



Consiglio Nazionale
delle Ricerche



Exploration of the marine environment From North Sea to Antarctica: let's discover a world of new compounds

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ERA-MBT Final Conference, Oslo 20-21 Nov 2017



WHO priority pathogens list for R&D of new antibiotics

27 February 2017



Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant



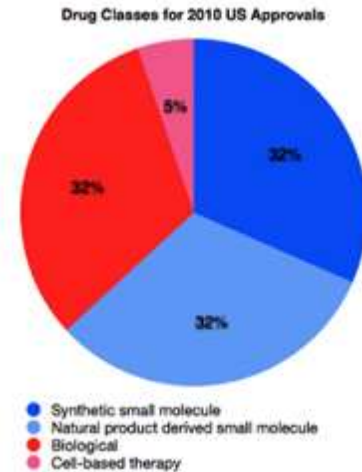
Exploitation of Marine resources as source for novel drugs.

Natural products and their analogs continue to play a prominent role in medicine, accounting for two-thirds of new antibacterial therapies approved from 1980 to 2010

Marine Organisms for Drugs Discovery

Majority of the marine natural products have been isolated from:

- Sponges (sea whips, soft coral)
- Tunicates
- Coelenterates
- Opisthobranch molluscs (nudibranchs)
- Echinoderms (starfish, sea cucumbers)
- Bryozoans (moss animals)
- Bacteria
- Fungi



