



# Newsletter

## *Magazine*

4<sup>th</sup> Issue May 2023

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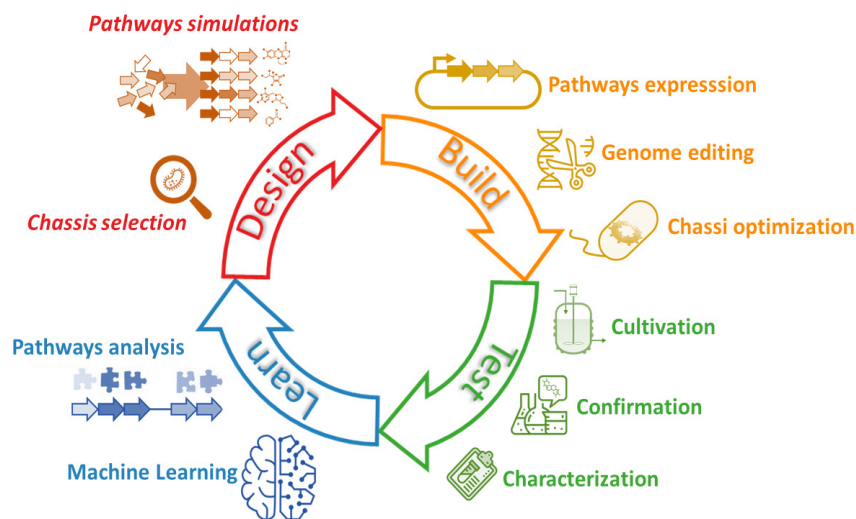


This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement No **101000794**.





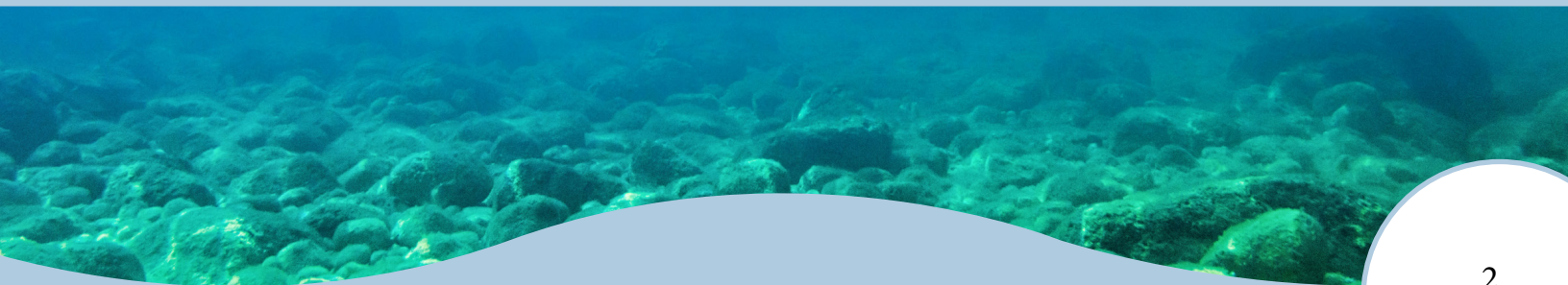
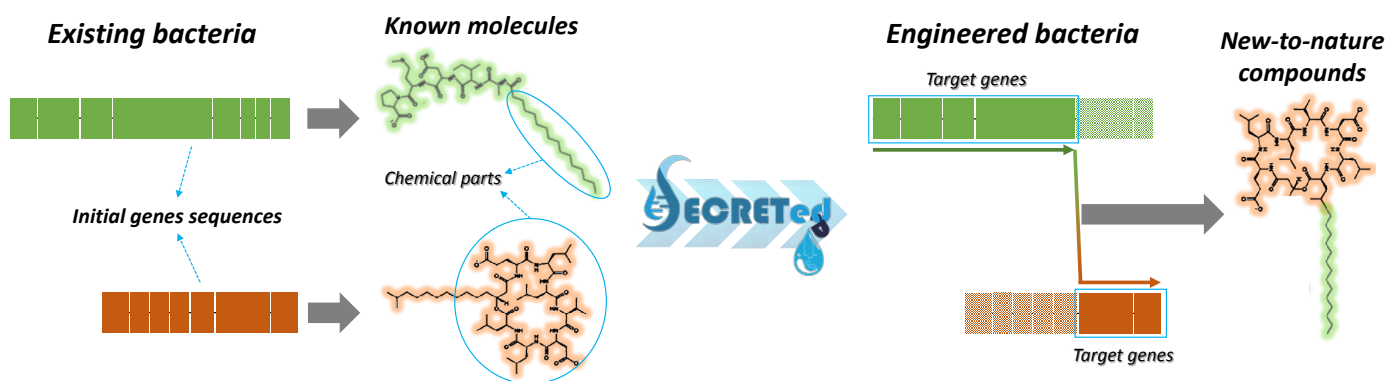
# SECRETed at a glance



