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

Q&A Consortium

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This Q&A has been prepared by the SECRETed experts and researchers, who hold extensive experience in the fields of microbiology, biotechnology, metabolic engineering, and machine learning. The purpose of this Q&A is to offer information and answers concerning the research & development activities of SECRETed as well as about prejudices related to the use of natural producers, engineered strains and their products in everyday life.

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What are microbial producers and why we exploit them?

Microbes are organisms that are too small to be seen by unaided eye. They include bacteria, fungi, protozoa, microalgae, and viruses. Microbes live in environments such as soil, water, food, and animal intestines, as well as in more extreme environments such as rocks, glaciers, hot springs, and deep-sea vents. To live in such diverse environments, microbes have to develop specialized strategies and physiologies. Said physiologies have been used since ancient times to make bread, cheese, yogurt and wine. Nowadays, we also employ them in a wide range of applications, from waste processing to pharmaceutical compound production. However, it is widely estimated that, to date, less than 1% of marine microbes have been successfully cultured limiting the diversity of discovered natural products. Microbial genomics and biotechnology research are critical for unveiling the hidden potential of microbial biodiversity.

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What are Siderophore compounds and what is their function in microorganisms?

Iron is essential for growth and health. Without it, we would all be in trouble! Bacteria and fungi (and especially the bad ones) also need iron to survive and is essential for successful infection. While iron is the 2nd most abundant element in the earth's crust, in many environments the level of freely available iron is low, while in the body levels of iron are extremely low so the bacteria and fungi must compete for this.

Where iron is low, the microorganisms, especially those that cause infection, have a secret weapon that they use to secure iron. They produce tiny magnets (known as siderophores or iron chelators), which they shoot out into the environment, where they scavenge the limited iron and then take it back inside them to help thrive, while humans or animals are infected.

Science is now exploring how to exploit the use of these tiny magnets, and the potential is immense.
Two examples of how this is being explored:

- the use of these magnets to help treat and fight infection
- detection of these magnets to help confirm the presence of an infection.

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How can we exploit siderophores against antibiotic resistance of infective microorganisms?

It is now widely accepted that we have a big problem with infective microorganisms that develop resistance to available antibiotics leading to many deaths. It has been predicted that by 2050, there will be more deaths from infection as result of antibiotic resistance than from all cancers combined, unless we act now. So, we need new and better ways to help fight infections. This is where science is exploiting the magnets and turning them against infective microorganisms.

If you can treat an infection with a stronger magnet – than the one that the microorganisms cannot use – then you can starve the infection and prevent it for taking hold.

Another solution is based on the strategy of Greeks in Troy, where they build a Trojan horse, only that in this case we join a "magic bullet" (for example, an antibiotic or other toxic agents) to a magnet that the microorganism will use. This tricks the microorganism into taking back what it thinks is its own magnet, but this time there is a surprise attached, which effectively kills the microorganism.

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How can we use of siderophores to detect infections?

The faster you detect an infection, the sooner it can be treated. While most infections can be detected, it is not always easy, or fast enough, or both. New and better ways are always under investigation. The fact that most microbes make siderophores (magnets), if you can detect the magnet, you could detect the infection. For example, one infection of increasing importance that has been recognised is the detection of the fungus Aspergillus, which caused up to 30% of deaths in the Intensive Care Units during the recent Covid-19 pandemic. Detection of Aspergillus requires x-rays, scanners, analysis of fluid from lungs, and blood tests that are less useful. However, all of these are difficult, or invasive, and potentially dangerous for those exposed to patients, especially when the patient is all hooked up and on a ventilator. But what if you could take a urine sample in which the "magnet" made by Aspergillus can be easily detected in matter of minutes. This is now possible!

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What are other applications of siderophores?

These are just two examples of how science is now exploring how we might exploit the use of siderophores. Scientists are looking for many other applications, such as using these as vaccines to help fight infection, as novel fertilizers enhancing plant growth or preventing infection in agricultural crops. In clinical conditions, where iron can be toxic, even fatal, these novel magnets can be used to mop up and remove the excess iron preventing toxicity or helping treat cancer. These application and more are all under active investigation.

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What are biosurfactants and which is their function?

Biosurfactants are chemical compounds obtained from microorganisms that hold specific structure and properties. Biosurfactants have two edges (parts), where the one is hydrophilic, while the other is hydrophobic; namely, the first favors and the other opposes water. Due to this structure, they are also called amphiphilic compounds and they tend to be formulated as spheres (like micelles or liposomes), when dissolved in water, holding their hydrophobic parts inside the sphere and letting the hydrophilic parts outside.

