



Newsletter

Magazine

5th Issue November 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**.



What is SECRETed

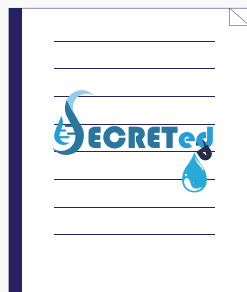


Overview

[SECRETed](#) is an 48-month EU Funded research project (Grant agreement ID: 101000794) oriented to the biodiscovery of novel industry-driven biosurfactants and amphipathic siderophores scaled up “from lab to application”. The [SECRETed](#) project entered the 3rd year having generated numerous promising results taking advantage of AI-driven biotechnology, experimental and pilot production of bio-based resources.

OBJECTIVES

- Unlock the potential of **marine & extremophilic bacteria** (4 microbial collections) for tailor made amphiphilic molecules.
- **Biochemicals database**: molecular structures, physicochemical characteristics, bioactivities, metabolic pathways, Biosynthetic Gene Clusters (BGCs).
- **“Mix and Match”** integrated with metabolic and process engineering for the **selection of BGCs** producing new-to-nature molecules.
- Implementation of **novel biosurfactants** in nano-encapsulation of ingredients with pharmaceutical and nutritional activity.
- **New siderophore** formulations to enhance diagnostics and therapeutic potential.
- **Pilot plant scale** fermentation of developed microbial strains (TRL-6)
- **Environmental, economic and social perspectives.**



[12 open access scientific publications](#)

[5 Public deliverables](#)



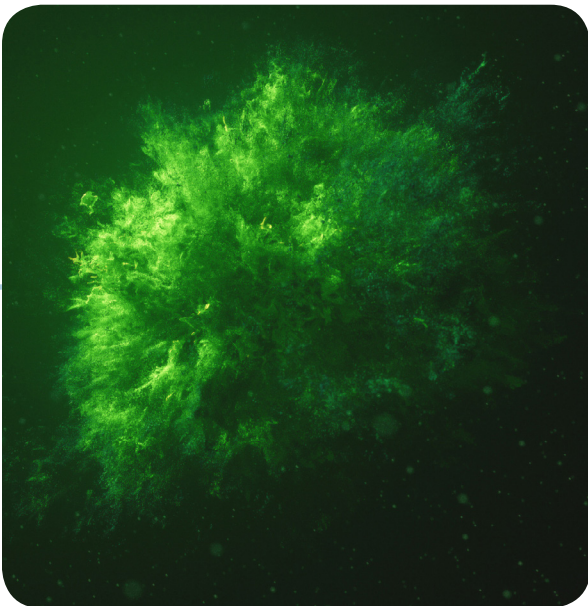
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[SECRETed is a founding member of the AIMS Cluster](#)



What is SECRETed

Biosurfactants



Surfactants are a very diverse group of lipids with a common amphiphilic nature (hydrophilic and hydrophobic domains within the same molecule) that appear lower interfacial tension allowing the solubilisation of hydrophobic substances in water. Biosurfactants are produced by microorganisms offering reduced environmental impacts and dependency on fossil resources. They organise themselves in self-assembled supramolecular structures (micelles, liposomes or microemulsions) capable of protecting and working as a carrier of active ingredients enabling their targeted and controlled (bio)activity in cosmetic, nutritional and pharmaceutical applications. Moreover, biosurfactants exhibit a higher activity at lower concentrations compared with many synthetic surfactants, showing very low critical micelle concentration (CMC), low toxicity, high biodegradability and tolerance to extreme conditions like high temperature, salinity and extreme pHs.

Siderophores

Siderophores bond and transport Fe^{3+} ions featuring their activity for biological systems. Marine and extremophilic bacteria have been recently identified as a prolific source for the discovery and production of novel amphiphilic molecules, which have the ability to chelate Fe^{3+} ions. Non-amphiphilic siderophores can also promote plant growth by inhibiting pathogens' growth and apply in medical treatments, like against bacterial infections, iron demanding tumor cells and as antibiotics uptake facilitators. Modifications on the molecular structure of known siderophores can further improve diagnostic applications or their therapeutic potential. Siderophores-antibiotics conjugation formulas constitute the major application, where siderophores act as a "Trojan Horse" to facilitate antibiotics' uptake by traversing the cell membrane.

