SynBio 4Flav

synbio4flav.eu

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August 2023

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Summary

Providing a path for the standardised production of flavonoids

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Invisible allies have played a decisive role in generating the rich environment that supports human life on Earth. All visible life forms evolved in intimate proximity with microorganisms and benefit from engaging in multiple symbiotic relationships with them.

Food was made durable, more nutritious and tastier through the activity of microbial cultures for thousands of years. Cheese, bread, beer, wine, and many other culinary specialties - as part of our cultural heritage - are the result of processes that enabled storage and transport of otherwise quickly perishable food. They have been manufactured with the help of microbial communities in the process of fermentation.

Today, as the expansion of industrial agriculture is increasingly exhausting the planetary capacity to regenerate, the oldest biotechnical tool of fermentation, enhanced by advances in life sciences, could provide a sustainable route to tackle the global bottlenecks that emerged with a rapidly growing population.

Contents

The European research project SynBio4Flav builds on the principle of co-production that is prevalent in natural ecosystems.

SynBio4Flav establishes artificial microbial communities, Synthetic Microbial Consortia (SMC), where microbes are enabled to co-produce complex substances, in particular flavonoids, valuable bioactive substances that are naturally produced by plants.

Flavonoids are currently obtained by extraction from plants, yielding only small quantities. This resource-intensive production system represents an economic and ecological barrier, not only to their applications on larger scale, but also to fundamental research on their promising properties.

The vision of SynBio4Flav reaches beyond the production of flavonoids. Through variations in the composition of synthetic microbial consortia, microbial cell factories have the potential to produce virtually any organic substance.

Indeed, microbial biomanufacturing promises to become a key player in meeting the needs of a growing world population and enabling the preservation of precious natural resources at the same time.

Animation link

photo: Bruno Stubenrauch

RADICAL VISION

Revolutionary in this project is that we are trying to change completely the paradigm in the synthesis of complex chemicals.

Juan Nogales, project coordinator, CSIC - Spanish National Research Council, Spain

The difference is that we work at the nanoscale and our machines are biological elements, enzymes and microorganisms.

Ewa Huszcza, Wrocław University of Environmental and Life Sciences, Poland

photo: Bruno Stubenrauch

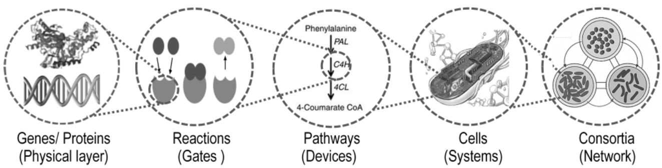
SYNTHETIC BIOLOGY

Synthetic biology is a rapidly advancing field in life sciences that applies engineering principles to biological systems with the goal to enhance their efficiency or develop novel organisms with desired complex functions - such as microorganisms producing flavonoids that are naturally produced by plants.

Driven by groundbreaking developments in information and computer sciences, genetics, biochemistry, molecular biology, systems biology, genome sequencing and editing techniques, synthetic biology opens up a huge space for a wide range of applications. Synthetic biology is expected to disrupt production industries across multiple sectors, medicine, food and agriculture, material science, environmental remediation, and shows the potential to deliver co-benefits for tackling global challenges such as climate change, food security, affordable global healthcare, providing novel business opportunities.

By developing and applying cutting-edge synthetic biology tools, SynBio4Flav aims to provide a cost-effective microbial production of complex chemicals.

SynBio4Flav expanded the scope of synthetic biology, addressing all core engineering principles which support efficiency and scaling: abstraction, decoupling and standardisation. SynBio4Flav deepened the concept of **decoupling** and contributed to **standardisation** on many levels by developing and advancing libraries, platforms and tools that enable the interchangeable combination of DNA parts at various levels of complexity, covering the whole chain of synthetic biology hierarchy **abstraction**, from genes and proteins to enzymatic reactions, metabolic pathways, to organisms (cells) and microbial consortia.

