Newsletter

Magazine

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In this Issue

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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: A 4-and-a-half Year Journey of Innovation

From Marine Exploration to Scientific Discovery

As SECRETed reaches its completion, the project reflects on an ambitious four-and-a-half year endeavour that has significantly advanced Europe's scientific and technological capabilities in marine biotechnology. From the outset, SECRETed set out to explore one of the planet's least understood, and most promising, biological frontiers: the unique microorganisms thriving in marine and extreme environments. These organisms have evolved extraordinary biochemical pathways that make them ideal candidates for producing novel, high-performance natural compounds.

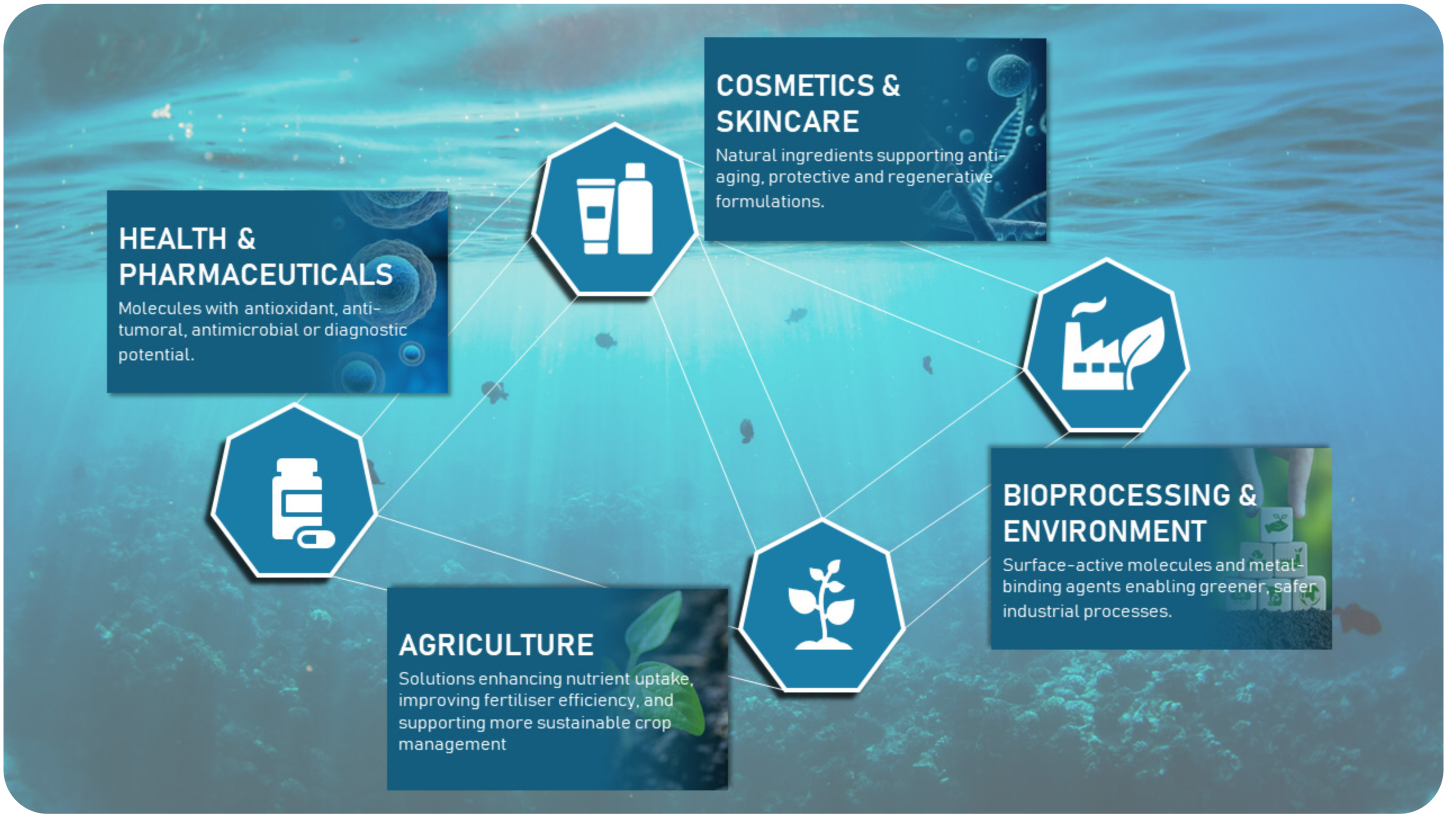
To harness this potential, the project brought together an exceptional consortium of universities, research institutes, technology developers, and industrial partners across Europe. This interdisciplinary alliance made it possible to connect advances in microbiology, biotechnology, artificial intelligence, sustainable engineering, and industrial scale-up into one unified innovation pipeline. The result is a comprehensive framework that spans the full journey from microbial discovery to real-world demonstration, an achievement that positions SECRETed as a landmark initiative in the European research landscape.

SECRETed systematically explored the biochemical diversity of more than 5,000 microbial strains, many originating from marine ecosystems and extreme habitats such as saline lakes, hydrothermal environments, and polar regions. The unique adaptations of these organisms provided fertile ground for discovering producers of biosurfactants and siderophores, two classes of natural compounds with significant potential for pharmaceuticals, cosmetics, agriculture, food systems, and environmental technologies.

A defining feature of the project was the integration of advanced computational modelling and machine-learning tools. Predictive algorithms supported the prioritisation of promising microbial strains, informed metabolic engineering decisions, and guided the optimisation of production conditions. This digital component streamlined the discovery process, reducing research time and increasing accuracy throughout the value chain.

From Laboratory Innovation to Industrial Validation

Building on these discoveries, SECRETed implemented a complete development pipeline that advanced microbial findings through a series of interconnected technological steps. This began with bioprospecting and microbial screening, where high-throughput assays were combined with AI-driven ranking tools to identify the most promising strains. Genomic and metabolic analyses then mapped the pathways responsible for biosurfactant and siderophore production, providing the foundation for systems metabolic engineering aimed at enhancing strain performance through genetic and biochemical optimisation. Fermentation processes were subsequently developed and refined, progressing from controlled laboratory cultivation to pilot-scale production. These efforts were complemented by detailed chemical characterisation using advanced analytical techniques to determine molecular structure and functionality.



COSMETICS & SKINCARE

Natural ingredients supporting anti-aging, protective and regenerative formulations.

HEALTH & PHARMACEUTICALS

Molecules with antioxidant, anti-tumoral, antimicrobial or diagnostic potential.

