Newsletter

Magazine

5th Issue November 2023

In this Issue

To gain access to our forthcoming Newsletters subscribe through our website.
Check the previous issues of Newsletter HERE

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

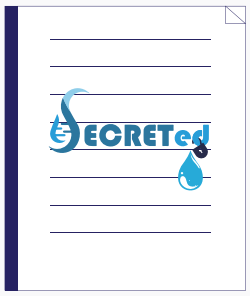
What is SECRETed

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.**

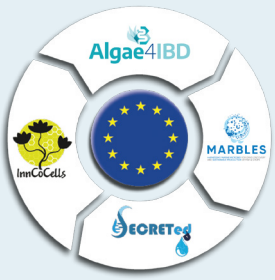


12 open access scientific publications

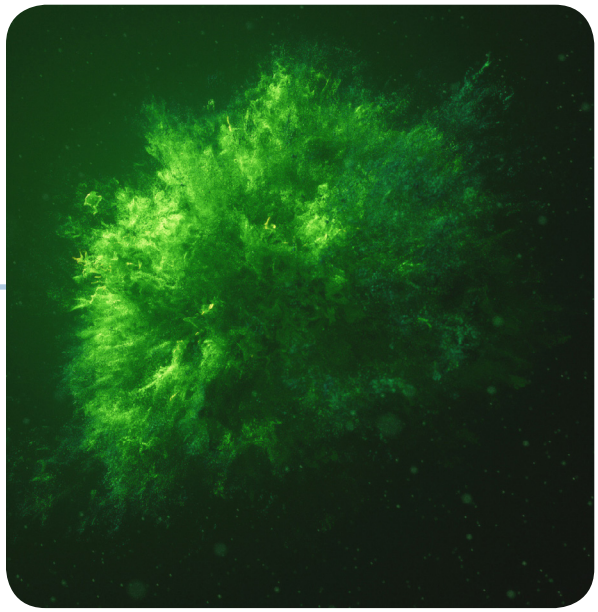
5 Public deliverables

Join our Listed Stakeholders' group

SECRETed is a founding member of the AIMS Cluster



What is SECRETed



Biosurfactants

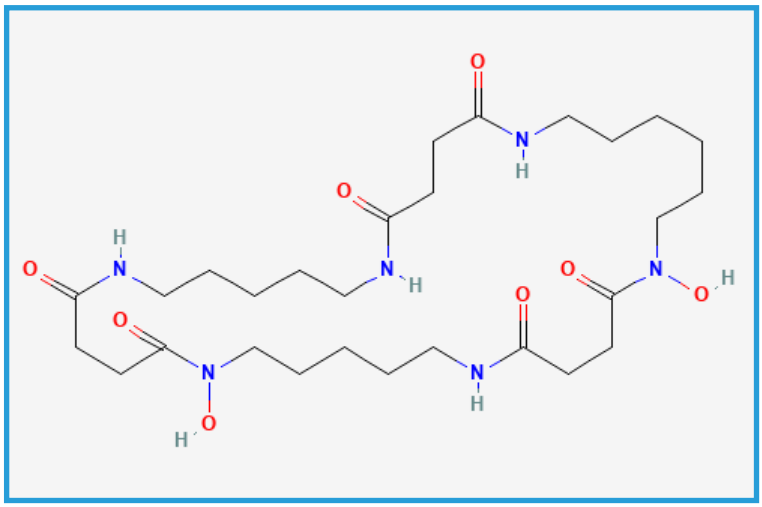
Surfactants are a very diverse group of lipids with a common amphiphilic nature (hydrophilic and hydrophobic domains within the same molecule) that appear lower interfacial tension allowing the solubilisation of hydrophobic substances in water. Biourfactants are produced by microorganisms offering reduced 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