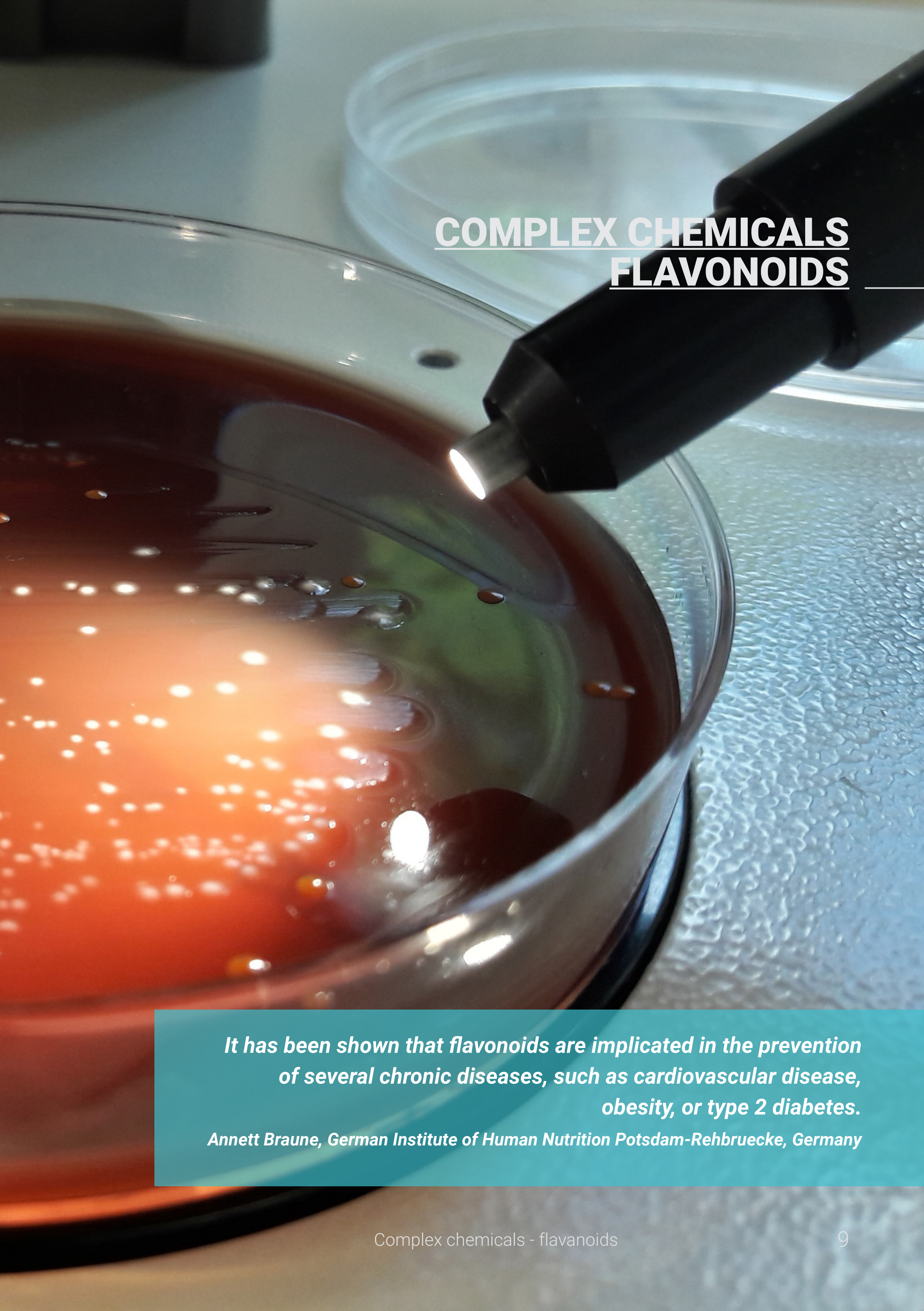


Flavonoids are a wide category of polyphenolic compounds that naturally occur in plants. They are synthesised within plants in response to environmental challenges and opportunities. Flavonoids protect plants against the damaging effects of UV radiation and harmful invasions of microbes or plant feeding insects and play a key role in beneficial symbiotic relationships with different species. Flavonoids are involved in the formation of colours, tastes and fragrances of fruits and flowers to attract insects, birds and other animals for the dispersal of seeds and pollen.

Their protective as well as colouring and taste-related properties are considered a huge potential for applications in the pharmaceutical, food and beverages, nutraceutical, cosmetics and animal feed industries.

Numerous scientific publications report on the potential of flavonoids for the prevention and treatment of widespread human diseases such as cancer, cardiovascular diseases, type 2 diabetes or Alzheimer's. Flavonoids research is presenting specifically promising results for the combined application with conventional treatment of cancer reporting less negative side effects for the patients. With their antioxidant and anti-inflammatory properties, flavonoids are expected to play an important role especially in future prevention and treatment of degenerative, age-related diseases.

credit: German Institute of Human Nutrition Potsdam-Rehbruecke



COMPLEX CHEMICALS
FLAVONOIDS

It has been shown that flavonoids are implicated in the prevention of several chronic diseases, such as cardiovascular disease, obesity, or type 2 diabetes.

Annett Braune, German Institute of Human Nutrition Potsdam-Rehbruecke, Germany

credit: German Institute of Human Nutrition Potsdam-Rehbruecke

Apart from medicinal and pharmaceutical applications, flavonoids are studied for their potential usage in the food and beverage industries, as nutraceuticals, food supplements or ingredients in functional food. One of the top demands for food and in particular the beverage industry is to lower the caloric intake, especially of sweet carbohydrates in response to the high prevalence and dramatic increase of obesity and type 2 diabetes. The taste-modulating properties and bitter-masking effects of several flavonoids are expected to provide potent solutions to this challenge.

Industrial applications are often limited by the restricted availability of flavonoids. Market suppliers are constrained by a narrow range of suitable plant sources containing only low concentrations of desired flavonoids. As a result, large amounts of plant biomass are needed to obtain small amounts of pure flavonoids. Long culture periods, climate-related and soil-dependent cultivation requirements, costly and resource-intensive extraction and purification processes hinder not only the commercial expansion of the flavonoid market but also research and development.

Alternative chemical synthesis of flavonoids is not feasible due to their complex chemical structure.

Heterologous biosynthesis in engineered microbial systems that mimics the complex biosynthetic pathways within plants may close the growing gap between demand and supply of functional flavonoids.

SynBio4Flav engages in the development of a versatile and optimised biosynthetic process to deliver flavonoids on industrial scale.

Animation link

SynBio4Flav represents a groundbreaking approach based on distributed catalysis unlocking the synthesis of complex chemicals using synthetic microbial consortia.

Instead of optimising the complex biosynthetic pathways within a single microbial host, different steps in flavonoid biosynthesis are distributed among several microbial strains that are optimised for certain enzymatic reactions and coupled in carefully orchestrated sequences of biochemical events within the microbial network.

The use of synthetic microbial communities over single strains has several advantages. Beyond the possibility to construct optimised strains for each enzymatic step, the distributed catalysis implies less genetic transformation per strain, decreasing the complexity of the intervention.

Moreover, the stability of the consortia is achieved by synthetic cross-feeding relationships between the different strains. The complementary resource distribution represents a step towards circular resource management, as metabolic by-products of one strain function as important resources for other.

Those synthetic microbial consortia could be even expanded to mimic the full plant-based flavonoid biosynthesis by integrating an autotrophic module (as validated in the EU project Living Architecture, grant no. 686585) to supply the heterotrophic module with sugars derived from photosynthesis.

credit: Chalmers University of Technology

DISTRIBUTED CATALYSIS

MODULARITY

Breaking down the complex biosynthetic pathways into standardised specific parts and transferring them to microbial producers, introduces the flexibility and robustness of a modular system. It enables a virtually infinite number of combinatorial variations of reassembling the standardised bio-modules in a plug-and-play manner. The SynBio4Flav approach offers the capacity to deliver a large number of outcomes with a restricted number of modules.

The modular concept opens up a huge space beyond the biosynthesis of flavonoids to produce a plenitude of complex natural and new-to-nature substances on large scale.

The functional modules

Genes from different origins can be rationally assembled and optimised into new-to-nature synthetic pathways to widen the fields of applications. The genes encoding the enzymatic reactions for flavonoid synthesis in plants are well characterised. The flavonoid pathways for biosynthesis were split into three minimal functional modules: the precursor production module, the flavonoid assembly module and the module for flavonoid functionalisation.