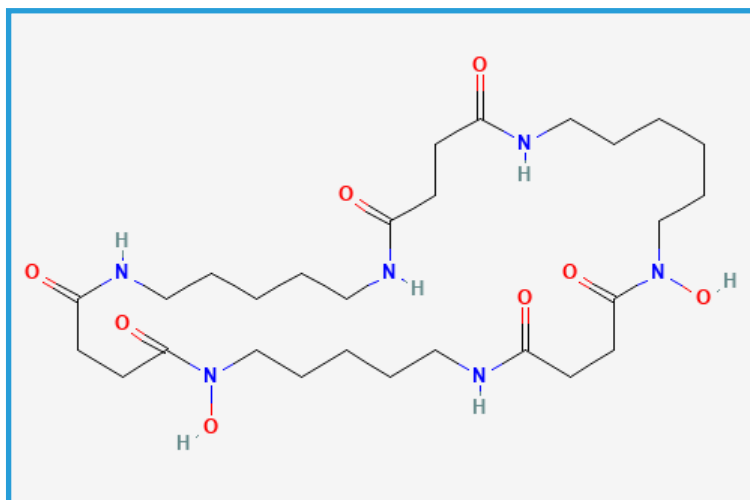


Achievements

1

Strains producing recombinant siderophores and surfactants

The [Imperial College London](#) continued the work of assembling pathways for the recombinant production of siderophores and surfactants. With the support of the database developed by IDENER, there have been identified bacterial gene clusters of appropriate length and cofactor requirements with the potential of producing novel molecules of interest belonging to the enacyloxin and desferrioxamine families. DNA fragments containing the corresponding genes for both sets of pathways have been generated and are being cloned. We expect to fully assemble the pathway in the coming weeks. In parallel, [Imperial College London](#) has continued the optimisation of strains using a directed evolution approach. Selection experiments monitored by Next-Generation sequences rendered a list of mutations in *Pseudomonas Putida* that are expected to correlate with higher production capabilities. These individual mutations (and their combinations) are being reconstructed in the original strain using a CRISPR-based gene editing method and our preliminary results indicate that some of the resulting strains can grow faster than the wild-type.



2

Biosynthetic Gene Clusters database for tailor-made compounds

Utilizing modern bioinformatics tools, the [EKUT research team](#) linked compounds to gene clusters, deepening our understanding of their operations. This insight aids the design of genetic sequences for specific compound production. Recently, we explored theoretical approaches to create new compounds by modifying existing gene clusters with chosen genetic elements. Currently, we're expanding our database of synthetic BGC combinations. Additionally, a genome mining analysis identified ~5366 potential siderophore producers across the Bacterial kingdom. We had the opportunity to present our initial findings as posters at the ISMB/ECCB 2023, VAAM Annual Conference 2023, and VAAM Symposium for Biology of Bacterial Natural Producers.

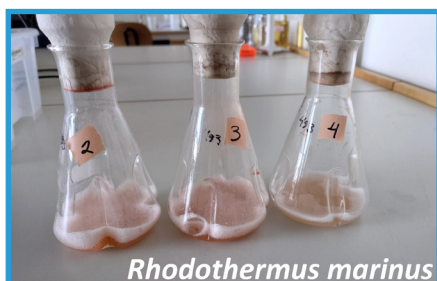
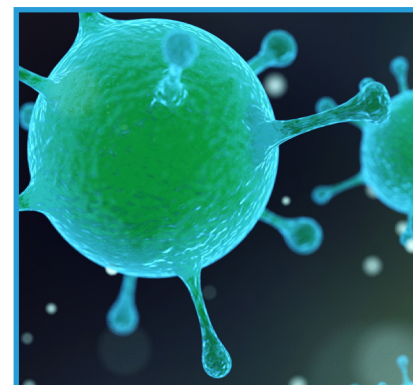


