



# Newsletter

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## In this Issue

1. SECRETed at a glance
2. End-user applications
3. Pathway towards industry-driven compounds
4. Achievements
5. SECRETed meetings
6. Website and Social media
7. Dissemination activities
8. The SECRETed consortium

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# SECRETed at a glance

The SECRETed project is aligned with the development of novel biosurfactants and siderophore compounds with custom-made properties taking advantage of aquatic micro-organisms.

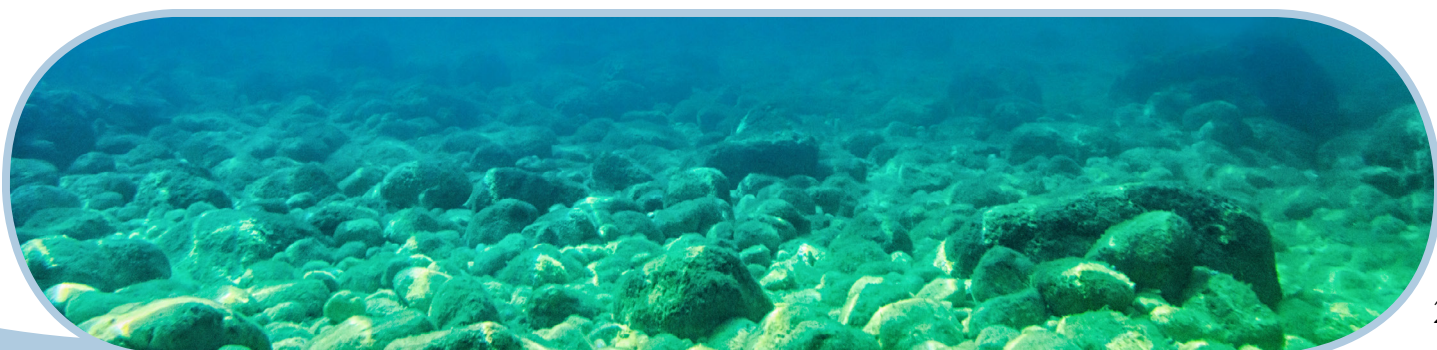
The large demand for surfactants has led to the need of utilizing biorenewable sources (microorganisms) in order to substitute fossil based synthetic surfactants and reduce environmental impacts and fossil resources dependency. In line with this, marine and extremophilic bacteria have been recently identified as a prolific source for the development of biosurfactants and siderophores.

**Biosurfactants** (bio-based surface active agents) are a group of lipids featuring both a hydrophilic and hydrophobic nature within the same molecule, enabling the solubilisation of hydrophobic substances in water. They form supramolecular structures, like micelles, liposomes or microemulsions and play an important role in household and industrial cleaning, cosmetics, nutritional and pharmaceutical industries.

**Siderophores** are capable of binding (chelating) and transporting  $\text{Fe}^{3+}$  ions featuring an outstanding activity in biological systems, like plants and blood. They are capable of inhibiting growth of plant pathogens, promoting bioremediation of heavy metals in contaminated soils, and featuring multiple effects in medical treatments (iron overload after blood transfusion, bacterial infections, antibiotics uptake facilitator and iron demanding tumour cells).

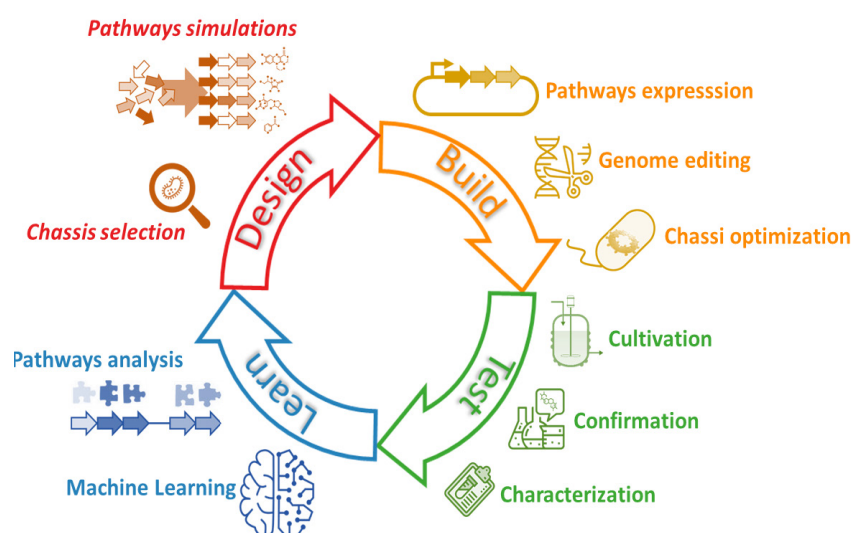
The SECRETed project focuses on screening already collected marine and extremophilic microorganisms to develop novel hybrid molecules with tailor-made properties obtained from the combination of biosynthetic genes. Machine Learning algorithms will be employed to reveal the genetic mechanisms responsible for biosurfactants and siderophores biosynthesis in order to expand the chemical diversity of bio-based compounds.