These functional modules were transferred into the cells of selected microbial species that do not naturally co-exist. Pathway performance was optimised within the metabolic context of each microbial host.

Precursor production module

The widely used bacterial workhorses *Escherichia coli* and *Pseudomonas putida* were successfully engineered by CSIC to overproduce key flavonoid precursors, such as aromatic amino acids, phenylpropanoids, and malonate, at a gram-scale. Positive interactions between different species drove higher malonate production titres underpinning the proof-of-concept of synthetic microbial consortia as efficient biocatalyst. The different microbial species are coupled by cross-feeding relationships, supporting nutrient supply.

Flavonoid assembly module

For flavonoid assembly, two independent microbial cell factories were developed in parallel to explore the potential of alternative hosts:

Chalmers University of Technology successfully advanced and optimised *Saccharomyces cerevisiae* (baker's yeast) as producer of flavonoids. Yeast, as a model eukaryotic cell factory, was anticipated to easier deal with enzymes from plants.

University of Oviedo optimised *Streptomyces albus* as prokaryotic alternative. Metabolic engineering in *S. albus* led to over 450 times enhancement in production titres.

Larger pathways were implemented in yeast and those requiring less enzymatic steps were introduced in *S. albus.*

Flavonoid functionalisation module

The huge functional diversity of flavonoids arises through a wide variety of modification reactions along their common carbon skeleton, such as glycosylation, hydroxylation or methoxylation, referred to as decorative modifications or functionalisation, naturally occurring in plants.

Numerous scientific studies have shown that decorating the flavonoid skeleton at specific positions with hydroxyl groups (hydroxylation) enhances their bioactivity, especially antioxidant properties, while the attachment of sugar residues (glycosylation) enhances molecular stability, water solubility and strongly affects the bioavailability.

SynBio4Flav created libraries of glycosylating enzymes covering enzymes originating from plants, fungi and bacteria including species from the human gut.

The diverse bacteria present in the intestine are able to metabolise a plethora of compounds ingested with diet, including plant-derived bioactive flavonoids. The team at the German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE) has been focusing on identification of flavonoid-modifying enzymes from human gut bacteria that were evaluated in collaboration with project partners. The transport of glycosylated and non-glycosylated flavonoids by intestinal bacteria was examined by DIfE with the aim to identify transporters that are involved in flavonoid uptake and release.

To increase the portfolio of functionalising modules within the SynBio4Flav context, novel enzymes from wild fungal strains were searched by the project team of Wrocław University of Environmental and Life Sciences, and integrated in the library of optimised glycosylation modules, also addressing flavonoid hydroxylation modules.

The University of Oviedo has been involved in the generation of tens of recombinant strains for the heterologous production of various flavonoids including members of flavanones, flavones, dihydroflavonols, chalcones and dihydrochalcones. These molecules possess biomedical and agro-industrial applications as anti-tumour, anti-microbial, antioxidant or anti-inflammatory bioactives.

A library of *E. coli* strains producing high levels of activated sugars suitable for flavonoid glycosylation was engineered and validated by CSIC.

The synthesis of a variety of functionalised flavonoids, which are challenging to extract from plants or to produce chemically, has been achieved during the project duration.

You have a modular system, you can use it in a plug-and-play manner, which means you can also adapt faster to changing requirements.

Lars Milke, Symrise, Germany

We developed a very efficient, high throughput screening method combined with a robot system and microfluidics.

Chenghai Sun, Greifswald University, Germany

The huge effort to optimise the performance of enzymes and synthetic pathways up to microbial consortia requires laboratory automation as well as computer assisted design methods.

photo: Bruno Stubenrauch

AUTOMATION AND MODELLING

We have developed a machine learning algorithm that can tell us how efficiently and how fast an enzyme works.

Traditionally, you would have to test this in the lab, which can take days or even weeks. With the help of this machine learning algorithm, you can do that in fractions of a second.

Eduard Kerkhoven, Chalmers University of Technology, Sweden

IN SILICO DESIGN

Bioinformatic technologies have been key elements within project development.

In silico optimisation guided design processes by providing valuable models to identify potential bottlenecks and the most promising strategies for improvement, thereby significantly reducing the experimental workload in the laboratories.

Computational modelling has been used to simulate and optimise the dynamic behaviour of metabolic systems at all levels of complexity: new synthetic genes, enzymatic reactions, pathways, the metabolism of organisms and of synthetic microbial consortia.

Models were either developed from scratch or, if accessible, developed further and continuously verified by experimental measurements in the laboratories to iteratively improve their reliability.

Derived from biochemical databases, a compilation of the current biochemical knowledge on flavonoids biosynthesis was reconstructed in a standardised and accessible format, which allows the computation of pathway performance in the context of heterologous microorganisms.

High-quality genome-scale metabolic models (GEMs) were generated and continuously improved for all microorganisms involved.

At systems level, in silico SMCs designs were constructed, based on the behaviour of its individual cell components. The understanding of deviations between predicted behaviour and in vivo performance as direct consequence of the microbial community level was driving rounds of design and implementation.

Chalmers University of Technology applied artificial intelligence to predict how fast enzymes work. A computer-aided design platform for flavonoid biosynthesis using microbial consortia was developed by CSIC. Computational tools and platforms of the partners CSIC and Chalmers University of Technology have been further developed and applied to optimise the distribution of metabolic fluxes.

GECKO (Genome-scale model with Enzymatic Constraints using Kinetic and Omic data) is a platform developed by Chalmers University of Technology. It incorporates catalytic efficiencies in enzyme-constrained models of metabolism.

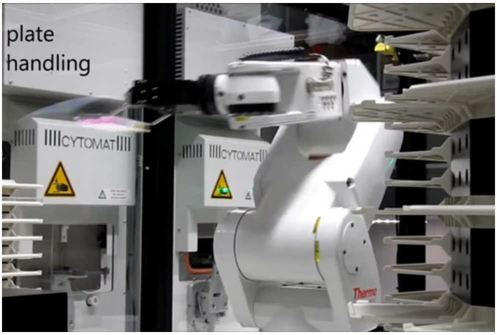
FlyCop (Flexible sYnthetic Consortium OPtimisation) is a computational framework developed by CSIC for modelling the metabolic behaviour of microbial communities and to allow the optimal in silico design for assembling cell-systems into SMCs.