Due to their amphiphilic nature, biosurfactants in solutions tend to absorb at the interface of two different phases, such as water and oil, reducing interfacial tension between the two different phases. The ability to change the surface or interfacial tension properties of a fluid is the key characteristic that allow biosurfactants to form and stabilize microemulsions.

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What are the applications of surfactants and how can we take advantage of them?

Biosurfactants, being able to reduce the interfacial tension between two different phases, can find applications in various fields, such as food, nutraceuticals, cosmetics, pharmaceuticals and agriculture. As an example, the abovementioned property is extensively exploited in the field of encapsulation, intended as the action of entrapping an active ingredient inside a protective membrane. In this context, biosurfactants are used to create stable emulsion in which hydrophobic and hydrophilic active ingredients are solubilized (i.e., oil in water emulsion and water in oil emulsion). Since almost all encapsulation techniques, such as spray dry, coacervation, etc., start with the creation of an emulsion, the use of biosurfactants is fundamental to stabilize the emulsion at the base of the process and ensure the right processing of the active ingredient within the final encapsulate.

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How we exploit biosurfactants in SECRETed applications (cosmetics, fertilizers, food supplements)?

Since biosurfactants can find application in different sectors, we selected those that are most promising in terms of their specific properties; in particular, the new biosurfactants of SECRETed that will be used for the encapsulation of the following active ingredients:

- Coenzyme Q10, a known antioxidant, for a nutraceutical application, thus as a food supplement, and also for cosmetic application as an antioxidant and anti-aging agent.
- Oregano essential oil, for the application in the agrochemical field for its antifungal properties.
- siRNA (provided from Sylentis) for pharmaceutical purpose.

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What is achieved by using the SECRETed formulations in pharmaceutical products?

Iron is vital for almost all living organisms by participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. Disorders of iron metabolism are among the most common diseases of humans and encompass a broad spectrum of diseases with diverse clinical manifestations, ranging from anemia to iron overload and, possibly, to neurodegenerative diseases. Iron levels in body tissues must be strictly controlled, though, as too much iron can cause tissue damage by creating free radicals, which can harm cells. Among these pathologies that can be associated with iron toxicity are some ophthalmologic pathologies, where iron may exacerbate different eye diseases. Therefore, it is plausible that reducing cellular or body iron stores could influence disease pathogenesis, so it is logical to consider the iron chelators' potential protective role in the various ophthalmic diseases. Sylentis plans to use iron chelators' to control iron-mediated toxicity as protective compounds for treatment of further ophthalmological iron related disease-conditions with chelators' – formulated siRNAs.

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How do we use biosurfactants in new medical therapies to increase their performance?

Surfactants have an amphiphilic character that permits self-assemble molecules of surfactant into variations of nanostructures from spherical/rod likes micelles to lamellar sheets. In order to efficiently deliver the RNAi drug to the tissues of interest and increase bioavailability, it is necessary to specifically formulate the RNAs to increase stability and bioavailability in the tissues. The development of these formulations requires the use of positively charged surfactants that allow the RNA (negatively charged) and other components that are part of the formulation to be complexed. Surfactants can be used as adjuvants, solubilisers or stabilisers of emulsions and formulations and can help to endosomal escape and improved efficiency of cargo delivery. Synthetic products are preferred to products of natural origin for quality reason, but for some of the industrial compounds may be able to activate immunostimulatory pathways even in the absence of other immune signals, harboring immunogenicity problems. Additionally, many of the synthetic surfactants are not properly metabolized and eliminated, even entering the lipid metabolism pathways and even associating with tissue structures. This is the case of DPPC. It is essential to find new completely biodegradable surfactants that, once they have performed their function, are rapidly metabolized in the body and excreted. Sylentis expects to find new surfactants that will allow the development of highly efficient and completely biodegradable formulations that can perform their function and be rapidly metabolized without producing alterations in the target tissues. Sylentis also hopes to find innovative surfactants that can be used in different formulation approaches for nucleic acids.

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What are marine ecosystems and how we take advantage of them without harming them?

The marine environment covers around 70% of the earth planet and represents a huge prosperous existing resource. Its diverse and often hostile physico-chemical features (e.g. low temperature, limited light access, high salinity, high pressure) allowed the evolution of peculiar ecological niches, incomparable with the terrestrial ecosystem. The efforts done by research community in exploring these hidden habitats, are progressively uncovering the presence of unknown microorganisms, which have evolved unique metabolic and genetic pathways for the production of uncommon metabolites. Many recent European projects are focusing their attention on microorganisms, improving their cultivability and exploiting their potential applications, as they are a still poorly explored resource for drug discovery and represent the possibility of obtaining a continuous source of bioactive compounds, without impacting the environment, avoiding the use of huge quantities of marine organisms.