BIOPROCESSING & ENVIRONMENT

Surface-active molecules and metal-binding agents enabling greener, safer industrial processes.

AGRICULTURE

Solutions enhancing nutrient uptake, improving fertiliser efficiency, and supporting more sustainable crop management

The project further conducted formulation and application studies to ensure the compounds met real-world requirements in cosmetics, pharmaceuticals, agriculture and biotechnology. Finally, multi-sector validation demonstrated the technical feasibility and practical added value of these solutions across several industrial domains.

Through its integrated development pipeline, SECRETed demonstrated how marine-derived compounds can be translated into practical solutions across multiple sectors. In the health and pharmaceutical fields, the project identified molecules with strong antioxidant, anti-tumoral, antimicrobial and even diagnostic potential. The cosmetics and skincare sector benefited from natural ingredients capable of supporting anti-aging effects, protection, and regenerative properties. In agriculture, the work led to approaches that improve nutrient uptake, boost fertiliser efficiency and promote more sustainable crop management practices. Finally, for bioprocessing and environmental use, SECRETed validated surface-active molecules and metal-binding agents that can help make industrial processes cleaner, safer and more resource-efficient.

Throughout this process, SECRETed remained committed to sustainability. Comprehensive life-cycle assessments, resource-efficiency analyses, and techno-economic evaluations ensured that each step aligned with the environmental and economic expectations of modern bio-based industries. This holistic perspective supported the development of solutions that contribute to Europe’s circular economy goals and the European Green Deal.

By integrating scientific innovation, advanced digital tools, industrial orientation and sustainability assessment, SECRETed demonstrated how next-generation marine biotechnology can drive Europe’s transition toward safer, greener and more resilient value chains. The methodologies, datasets and integrated workflows established through the project now serve as a strong foundation for future research, policy development and industrial-scale innovation well beyond SECRETed’s lifetime.

Recent Publications

Abstract

Rhamnolipids are a class of glycolipids known for their surface and emulsifying activity. These molecules, produced by a few Gram-negative genera, mostly Pseudomonas, offer natural alternatives to synthetic surfactants in different industrial fields. This study examines the emulsifying and encapsulation performance of Rhamnolipids derived from the marine Antarctic bacterium Pseudomonas gessardii M15, comparing its emulsification ability and stability with those of commercial surfactants, Sodium dodecyl sulfate (SDS) and sucrose esters (SE), under extreme conditions of temperature and pH.

The Rhamolipids were used to encapsulate Coenzyme Q10 with Arabic gum as the carrier matrix. Rhamnolipids exhibited surface and emulsifying activity comparable to that of SDS and superior to SE at neutral and basic pH levels. Their performance declined under acidic conditions, whereas exposure to 90 ◦C had no significant effects. The encapsulation efficiency of Coenzyme Q10 was significantly higher in the case of Rhamnolipids, with a percentage of encapsulated compound of 99.6 ± 0.2%, compared to the 38.2 ± 7.1% found when SDS was used. Rhamnolipids extracted from Pseudomonas gessardii M15 exhibit strong potential as a natural surfactant, particularly in formulations that require thermal stability and effective encapsulation. These findings support its use as a sustainable alternative to synthetic agents in diverse industrial settings.

DOI: 10.3390/ biom15101451

Evaluation of the Emulsification Properties of Marine-Derived Rhamnolipids for Encapsulation: A Comparison with Commercial Surfactants

biomolecules

MDPI

Article

Evaluation of the Emulsification Properties of Marine-Derived Rhamnolipids for Encapsulation: A Comparison with Commercial Surfactants

Sara Gorrieri [1,†], Carmine Buonocore [2,†], Giulia Donà [3], Chiara Pezzoli [4], Martina Vakarelova [3], Daniela Coppola [2], Fortunato Palma Esposito [2], Donatella de Pascale [2], Gerardo Della Sala [2], Francesca Zanoni [3,*] and Pietro Tedesco [2,*]

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Abstract

Rhamnolipids are a class of glycolipids known for their surface and emulsifying activity. These molecules, produced by a few Gram-negative genera, mostly Pseudomonas, offer natural alternatives to synthetic surfactants in different industrial fields. This study examines the emulsifying and encapsulation performance of Rhamnolipids derived from the marine Antarctic bacterium *Pseudomonas gessardii* M15, comparing its emulsification ability and stability with those of commercial surfactants, Sodium dodecyl sulfate (SDS) and sucrose esters (SE), under extreme conditions of temperature and pH. The Rhamolipids were used to encapsulate Coenzyme Q10 with Arabic gum as the carrier matrix. Rhamnolipids exhibited surface and emulsifying activity comparable to that of SDS and superior to SE at neutral and basic pH levels. Their performance declined under acidic conditions, whereas exposure to 90 °C had no significant effects. The encapsulation efficiency of Coenzyme Q10 was significantly higher in the case of Rhamnolipids, with a percentage of encapsulated compound of 99.6 ± 0.2%, compared to the 38.2 ± 7.1% found when SDS was used. Rhamnolipids extracted from *Pseudomonas gessardii* M15 exhibit strong potential as a natural surfactant, particularly in formulations that require thermal stability and effective encapsulation. These findings support its use as a sustainable alternative to synthetic agents in diverse industrial settings.

Keywords: rhamnolipids; glycolipids; biosurfactants; encapsulation; micelles; coenzyme Q10

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1. Introduction

Surfactants are a class of molecules used in various sectors, including the cleaning, food, agrochemical, and pharmaceutical industries, with a growing demand. These are a broad group of molecules characterized by an amphiphilic nature, which gives them the capacity to position at interfaces (water/oil and water/air), stabilizing them [1,2]. Therefore,

Biomolecules **2025**, 15, 1451 https://doi.org/10.3390/biom15101451

Recent Publications

Automated genome mining predicts structural diversity and taxonomic distribution of peptide metallophores across bacteria

Abstract

Microbial competition for trace metals shapes their communities and interactions with humans and plants. Many bacteria scavenge trace metals with metallophores, small molecules that chelate environmental metal ions. Metallophore production may be predicted by genome mining, where genomes are scanned for homologs of known biosynthetic gene clusters (BGCs). However, accurately detecting non-ribosomal peptide (NRP) metallophore biosynthesis requires expert manual inspection, stymieing large-scale investigations. Here, we introduce automated identification of NRP metallophore BGCs through a comprehensive algorithm, implemented in antiSMASH, that detects chelator biosynthesis genes with 97% precision and 78% recall against manual curation. We showcase the utility of the detection algorithm by experimentally characterising metallophores from several taxa. Highthroughput NRP metallophore BGC detection enabled metallophore detection across 69,929 genomes spanning the bacterial kingdom. We predict that 25% of all bacterial non-ribosomal peptide synthetases encode metallophore production and that significant chemical diversity remains undiscovered. A reconstructed evolutionary history of NRP metallophores supports that some chelating groups may predate the Great Oxygenation Event. The inclusion of NRP metallophore detection in antiSMASH will aid non-expert researchers and continue to facilitate large-scale investigations into metallophore biology.