Achievements

3

Siderophores against antibiotic resistance bacteria

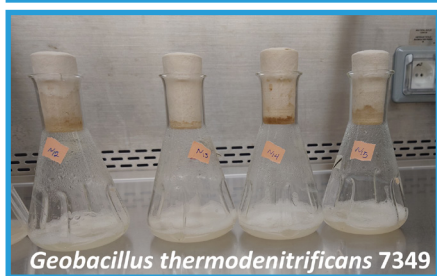
Health care systems worldwide are rapidly running out of ways to treat infections due to the overuse of most antibiotics and the growing resistance developed by many bacteria. New and novel ways to treat infections are required, and [Accuplex Diagnostics](#) is beginning to show one possible solution to address the problem of resistance. Fungi were the source of the first known antibiotic, penicillin. Accuplex Diagnostics is now showing that other fungal molecules, referred to as siderophores (think about them like tiny magnets) are also effective in treating infections. Most microbes need iron to grow and survive, so if you can stop a bacteria getting iron, you can stunt or stop its growth. [Accuplex Diagnostics](#) has shown that one fungal specific siderophore can reduce or eliminate growth of *Klebsiella pneumoniae* and *Acinetobacter baumannii*, which are two key bacteria high in the list of bacteria with resistance to all existing antibiotics. Accuplex Diagnostics is now exploring other new and novel siderophores identified in SECRETed.



4

Biosurfactants production from thermophiles

The production of biosurfactants by thermophiles (*Rhodothermus marinus* and *Geobacillus thermodenitrificans* 7349) collected from hot springs in Iceland has been examined and optimized by [LUND university](#). The production was tested in a defined medium supplemented with glucose as sole carbon source in batch and fed-batch mode resulting in biosurfactants yields of >0.2g/g glucose. A method was developed to analyse produced biosurfactants (rhamnolipids and lipopeptides) by HPLC and MS using CAD detector. Liquid-liquid extraction was used and optimized for the extraction and purification of the produced biosurfactants. Moreover, the production of siderophore compounds by the examined thermophiles has also been evaluated using fluorescence spectrometry, while the CMC values of produced rhamnolipids are determined using fluorescence probes as an advanced method.

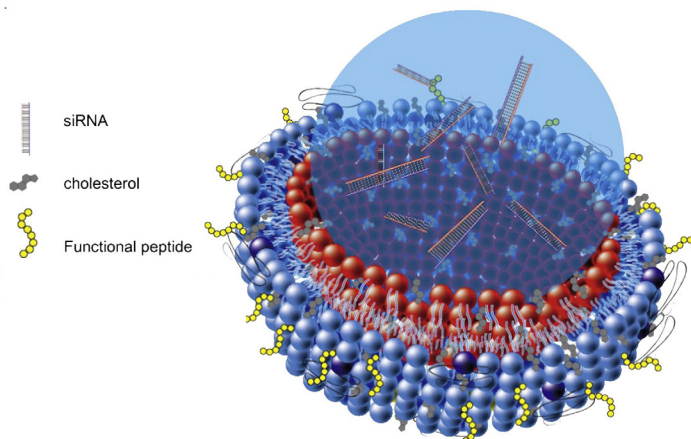


5

SYLENTIS

[SYLENTIS](#) is collaborating with Sphera Encapsulation towards the development of RNAi-based drug delivery systems that are scalable, and industrially developable and use biodegradable and sustainable compounds. [SYLENTIS](#) as an end-user in SECRETed is interested in the development of delivery systems that allow future targeted drug delivery to cells of the ocular system and particularly the retina. SYLENTIS is working on several diseases

Achievements



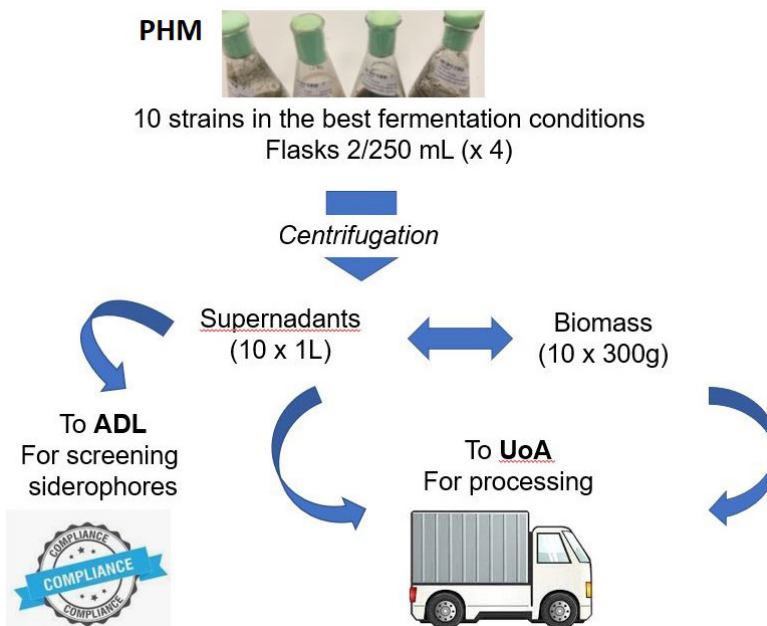
that involve ocular toxicity processes associated with iron accumulation in the retina and aims to identify, isolate, produce and purify surfactants, siderophores and biodegradable substances with peptidomimetic function that can be part of the final formulations.