SECRETed is an EU Funded H2020 project that aims to unlock the potential of aquatic biotechnology for the development of industry-driven compounds for end-user applications in the fields of pharmaceuticals, cosmetics, food supplements and agriculture.

The project follows an iterative Design-Build-Test-Learn approach to reverse engineer Biosynthetic pathways and develop microbial strains for the production of biosurfactants and siderophores with tailor-made properties.

The amphiphilic nature of biosurfactants and marine siderophores provides an exciting opportunity to develop methods of biosynthesis that would enable the exchange of their chemical parts towards the development of new-to-nature compounds.





# SECRETed at a glance

## The project is structured over 10 work packages (WP):

- WP1:** Ethics requirements  
*Leader: IDENER*
- WP2:** Microbial collections screening  
*Leader: MATIS*
- WP3:** Databases integration & Industry-driven designs  
*Leader: IDENER*
- WP4:** Systems metabolic engineering for microbial platform optimization  
*Leader: University of Sevilla*
- WP5:** Lab-scale fermentation & chemical characterization of compounds  
*Leader: University of Athens*
- WP6:** Pilot systems for process optimization  
*Leader: Bio Base Europe Pilot Plant*
- WP7:** Proof of concept for end users' applications  
*Leader: Sphera Encapsulation*
- WP8:** Sustainability assessments  
*Leader: Blue Synergy*
- WP9:** Dissemination & exploitation  
*Leader: EXELISIS*
- WP10:** Management & Coordination  
*Leader: IDENER*





# Achievements

## 1 SideroTec HiSen™ assay

[Accuplex Diagnostics](#) successfully completed and launched (in 2023) the new SideroTec HiSen™ assay. As a response of microbial expression, excess siderophore contamination has potential for generation of inflammation. The test detects siderophores and iron chelation molecules at levels as low as 5nM; that is 100 times more sensitive than any other assay. The SideroTec HiSen™ assay has been recently used to detect siderophore contamination in new biobased therapeutic drug treatment under investigation with the FDA.



Figure 1: The SideroTec HiSen™ assay

## 2 Fungal siderophore

[Accuplex Diagnostics](#) demonstrated the use of fungal siderophores as novel antimicrobial tool to combat the growing incidence of antimicrobial resistance. The plan is to also focus on other novel siderophores over the following 2 years of SECRETed project.

## 3 Biosurfactants and siderophores process development

At [BBEPP](#), fermentation and down-stream purification protocols were developed and optimized for the production of biosurfactants and siderophores produced by marine and extremophilic microorganisms. The most promising strains were cultivated, while optimization of the fermentation strategy resulted in sufficient production yields which enabled further analysis of desired properties by the project end-users ([PharmaMar](#), [Accuplex Diagnostics](#), [Sylentis](#) and [Sphera Encapsulation](#)).

## 4 Systems biology

The microbial production of siderophores and biosurfactants is encoded within biosynthetic gene clusters (BGCs); namely, groups of closely located genes that encode the production of enzymes responsible for their synthesis and regulation. The research group of [Translational Genome Mining for Natural Products](#) (Eberhard Karls Universität Tübingen) focused on building a BGCs database linked to the SECRETed chemicals database and connecting genes to the bioactive moieties in the compounds of interest. The research group collected all publicly available information and built a database with 255 BGCs that encode the biosynthesis of 361 unique compounds (176 siderophores and 185 biosurfactants). Apart from the genetic information, this database contains the producers' information, the biosynthetic pathway (where known), the types of chelating groups in each molecule, as well as external links to NCBI, PubChem, NPAtlas, and MiBIG.