DOI: 10.7554/eLife.109154.1.sa2

eLife

Microbiology and Infectious Disease
Computational and Systems Biology

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Automated genome mining predicts structural diversity and taxonomic distribution of peptide metallophores across bacteria

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eLife Assessment

This **important** and **compelling** study establishes a robust computational and experimental framework for the large-scale identification of metallophore biosynthetic clusters. The work advances beyond current standards, providing theoretical and practical value across microbiology, bioinformatics, and evolutionary biology.

https://doi.org/10.7554/eLife.109154.1.sa2

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Zachary L Reitz *et al.*, 2025 eLife. https://doi.org/10.7554/eLife.109154.1 1 of 26

Recent Publications

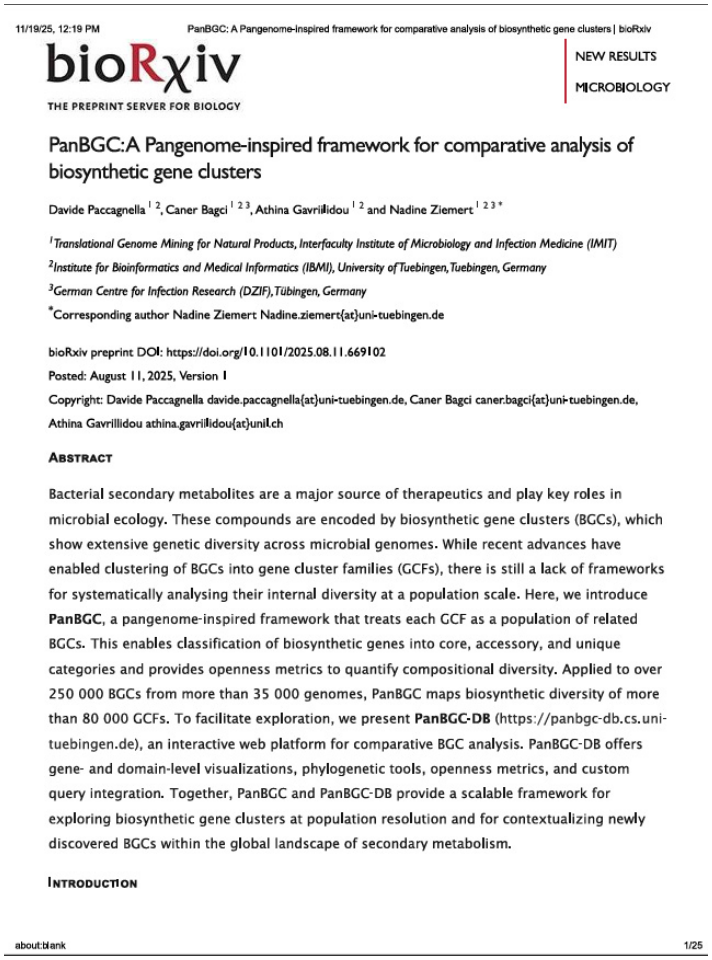
Abstract

PanBGC: A Pangenome-inspired framework for comparative analysis of biosynthetic gene clusters

Bacterial secondary metabolites are a major source of therapeutics and play key roles in microbial ecology. These compounds are encoded by biosynthetic gene clusters (BGCs), which show extensive genetic diversity across microbial genomes. While recent advances have enabled clustering of BGCs into gene cluster families (GCFs), there is still a lack of frameworks for systematically analysing their internal diversity at a population scale. Here, we introduce PanBGC, a pangenome-inspired framework that treats each GCF as a population of related BGCs.

This enables classification of biosynthetic genes into core, accessory, and unique categories and provides openness metrics to quantify compositional diversity. Applied to over 250 000 BGCs from more than 35 000 genomes, PanBGC maps biosynthetic diversity of more than 80 000 GCFs. To facilitate exploration, we present PanBGC-DB (https://panbgc-db.cs.uni-tuebingen.de), an interactive web platform for comparative BGC analysis. PanBGC-DB offers gene- and domain-level visualisations, phylogenetic tools, openness metrics, and custom query integration. Together, PanBGC and PanBGC-DB provide a scalable framework for exploring biosynthetic gene clusters at population resolution and for contextualising newly discovered BGCs within the global landscape of secondary metabolism.

DOI: 10.1101/2025.08.11.669102



11/19/25, 12:19 PM PanBGC: A Pangenome-inspired framework for comparative analysis of biosynthetic gene clusters | bioRxiv

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MICROBIOLOGY

PanBGC:A Pangenome-inspired framework for comparative analysis of biosynthetic gene clusters

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ABSTRACT

Bacterial secondary metabolites are a major source of therapeutics and play key roles in microbial ecology. These compounds are encoded by biosynthetic gene clusters (BGCs), which show extensive genetic diversity across microbial genomes. While recent advances have enabled clustering of BGCs into gene cluster families (GCFs), there is still a lack of frameworks for systematically analysing their internal diversity at a population scale. Here, we introduce **PanBGC**, a pangenome-inspired framework that treats each GCF as a population of related BGCs. This enables classification of biosynthetic genes into core, accessory, and unique categories and provides openness metrics to quantify compositional diversity. Applied to over 250 000 BGCs from more than 35 000 genomes, PanBGC maps biosynthetic diversity of more than 80 000 GCFs. To facilitate exploration, we present **PanBGC-DB** (https://panbgc-db.cs.uni-tuebingen.de), an interactive web platform for comparative BGC analysis. PanBGC-DB offers gene- and domain-level visualizations, phylogenetic tools, openness metrics, and custom query integration. Together, PanBGC and PanBGC-DB provide a scalable framework for exploring biosynthetic gene clusters at population resolution and for contextualizing newly discovered BGCs within the global landscape of secondary metabolism.