Ziconotide Prialt™
Azur Pharma



Severe Chronic pain

Ecteinascidin-743
Trabectedin
Yondelis™,
PharmaMar/Johnson

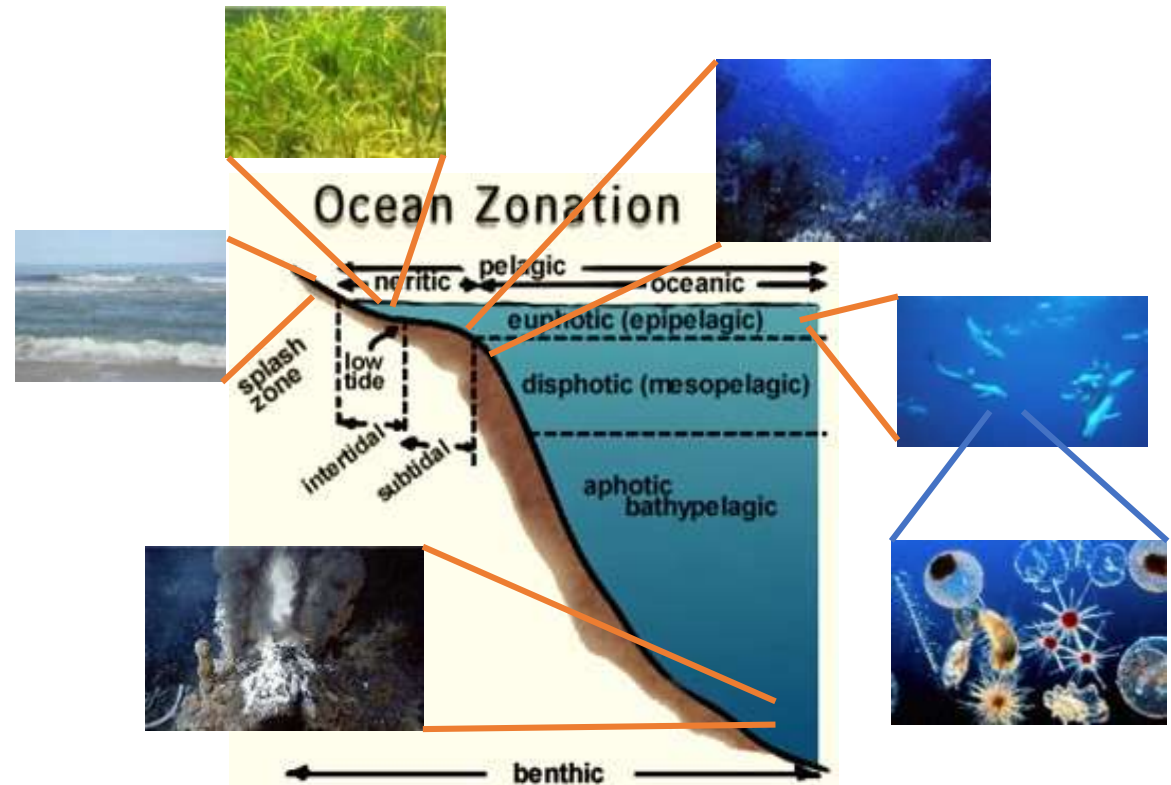


Anti-cancer

FDA approved	Disease area
Brentuximab vedotin	Cancer
Cytarabine (Ara-C)	Leukemia
Eribulin Mesylate (E7389)	Breast Cancer
&3-acid ethyl esters	Hypertriglyceridemia
Vidarabine (Ara-A)	Herpes Simplex Virus

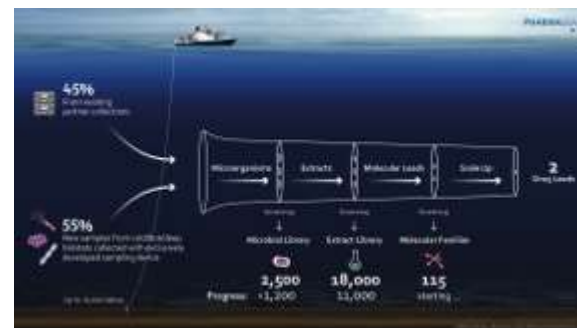
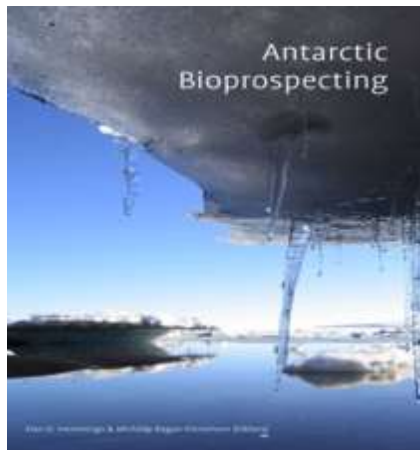
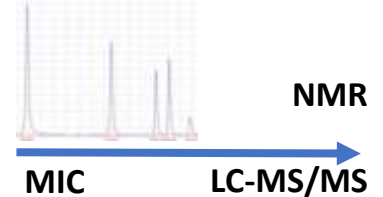
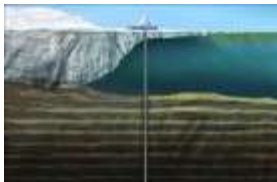
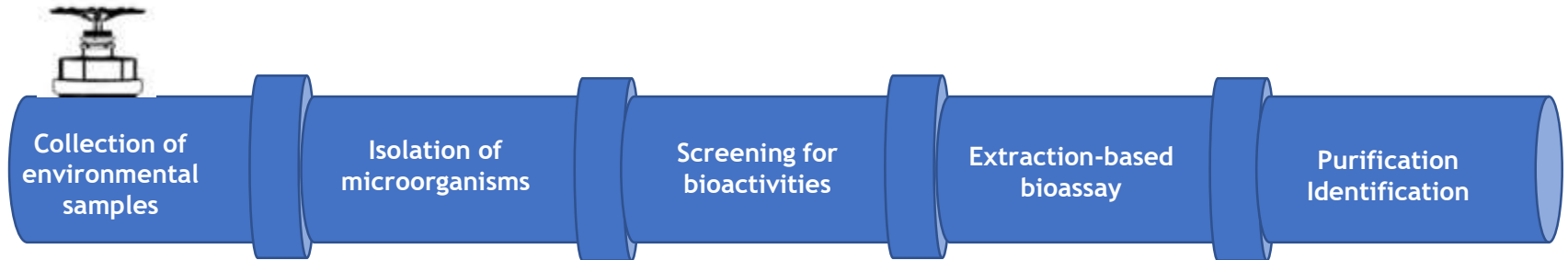
Why marine environment as source of new drugs?