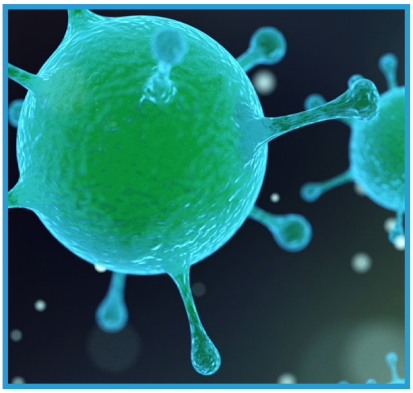
The Imperial College London continued the work of assembling pathways for the recombinant production of siderophores and biosurfactants. With the support of the database developed by IDENER, there have been identified bacterial gene clusters of appropriate length and cofactor requirements with the potential 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

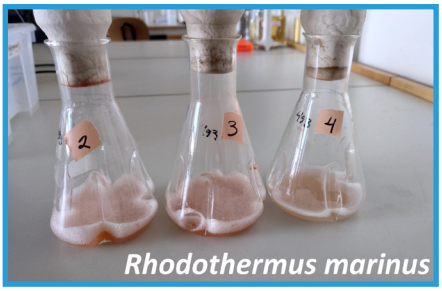
3 Siderophores against antibiotic resistance bacteria



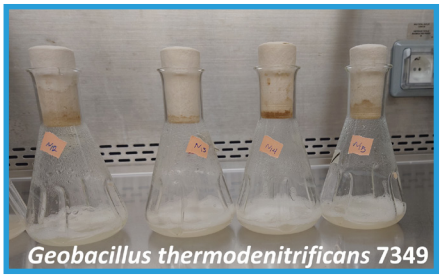
In nature, iron availability is low and is a key nutritional requirement for growth and infection. Most bacteria produce iron chelation molecules (siderophores), which scavenge for surrounding iron and bring this back inside the bacterium, where is used for growth. Adding siderophores from other sources has the potential to compete and deprive the bacteria of this essential iron. Accuplex Diagnostics have identified siderophores secreted by the fungal Aspergillus fumigatus as a way to help prevention of growth of two bacteria, Klebsiella pneumonia and Acinetobacter baumannii, both of which have well established resistance to existing antibiotics. The siderophore Triacetylfusarinine C had a significant impact on the growth of both bacteria after 12-24 hours of exposure. Accuplex Diagnostics is now exploring other novel naturally occurring and genetically restructured siderophores from marine microbes discovered and generated in context of SECRETed.

Publications:

1. Prolonged subculturing of Aspergillus fumigatus on Galleria extract agar results in altered virulence and sensitivity to antifungal agent (https://www.mdpi.com/2073-4409/12/7/1065)
2. Gliotoxin-mediated bacterial growth inhibition is caused by specific metal ion depletion (https://www.nature.com/articles/s41598-023-43300-w)



Rhodothermus marinus



Geobacillus thermodenitrificans 7349

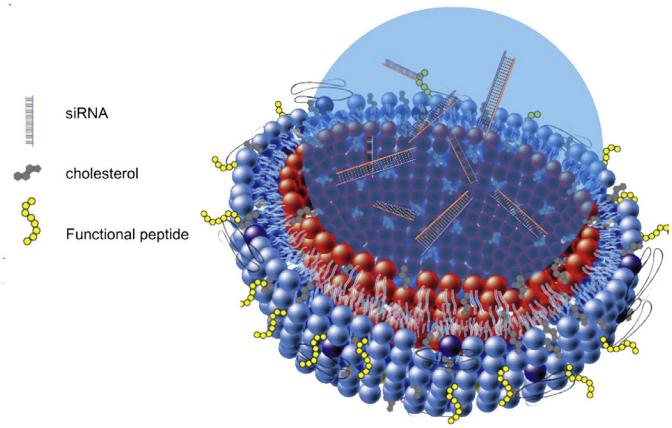
4 Biosurfactants production from thermophiles

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 and the maximum yield of the produced biosurfactants was 0.22 g/g glucose for R*hodothermus marinus* and 0.28 g/g glucose for *Geobacillus thermodenitrificans* 7349. A method was developed to analyse produced biosurfactants (rhamnolipids and lipopepdids) 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 probs as an advanced method.