INTRODUCTION

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Recent Publications

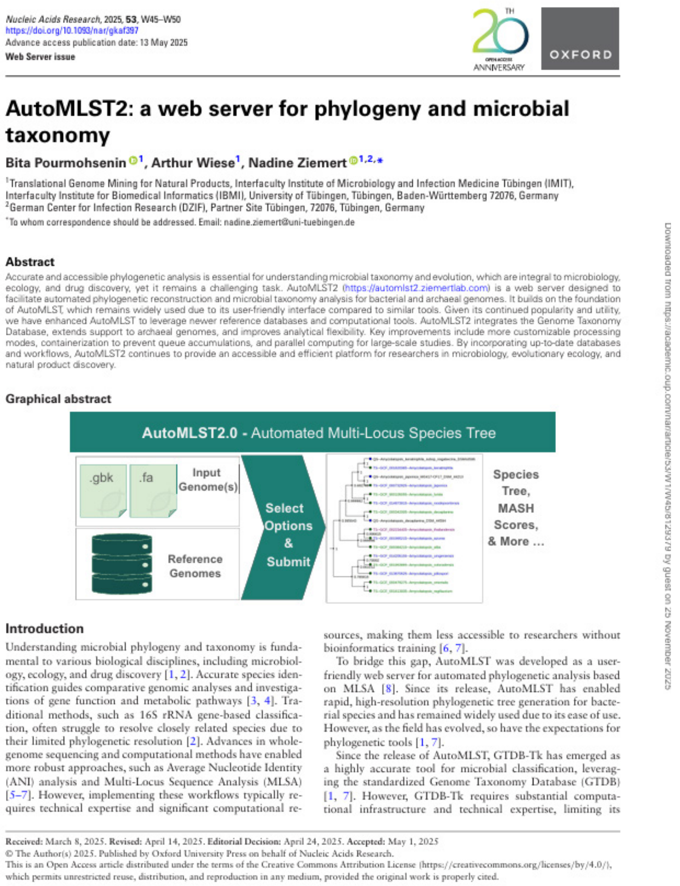
AutoMLST2: a web server for phylogeny and microbial taxonomy

Abstract

Accurate and accessible phylogenetic analysis is essential for understanding microbial taxonomy and evolution, which are integral to microbiology, ecology, and drug discovery, yet it remains a challenging task. AutoMLST2 (https://automlst2.ziemertlab.com) is a web server designed to facilitate automated phylogenetic reconstruction and microbial taxonomy analysis for bacterial and archaeal genomes. It builds on the foundation of AutoMLST, which remains widely used due to its user-friendly interface compared to similar tools. Given its continued popularity and utility, we have enhanced AutoMLST to leverage newer reference databases and computational tools.

AutoMLST2 integrates the Genome Taxonomy Database, extends support to archaeal genomes, and improves analytical flexibility. Key improvements include more customisable processing modes, containerisation to prevent queue accumulations, and parallel computing for large-scale studies. By incorporating up-to-date databases and workflows, AutoMLST2 continues to provide an accessible and efficient platform for researchers in microbiology, evolutionary ecology, and natural product discovery.

DOI: 10.1093/nar/gkaf397



Nucleic Acids Research, 2025, **53**, W45–W50
https://doi.org/10.1093/nar/gkaf397
Advance access publication date: 13 May 2025
Web Server issue

AutoMLST2: a web server for phylogeny and microbial taxonomy

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Abstract

Accurate and accessible phylogenetic analysis is essential for understanding microbial taxonomy and evolution, which are integral to microbiology, ecology, and drug discovery, yet it remains a challenging task. AutoMLST2 (https://automlst2.ziemertlab.com) is a web server designed to facilitate automated phylogenetic reconstruction and microbial taxonomy analysis for bacterial and archaeal genomes. It builds on the foundation of AutoMLST, which remains widely used due to its user-friendly interface compared to similar tools. Given its continued popularity and utility, we have enhanced AutoMLST to leverage newer reference databases and computational tools. AutoMLST2 integrates the Genome Taxonomy Database, extends support to archaeal genomes, and improves analytical flexibility. Key improvements include more customizable processing modes, containerization to prevent queue accumulations, and parallel computing for large-scale studies. By incorporating up-to-date databases and workflows, AutoMLST2 continues to provide an accessible and efficient platform for researchers in microbiology, evolutionary ecology, and natural product discovery.

Graphical abstract

Introduction

Understanding microbial phylogeny and taxonomy is fundamental to various biological disciplines, including microbiology, ecology, and drug discovery [1, 2]. Accurate species identification guides comparative genomic analyses and investigations of gene function and metabolic pathways [3, 4]. Traditional methods, such as 16S rRNA gene-based classification, often struggle to resolve closely related species due to their limited phylogenetic resolution [2]. Advances in whole-genome sequencing and computational methods have enabled more robust approaches, such as Average Nucleotide Identity (ANI) analysis and Multi-Locus Sequence Analysis (MLSA) [5–7]. However, implementing these workflows typically requires technical expertise and significant computational resources, making them less accessible to researchers without bioinformatics training [6, 7].

To bridge this gap, AutoMLST was developed as a user-friendly web server for automated phylogenetic analysis based on MLSA [8]. Since its release, AutoMLST has enabled rapid, high-resolution phylogenetic tree generation for bacterial species and has remained widely used due to its ease of use. However, as the field has evolved, so have the expectations for phylogenetic tools [1, 7].

Since the release of AutoMLST, GTDB-Tk has emerged as a highly accurate tool for microbial classification, leveraging the standardized Genome Taxonomy Database (GTDB) [1, 7]. However, GTDB-Tk requires substantial computational infrastructure and technical expertise, limiting its

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Publications

FULL LIST OF PUBLICATIONS

1 Combining OSMAC Approach and Untargeted Metabolomics for the Identification of New Glycolipids with Potent Antiviral Activity Produced by a Marine Rhodococcus

2 Antiviral Activity of the Rhamnolipids Mixture from the Antarctic Bacterium Pseudomonas gessardii M15 against Herpes Simplex Viruses and Coronaviruses

3 Applicability of Control Materials To Support Gene Promoter Characterization and Expression in Engineered Cells Using Digital PCR

4 Novel Insights on Pyoverdine: From Biosynthesis to Biotechnological Application

5 Medium development and production of carotenoids and exopolysaccharides by the extremophile Rhodothermus marinus DSM16675 in glucose-based defined media

6 Dihydroauroglaucin Isolated from the Mediterranean Sponge Grantia compressa Endophyte Marine Fungus Eurotium chevalieri Inhibits Migration of Human Neuroblastoma Cells

7 MIBiG 3.0: a community-driven effort to annotate experimentally validated biosynthetic gene clusters

8 Production of selenium nanoparticles occurs through an interconnected pathway of sulphur metabolism and oxidative stress response in Pseudomonas putida KT2440