- ❖ A broad range of different habitats
- ❖ Peculiar conditions (high pressure, salinity, extreme temperatures, darkness)
- ❖ Competition, defence, communication



In order to survive to these peculiar and varied environments, marine organisms developed enormous quantity of different compounds, especially **secondary metabolites with unique biological activities**

Drug Discovery pipeline



Our samples...from Italy to Antarctica



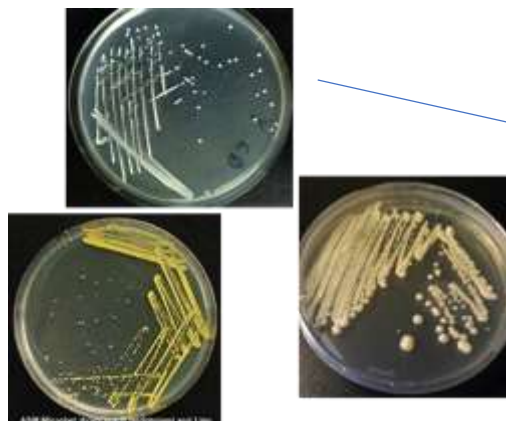
Mussels, Algae
(Canary Islands)



Marine fungi
(Elba Island)



Sponges
(Antartica / Barents Sea
Rio Formosa, Faro)



Microorganisms

(Antarctica and Barents Seas, Rio Formosa, Faro)

Deep Sea –5000m,
South Shetland
trench





Sampling in the Ross sea, Antarctica 2015-2016



Antarctic sub-sea sediments

-46 m of depth



Baia Terranova,
Ross Sea



In the framework of the PNRA project.

Pharmadeep expedition December 2015

South Shetland Trench, Antarctica: -5000 m



The aim of the mission was to collect sediments and animals from South Shetland Trench, Antarctica

- 5 Stations: - 5000 m
- 4000 m
- 3000 m
- 2100 m
- 1000 m

Partner organization	Supervisors
1 – UNIABDN, Aberdeen UK COORDINATOR	Alan Jamieson
2- SZN, Naples, IT	Adrianna Ianora
3- IBP-CNR, Naples, IT	Donatella de Pascale
4- University of Barcelona, SP	Conxita Avila
5- Nicolaus Copernicus University, PO	Patrycja Golinska
6- University of Chile, Chile	Juan Asenjo

Gravity Core



Up to 5 meters of sediments



Multicore

40 cm of sediments

The instruments were connected to an iron wire of 6000 meters and released in the sea



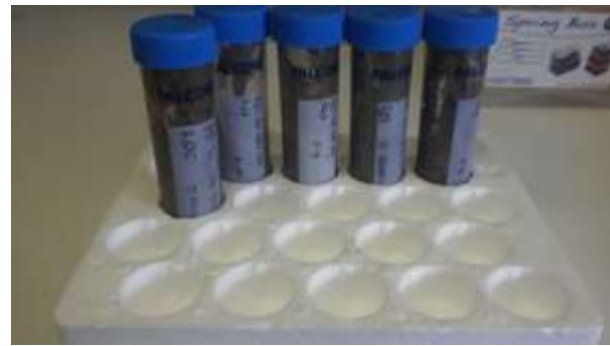
R/V Biosperides



Barkut expedition June 2015 Barents Sea



R/V Helmer Hannsen Vessel



Sampling in 9 different sites in the Barents Sea close to Oil rigs

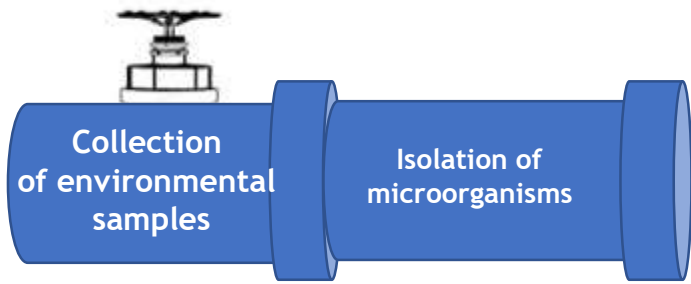


Exploring the unexplored: from new microbes to novel bioactive compounds



Two major activities have been identified:

- The sampling of marine organisms (**sponges, tunicates, bryozoan, fungi and bacteria**) that have been carried out at the CCMAR (Centre of Marine Sciences, Faro, Portugal), RIA FORMOSA lagoon.
- The metagenomic analysis of the novel microbial strains isolated during the first activity at DSMZ, Berlin (Germany).