With the goal to enable the in silico design of functionalised flavonoids a-la-carte, libraries of DNA parts and pathways of optimised microorganisms and synthetic microbial consortia have been established as part of a web-based pipeline.

What impressed me the most is how we are approaching to really use data to design metabolic pathways.

For several decades, that was really a trial-and-error approach. Let's stick together a couple of promoters and enzymes and keep fingers crossed that something is going to get out of it. Now, we have a more data-driven approach.

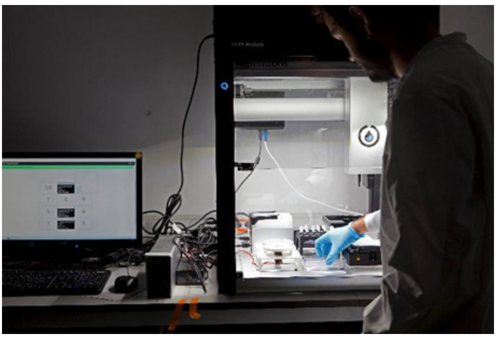
Davide de Lucrezia, Explora Biotech, Italy



Robotic platform LARA
credit: University Greifswald
screenshot: https://lara.uni-greifswald.de/

Robotic platform LARA
credit: University Greifswald
screenshot: https://lara.uni-greifswald.de/

Chi.Bio system configured for flavonoid production
with synthetic microbial consortia,
CSIC, Madrid
photo: Bruno Stubenrauch



Opentrons OT-2 set up for a high-throughput
experiment using a 96-well plate,
CSIC, Madrid
photo: Bruno Stubenrauch

LABORATORY AUTOMATION

Laboratory automation was implemented in partner laboratories at various scales depending on their feasibility.

To speed up the search for optimal strains, specific High-Throughput Screening (HTS) processes were applied.

The fully automated robotic platform LARA, developed by Greifswald University, was made available to the consortium partners, and adopted for specific project requirements. LARA substantially facilitates enzyme engineering and high-throughput protein screening enabling all required steps including statistical evaluation of the experimental data and documentation in automated processes.

During the project duration, LARA was updated with HT combinatorial pathways and synthetic protein complexes assembly SynBio-tools as well as with metabolite biosensors-based HTS methods allowing pathway optimisation.

A microfluidic system enables high-throughput screening of very large variant libraries using fluorescence-activated cell sorting and droplet sorting. Specific synthetic biosensors have been developed to comply with the high- and ultra-high-throughput screening methods.

CSIC laboratory has implemented a fully automated robotic platform configured to run flavonoid production assays with synthetic microbial consortia. The system combines heating, stirring, liquid handling, spectrometry, and optogenetics modules into a single set-up able to run experiments for weeks without user intervention. CSIC also used a liquid handling robot, a pipetting platform designed to automate time-consuming sample dilution, reagent distribution, mixing, etc.

ADM Biopolis has generated novel high-throughput screening platforms, to evaluate and characterise the nutritional and health protection functionalities of selected ingredients in *Caenorhabditis elegans* model.

SynBio4Flav has played a significant role in standardisation within synthetic biology.

A comprehensive set of standardised synthetic biology tools was developed for genome editing, optimisation of biochemical catalysis, and assembly of three-dimensional microbial consortia.

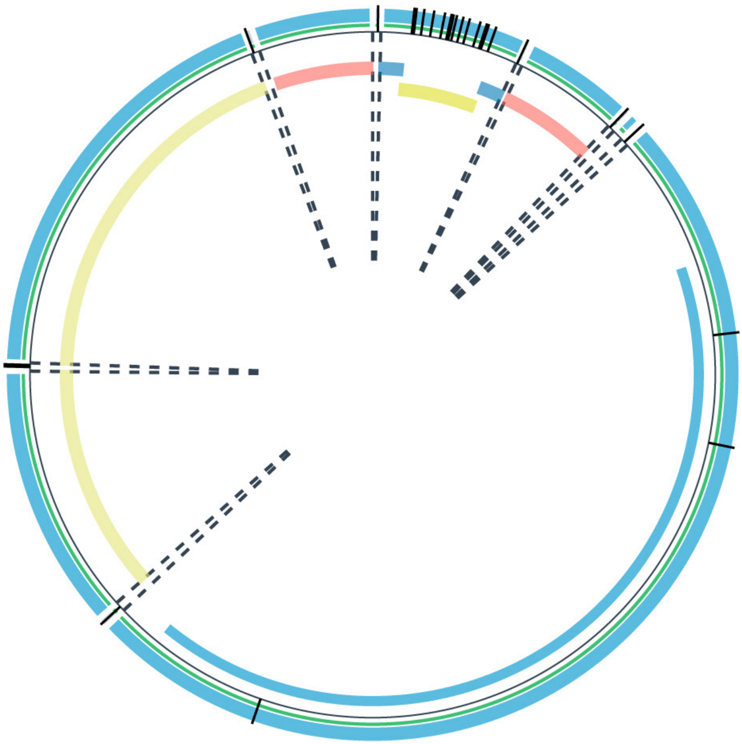
The ambition of the project partners reaches beyond the scope of this project, towards the establishment of a universal and standardised platform for predictable performance, containing hundreds of functional modules suitable to explore the full combinatorial space of biosynthesis of complex metabolites accessible to industrial bioprocesses.

credit: Chalmers University of Technology

STANDARDISATION PLATFORMS TOOLS

I can't stress enough how important standardisation is in synthetic biology. If you don't have a standard, if you don't have data, if you don't have a methodology, what you're doing is rule of thumb. DOULIX specifically addresses this issue by providing a fair compliant database of digital sequences and related data.

