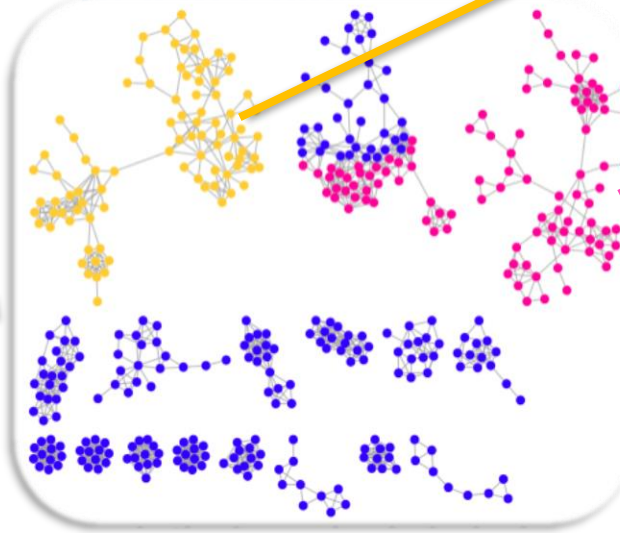
MeOH 50%

MeOH 100%
MeOH/TFA

ANTIBACTERIAL ACTIVITY

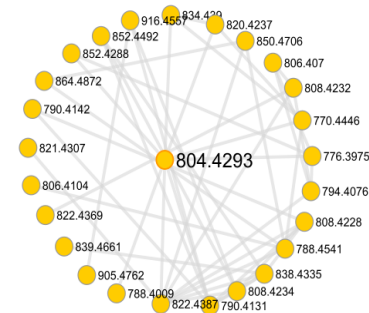
FRACTION	VALUES of MIC (ug/ml)	
	<i>S. aureus</i> 6538p	<i>L. monocytogenes</i>
H ₂ O	-	-
MeOH 50%	31	-
MeOH 50%	1,9	1,9
MeOH 100%	31	-
MeOH 100%	105	-

MOLECULAR NETWORK from LC-MS/MS data

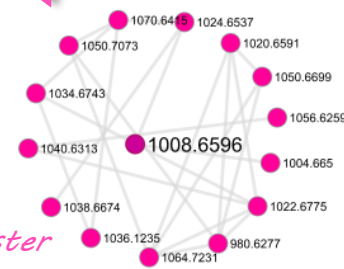


MZmine GNPS Cytoscape

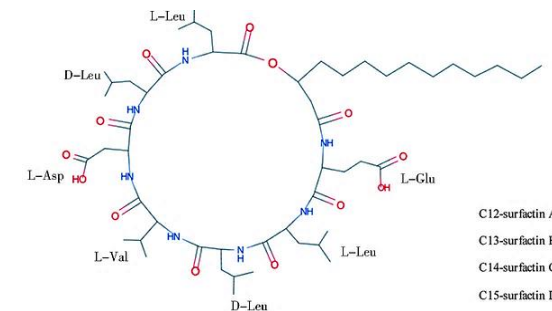
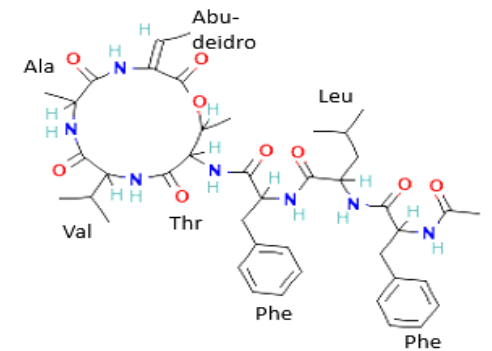
TL₁₁₉ Cluster