9 Water potential governs the effector specificity of the transcriptional regulator XylR of Pseudomonas putida

10 Evaluation of Antimicrobial Properties and Potential Applications of Pseudomonas gessardii M15 Rhamnolipids towards Multiresistant Staphylococcus aureus

11 Prolonged Subculturing of Aspergillus fumigatus on Galleria Extract Agar Results in Altered Virulence and Sensitivity to Antifungal Agents

12 New Imidazolium Alkaloids with Broad Spectrum of Action from the Marine Bacterium Shewanella aquimarina

13 Gliotoxin-mediated bacterial growth inhibition is caused by specific metal ion depletion

14 Integrated genome and metabolome mining unveiled structure and biosynthesis of novel lipopeptides from a deep-sea Rhodococcus

15 Novel Insights into the Nobilamide Family from a Deep-Sea Bacillus: Chemical Diversity, Biosynthesis and Antimicrobial Activity Towards Multidrug-Resistant Bacteria

16 MIBiG 4.0: advancing biosynthetic gene cluster curation through global collaboration

17 Evaluation of the Emulsification Properties of Marine-Derived Rhamnolipids for Encapsulation: A Comparison with Commercial Surfactants

18 Automated genome mining predicts structural diversity and taxonomic distribution of peptide metallophores across bacteria

19 PanBGC: A Pangenome-inspired framework for comparative analysis of biosynthetic gene clusters

20 AutoMLST2: a web server for phylogeny and microbial taxonomy

Events, Outreach & Key Engagements

International Summer School on Natural Products
June 29 – July 4, 2025

The 6th edition of the International Summer School on Natural Products (ISSNP), held in Naples, Italy, offered an intensive learning environment for PhD candidates and early-career researchers working in the field of natural products. Co-organised by SZN, partner of the SECRETed consortium, this edition continued the school's long-standing mission to expand the global community established in 2015 by promoting interdisciplinary training and strengthening cross-sector collaboration. The programme placed particular emphasis on interactive and hands-on learning, featuring sessions on genome mining, molecular docking, large-scale bioinformatic data analysis and other emerging research approaches. Participants took part in team-based problem-solving, guided discussions and applied exercises designed to build practical skills and support future research careers.

Interdisciplinary Advances in Natural Products Research: From Ecosystems to Molecular Targets
September 23-24, 2025

Stazione Zoologica Anton Horn contributed to a two-day workshop in Bacoli (Napoli, Italy), an event dedicated to advancing interdisciplinary research on natural bioactive compounds.SZN's participation included a presentation by Gerardo Della Sala titled "Decoding the biosurfactant repertoire in marine extremophiles through metabologenomics", which showcased innovative insights into the metabolic capabilities of extremophilic microorganisms. The workshop fostered collaboration among chemists, biologists, bioinformaticians and ecologists, promoting a sustainable, biodiversity-conscious approach to natural products research.

ISSSB-International Summer School on Systems Biology
October 27-30, 2025

Stazione Zoologica Anton Horn hosted ISSSB 2025 held in Napoli, Italy. During the event, SZN delivered a presentation on "Linking genomics and metabolomics to explore the metabolic diversity of marine bacteria", highlighting how integrated omics approaches can reveal new biochemical pathways and bioactive compounds of relevance to marine biotechnology.

Events, Outreach & Key Engagements

Biosurfactants 2025 international conference September 10-12, 2025

Stazione Zoologica Anton Horn participated in the Biosurfactants 2025 International Conference in Aachen, Germany, a key global meeting point for experts in sustainable surfactant technologies. The team presented the work “Unlocking the production of new rhamnolipids mixtures through pathway engineering and heterologous expression in Pseudomonas putida,” demonstrating advanced strategies for expanding the diversity and yield of bio-based surfactants.

REDEX2025: XIX Meeting of the National Network of Extremophile Microorganisms October 14-16, 2025

The University of Seville (USE), as a key partner of the SECRETed project, participated in the XIX Meeting of the National Network of Extremophile Microorganisms (REDEX 2025), held in Huelva, Spain on 14–16 October 2025. USE engaged in scientific exchanges with national and international research groups, contributing its expertise in extremophilic bacteria and marine microbiology—areas central to SECRETed’s objectives.

LMB Graduate Life Sciences Symposium July 10, 2025

José Zarco Jiménez from Imperial College London (ICL) took part in the international symposium co-organised by the MRC Laboratory of Molecular Biology and the Institut Pasteur. He presented “Euk.oli: A eukaryotic-inspired prokaryotic model for orthogonal resource allocation,” contributing SECRETed’s perspective to a programme spanning structural biology, immunology, neuroscience, and cell biology.

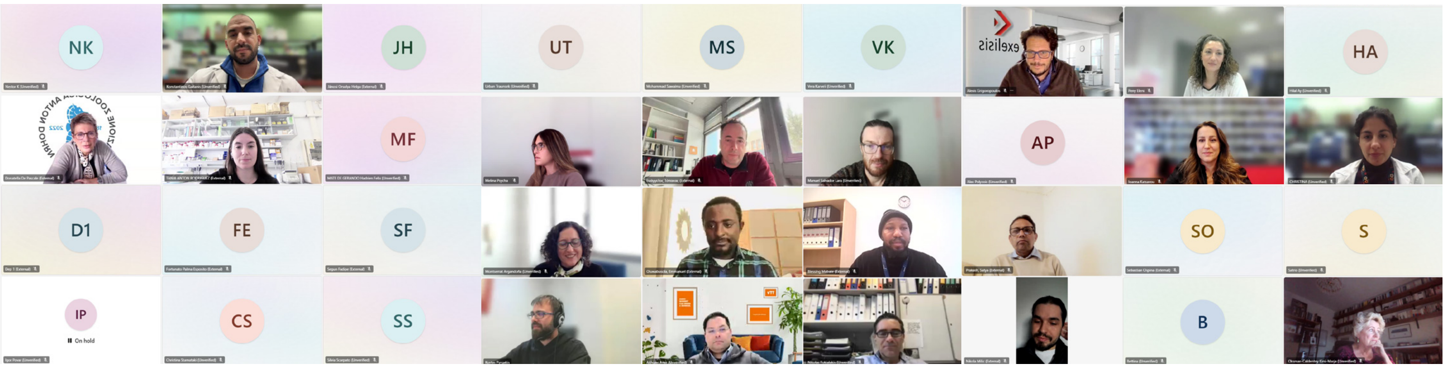
Events, Outreach & Key Engagements

8th General Assembly
May 26-27, 2025

The 8th General Assembly of the SECRETed Project (H2020) took place on 26–27 May 2025 in Athens, bringing partners together for two days of focused discussions and progress reviews. Work Package leaders presented key achievements and next steps, while a networking dinner supported team cohesion. Day 2 centered on monitoring and internal evaluation to ensure strong progress toward final objectives. The assembly also featured virtual contributions from the AIMS Cluster projects, MARBLES, InnCoCells, and Algae4IBD, strengthening cross-project collaboration.