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What are the benefits of exploiting natural microbial producers?

Research on unexplored bacterial metabolisms is related to unveiling the high potential that their specialized physiologies possess. The diverse metabolisms of marine and extremophilic bacteria contribute both to i) the production of new green, low-emission, nature-based chemicals and ii) defining new raw renewable materials to employ post-consumer wastes in a cost-effective manner. In this way, natural microbial producers can contribute to the "circularity" and "defossilisation" of major economic sectors, including the chemical industry. That is crucial to reduce greenhouse emissions and revert climate change.

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Is SECRETed focusing on modifying micro-organisms or the chemicals that they produce?

Both, SECRETed aims to reutilize unexplored microbial collections to discover new compounds that satisfy the requirements of industry and eventually those of consumers. To complement this approach, Machine Learning algorithms (ML) are deployed to reveal the genetic mechanisms, which are responsible for their biosynthesis and to expand the chemical diversity of such bio-based compounds. New strains can then be designed, built and tested in an iterative process for the development of viable and sustainable industrial processes. Thus, both natural and new-to-nature compounds will be evaluated regarding their potential use in the agrochemical, cosmetic, nutrition and health industry.

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Why do we need engineered strains? Cannot we directly use natural producers as they are?

Natural strains may not be the best ones for production. Sometimes we do not know how to culture and propagate the organisms, which can have a poor performance in production settings. Other times the organisms might be harmful for humans, or may require conditions that are not compatible with the production process (for example, high salt that corrodes steel tanks). In lab and industrial settings alike we tend to work with a handful of model organisms that we know how to culture and are certified as safe. It is also very important that these organisms can be easily genetically modified, given that we need to introduce new pathways or make them more efficient. Environmental organisms are normally not domesticated and not suitable for these purposes.

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Genetic Modified Organisms. Is it safe to use them? Can we consume them or their products?

Absolutely, even though it is true, we use genetically modified organisms for production, the products themselves are not different from those identified in nature. This is a key point: the product – the food supplement for example – is identical. In fact, even from a legal perspective in most countries (including the EU) the products are not considered 'Genetically Modified' once they are purified and removed from the engineered strain.

What we do is to take advantage of the technology to improve its production so that we can obtain higher yields of the desired molecules, therefore reducing their cost and improving the sustainability of the process.

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Which is the origination of the microorganisms used in SECRETed?

SECRETed involves 4 partners with large experience and strains databases to be exploited in contexts of the project activities. Specifically:

- PharmaMar holds thousands (>5000) extracts from marine isolated strains collected in national water of Atlantic Ocean.
- MATIS holds thousands (>5000) thermophiles and marine bacterial strains collected from Iceland coasts and thermal vents.
- SZN owns around 500 valuable and unexploited bacterial strains and their extracts, collected in Antarctica, Arctic Sea, and north EU waters.
- USE will research species from public collections in Germany (DSMZ) and Spain (CECT).

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How safe is to modify organisms in the laboratory? Is it possible that the organisms escape from laboratories and get harmful for human or the environment?

The consortium is using classical proven production organisms (e.g. E.coli and Bacillus) and extremophiles organisms, psychrophiles, piezophiles, halophiles and thermophiles as host/chassis species in research. The consortium will comply with national and European rules and regulations regarding the development and use of genetically modified organisms (GMO), and the containment of GMOs. Also, as regards extremophiles, it is very unlikely that they would be harmful to human and the environment due to the strict adaptations of such species to extreme environmental conditions. They are not pathogenic and are largely naturally contained as their habitats are confined on a global scale. Furthermore, the target chemicals are natural compounds and as such they are not harmful to other organisms, rather the opposite, as they are secreted and therefore unlikely to give modified strains, a competitive edge in the complex biological relationship-matrix of an extreme biotope.

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How can algorithms assist in confronting the SECRETed challenges and contribute to laboratory research?