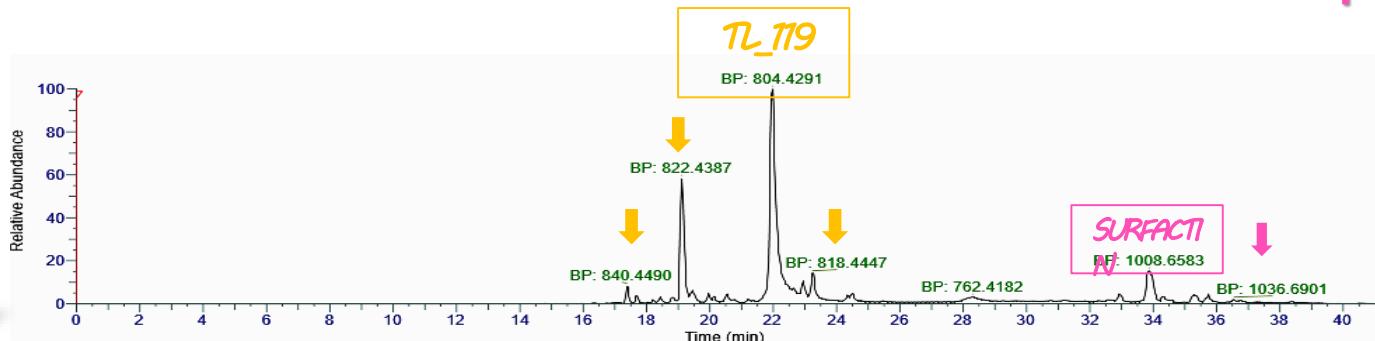
Surfactin Cluster



Antibiotic TL₁₁₉ and many NEW analogues

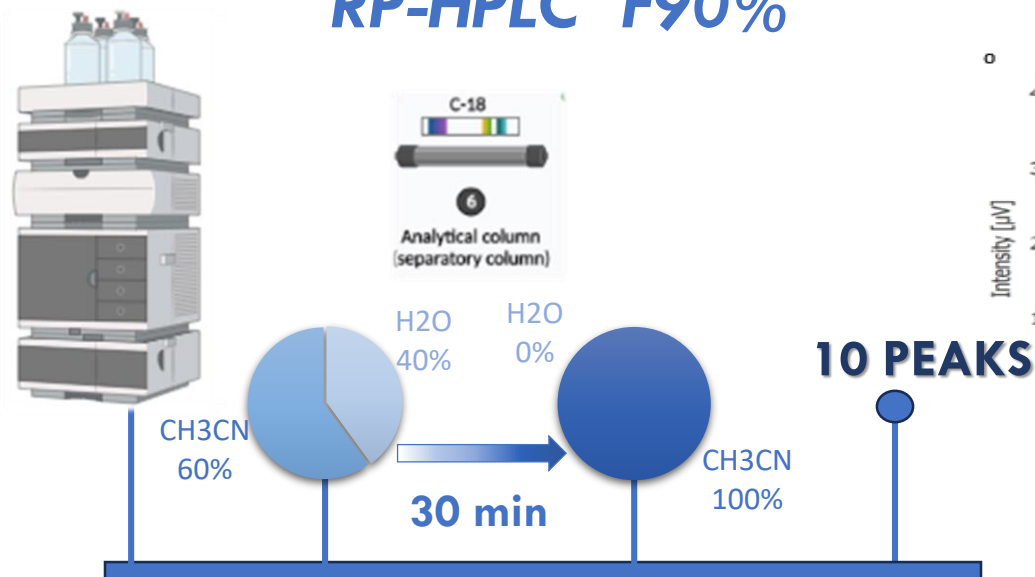


Potential Novel Lipopeptides

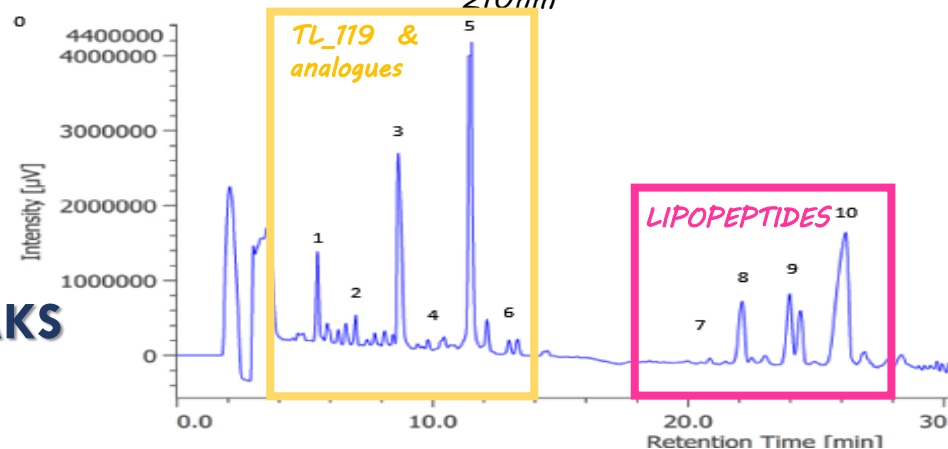


PURIFICATION & ISOLATION

RP-HPLC F90%



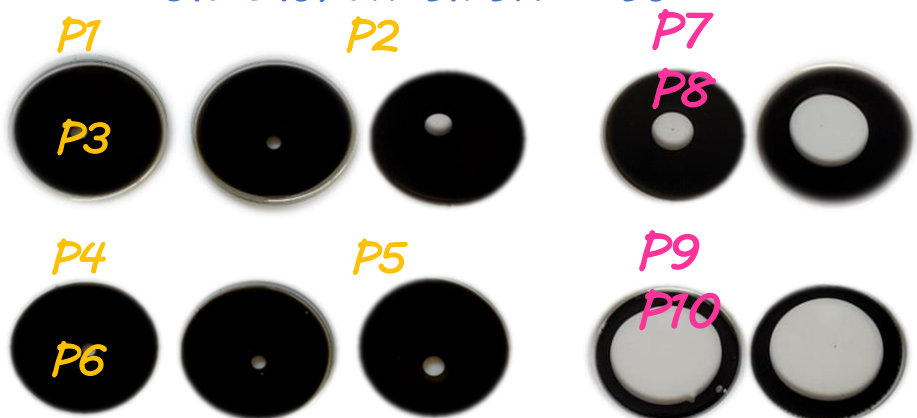
CHROMATOGRAM- UV 210nm



MASS SPECTROMETRY ANALYSIS

PEAKS	[M+H] ⁺	COMPOUND
P1	840,4498	Potential Novel Nobilamide
P2		Nobilamide analogues
P3	822,4397	Nobilamide A
P4		TL_119 analogues
P5	804,4301	TL_119
P6	818,4439	Nobilamide E & TL_119 new analogues
P7		Surfactin analogues
P8	1008,6578	Surfactin C13
P9	1022,6754	Surfactin C14
P10	1036,6907	Surfactin C15