SECRETed Final webinar
November 27, 2025

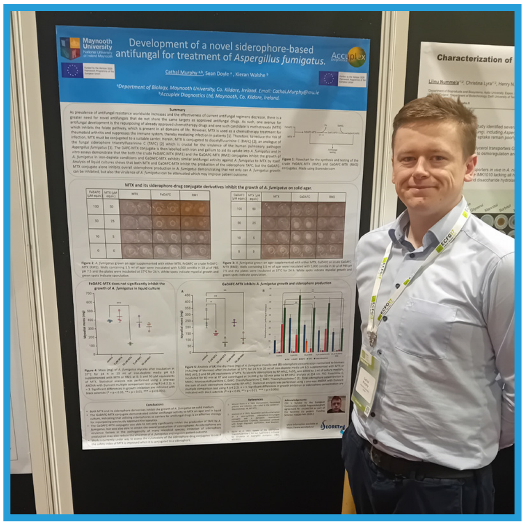
The Final SECRETed Webinar on 27 November 2025 brought together 105 participants from 62 organisations to present the project's closing scientific results. Featuring eight expert talks, including invited speaker Prof. Evangelos Topakas (National Technical University of Athens), the event showcased key advances in computational discovery, biosurfactants, extremophile-based production, cosmetic bioprocessing, deep-sea metabolites, and sustainable DHA pathways. With active Q&A and contributions from AIMS Cluster partners, the webinar demonstrated the relevance of SECRETed's findings for health, agriculture, and environmental applications, marking a strong conclusion to the project's dissemination efforts.



Project Achievements & Legacy

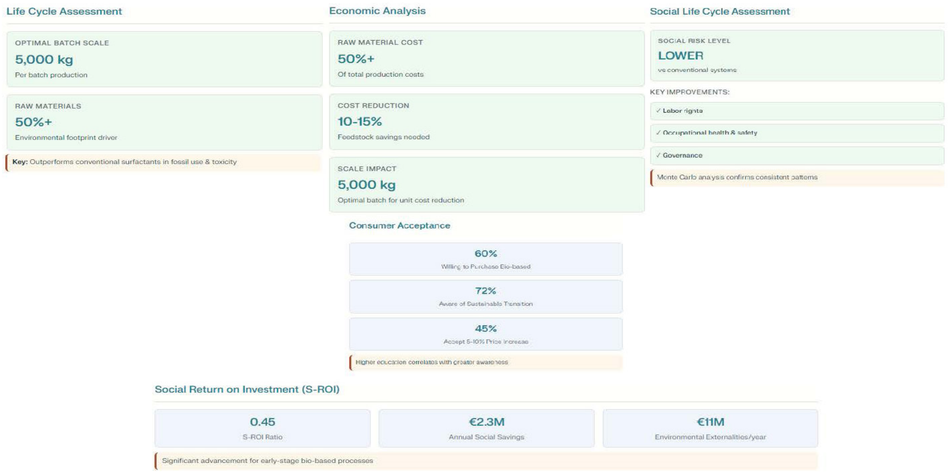
1 Breakthroughs in Siderophore Detection and Utilisation

While key deliverables were a bridge too far, Acculplex Diagnostics Ltd nonetheless had a very successful participation in the SECRETED project. Key outputs include development and launch of a high-sensitivity assay for the detection of siderophores, now being used as a new tool for monitoring water quality, initially in the cosmetics industry. In addition, a proof-of-concept trojan horse siderophore antifungal was demonstrated, as well as a siderophore-polymer complex, designed for use as an antimicrobial but found to have significant potential for application in gallium recovery, a key rare metal needed by the microprocessor industry. Other additional outputs included a Master's degree, 3 publications/manuscripts, 3 posters and a presentation at a conference in Poland, and acquiring a substantial insight into siderophore discovery and analysis.



2 LCSA Highlights Advantages of Bio-Based Surfactants

Blue Synergy has successfully finalised its activities, delivering a comprehensive Life Cycle Sustainability Assessment (LCSA) that integrates environmental, economic, and social evaluations with regulatory analysis. The environmental Life Cycle Assessment (LCA) demonstrated that rhamnolipids outperform conventional surfactants regarding fossil resource use and toxicity, identifying a production scale of 5,000 kg per batch as the optimal threshold for maximising both environmental benefits and cost efficiency. While raw materials remain the dominant driver for both environmental footprint and economic costs, exceeding 50% of total expenditure, the analysis confirmed that scale-up strategies significantly reduce unit costs. On



Life Cycle Assessment

OPTIMAL BATCH SCALE
5,000 kg
Per batch production

RAW MATERIALS
50%+
Environmental footprint driver

Key: Outperforms conventional surfactants in fossil use & toxicity

Economic Analysis

RAW MATERIAL COST
50%+
Of total production costs

COST REDUCTION
10-15%
Feedstock savings needed

SCALE IMPACT
5,000 kg
Optimal batch for unit cost reduction

Consumer Acceptance

60%
Willing to Purchase Bio-based

72%
Aware of Sustainable Transition

45%
Accept 5-10% Price Increase

Higher education correlates with greater awareness

Social Life Cycle Assessment

SOCIAL RISK LEVEL
LOWER
vs conventional systems

KEY IMPROVEMENTS:
✓ Labor rights
✓ Occupational health & safety
✓ Governance

Monte Carlo analysis confirms consistent patterns

Social Return on Investment (S-ROI)