The huge development of Machine Learning (ML) in Cheminformatics and Synthetic Biology tools in the last decade is assisting our approach. Those algorithms contribute to databases inspection and data collection, facilitating the construction of a unique microbial compounds space comprehending molecular structures, physicochemical characteristics, associated bioactivities and genetic mechanisms responsible for their biosynthesis. Clustering of data facilitates the implementation of algorithms that “fill in the gaps” of experimental knowledge. The multi-dimensional characterization of chemical species guides the discovery of new compounds with specific molecular features. This database can be used for training machine learning algorithms to predict the compounds from Biosynthetic Gene Clusters (BGC) data and vice versa. These algorithms can be extended to mix genes of different BGCs from distinct organisms into new-to-nature BGCs that potentially encode the production of new-to-nature compounds with desired traits. Finally, when needed, specific molecular features can also be improved by simulating virtual compounds. Their synthesis is guided by algorithms, which let us discover the genetic combinations towards industry-driven, tailor-made and new-to-nature compounds production. The predicted BGC/compound pairs can then be tested in the laboratory.

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How SECRETed can reduce pressure on harnessing wild microbial populations?

Despite the progress in isolation and cultivation technology, it is estimated that only 1% of bacteria can be successful cultivated in laboratory conditions and this significantly impacts the discovery of natural products. SECRETED helps to overcome this limitation by focusing also on already available microbial collections and genomes, therefore avoiding the potential loss of biodiversity. The in-silico identification of biosynthetic gene clusters, combined with the heterologous expression in suitable hosts, shifts the focus on microbial industrial producers, therefore reducing the pressure on wild-type microbial population. The success of this strategy could further stimulate omics-based research, thus minimizing human impact on marine ecosystems.

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What are the benefits from the use of bio-based products of SECRETed?

In recent decades, the large demand for surfactants has led to an increasing interest in obtaining them from renewable sources, in order to reduce the impact on the environment and the dependency on fossil resources. Moreover, biosurfactants typically exhibit a higher activity at lower concentrations compared to many synthetic surfactants, showing very low critical micelle concentration (CMC), low toxicity, high biodegradability and tolerance to extreme conditions such as high temperature values, extreme pHs, and high salinity. Biosurfactants are also endowed with important biological activities, which make them attractive from an environmental point of view.

Specifically, for:

i) their use in agriculture as a biocontrol agent thanks to their insecticide activity;
ii) their application in bioremediation, assisting hydrocarbon pollutant biodegradation and metal sequestering;
iii) and as biobased products, replacing fossil fuel-based chemicals, for use in the chemical, food processing, food additives, cosmetics, and pharmaceutical industries for their emulsifying, foaming, dispersant, and antiadhesive activities.

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Who could be the potential stakeholders of SECRETed and which are the main messages that SECRETed provides to them?

The SECRETed project aims to reach stakeholders from several groups:, the scientific community, industrial stakeholders, European and national policy bodies and public stakeholders and potential investors as well as the general public which is continuously updated about SECRETed activities. The main messages provided to each stakeholders group are as follows:

- Pharmaceutical and cosmetics industry. They will be informed about the capabilities that new biologically produced amphiphilic molecules have, especially those with direct industrial applications.
- Biotechnology industry and biorefineries. New bio-based value chains and substitution of fossil-based products with biological-based routes.
- Waste management and recycling industries. Their interest in finding new waste valorization routes to reduce the environmental impact can be satisfied through the biotechnological production of biosurfactants and siderophores.
- Policymakers. They can promote the circular economy through new advances in biosurfactants and siderophore usage standardization.
- Consumers and Consumer associations. SECRETed provides proof of compounds production and their potential use to improve wellbeing and to reduce the environmental impact of their synthetic counterparts, together with assuring and demonstrating the safety of both the process and the products.

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What is the contribution of SECRETed to the Sustainable Development Goals set by the United Nations (UN)?

The outcomes of SECRETed directly contribute to the Sustainable Development Goals of UN through several activities and outcomes that offer solution concerning:

- SDG 2 – Zero Hunger, by proposing new biosurfactant formulations capable of encapsulating compounds to avoid phytopathogens proliferations.
- SDG 6 – Clean Water and Sanitation, by using novel siderophores capable to be used as plant-promoting factors and for plant pathogens control.
- SDG 8 – Inclusive and sustainable economic growth and SDG 9 – Sustainable Industrialization, by introducing economically promising industrial biotechnology solutions (bioprocess scales) leading to highly specialized quality jobs.
- SDG 12 – Responsible Consumption and Production, by preparing cultivation and metabolic engineering protocols for safe and sustainable production as well as finding more efficient routes to replace synthetic chemicals used in markets.
- SDG 13 – Climate Action, by using bio-based surfactants from raw feedstocks, diminishing petrochemical dependency, and contributing to circular economy.
- SDG 14 – Life Below Water and SDG 15 – Life on Land: by using biosurfactants that facilitate microbial crude oil degradation and siderophores employed for bioremediation of soils by chelating heavy metals.

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