SYLENTIS, in collaboration with IDENER, has been working on finding biosurfactants, siderophores, and peptidomimetics that have a tropism for retinal cells and will be used as components in future nanoparticles that can specifically and efficiently deliver nucleic acids to the ocular cells of interest (retina), as well as control the iron-mediated toxicity of these pathologies.

6

Screening bacteria strains for compounds with cytotoxic activity

The production of 10 selected bacteria strains has been scaled-up and pre-screened by **PharmaMar** for the detection of siderophores of interest. Supernatants and produced biomass has been shared within SECRETed for subsequent isolation and identification of siderophores. PHM has also re-screened samples of rhamnolipids (produced by BBEPP) by pooling the samples and following an extraction procedure with ethyl acetate and methanol. The high cytotoxic activity was confirmed.

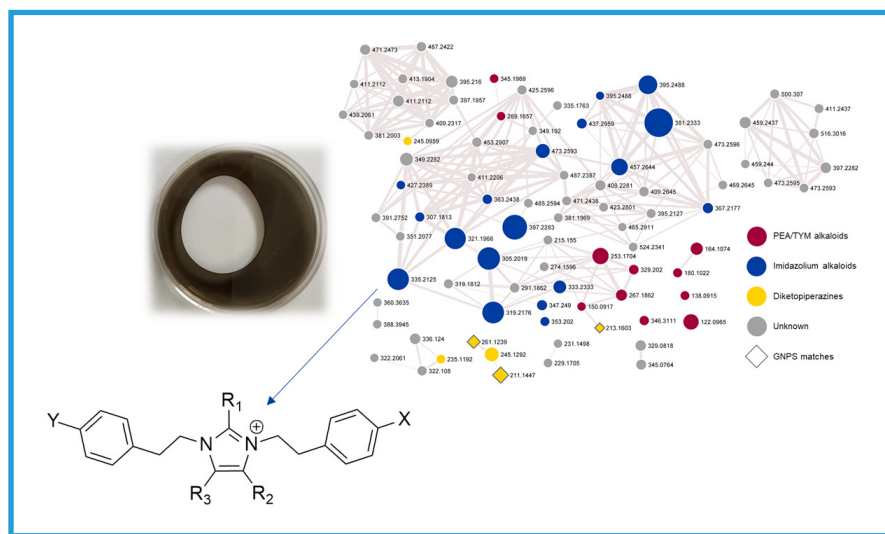


Achievements

7

Screening bacteria for biosurfactants and siderophore compounds

Combining the One-Strain-Many-Compounds (OSMAC) approach and tandem mass spectrometry molecular networking, [Stazione Zoologica Anton Dohrn \(SZN\)](#) has recently discovered a new family of cationic biosurfactants, i.e. imidazolium alkaloids from the halophilic bacterium *Shewanella aquimarina*. These



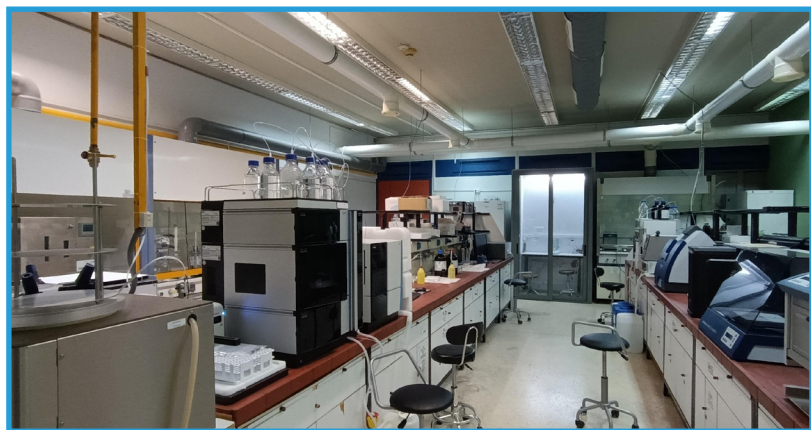
molecules displayed a broad spectrum of action, as exerting antibacterial activity against antibiotic-resistant *Staphylococcus aureus* clinical isolates, synergistic effects with antibiotics included in clinical settings, antiviral activity against viruses with and without envelope, and anthelmintic activity against *Caenorhabditis elegans*. [SZN](#) has also expanded the biosurfactant lipopeptide family from the deep-sea *Rhodococcus* sp.I2R through the detection of approximately 20 congeners, shedding light on the architecture of their NRPS biosynthetic pathway. The MS-based