Databases inspection and collected data will result in a unique microbial amphiphilic (holding both hydrophilic and lipophilic properties) compound space to assist in comprehending and investigating molecular structures, physicochemical characteristics, bioactivities, genetic mechanisms and associated biosynthetic gene clusters for compounds biosynthesis with desired properties. The engineered combinations of genetic elements will be tested and validated resulting in the development of sustainable strains designs for industrial applications.



# SECRETed at a glance

SECRETed systems metabolic engineering, experimental, Machine-Learning and bioprocess development activities following an iterative methodology including Design, Build, Test and Learn analysis stages (Figure 1) to develop industry-driven and new-to-nature strain designs for the production of biosurfactants and siderophores.



- WP1: Ethics requirements (IDE R&D)
- WP2: Microbial collections screening (MATIS)
- WP3: Databases integration and Industry-driven designs (IDE R&D)
- WP4: Systems metabolic engineering for microbial platform optimization (USE)
- WP5: Lab-scale fermentation and chemical characterization of compounds (UoA)
- WP6: Pilot systems for process optimization (BBEPP)
- WP7: Proof of concept for end users' applications (SE)
- WP8: Sustainability assessments (Blue Synergy)
- WP9: Communication, dissemination and exploitation (EXELISIS)
- WP10: Management and Coordination (IDE R&D)

*The SECRETed project brings a multi-disciplinary team integrating expertise from different disciplines.*

- Microbiology, Systems and Synthetic Biology: IDENER R&D (Project Coordinator), University of Sevilla, Imperial College London, Lund University, Matis Ohf, Stazione Zoologica Anton Dohrn, Eberhard Karls Universitaet Tuebingen
- Natural product chemistry: University of Athens, Stazione Zoologica Anton Dohrn, Eberhard Karls Universitaet Tuebingen
- Industrial testers: Sylentis, Pharma Mar, Accuplex Diagnostics, Sphera Encapsulation
- Industrial bioprocessing: BIO BASE EUROPE PILOT PLANT
- Sustainability assessments: Blue Synergy
- Market analysis and knowledge exchange: EXELISIS

# End-user Applications

## Biosurfactants (encapsulation agent)

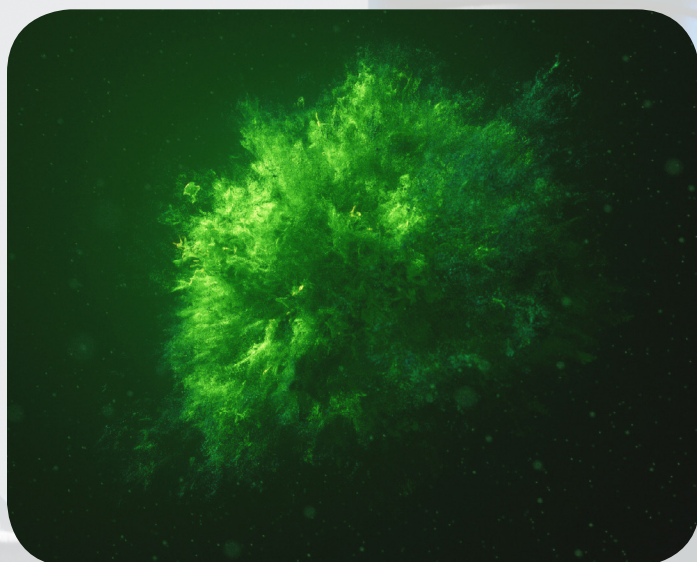
- anti-aging and anti-wrinkle ingredients to increase bioavailability - cosmetics
- bio-pesticides (essential oils) with controlled release - agro-chemicals
- RNAi encapsulation and delivery features of selected biosurfactant-based formulations - pharmaceuticals