Achievements

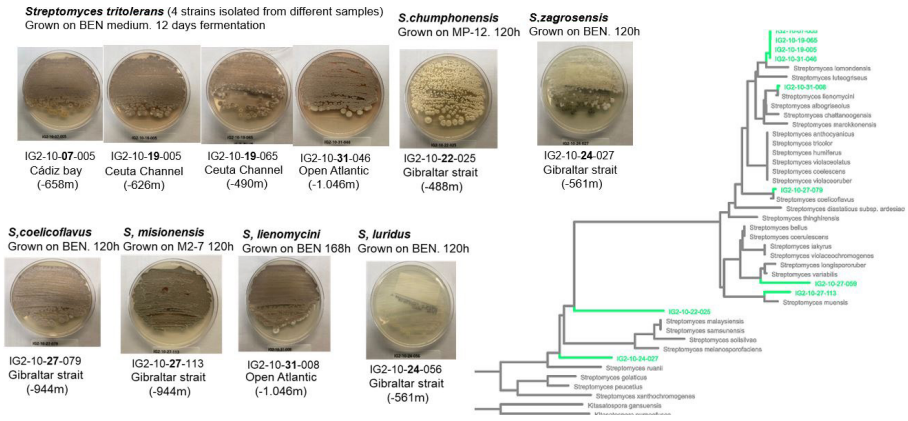
5 SYLENTIS

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 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

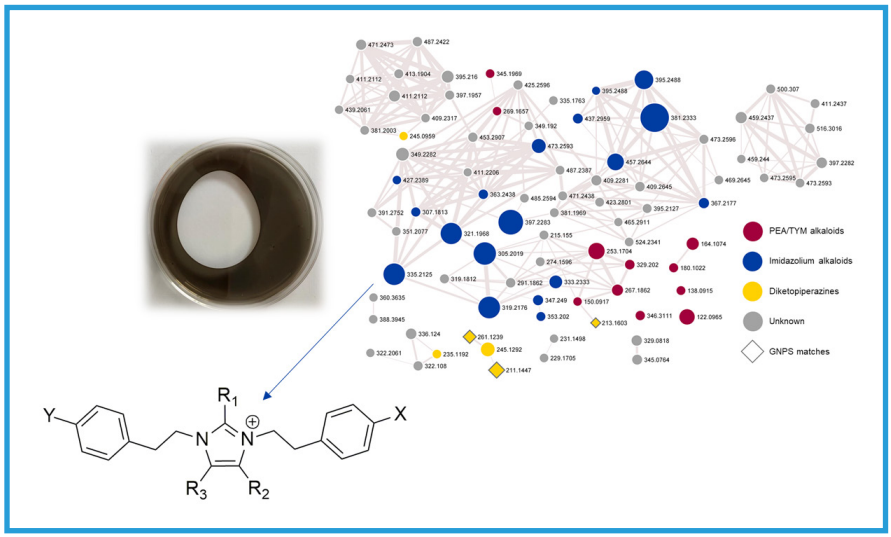


PharmaMar (PHM) scaled-up 10 selected actinobacteria for getting enough material for isolation of possible new siderophores. The supernatants of the culture were screened for confirmation and quantification of siderophores using of the Siderotec HISENS screening product of Accuplex Diagnostics. PHM has also performed antitumor screening and chemical de-replication of extracts of Rhamnolipids sent by Bio Based Europe Pilot Plant. In this occasion, the four samples were pooled due to their identical chemical de-replication (HPLC-MS), and extracted with ethyl acetate and methanol confirming the high cytotoxic effect.

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 anthelminthic 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 ethyl acetate 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.

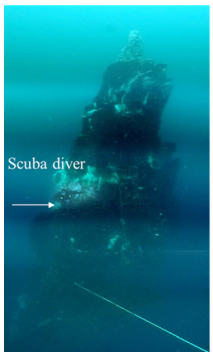
Achievements

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 trains 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.