Davide de Lucrezia, Explora Biotech, Italy



graphic: Explora Biotech

SYNTHETIC BIOLOGY PLATFORMS

SynBio4Flav contributed to several standardised platforms:

SEVA - the Standard European Vector Architecture platform, originally developed by CSIC and then further optimized in collaboration with Explora Biotech

Golden Standard - unifying the available MoClo models and providing a platform that is applicable to multiple microbial strains - CSIC and Wroclav University of Environmental and Life Sciences, in collaboration with several European project partners

DOULIX - the SEVA-based toolkit for synthetic biology applications, developed by Explora Biotech in cooperation with CSIC

Standard-GEM - Standardisation of genome-scale metabolic models GEMs, by CSIC and Chalmers University of Technology

Standard European Vector Architecture platform SEVA

SEVA is a web-based synthetic biology platform that was implemented in the Molecular Environmental Microbiology Laboratory of the Centro Nacional de Biotecnología (CSIC). SEVA aims to assist the choice of optimal plasmid vectors as genetic tools for de-constructing and re-constructing complex prokaryotic phenotypes.

Within the SynBio4Flav project, the platform was extended to version SEVA 4.0. by including new functionalised plasmids and standardised DNA assembly tools such as Golden Standard.

https://seva-plasmids.com

Golden Standard
Modular cloning assembly tool

Collaborative efforts between SynBio4Flav partners and other European research projects led to the development of Golden Standard, a versatile and efficient synthetic biology tool for standardised modular cloning that unifies and harmonises various Modular Cloning (MoClo) systems.
Most of the available MoClo systems were specifically designed to engineer single species. Golden Standard extends the frequently incompatible organism-specific MoClo systems to applications with any type of microorganism, enabling interoperability between the different systems.
Golden Standard aims at the construction of reusable and interchangeable part libraries and the development of standard protocols supporting rapid and high-throughput combinatorial assemblies, facilitating systematic performance optimisation of a given metabolic pathway with limited available parts. By adopting the standardised vector structure provided by SEVA, Golden Standard allows not only modularity in the assembly process, but also modularisation of the host vectors.
A web-portal has been established that hosts the Golden Standard database and provides assistance for in silico design, promoting community-driven development.

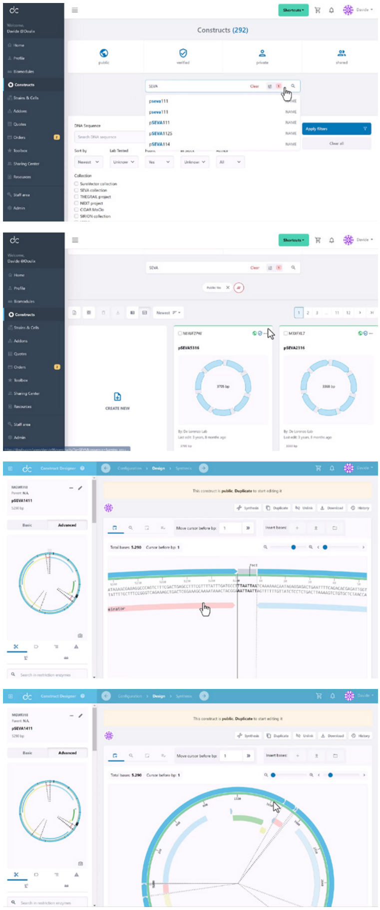
DOULIX - Double Helix technologies

DOULIX is the first toolkit for synthetic biology applications that provides an end-to-end solution to move from design to synthesis within one single cloud-based application on the base of SEVA. It allows the design of customised synthetic biology constructs choosing among hundreds of standard biological parts using several technologies for assembly.

DOULIX has been built with the contribution or via commission over several years, and in partnership with several European consortia including the SynBio4Flav project and the EU-funded project Living architecture (Grant nr. 686585) which provided the first proof-of-concept of how to combine and pre-program different microbial communities.

DOULIX can deliver disruptive value to the next generation of bioentrepreneurs because it enables start-ups to really kick-start their research by granting them access to a fair compliant database of digital sequences and related data, but it is also valuable for more mature companies, allowing them to standardise data and harmonise results in order to accelerate R&D.

https://doulix.com/



credit: Explora Biotech
screenshots: https://doulix.com/

Standard-GEM: standardisation of open-source genome-scale metabolic models

Genome-scale metabolic models (GEMs) are created for understanding and simulating the complex dynamics within metabolic systems, incorporating all accessible metabolic information on a biological system. As they are continuously maintained by multiple actors, common principles are essential to ensure reliability and reproducibility of the models. Standard-GEM responds to the need for open science data management and applies FAIR principles (Findable, Accessible, Interoperable, Reusable) to GEMs through the implementation of open-source standards.

"Standard-GEM facilitates the reuse of GEMs across web services and platforms in the metabolic modelling field and enables automatic validation of GEMs. The use of this template for new models, and its adoption for existing ones, paves the way for increasing model quality, openness, and accessibility with minimal effort."

The documentation process is supported by a fixed folder structure, the use of a git-based workflow for version control and reliance on repository hosting. To reduce the burden of maintaining and checking open-source GEMs, an automated validation pipeline has been generated.

"By adopting the workflow described by Standard-GEM, the curation history of genome-scale and other metabolic models can be transparently reviewed in the git repository, thus facilitating continued community contributions and enabling GEMs to evolve from being research outputs to acting like an infrastructure."

Source:
standard-GEM: standardisation of open-source genome-scale metabolic models
https://doi.org/10.1101/2023.03.21.512712
March 23, 2023

Extrasynthese
photo: LIQUIFER

ANALYTICAL STANDARDS

Reference materials are purified substances used as standards to calibrate quality control operations or to calibrate research experiments to give companies, industries, academic labs an assessment of the results they provide and on the quality of the products they make.