Isolation of microorganisms

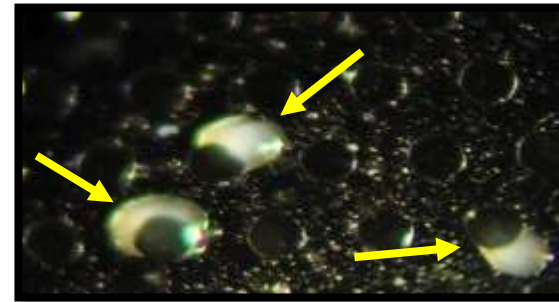
Classic isolation techniques



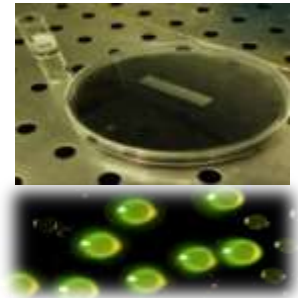
Different media, temperatures, incubation time

Screening and selection of the best antimicrobial producers

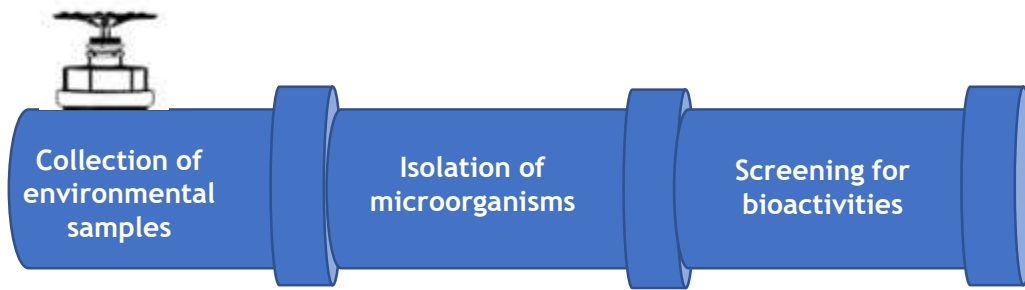
Novel isolation techniques with MicroChip culture



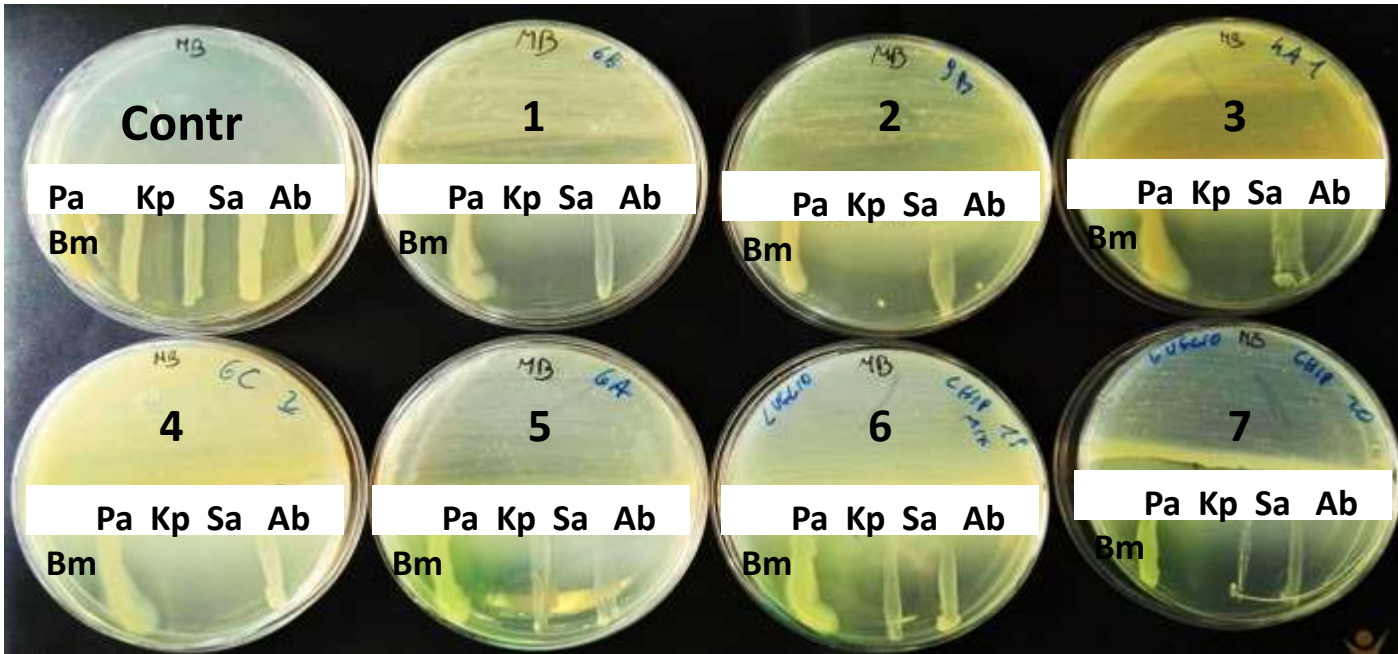
Dr. Colin J. Ingham, University of Wageningen