FUNCTIONAL SCREENING OIL DISPLACEMENT TEST

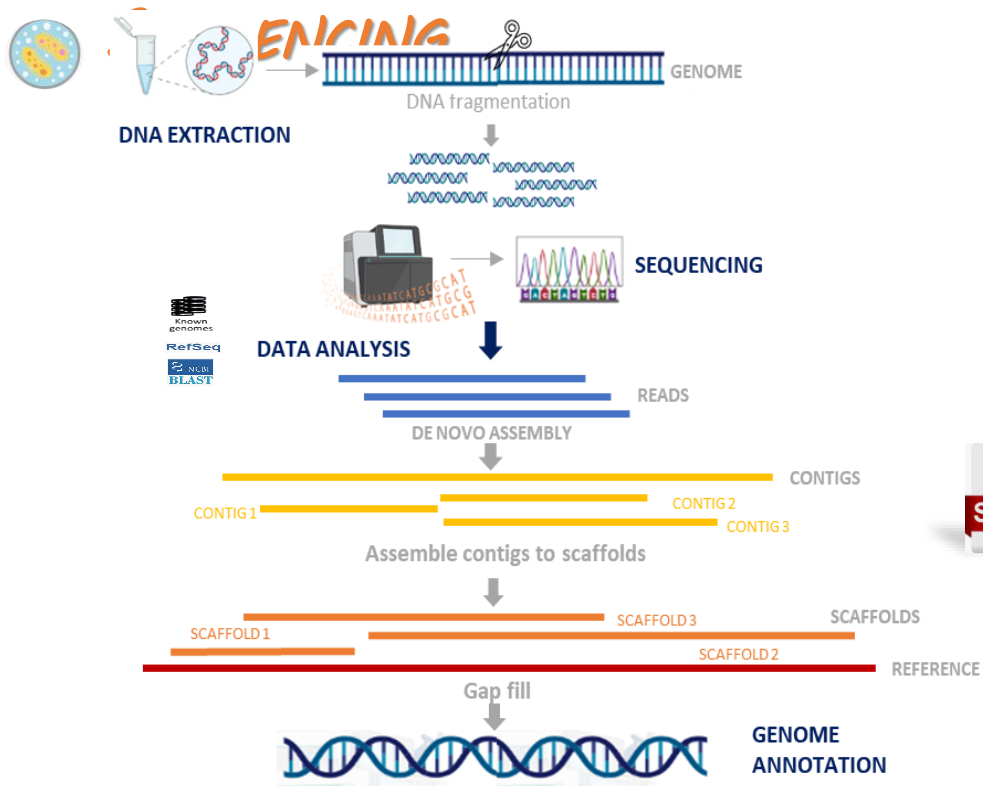


ANTIBACTERIAL ACTIVITY

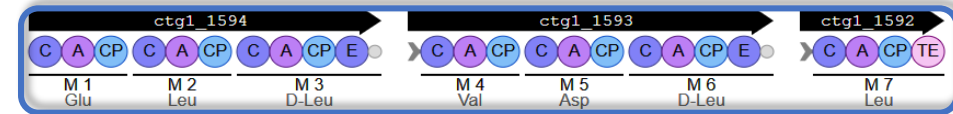
	VALUES of MIC (ug/ml)					
PEAKS	<i>S. aureus</i> 6538p	<i>L. monocytogenes</i>	<i>S. aureus</i> 6538	<i>S. aureus</i> MRSA	<i>S. Aureus</i> Res-MACROLIDE	<i>S. Aureus</i> Res-QUINOLONE
P4	3,8	7,75	7,7	62	62	15,5
P5	0,25	0,25	0,25	3,8	1,9	0,9
P6	15,5	7,75	31	-	-	-
P9	62	-	31	-	-	-
P10	15,5	-	15,5	-	-	-

GENOME MINING

BCP32 WHOLE GENOME



SURFACTIN BGCs - NRPS



Select genomic region:



Identified secondary metabolite regions using strictness 'relaxed'

Region	Type	From	To	Most similar known cluster	Similarity
Region 1.1	NRPS  , betalactone 	1	26,443	fengycin 	NRP 80%
Region 1.2	NRPS  , transAT-PKS  , PKS-like  , T3PKS 	136,437	242,887	bacillaene 	Polyketide+NRP 100%
Region 1.3	NRPS 	390,469	436,064		
Region 1.4	terpene 	853,966	874,319		
Region 1.5	NRPS 	1,592,575	1,654,529	surfactin 	NRP:Lipopeptide 82%
Region 1.6	RiPP-like 	2,114,971	2,127,154		
Region 1.7	other 	2,332,322	2,373,740	bacilysin 	Other 100%
Region 1.8	sactipeptide 	2,375,620	2,397,232	subtilosin A 	RiPP:Thiopeptide 100%
Region 1.9	NRP-metallophore  , NRPS 	2,923,735	2,975,509	bacillibactin 	NRP 100%
Region 1.10	T3PKS 	3,875,741	3,916,838	laterocidine 	NRP 5%
Region 1.11	terpene 	3,964,624	3,986,522		
Region 1.12	NRPS 	4,053,554	4,100,386	plipastatin 	NRP 61%
Region 2.1	NRPS 	1	3,598		
Region 3.1	NRPS	1	5,826	pelgipeptin	NRP 37%



ANALYSIS OF
BIOSYNTHETIC GENE
CLUSTERS (BGC)

FUTURE PERSPECTIVES

✓ In conclusion, in the framework of SECRETed project, we have screened 300 marine extremophilic strains for BSs. We have selected *Bacillus* sp. BCP32 (deep sea) for the production of novel lipopeptides and antimicrobials.

»» All peaks will be tested in Anti-cancer and Anti-biofilm assays

»» Structural elucidation and characterization of the pure active molecules produced by BCP32.

»» Identification of the biosynthetic gene cluster encoding for TL_119 and its analogues.



THANK YOU FOR YOUR KIND



ATTENTION



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Stazione
Zoologica
Anton Dohrn
Napoli



BlueRemediomics



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