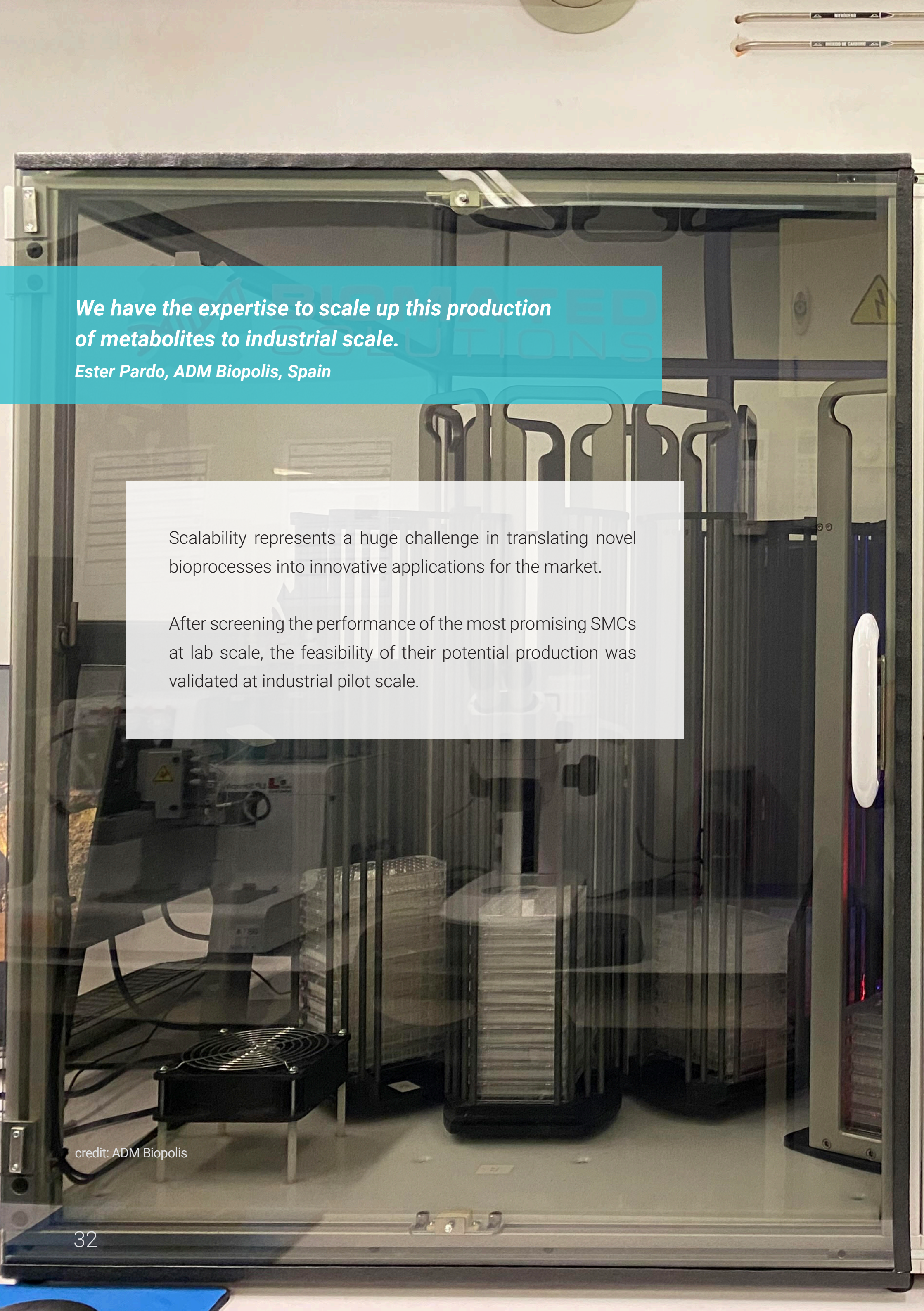
René de Vaumas, Extrasynthese, France

Extrasynthese provides analytical standards of bioactive compounds in plants that serve as reference material for plant-related academic research and industries, to verify the quality and authenticity of the source material, and the potency, the bioactivity of the finished products. Traditionally, the target substance is extracted from plant material and treated in iterative purification processes to reach the highest level of purity. If feasible, chemical synthesis is applied, but novel biotechnologies based on synthetic biology hold the potential to complement and replace traditional extraction/synthesis practices.

Moreover, biotransformation gives an effective access to new-to-nature substances, similar to natural products, with potentially enhanced properties: bioactivity, bioavailability, stability, solubility, … opening opportunities to design and develop new ingredients obtained by versatile and sustainable processes.

… to expand what is called the chemical space of what is accessible to us for developing ingredients

René de Vaumas, Extrasynthese, France



We have the expertise to scale up this production of metabolites to industrial scale.

Ester Pardo, ADM Biopolis, Spain

Scalability represents a huge challenge in translating novel bioprocesses into innovative applications for the market.

After screening the performance of the most promising SMCs at lab scale, the feasibility of their potential production was validated at industrial pilot scale.

credit: ADM Biopolis

SCALING-UP

Our main interest is to get enhanced production levels of the final flavonoids using this novel biotechnological approach.

Felipe Lombó, University of Oviedo, Spain


AG-103
PUSH
VELP SCIENTIFICA
MSL 25 DIGITAL
Magnetic Stirrer

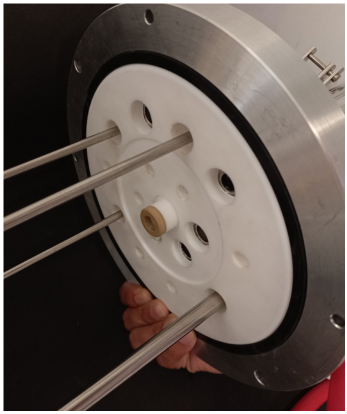
credit: CSIC

BIOREACTOR DESIGN

The novel bioreactor design, developed by CSIC and Liquifer, based on semipermeable ceramic membranes and suitable for the growth of microbial consortia was built and validated.

The bioreactor design empowers scalability through its design approach. Semipermeable ceramic membranes selectively separate bioreactor chambers for specific metabolites whilst allowing the transfer of others. This design approach facilitates extraction and further downstream processes.

Ceramic membranes that meet the respective permeability requirements could be successfully identified from a wide range of ceramic samples manufactured by variations in firing temperature and clay composition. Two bioreactors were combined and implemented. The first selectively separates the precursor chamber from the assembly chamber, and the second comprises the synthon chamber and the functionalisation chamber.



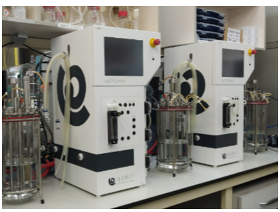
3D printed stand and ceramic membrane,
Bioreactor lid,
credit: CSIC

Scaling-up bioreactor volume

ADM Biopolis optimised the SMC-based fermentation process in 48-well microbioreactors at lab scale. Once conditions and fermentation parameters, such as nutrient concentration, temperature, pH value, etc, were established for individual microorganism, fermentation of SMC was optimised.