Optimisation of the production conditions (OSMAC approach, ...) and formation of an extracts library



Cross-streak against MDR bacteria



- Some of isolates show an inhibitory activity against MDR bacteria:

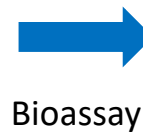
Pseudomonas aeruginosa
Klebsiella pneumoniae
Staphylococcus aureus
Acinetobacter baumannii
Burkholderia metallica



From an active crude extract....to purified active compounds



Positive extract

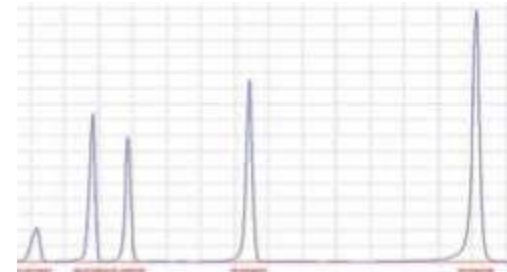


[1 mg/mL]	<i>Pseudomonas</i>			<i>Pseudoalteromonas</i>			<i>Psychrobacter</i>			<i>Arthrobacter</i>	
%inhibition	BTN 1	BTN 2	BTN 15	BTN 3	BTN 19	BTN 21	BTN 4	BTN 5			
<i>B. diffusa</i>	90	75	77	43	45	70	63	77			
<i>B. metallica</i>	90	70	71	32	30	53	64	77			
<i>B. cenocepacia</i>	90	78	87	84	64	60	55	84			
<i>B. latens</i>	90	53	75	55	43	40	68	56			
<i>B. anthina</i>	90	38	60	30	25	46	50	38			

Positive fractions



UPLC



Bioassay

Positive peaks

**LC-MS/MS
NMR**



Fractionation (SPE extraction)

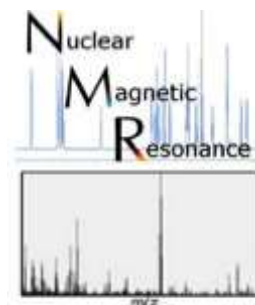
**IDENTIFICATION
OF COMPOUNDS**



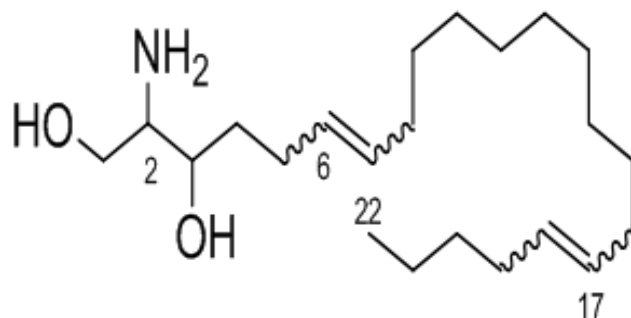
Absciex qtrap 4500

- ❖ *Pseudomonas aeruginosa* (PA01 - 4596 - 4598)
- ❖ *Klebsiella pneumoniae* (K6)
- ❖ *Staphylococcus aureus* (6538P - 4709 - 4713)
- ❖ *Acinetobacter baumannii*
- ❖ *Burkholderia cepacia* complex
- ❖ *Achromobacter xylosoxidans* (4630 - 4631)
- ❖ *Stenothropomonas maltophilia* (4671 - 4673)
- ❖ *Francisella tularensis*