# Achievements

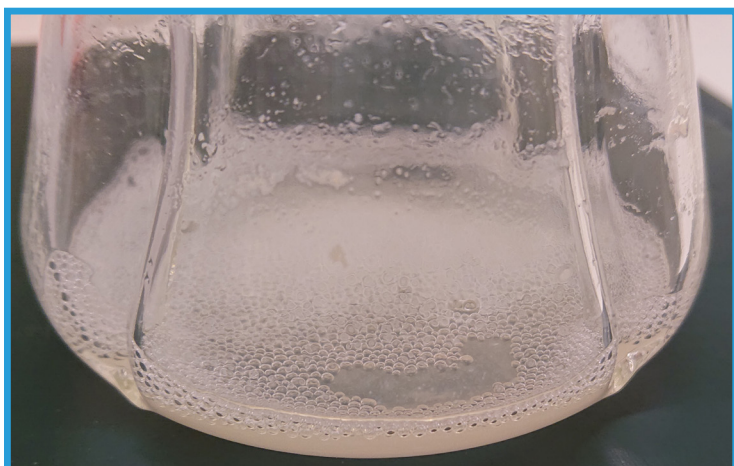


Figure 2: Biosurfactants (foam) by thermophiles

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## Biosurfactants Production

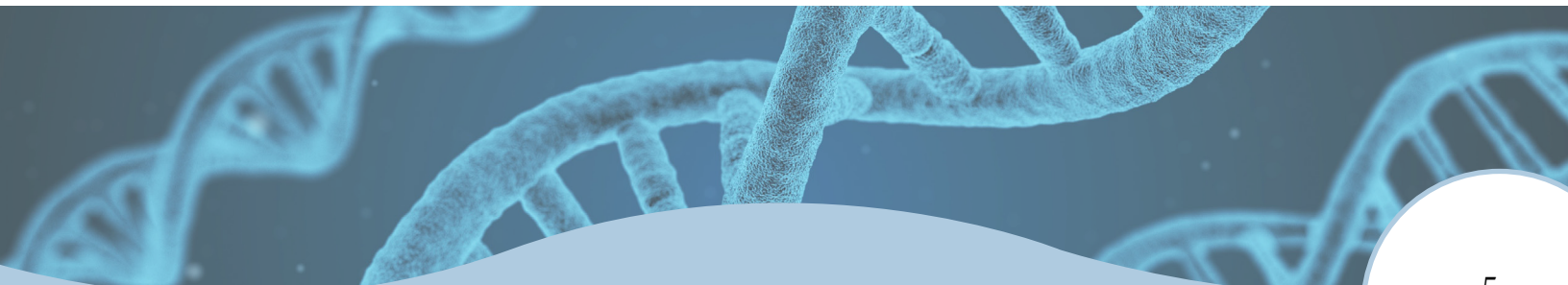
The research group of [prof. Eva Karlsson](#) (LUND University) has achieved significant milestones in advancing production, purification, and characterization of biosurfactants with the aim to expand the knowledge surrounding their properties. Lund university has screened >100 thermophilic strains; optimized cultivation conditions for increased biosurfactants yields; and developed isolation and purification methods from the cell-free culture broth. HPLC and mass spectrometry analyses indicated the existence of several mono-rhamnolipids (Rha-C8-C10, Rha-C10-C10, and Rha-C10-C12).