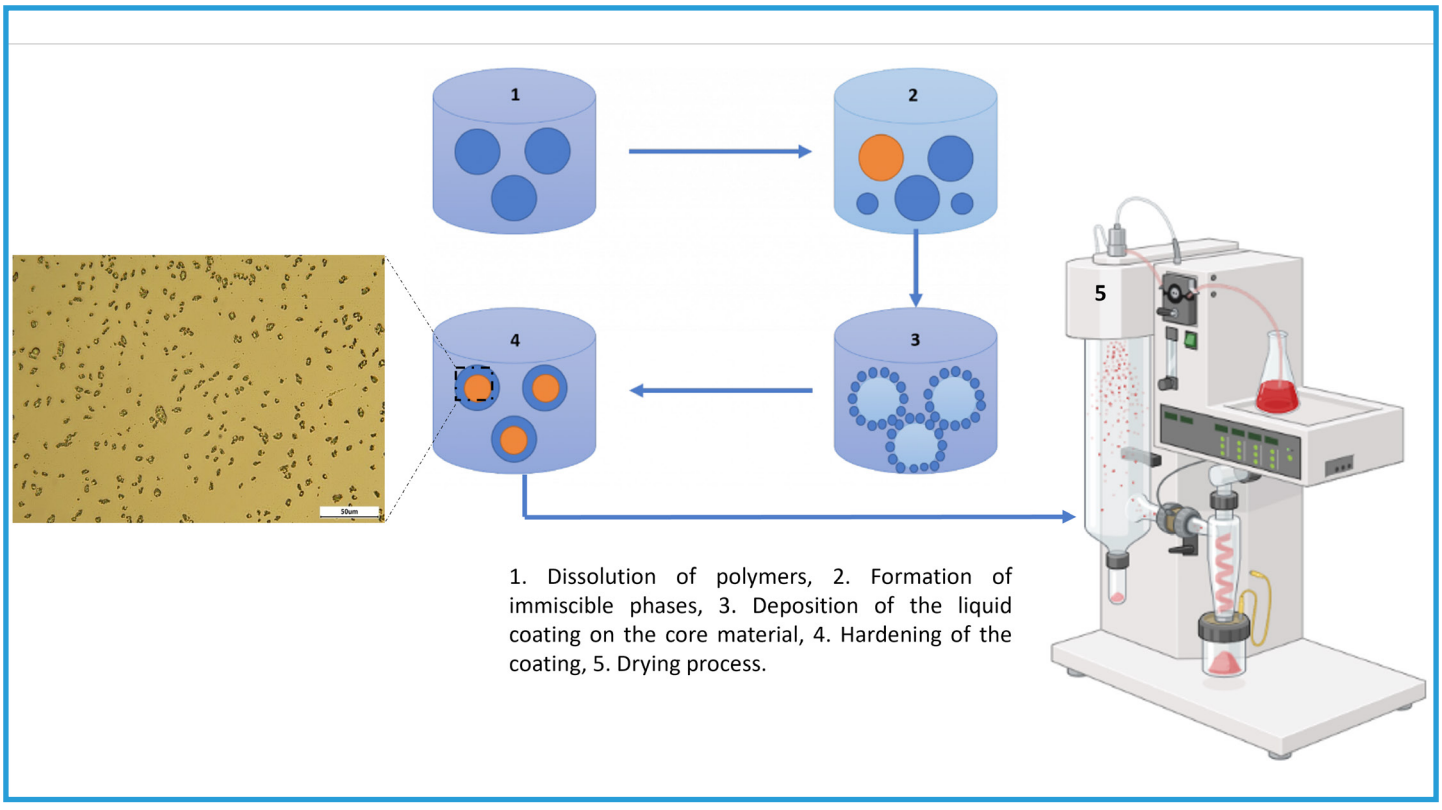
11 Biopesticide microparticles: encapsulation of oregano essential oil