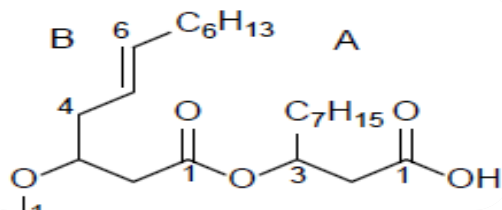
Recently isolated bioactive compounds



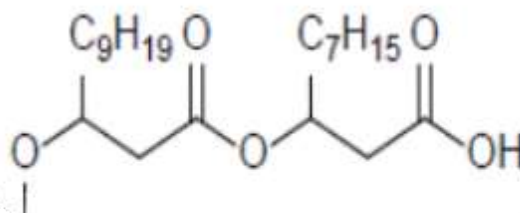
Bioactive sphingadienine from a symbiotic marine fungus



Rhamnolipids from Antarctica *Pseudomonas* sp. BTN1



Rha



Rha

PHARMASEA



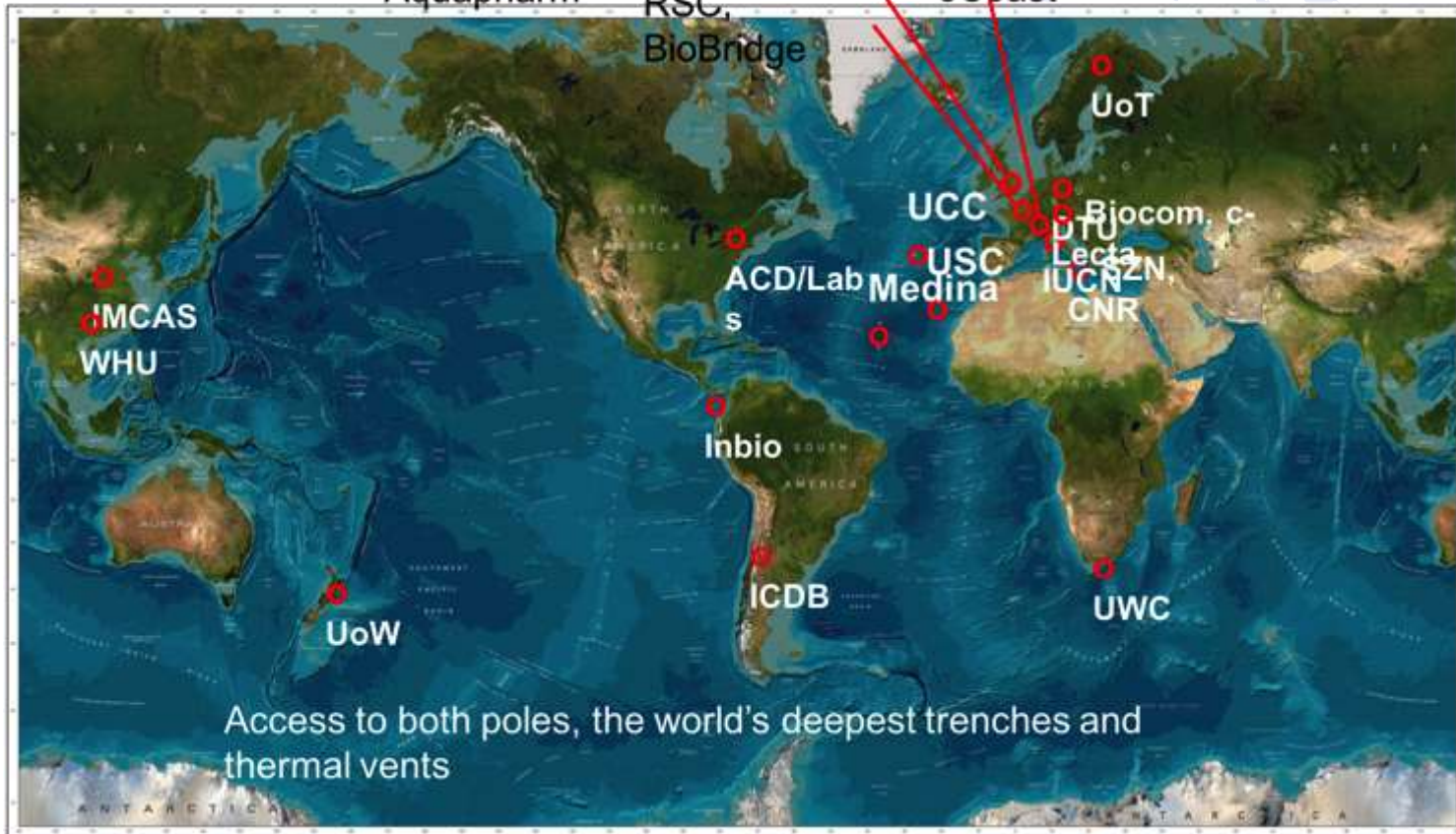
**KBBE.2012.3.2-01: Increasing Value and Flow
in the Marine Biodiscovery Pipeline**
24 partners - EU funding: 9M€