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## Heterologous production of rhamnolipids

The marine research institute of [Stazione Zoologica Anton Dohrn](#) in Naples holds a wide collection of extremophilic bacteria and has set-up fast screening of biosurfactants and siderophores producing strains. Secondary screening and analyses (chemical and genetic) resulted in bioactive molecules and their associated genes of production. A top-20 strains passed the evaluation for further genome sequencing and identification of biosynthetic gene clusters. Outcomes:

- Saccharide esters from marine strains (*Rhodococcus* I2R) were identified.
- Optimised protocol for production and purification of rhamnolipids.
- Biomolecules with antiviral and antimicrobial activity were identified.
- Development of microbial chassis and protocols for heterologous production of biosurfactants and siderophores.



# Achievements

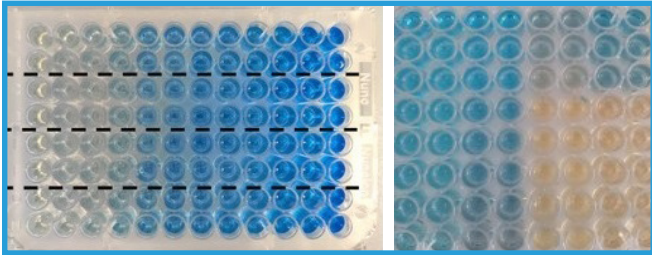


Figure 3: Detection and quantification of biosurfactants (left) and siderophores (right) in halophilic bacteria

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## Synthetic biology and systems metabolic engineering

The University of Sevilla ([prof. Montserrat Argandoña Bertran](#)) optimized several methods for the detection and quantification of biosurfactants and siderophores in halophilic bacteria avoiding interference of high salinity and media components. Cultivation conditions for increasing siderophore yields have been also optimized. From the strain collection of University of Sevilla, > 30 novel siderophores and biosurfactants producing strains from > 20 genera have been selected increasing hopes for new chemical structures and properties. > 15 strains with potential new BGCs have been found.

The contributing partners have been also focused on testing and the development of genetic tools to expand the genetic Toolbox available for halophilic, psychrophilic and thermophilic bacteria. Bacterial chassis have been selected and optimized for natural, engineered or heterologous production of target compounds. First new derivatives of biosurfactants from thermophilic and psychrophilic strains have been obtained by genetic engineering and they have been functionally characterized.

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## Dereplication

The Laboratory of Natural Products Chemistry ([National and Kapodistrian University of Athens](#)) worked on the microbial cultures' supernatants and biomasses of extremophilic bacterial strains as received by the University of Seville (SECRETed partner). The culture material was recovered via solvent extraction (multi-step resin-assisted procedure) and analysed against in-house libraries demonstrating the presence of biosurfactant and siderophore compounds as well as a plethora of unknown molecules sharing similar features.

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## Rhamno-lipids extract ([Sphera Encapsulation](#))

- Soluble in water; able to significantly lower surface tension to  $\approx 26$  mN/m.
- Very negative surface charge.
- 4 Rhamnolipids tested: good emulsifying properties comparable with commercial surfactants (Emulsion Index > 85%).
- Based on visual appearance of the emulsion and the size of the particles, the rhamnolipids show behavior comparable to SDS.

# Achievements

## 10 CoQ10 ENCAPSULATION

CoQ10, also known as ubiquinone, is a lipophilic molecule which plays a fundamental role in the mitochondrial respiratory chain. It is well known also as a major antioxidant; it was proven to be more effective than Vitamin E in the protection of lipids from peroxidation reactions. The use of CoQ10 is reported to improve immune functions and contribute to the treatment of cardiovascular diseases, various neurological disorders, diabetes, cancer and obesity. However, the use of this molecule is hindered by its strong hydrophobic character and low bioavailability. In addition, CoQ10 is a light sensitive molecule. [Sphera Encapsulation](#) is exploring the possibility to encapsulate CoQ10 through nano-emulsions to protect CoQ10 from degradation, control the release during digestion, and increase its bioavailability.