0.45
S-ROI Ratio

€2.3M
Annual Social Savings

€11M
Environmental Externalities/year

Significant advancement for early-stage bio-based processes

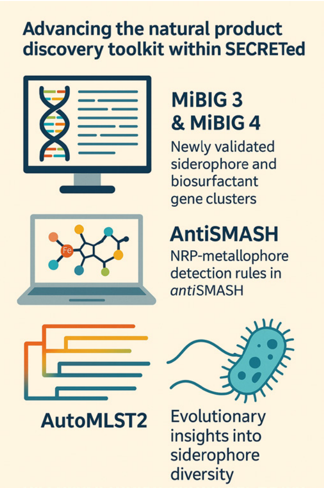
Project Achievements & Legacy

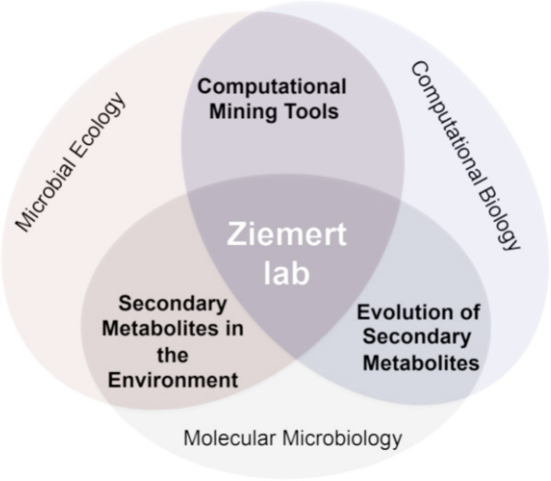
the social front, the Social LCA revealed lower risks compared to conventional systems, particularly concerning labor rights and governance, contributing to an integrated Social Return on Investment (S-ROI) ratio of 0.45. This S-ROI model highlighted approximately €2.3 million in annual social cost savings, providing a quantifiable measure of societal return for early-stage bio-based processes. Parallel efforts in consumer awareness, including workshops in Madrid and Seville and surveys across three countries, showed that while 60% of consumers are willing to purchase bio-based products, price sensitivity remains a key factor. These consolidated findings are now being prepared for scientific publication to support evidence-based decision-making in sustainable biomanufacturing.

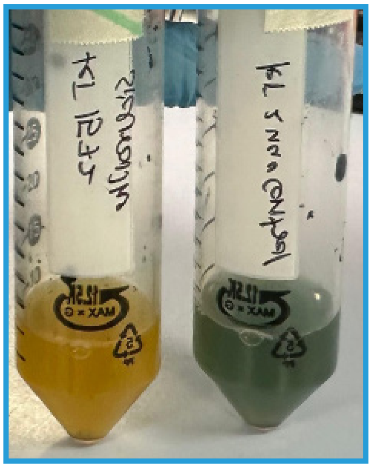
3

Enhanced Tools for Natural Product & Siderophore Discovery

The team of the University of Tübingen (EKUT) advanced the natural product discovery toolkit within SECRETed. They helped expand the community reference databases MiBIG 3 and MiBIG 4 by adding new, experimentally validated siderophore and biosurfactant gene clusters, strengthening the foundation for genome-guided bioprospecting. The team also contributed to new NRP-metallophore detection rules in AntiSMASH (with Wageningen University and UC Santa Barbara), improving the identification of siderophore pathways. By combining these tools with their large-scale phylogenomic workflow AutoMLST2, EKUT produced the first broad view of how siderophores are distributed and have evolved across bacterial diversity, offering valuable insights for future biotechnological applications.







4

Advances in Engineered Siderophore Biosynthesis

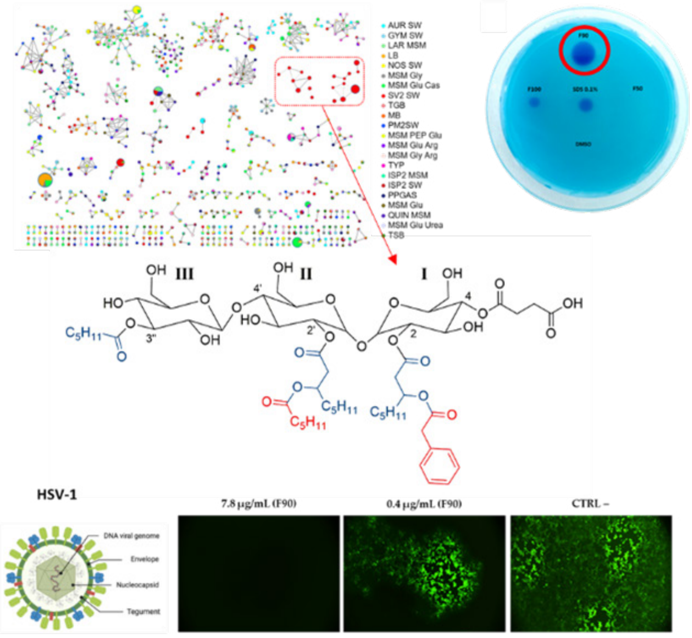
ICL has succeeded in the recombinant expression of pathways for the bioproduction of siderophores including some new-to-nature. Moreover, through the use of directed evolution approaches, they have managed to optimise the strains used for their production, reaching yields in the range of 0.5 g/l in lab scale tests.

Project Achievements & Legacy

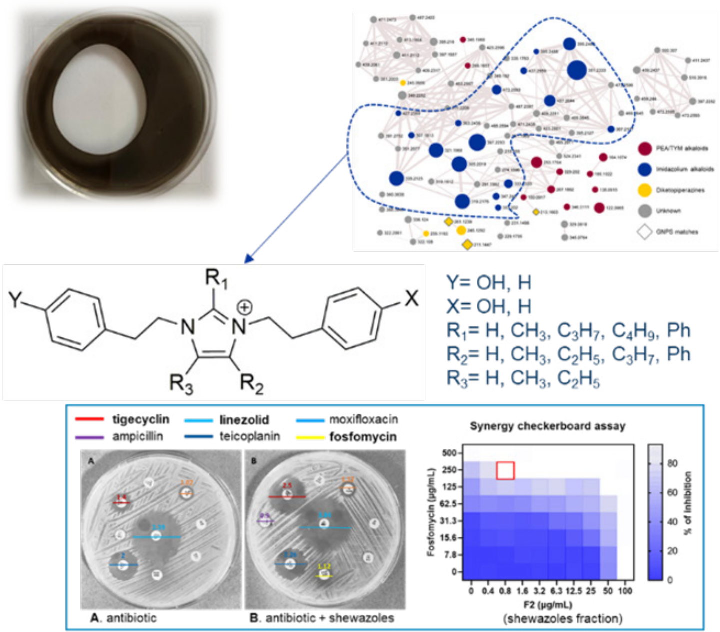
5 Novel Biosurfactants and Siderophores with Strong Therapeutic Potential

Through an integrated metabologenomics approach and bioassay-guided screening, the SZN group succeeded in the stereo-structural elucidation and biosynthesis characterisation of novel biosurfactants and siderophores belonging to five molecular families from extremophiles belonging to the SZN in-house strain collection. Among them, unprecedented succinoyl trehalolipids from the deep-sea bacterium Rhodococcus sp. I2R showed a potent antiviral effect against herpes simplex virus and human coronaviruses, and high antiproliferative activity in PC3 prostate cancer cell line. In addition, shewazoles from Shewanella aquimarina possess a broad spectrum of action, including (a) antibacterial activity against antibiotic-resistant Staphylococcus aureus clinical isolates, (b) synergistic effects with established antibiotics, (c) antiviral activity against viruses with and without envelopes (HSV-1, HcoV-229E, and PV-1).