## Siderophores

- treatments for eye diseases related to iron excess (siderosis) - pharmaceuticals
- antitumor activity; selected cancer cell lines (colon, lung, breast, pancreas) - oncology
- Trojan horse with antimicrobial activity when combined with antibiotics - biotechnology

## Other uses include the delivery of compounds in nano scale

- protecting the compound from environmental degradation (food industry)
- increasing absorption in target tissue (health industry)
- improving manipulation of lipophilic molecules (household care applications).



# Pathway towards industry-driven compounds

## 4 Different Aquatic Microbial Collections

1. PHM will screen 5000 cell extracts from marine microbial samples located in deep-sea sediments (Canary Islands and Cadiz Gulf, national waters)
2. MATIS & LUND will cover the analysis of 200 thermophiles and marine bacteria strains from the Iceland coasts and thermal vents
3. SZN will screen 250 psychrophilic and piezophilic strains from Arctic and Antarctic deep sea (3000m)
4. USE will address at least 50 model-targeted halophilic strains from European public microbial collections (DSMZ/CECT)

The SECRETed project will unlock the potential of marine and extremophilic bacteria that are used as sources for the development of tailor-made biosurfactants and siderophores. A key capability of biosurfactants and marine siderophores biosynthesis is the exchange of their hydrophobic and hydrophilic chemical parts enabling the development of new-to-nature compounds (Figure 2). In this

scope, thermodynamic and experimental analyses will be processed by Quantitative Structure Property Relationships models (QSPRs) in order to guide the development of industry-driven strain designs prepared for the synthesis of chemical formulations with desired physicochemical properties.

The selected strains will be genetically dereplicated to identify the genomic sequencing and further evaluate their abilities for compounds biosynthesis. Machine Learning algorithms will associate the molecular structures and their characteristics with metabolic pathways to explore engineered microbial physiologies producing molecules of interest through a 'Mix and Match' approach.

The in silico predictions will be validated through Synthetic and Systems Biology tools and the expression of proposed genetic sequences will be experimentally tested and explored in pilot-scale scenarios preparing the processing conditions for industrial cultivation of microbial hosts.

Bacteria are investigated and identified based on their capability of producing desired compounds by considering general parameters, like critical micelle concentration, Krafft temperature and ligand affinity.

The target genes – these are associated with the biosynthesis of newly discovered molecules and/or chemicals parts (Figure 2) – will be characterized to reveal the link between the Molecular and Gene Cluster Families. To enable isolation of known molecules, dereplication techniques (Nuclear Magnetic Resonance and liquid-chromatography-mass spectrometry) are used in order to profile and quantify new natural products within complex mixtures and to develop the chemical space. In this space, the molecules are placed with respect to properties of corresponding Molecular Families highlighting a clear structural signature.

The SECRETed projects will broaden the research activities focusing on a wide range of 4 different aquatic microbial resources

# Pathway towards industry-driven compounds

A database of genetic clusters and subclusters will be developed for the biosynthesis of the target compounds. Experimental work of addressed bacteria and thermophiles will provide the desired biological pathways and gene clusters preparing a pipeline for metabolic engineering. By these means, sequencing of genomes of organisms will be investigated towards the production of desired biosurfactants and siderophores structures, as presented in Figure 2.