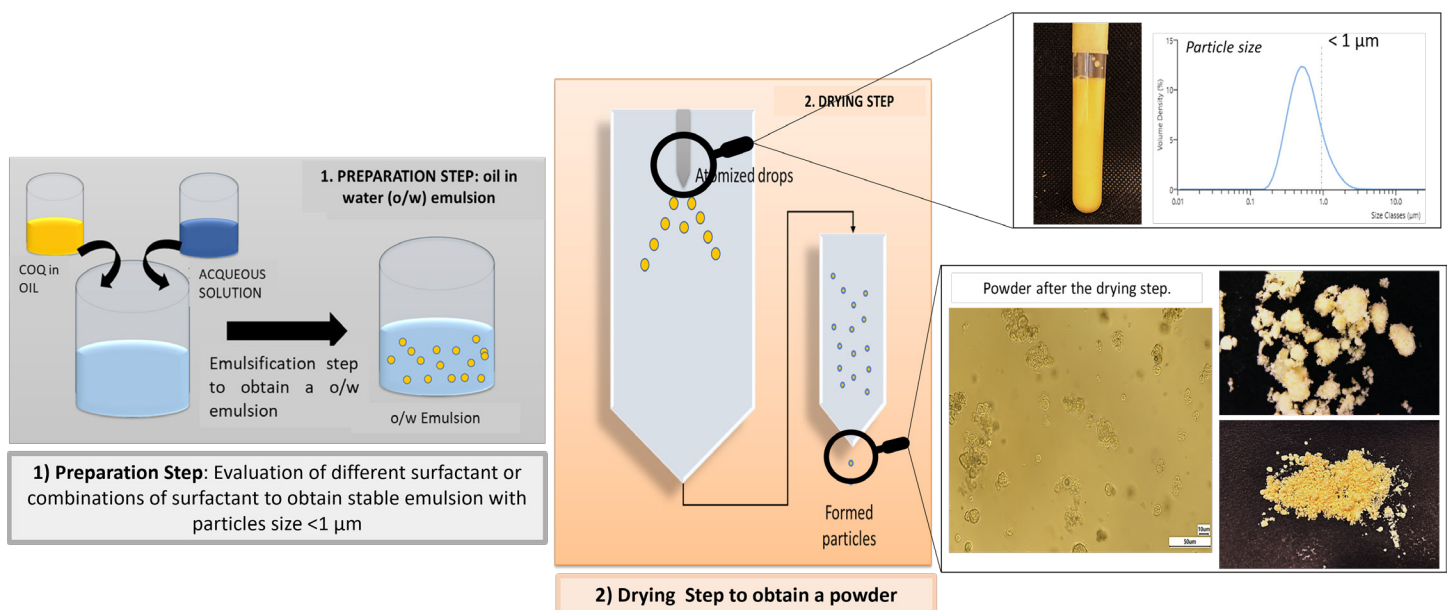


Figure 4: Encapsulation approach of CoQ10





# Achievements

## SCIENTIFIC ARTICLES

Title	Abstract
<p><u><a href="#">Dihydroauroglauclin Isolated from the Mediterranean Sponge <i>Grantia compressa</i> Endophyte Marine Fungus <i>Eurotium chevalieri</i> Inhibits Migration of Human Neuroblastoma Cells</a></u></p> <p><u><a href="#">Open Access Data</a></u></p>	<p>Cancer cell migration is a hallmark of the aggressiveness and progression of malignancies such as high-risk neuroblastoma. Given the lack of effective therapeutic solutions to counteract cancer progression, basic research aims to identify novel bioactive molecules with inhibitory potential on cancer cell migration.</p>
<p><u><a href="#">Water potential governs the effector specificity of the transcriptional regulator XylR of <i>Pseudomonas putida</i></a></u></p>	<p>The biodegradative capacity of bacteria in their natural habitats is affected by water availability. In this work, we have examined the activity and effector specificity of the transcriptional regulator XylR of the TOL plasmid pWW0 of <i>Pseudomonas putida</i> mt-2 for biodegradation of m-xylene when external water potential was manipulated with polyethylene glycol PEG8000.</p>
<p><u><a href="#">Production of selenium nanoparticles occurs through an interconnected pathway of sulphur metabolism and oxidative stress response in <i>Pseudomonas putida</i> KT2440</a></u></p>	<p>The soil bacterium <i>Pseudomonas putida</i> KT2440 has been shown to produce selenium nanoparticles aerobically from selenite; however, the molecular actors involved in this process are unknown. Here, through a combination of genetic and analytical techniques, we report the first insights into selenite metabolism in this bacterium.</p>
<p><u><a href="#">Evaluation of Antimicrobial Properties and Potential Applications of <i>Pseudomonas gessardii</i> M15 Rhamnolipids towards Multiresistant <i>Staphylococcus aureus</i></a></u></p>	<p><i>Staphylococcus aureus</i> is a Gram-positive opportunistic human pathogen responsible for severe infections and thousands of deaths annually, mostly due to its multidrug-resistant (MDR) variants. The cell membrane has emerged as a promising new therapeutic target, and lipophilic molecules, such as biosurfactants, are currently being utilized.</p>
<p><u><a href="#">MIBiG 3.0: a community-driven effort to annotate experimentally validated biosynthetic gene clusters</a></u></p>	<p>With an ever-increasing amount of (meta)genomic data being deposited in sequence databases, (meta)genome mining for natural product biosynthetic pathways occupies a critical role in the discovery of novel pharmaceutical drugs, crop protection agents and biomaterials. The genes that encode these pathways are often organised into biosynthetic gene clusters (BGCs).</p>