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

Achievements

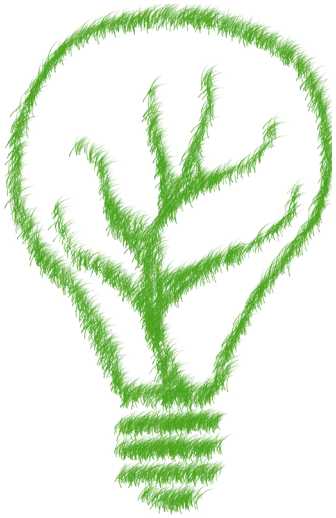
the market, biopesticides are emerging as a new tool to counter the massive use of synthetic pesticides. Biopesticides can be formulated as microparticles with higher water solubility, protective action against environmental degradation, and a higher penetration rate. Among them, oregano essential oil 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 to encapsulate oregano essential oil through complex coacervation and spray drying using natural polymers as wall material and trying to substitute synthetic surfactant with Rhamnolipids (RL) obtained from Pseudomonas Gessadii. Rhamnolipids were used in the coacervation process and were compared with the emulsifying activity of the synthetic surfactant Sodium dodecyl sulfate (SDS). Performances were evaluated in terms of encapsulation efficiency and particle size. RL shown to have encapsulation capacities comparable with the commercial surfactant, i.e., 94,0 ± 0,09 % and 91,46 ± 11,70 % for SDS and RL respectively. The sizes were also comparable with those obtained with commercial surfactant, i.e., 6.98 ± 1.03 µm and 9.15 ± 0.57 µm for SDS and RL, respectively. These results confirmed the emulsifying activity of the tested extract and the possibility to use them in order to substitute synthetic surfactants such as SDS.



1. Dissolution of polymers, 2. Formation of immiscible phases, 3. Deposition of the liquid coating on the core material, 4. Hardening of the coating, 5. Drying process.

Achievements

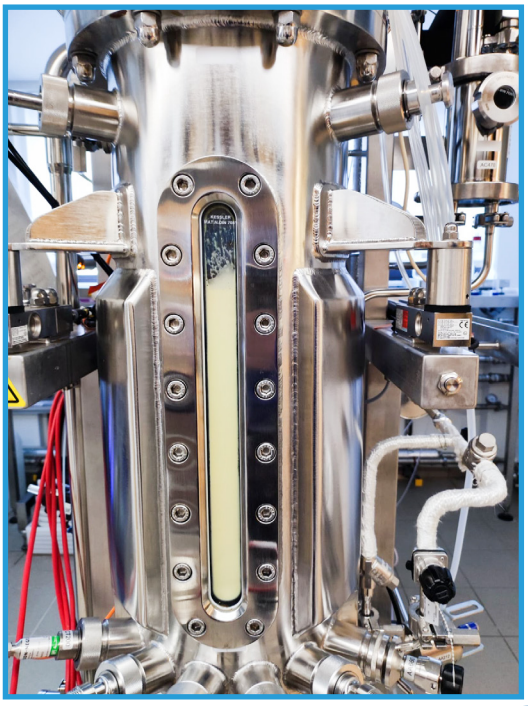


12 Life Cycle Assessment (LCA)

The goal is to reduce environmental burdens through the adoption of innovative bio-based surfactants. The Blue Synergy team defined the scope of LCA 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 and cross-referenced with reputable databases (Ecoinvent 3.8 and Agri-footprint 6.0). The LCA assumptions, baselines and benchmarks are also identified to compare and showcase the potential benefits and feasibility of the SECRETed biosurfactants across various industrial sectors. In our pursuit to replace conventional surfactants such as Span 80, Tween 80, and Sodium dodecyl sulfate with the innovative SECRETed biosurfactants, we have invested substantial resources in selecting the most appropriate baselines for each LCA. Our preliminary results are promising, indicating a potential of 5-10% reduction in environmental impact when the SECRETed biosurfactants are utilized, marking a significant step towards achieving our sustainability objectives.

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 asses more purification techniques to find the best purification flow for the SECRETed processes for further scale-up to 150L in 2024."