approach set up by SZN for targeted identification of siderophores, uncovered a *Tsukamurella* strain as a producer of mirubactins, well-known iron-chelating molecules, including a new congener.

8

Recovery of biosurfactants and siderophores from fermentation supernatants

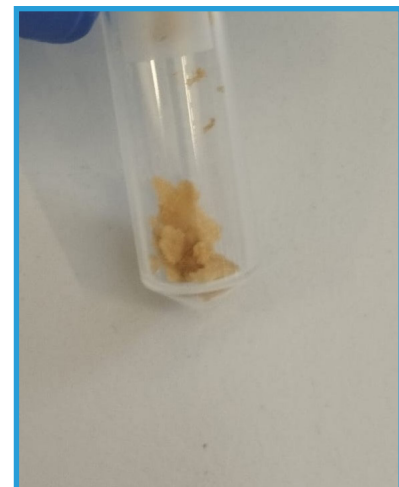
[University of Athens](#): Extracts of supernatants obtained through the liquid-liquid and multi-step XAD resin-assisted extraction techniques, (utilizing ethylacetate and methanol respectively), have undergone fractionation processes using molecular exclusion chromatography, and centrifugal partition chromatography (CPC) techniques. The most promising fractions are currently undergoing purification procedures, followed by comprehensive analyses using mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) techniques, to elucidate the intricate structures of the compounds present.



Achievements

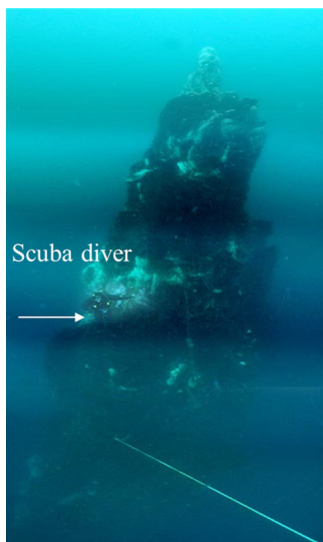
9 Screening of biosurfactant and siderophore producers (halophilic strains)

The [University of Sevilla](#) has expanded their halophilic strain collection and completed the screening of potential biosurfactant and siderophore producers. A great number of strains have been positive for production of bioactive compounds, some of which being outstanding producers. With the aim of increasing biosurfactant and siderophore yields, the influence of different factors on their production has been analysed. Following media optimization, lab-scale experiments have been carried out to adapt halophilic culture media to bioreactor requirements. First purification steps have been done to obtain extracts of the bioactive metabolites. The analysis of these extracts corroborated biosurfactant and/or siderophore activity for some strains.



10 Screening thermophilic bacteria for functional assays for siderophores and biosurfactants

[MATIS](#) has been finishing screening of strains of thermophilic bacteria from coastal hot springs. More than 200 strains were screened with one or more functional assays for siderophores and biosurfactants in collaboration



with LUND university and Accuplex Diagnostics. Their genomes were sequenced and were screened for relevant gene clusters with the bioinformatic program AntiSmash in collaboration with EKUT university. A number of positive strains have been selected for improvement of growth and production of targeted biomolecules for analysis of structure and properties. A large metagenome microbial

sequence library, from the sea around Iceland is currently being screened for siderophore and biosurfactant gene clusters in collaboration with IDENER.