# Achievements

## SCIENTIFIC ARTICLES

Title	Abstract
<p><u><a href="#">Medium development and production of carotenoids and exopolysaccharides by the extremophile <i>Rhodothermus marinus</i> DSM16675 in glucose-based defined media</a></u></p> <p><u><a href="#">Open Access Data</a></u></p>	<p>Two defined media were initially prepared: one including a low addition of yeast extract (modified Wolfe's medium) and one based on specific components (defined medium base, DMB) to which two amino acids (N and Q), were added. Cultivation trials of <i>R. marinus</i> DSM 16675 in shake flasks, resulted in maximum cell densities (OD620 nm) of <math>2.36 \pm 0.057</math>, cell dry weight (CDW) <math>1.2 \pm 0.14</math> mg/L, total carotenoids <math>0.59 \times 10^{-3}</math> mg/L, and EPSs <math>1.72 \pm 0.03</math> mg/L using 2 g/L glucose in DMB.</p>
<p><u><a href="#">Combining OSMAC Approach and Untargeted Metabolomics for the Identification of New Glycolipids with Potent Antiviral Activity Produced by a Marine Rhodococcus</a></u></p> <p><u><a href="#">Open Access Data</a></u></p>	<p>Natural products of microbial origin have inspired most of the commercial pharmaceuticals, especially those from Actinobacteria. However, the redundancy of molecules in the discovery process represents a serious issue. The untargeted approach, One Strain Many Compounds (OSMAC), is one of the most promising strategies to induce the expression of silent genes, especially when combined with genome mining and advanced metabolomics analysis. In this work, the whole genome of the marine isolate <i>Rhodococcus</i> sp. I2R was sequenced and analyzed by antiSMASH for the identification of biosynthetic gene clusters.</p>
<p><u><a href="#">Applicability of Control Materials To Support Gene Promoter Characterization and Expression in Engineered Cells Using Digital PCR</a></u></p> <p><u><a href="#">Open Access Data</a></u></p>	<p>The use of standardized components and processes in engineering underpins the design-build-test model, and the engineering of biological systems is no different. Substantial efforts to standardize both the components and the methods to validate the engineered biological systems is ongoing. This study has developed a panel of control materials encoding the commonly used reporter genes GFP and RFP as DNA or RNA molecules.</p>

Read all our Publications here!



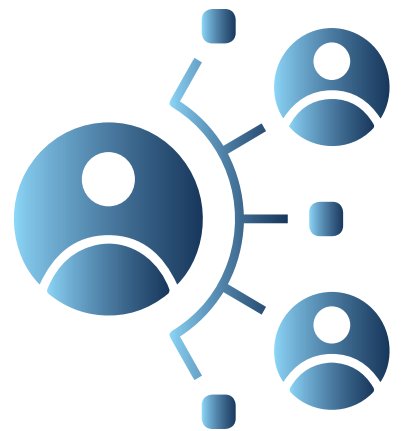
## Get connected



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### Become a listed stakeholder and benefit from

- Specific dissemination scientific material
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- Promotion through SECRETed website and social media
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# Dissemination activities



On the 30<sup>th</sup> of March 2023, [Bio Base Europe Pilot Plant](#) organized [“The Tech4Biowaste Database”](#) workshop. The Tech4Biowaste database is the first and only open-source database for biowaste conversion technologies. Grab this unique, free and online opportunity to check it out under the guidance of its creators.