PharmaSea Partners

UniAbdn, DeepTek,
Aquapharm RSC,
BioBridge

KULeuven,
eCoast



Access to both poles, the world's deepest trenches and thermal vents



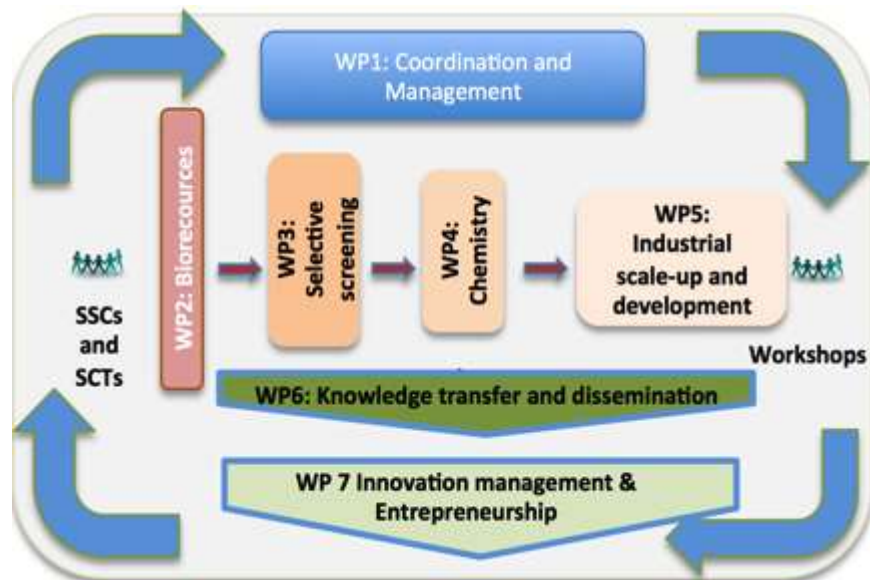
H2020-MSCA-RISE-2015-2019 OCEAN MEDICINES