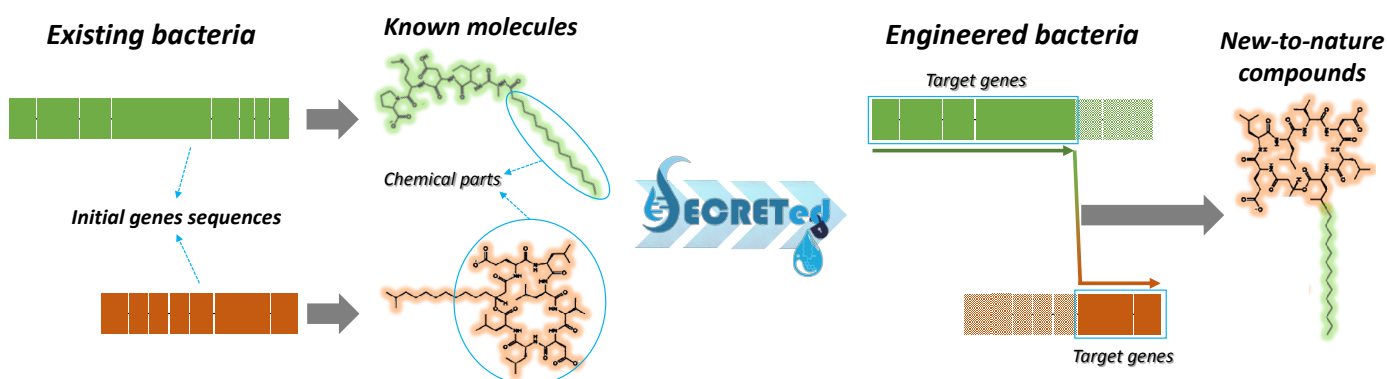


Figure 2: Synthesis of target genes for tailor-made compounds biosynthesis

Use of biosurfactants and amphiphilic siderophores as **antitumor agents**.

Siderophores-antibiotic conjugation formulas have been the major application to facilitate the **uptake of antibiotics** across the cell membrane, acting as a “Trojan Horse”.

Retrosynthetic in silico tools will be employed to evaluate the performance of target compounds produced by host metabolisms of different microorganisms. As a result, optimal genes combinations, with minimized undesired effects, will be identified for the production of biosurfactants and siderophores with characteristics that meet the needs of the end-users applications (cosmetics, pharmaceuticals and agro-chemicals).

# Pathway towards industry-driven compounds

Less than 1% of marine microbes have been successfully cultured, limiting the diversity of discovered natural products. The SECRETed project will fill the gaps of the underlying biosynthetic pathways leading to the production of novel bio-based compounds.

The real-life expression of genetic combinations proposed by modelling activities will be validated in host microorganisms (e.g. *E. coli*, *Bacillus*, *Streptomyces*, *Thermus thermophilus*, *Rhodothermus marinus*, *Chromohalobacter salexigens*) and the production performance will be tested and evaluated in terms of biosurfactants and siderophores quality and quantity.

Genome-based metabolic networks will contribute to the optimal design of new microbial scaffolds. In this contexts, Flux Balance Analysis (FBA) methods will be employed to further address technical specifications of the strains, their feedstocks and products and will account for cost-effective production and downstream processing. The prepared data will provide key strategies for the industrial friendly implementation of discovered microbial scaffolds and sustainable scaling up of bioprocessing. A pilot line will be developed to host the cultivation of optimized strain designs.

Coacervation and nano-encapsulation methods will be addressed to prepare compounds for end-user applications. The SECRETed project includes testing and product development tasks targeted to the utilization of biosurfactants and siderophores in multiple sectors including cosmetics, nutraceuticals, agrochemicals, pharmaceuticals and biotechnology. In this scope, the SECRETed end-users will test the proposed genetic combinations and the resulting compounds towards satisfaction of Industry-driven requirements.



***The goal of SECRETed is to improve the Technological Readiness of desired compounds and emerging technologies to TRL-6.***

# Achievements

## Integrative Management Platform

The Integrative Management Platform has been launched and includes:

- microbial amphiphilic compounds space
- structural properties and molecules identifiers
- molecular structures, physicochemical characteristics, bioactivities, genetic mechanisms
- Molecular Networks and associated features
- taxonomical and biological data

### 2178 unique compounds database

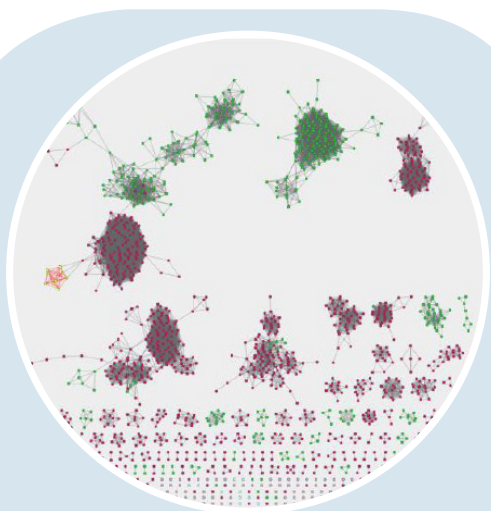
- ▶ 1606 biosurfactants
- ▶ 572 siderophores