Compounds	Class	Number of Congeners	Producer	Type	Function
trehalolipids	glycolipids	39	Rhodococcus sp. I2R	piezophile	biosurfactant
shewazoles	alkaloids	24	Shewanella aquimarina	halophile	biosurfactant
rhodoheptins	lipopeptides	20	Rhodococcus sp. I2R	piezophile	biosurfactant
rhodamides	lipopeptides	33	Rhodococcus sp. I2R	piezophile	biosurfactant
pyoverdines	non ribosomal peptides	4	Pseudomonas gessardii M15	psychrophile	siderophore



Succinoyl Trehalolipids from Rhodococcus I2R



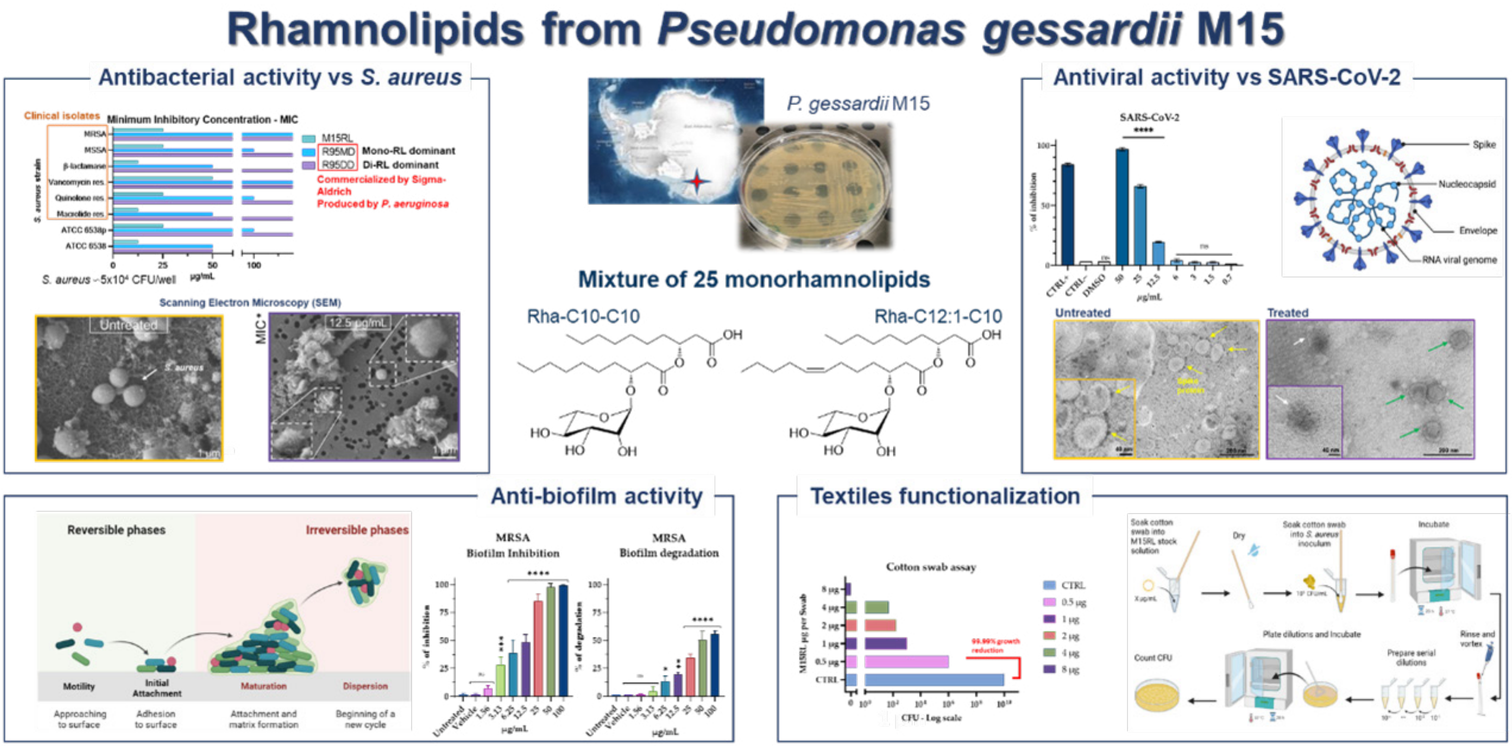
Shewazoles from Shewanella aquimarina

Project Achievements & Legacy

6

High-Efficacy Rhamnolipids for Medical and Antimicrobial Use

The SZN group explored the antiviral and antibacterial activity of the rhamnolipids mixture (M15RL) produced by the Antarctic bacterium Pseudomonas gessardii M15. M15RL induces complete inactivation of viruses belonging to Coronaviridae (HCoV-229E and SARS-CoV-2) and Herpesviridae families (HSV-1, HSV-2). In addition, M15RL has higher bactericidal activity against a panel of clinical multidrug-resistant isolates of S. aureus as compared to commercial rhamnolipids and can be an additive for wound dressings for chronic wounds, as being able to functionalise biomedical textiles for medical devices.



Acknowledging our Partners & Supporters

The SECRETed consortium extends heartfelt thanks to all project partners whose scientific expertise, technical contributions, and collaborative spirit have been instrumental in driving this four-and-a-half year initiative forward. Special appreciation is owed to the coordinating organisations and Work Package leaders whose leadership ensured each milestone was met with rigor and vision. We also wish to recognise our listed stakeholders, Society for Medicinal Plant and Natural Product Research, MANE, Kalsec, Croda and Welcare Industries, for their valuable support, engagement in dissemination activities, and important feedback on end-user applications. Finally, the consortium gratefully acknowledges the European Commission for funding, guidance, and trust in our mission. Together, these efforts have enabled SECRETed to leave a meaningful legacy and contribute to Europe's transition toward a more sustainable, innovation-driven future.

Thank you for your support all these years!

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