For that, culture media composition, temperature, inoculum strategy and carbon source addition were evaluated. Fermentation was run in stirred bench-top bioreactors from laboratory to pilot scale for further evaluation.

credit: ADM Biopolis



credit: ADM Biopolis

DOWNSTREAM OPTIMISATION

Downstream optimisation was validated by Extrasynthese and Symrise. Downstream processing of target compounds was optimised to generate draft specifications for application development. During the different purification states, extended analytical and sensorial tests were performed to optimise conditions.

Extrasynthese developed industrially scalable work-up methods of isolated flavonoids from fermentation broths, including extraction, purification and (bio-)synthetic modification technologies - with a focus on 'green' and food-compatible techniques, also paying special attention on recycling of solvents.

Symrise analysed the performance of the fermentation process in a pilot plant environment. Efficient downstream process parameters were identified, allowing for the separation of the flavonoid naringenin from biosynthetic byproducts in *S. cerevisiae* fermentation broth. Sensory evaluation of microbially produced flavonoids highlighted additional downstream process requirements.

credit:
University of Oviedo

credit: ADM Biopolis
screenshot:https://synbio4flav.eu/virtual-exhibition/biopolis/

PRODUCT EVALUATION

In vivo experiments were conducted using two animal models.

The University of Oviedo tested selected flavonoids in vitro and in vivo. The in vitro assays were carried out using three types of human colon cancer cell lines to quantify the anti-tumour activity of selected flavonoids. In a second round of experiments, the most promising flavonoid derivatives were tested in two in vivo experiments in rat models, one of them evaluating the anti-tumour effect of these compounds for colorectal cancer. In the second animal model, the anti-inflammatory effect for ulcerative colitis was tested as well. Both in vivo assays provided promising results of strong anti-tumour and anti-inflammatory activities for some flavonoids. These two diseases are very common in European populations, with colorectal cancer leading the cancer type statistics in the EU.

Pharmacological and nutraceutical properties were evaluated using the *Caenorhabditis elegans* model, to identify potential applications in the agri-food, feed, pharmaceutical and chemical industries. ADM Biopolis has developed a robust animal model based on the worm *C. elegans* to evaluate the in vivo effects of novel food or feed ingredients. Worms were treated with selected flavonoids and incubated and analysed in a WorMotel equipment, evaluating viability, fat deposition, motility and reproduction by means of artificial vision systems.

The effects of flavonoids depend on their bioavailability in the human body. These polyphenols are poorly absorbed and metabolised by human enzymes in the gastrointestinal tract after their intake. Thus, gut bacteria may play a crucial role in conversion of flavonoids and, thereby, affect their bioavailability and corresponding health effects. The German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE) studied the metabolisation by human gut microbiota of flavonoids produced in the SynBio4Flav project with a focus on new-to-nature compounds. For this, a defined mixture of eight relevant human gut bacteria, the SImplified HUman MIcrobiota (SIHUMIx) consortium, was used. In addition, individual gut bacterial species or derived enzymes were applied. Subsequently, metabolites formed from flavonoids by bacterial activities were analysed and conversion pathways identified.