### Biosurfactants

- > 437 compounds with CMC values
- > 42 with Emulsification indexes
- > 44 with Surface tension coefficients
- > Other properties supported: Antifungal, Antimicrobial act, Antitumoral, Bioremediation, Antiviral, Antimalarial.

### Siderophores

- > 52 compounds showing the ChromoAzuroil tests
- > 19 compounds indicating affinity to non-iron metals
- > Other properties supported: metal affinity, Antifungal, Antimicrobial act, Antitumoral, Bioremediation, Antiviral, Antimalarial



- ▶ *Display of Molecular Networks*
- ▶ *New molecular designs*
- ▶ *Biosynthetic gene clusters for compounds production*

## Handbook

Guidelines on standardization and organization of protocols for

- preparation and processing of samples
- sharing and further analysis
- defining data associated with target compounds

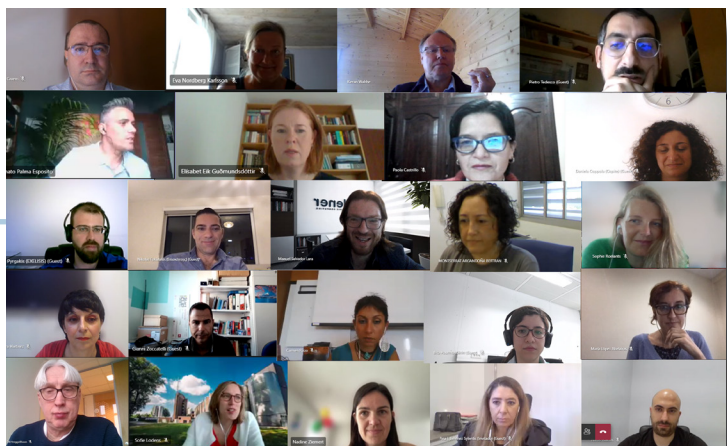
## Data for

- 450 bacteria (isolated by SZN)
- 50 phylogenetically diverse halophilic bacteria (isolated by USE)
- 1500 taxonomically diverse marine bacteria (isolated and extracted by PHM)
- 200 phylogenetically diverse marine bacteria (isolated by MATIS)

# SECRETed meetings

## Kick-off Meeting of SECRETed

The Kick-off meeting was held remotely due to COVID-19 Pandemic on the 28th of June 2021 and was hosted by IDENER R&D (project coordinator). The SECRETed partners presented their organisations and contributions to the Work Packages and associated Tasks. The EC Project Officer introduced the EC expectations that are mainly focused on the guidelines for project implementation.



## Meeting on microbial collection screening

The Meeting was held online, on 15th July, with the participation of Matís, PharmaMar, Accuplex Diagnostcs, Stazione Zoologica Anton Dohrn, Universidad de Seville, Lund University and Exelisis. The meeting focused on the following research steps concerning the selection of microbial collection and screening and characterization methods of siderophores and biosurfactants pathways.



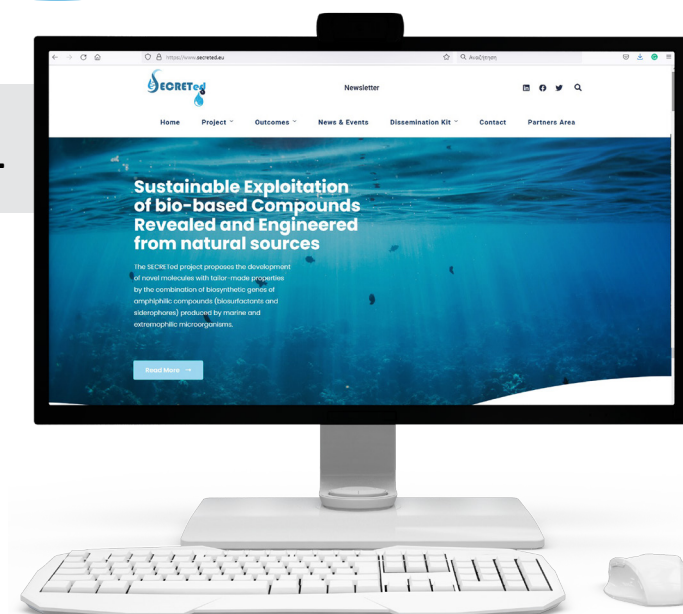


# Website and Social Media

**Website lunched on 28<sup>th</sup> August 2021**

Find more information about the SECRETed:

- Partners
- WPs
- Tasks
- News
- Digital Materials
- Deliverables
- Contact



Facebook Posts 23  
Likes 244  
Followers 70



Page Views 863  
Followers 182  
Reactions 351



Followers 67  
Profile Visits 6330  
Impressions 6653



# Dissemination Activities

## Dissemination Material

The dissemination material includes General information, infographics, and content to introduce the general public & specific audiences into the main objectives, State of the Art, Impact and implementation of the SECRETed project.



Poster  
Published on 27/08/2021



Roll-up  
Published on 6/10/2021



Flyer  
Published on 31/08/2021



Press Release  
Published on 12/11/2021



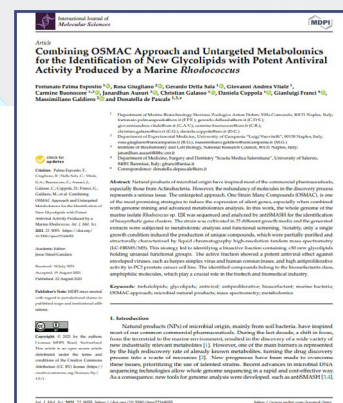
# Dissemination Activities

## Published Work

Palma Esposito F, Giugliano R, Della Sala G, Vitale GA, Buonocore C, Ausuri J, Galasso C, Coppola D, Franci G, Galdiero M, de Pascale D. Combining OSMAC Approach and Untargeted Metabolomics for the Identification of New Glycolipids with Potent Antiviral Activity Produced by a Marine Rhodococcus. International Journal of Molecular Sciences. 2021; 22(16):9055. <https://doi.org/10.3390/ijms22169055>



Giugliano, R.; Buonocore, C.; Zannella, C.; Chianese, A.; Palma Esposito, F.; Tedesco, P.; De Filippis, A.; Galdiero, M.; Franci, G.; de Pascale, D. Antiviral Activity of the Rhamnolipids Mixture from the Antarctic Bacterium *Pseudomonas gessardii* M15 against Herpes Simplex Viruses and Coronaviruses. *Pharmaceutics* 2021, 13, 2121. <https://doi.org/10.3390/pharmaceutics13122121>



## Dissemination Events

On the 9th of July, Blue Synergy participated and made a brief presentation of the SECRETed project objectives in the Horizon Europe Brokerage Event for Cluster 6: "Food, Bioeconomy, Natural Resources, Agriculture and Environment" Work Programme 2021-2022. <https://he-cluster6.b2match.io/>



In the context of the IUPAC|CCCE 2021 conference, Eberhard Karls Universität Tübingen co-organized with Queen's University (Canada), the Symposium of "Natural Products: From Discovery to Application". The event was focused on the discovery and development of Natural Products as therapeutics and brought together scientists from across a range of traditional disciplines including Chemical Synthesis, Analytical Chemistry, Computational Chemistry/Biology, Microbiology and Molecular Biology. <https://www.cheminst.ca/conference/ccce2021/program/program-overview/chemistry-for-health/>



# Dissemination Activities

## Upcoming Events

DATE	TITLE OF EVENT
March 2022	<a href="#">GRC conference for Marine Natural Products</a>
August 2022	<a href="#">70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA)</a>
September 2022	<a href="#">Biosurfactants   International Conference</a>



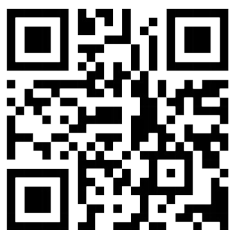


## The SECRETed consortium

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



Stazione  
Zoologica  
Anton Dohrn  
Napoli



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