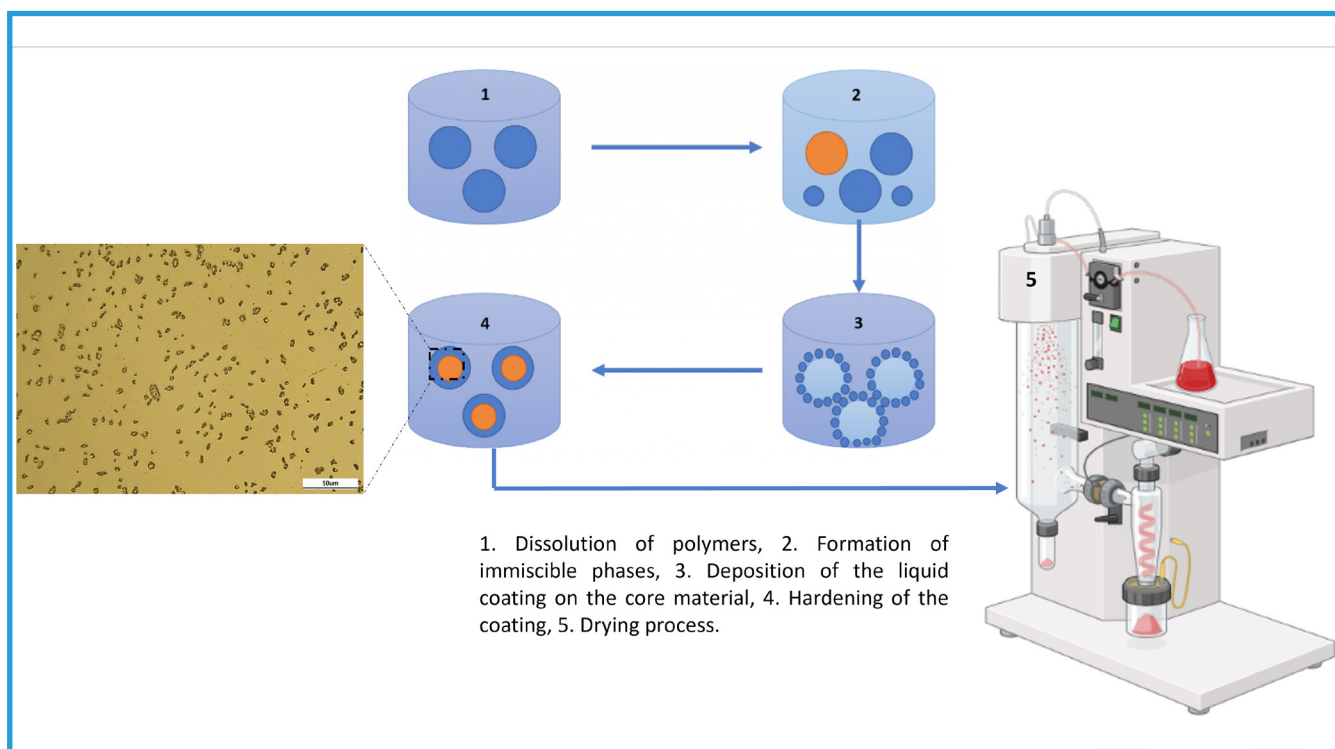
Achievements

11

Biopesticide microparticles: encapsulation of oregano essential oil

Synthetic pesticides are widely used in agrochemical field to increase crop yields, but they can have some adverse effects on the biosphere. They can weaken the soil by limiting its transpiration, damage other plants, and can lead to water pollution by intrusion of pesticides into water bodies. These effects can then be transmitted to animals and humans, resulting in serious diseases. Since there are still no safe natural-based solutions on the market, biopesticides are emerging as a new tool to counter the massive use of synthetic pesticides. Biopesticides can be formulated as microparticles with higher water solubility, protective action against environmental degradation, and a higher penetration rate. Among them, oregano essential oil is a valuable candidate as it has shown antibacterial and antimicrobial properties mainly due to its ability to break bacterial and fungal membranes and viral envelopes.

Sphera Encapsulation is evaluating the possibility of encapsulating oregano essential oil through complex coacervation and spray drying using natural polymers as wall material and trying to replace synthetic surfactants with biosurfactants developed in the SECRETed project. Crude extract containing a mixture of Rhamnolipids from *Pseudomonas Gessardii* behaved similarly to the commercial surfactant Sodium Dodecyl Sulfate (SDS) used as a reference, confirming that synthetic surfactants like SDS can be replaced by a bio-surfactants maintaining similar performance.





Achievements



12 Life Cycle Assessment (LCA)

The goal is to reduce environmental burdens through the adoption of innovative bio-based surfactants. The [Blue Synergy](#) team has meticulously defined the scope to ensure that our efforts are laser-focused on achieving this objective. They have also defined functional units and reference flows for each LCA scenario to ensure comparable results, relevant to our specific objectives. The invaluable Life Cycle Inventory of the SECRETed raw materials has been compiled along with LCA assumptions, baselines and benchmarks to compare and showcase the potential benefits and feasibility of the SECRETed biosurfactants across various industrial sectors.