Work plan and Consortium

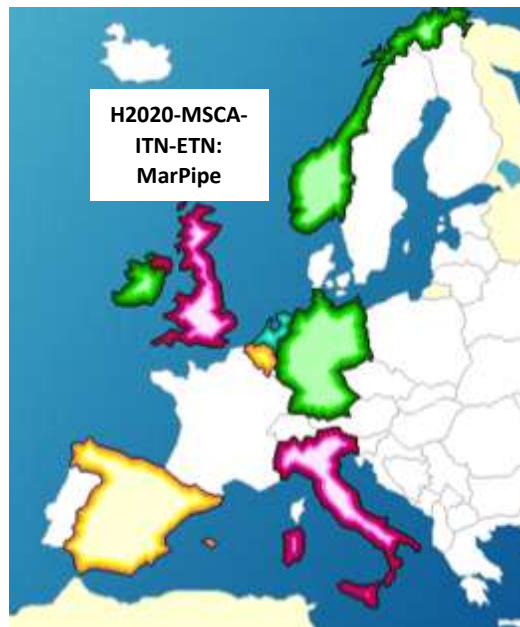
EU Funds: € 373.500,00

IBP-CNR Funds: € 49.500,00



Partner organization	Supervisors
1 – IBP-CNR, Naples, IT COORDINATOR	Donatella de Pascale
2- SZN, Naples, IT	Adrianna Ianora
3- UiT, Tromso, NO	Jeanette H Andersen
4- UNIABDN, Aberdeen UK	Marcel Jaspars
5- Fundacion MEDINA, Granada, SP	Fernando Reyes
7- UWC, South Cape, SA	Marla Trinidad
8- eCOAST, Ostenda, BE	Thomas Vanagt
9- Epi-C Srl, Naples, IT	Lucia Altucci
10- Sea4Us, Lisbon, PT	Pedro Lima

H2020-MSCA-ITN-ETN 2016-2020: MarPipe Improving the flow in the pipeline of the next generation of marine biodiscovery scientists



Partner organization	Supervisors
1 – IBP-CNR, Naples, IT COORDINATOR	Donatella de Pascale
2- SZN, Naples, IT	Adrianna Ianora
3- UiT, Tromso, NO	Jeanette H Andersen
4- UNIABDN, Aberdeen, UK	Marcel Jaspars
5- GEOMAR, Kiel, DE	Deniz Tasdemir
6- KU Leuven, BE	Peter de Witte
7- University College Cork	Alan Dobson
8- eCOAST, Ostenda, BE	Thomas Vanagt
9- Fundacion MEDINA, Granada, SP	Fernando Reyes
10- University of Wageningen, NL	Detmer Siphema
11- Italtitech, IT	Rolando Lorenzetti

EU funding requested: € 2.853.005,40
IBP-CNR funding: € 258.061,32

Partner organization	Supervisors
1 – Gent University, BE COORDINATOR	Katleen Raes
2- Ilvo, BE	Joan Robben
3- Chalmers University of Technology, SE	Ingrid Undeland
4- IBP-CNR, IT	Donatella de Pascale
5- NOFIMA, NO	Ragnhild Whitaker
6- Dublin Institute of Technology	Michelle Giltrap
7- Irish FishCanners Ltd	Karl Bonner



Three typical Shellfish by-products

ERA-NET, total funding: € 720.000

IBP-CNR funding: € 80.000

Objectives:

- 1) **Bivalves** have been identified as hosts for interesting **anti-inflammatory** compounds; particularly unusual fatty acids like furan fatty acids and “**novel**” omega-3’s. In crustaceans, anti-inflammatory properties have also been ascribed e.g. to astaxanthin and non hemorrhagic heparin-like compounds like sulphated polysaccharides.
- 2) **Antibacterial** effects- **antimicrobial peptides** are known to be present in the haemolymph of crustaceans. Molluscs and crustaceans possess numerous antimicrobial peptides because of their open circulatory system and their lack of an adaptive immune system. Over the past several years, many antimicrobial peptides have been found and characterized in crabs.
- 3) Mussel and Crab shells share a special capability to counter fouling even when submerged in sea water compared to ‘**inert**’ materials, such as wood, plastics or stone.
- 4) Plant horticultural stimulants: Chitin and its derivatives potentially improve plant crop health and yield when incorporated in the soil.

Take home message

- We established a biodiscovery pipeline for drug discovery of new compounds for pharmaceutical applications, also by modifying metabolic pathway of microorganisms.
- Sustainable production of new compounds from marine source.
- OSMAC approach.

Perspectives

Highlight the role of Blue Biotechnology at EU level as a tool for building up a community of scientists for the discovery of new drugs.

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e Proteine



Thank you for your attention



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RESEARCH INFRASTRUCTURE CLUSTER