Events

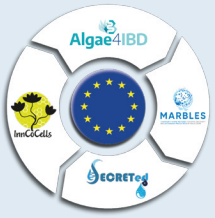
SECRETed & AIMS Cluster events

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 planed 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

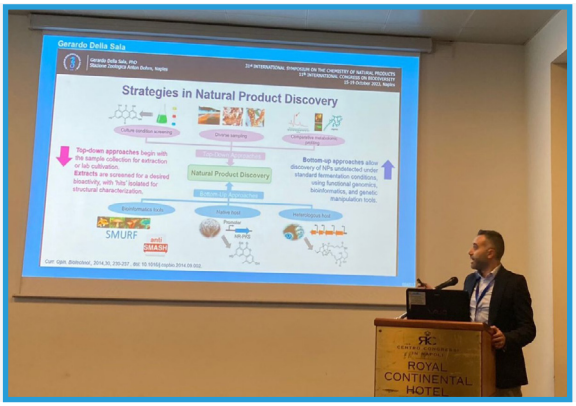
University of Seville participated in the 10th FEMS Congress of European Microbiologists. 9-13 July, 2023, Hamburg (Germany).

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



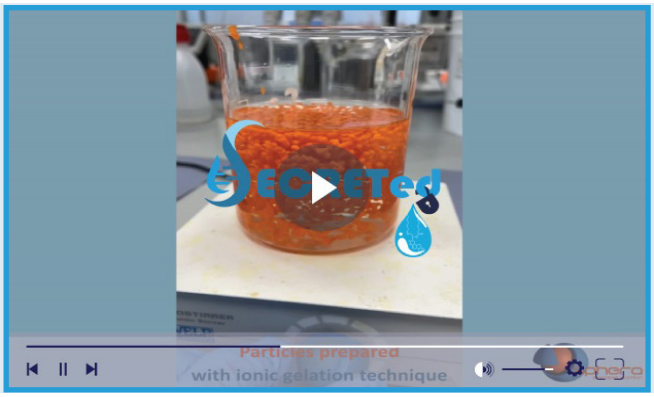
Conference

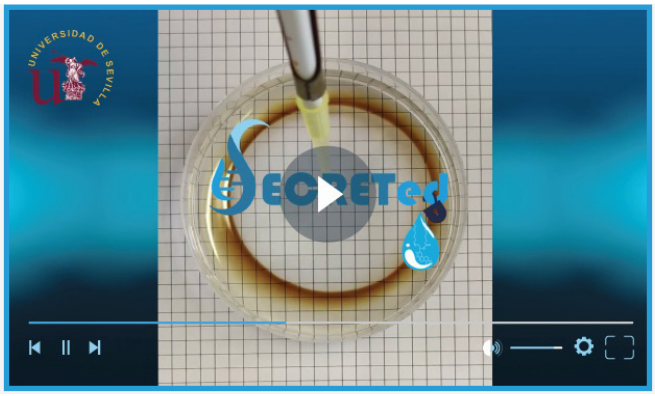
Stazione Zoologica Anton Dohrn participated in the 31st International Symposium on the Chemistry of Natural Products and 11th International Congress on Biodiversity.

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

Get connected

Subscribe to NEWSLETTER

Visit our Website

www.secreted.eu

Check out our Social Media

Reached 600 Followers

Mail Us

info@secreted.eu

Join our listed stakeholders' group

- Benefit from specific dissemination material
- Provide your feedback for custom applications to be considered in our research
- Promote your entity through our channels
- Enter the priority list of candidates to join the SECRETed Experts Advisory Board
- Lease directly with SECRETed experts

Contact Info

Subscribe to our NEWSLETTER

Project Coordinator
IDENER RESEARCH & DEVELOPMENT AGRUPACION DE INTERES ECONOMICO

Calle Early Ovington 24-8,
La Rinconada Sevilla, 41300, ES

info@secreted.eu

The SECRETed consortium

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

Visit our website and subscribe to our newsletter
www.secreted.eu

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