13 Scaling up biosurfactants production

[Bio Base Europe Pilot Plant](#) finished the lab scale bioreactor work mostly and is now testing the SECRETed processes at 30L scales. As such we are transferring the processes from glass bioreactors to bigger stainless steel bioreactors that are pressurised. As such, we are evaluating whether the processes in glass reactors perform the same in the stainless steel reactors. With these bigger volumes of fermentation broth we were also able to assess more purification techniques to find the best purification flow for the SECRETed processes for further scale-up to 150L in 2024.”





Events

SECRETed & AIMS Cluster events

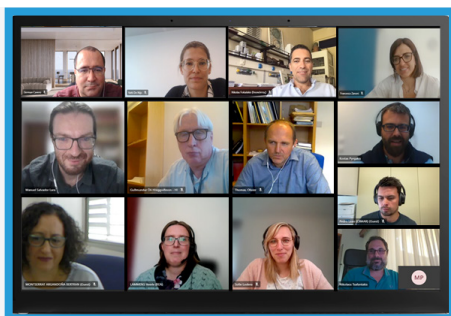


AIMS CLUSTER

[SECRETed](#) is a founding member of the AIMS Cluster. In the context of [XVII MaNaPro conference](#) (3 September 2023, Granada, Spain), the [AIMS Cluster](#) had a 2-hour session to present the activities of its members. All 4 projects of AIMS Cluster presented their key research activities and their 2-year progress, while a Q&A session with the audience followed.

Annual Meeting of AIMS Cluster

All 4 projects of the AIMS Cluster held the [2nd annual meeting of the cluster](#) in 22 May 2023 (Online). The project coordinators and dissemination managers of the project meet at least once a year to discuss about progress and complementarities among projects, as well as to evaluate and update their joint dissemination strategy.



SECRETed Review meeting

The [2nd Review Meeting](#) concerning the reporting period M12-M24 of SECRETed project was successfully completed on the 14th of September, 2023 (Online). The project coordinator (IDENER) and all work package leaders (IDENER, MATIS, USE, UoA, BBEPP, SE, BlueSynergy, EXELISIS) presented the project progress and received positive comments from the Project Officer and the reviewers.

4th General Assembly meeting

The [General Assembly meeting of SECRETed](#) was hosted by Bio Base Europe Pilot Plant and held in Ghent (Belgium) on 19-20 June, 2023. The project partners presented their 6-month advances and progress and planned next steps. SECRETed just entered the 3rd year summarising promising results including advanced strains, novel compounds and associated Biosynthetic Gene Clusters, and a strong biosurfactants and siderophores database.





Events

Conferences, events, YouTube Channel & Vlog



Research Society Event

[Accuplex Diagnostics](#) participated in the [The Irish Mass Spectrometry Society](#). 10 May 2023, Dublin (Ireland).

Conference

[University of Seville](#) presented SECRETed progress in the [National Conference of Spanish Society of Microbiology](#). 25-25 June, 2023, Burgos (Spain)



Summer School

[Stazione Zoologica Anton Dohrn](#) organised the [International Summer School on Natural Products](#) on the 2-7 July 2023, Maratea (Italy)

Congress

[University of Seville](#) participated in the [10th FEMS Congress of European Microbiologists](#). 9-13 July, 2023, Hamburg (Germany).



Website

On the 25th September 2023, the SECRETed research team from [Imperial College London](#) launched its official website: [iGEMx](#)



Events

Conferences, events, YouTube Channel & Vlog



Conference

[Stazione Zoologica Anton Dohrn](#) participated in the [31st International Symposium on the Chemistry of Natural Products and 11th International Congress on Biodiversity](#).

15-19 October 2023, Naples (Italy).

[Sphera Encapsulation - The Veronese factory of encapsulation](#)

- Mini Spray Dryer to obtain powders from emulsions
- Particles obtained with the ionic gelation technique
- Sphera Encapsulation premises



[Experiments at the University of Seville](#)

- Oil displacement for biosurfactants detection
- Halophilic bacteria for biosurfactants production
- Siderophores screening - Strains isolation



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The SECRETed consortium

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



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Zoologica
Anton Dohrn
Napoli



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