[Sphera Encapsulation](#) participated in the [Fi Europe](#) conference (6 December 2022). The event promoted alternatives and innovative solutions in the field of Food & Beverage, networking, and access to content of hot trends towards a sustainable food future.



[Blue Synergy](#) took part in the virtual event: [“Horizon Europe - Cluster 6 Calls 2023 - virtual Brokerage Event”](#), 19 December 2022. The network of National Contact Points for Horizon Europe - Cluster 6: Food, Bioeconomy, Natural Resources, Agriculture and Environment.

[Blue Synergy](#) participated in the [“GREENET Brokerage Event for HE Cluster 5”](#), 15 December 2022. The network of Horizon Europe CL5 National Contact Points organized a networking and brokerage event for proposals from the Work Programme of "Cluster 5: Climate, Energy and Mobility"





# Dissemination activities



[Blue Synergy](#) participated in the [“Horizon Europe - Industry 2023 Brokerage event”](#), 14 December 2022. NCP4Industry, the network of Cluster 4 - Industry National Contact Points organized a brokerage event with pre-arranged online Face2Face meetings complementing the European Commission's Horizon Europe Information Day on Cluster 4 - Digital, Industry & Space with a 2023 deadline.

[Bio Base Europe Pilot Plant](#) was in the [IBioIC's 9th Annual Conference](#) on 16 March 2023. IBioIC's Annual Conference is the largest industrial biotechnology conference in the UK and attracts an ever-growing cohort of key figures across policy, industry, research and academia.



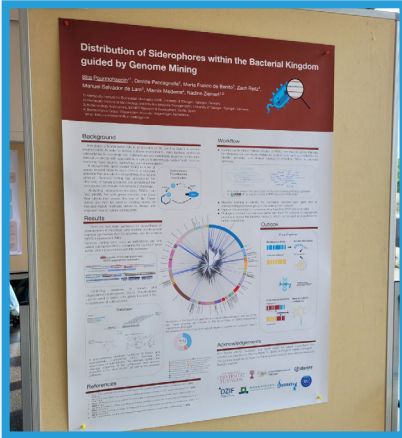
[University of Seville](#), partner of SECRETed project, joined the [“I edition of Multidisciplinary Conference on Science, Technology, Engineering and Mathematics \(STEM\) for scientific dissemination”](#) celebrated in Sevilla, on 14 February 2023.

[Stazione Zoologica Anton Dohrn](#) participated in [BIOPROSP 23](#) (14 March 2023) presenting the SECRETed research activities and its actual contributions towards responsible harnessing marine microorganisms to fully exploit the potential of aquatic biotechnology to produce novel molecules with industry-driven properties.





# Dissemination activities



[Eberhard Karls Universität Tübingen](#) presented a poster with the advances of SECRETed in the international [German Conference on Bioinformatics](#) (6 September 2022), which is devoted to all areas of bioinformatics and meant as a platform for the whole bioinformatics community.

[Eberhard Karls Universität Tübingen](#) was in the [“VAAM workshop of microorganisms-producing-natural-products”](#), which took place on 7 September 2022.

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International VAAM Workshop 2022  
Biology of Microorganisms Producing Natural Products

September 7 - 9, 2022 | Dortmund, Germany






# Contact Info

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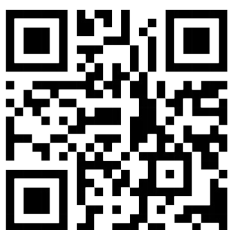


## The SECRETed consortium

Sustainable Exploitation of bio-based Compounds  
Revealed and Engineered from naTural sources



Stazione  
Zoologica  
Anton Dohrn  
Napoli



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