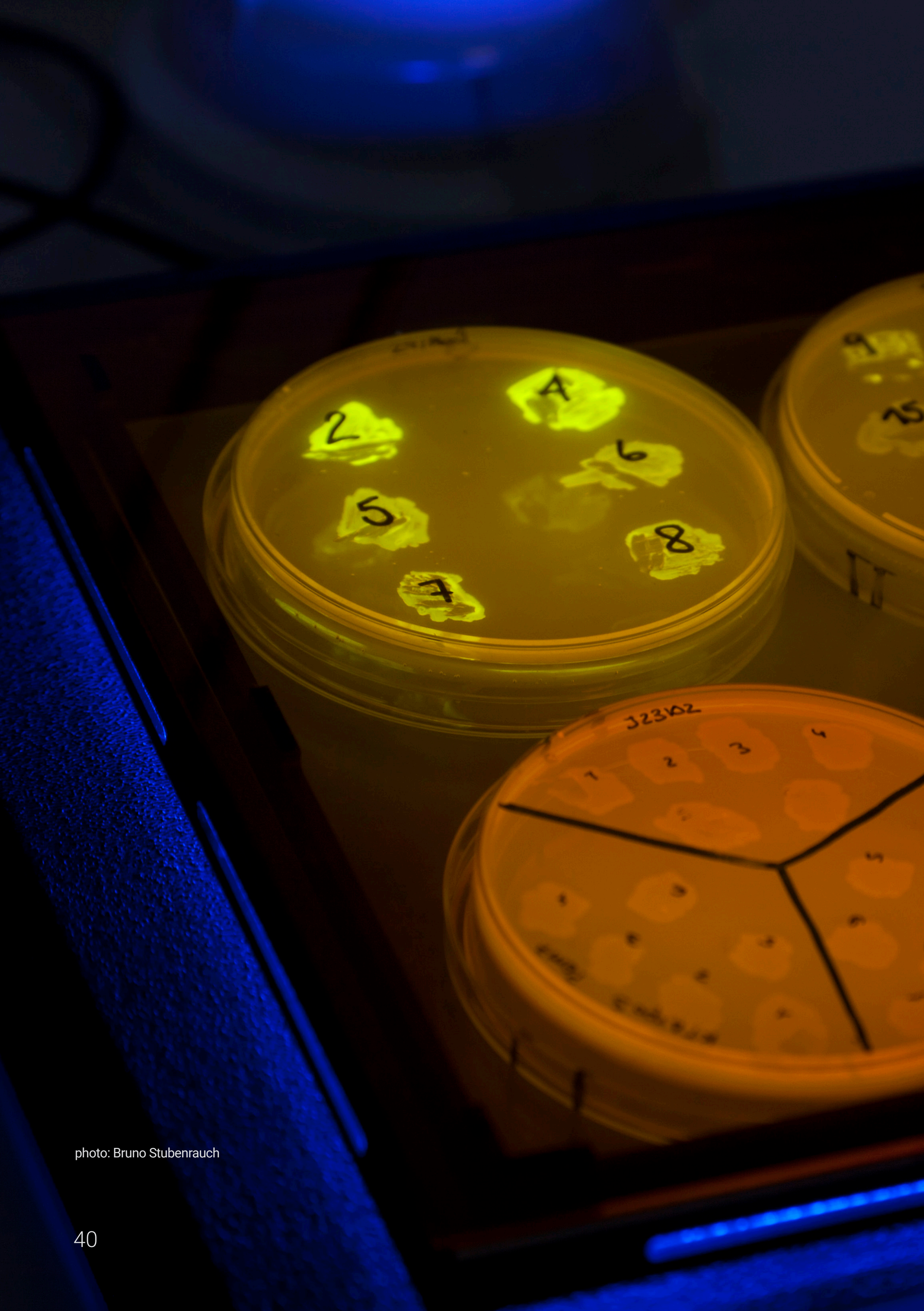
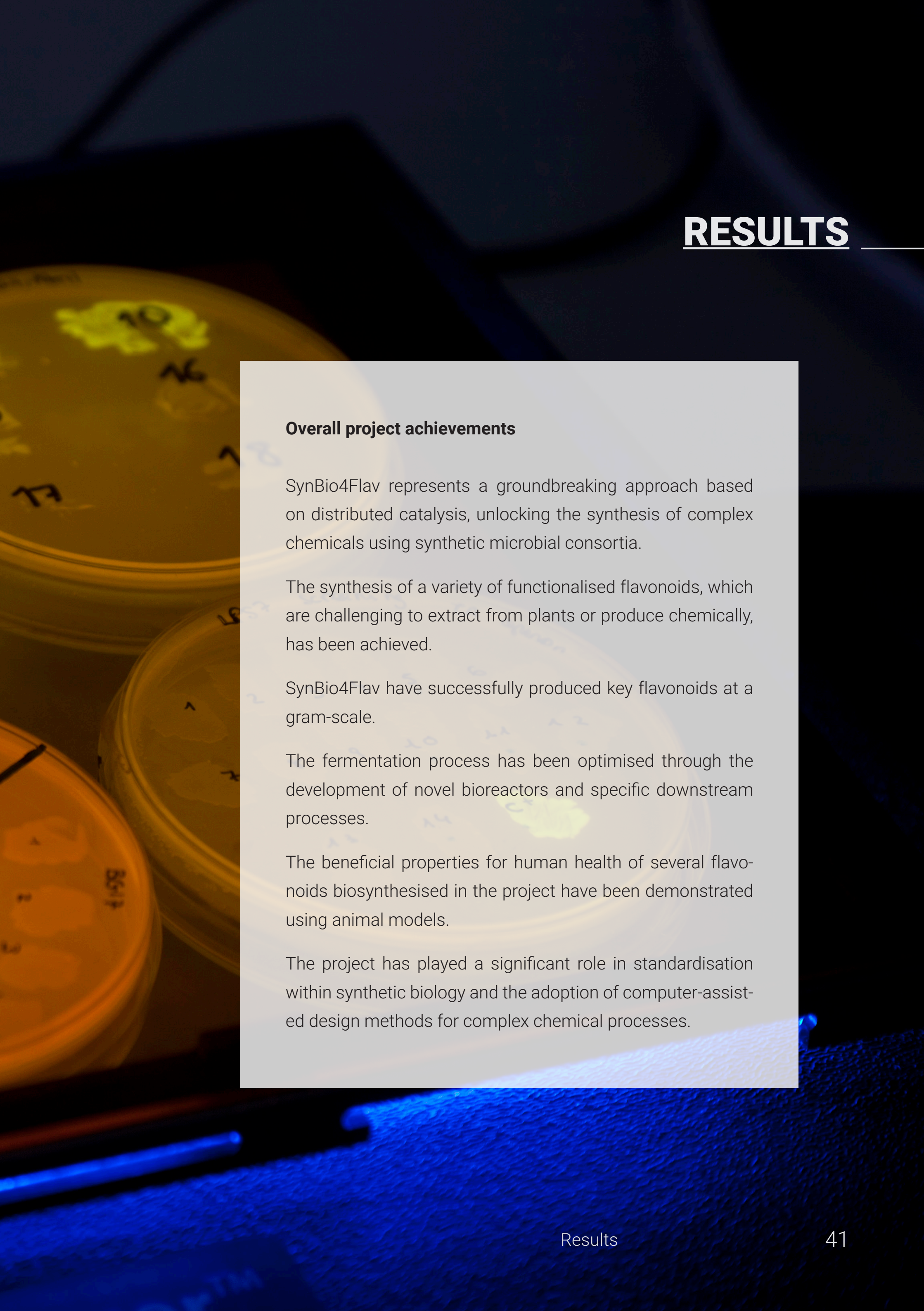


photo: Bruno Stubenrauch



RESULTS

Overall project achievements

SynBio4Flav represents a groundbreaking approach based on distributed catalysis, unlocking the synthesis of complex chemicals using synthetic microbial consortia.

The synthesis of a variety of functionalised flavonoids, which are challenging to extract from plants or produce chemically, has been achieved.

SynBio4Flav have successfully produced key flavonoids at a gram-scale.

The fermentation process has been optimised through the development of novel bioreactors and specific downstream processes.

The beneficial properties for human health of several flavonoids biosynthesised in the project have been demonstrated using animal models.

The project has played a significant role in standardisation within synthetic biology and the adoption of computer-assisted design methods for complex chemical processes.

A comprehensive set of standardised synthetic biology tools was developed for genome editing, optimisation of biochemical catalysis, and assembling three-dimensional microbial consortia.

Microbial strains were successfully engineered for the (over-)production of key flavonoid precursors, such as aromatic amino acids, phenylpropanoids, and malonate, at a gram-scale.

Positive interactions between different species drove higher malonate titres stabilising the proof-of-concept of synthetic microbial consortia as efficient biocatalysts.

Novel bioreactors based on semipermeable ceramic membrane and suitable for the growth of microbial consortia were built and validated.

A library of *E. coli* strains producing high levels of activated sugars suitable for flavonoid glycosylation was engineered and validated.

Naringenin, along with several glycosylated and hydroxylated derivatives, was de-novo synthesised using synthetic microbial consortia.

A computer-aided design platform for flavonoid biosynthesis using microbial consortia was developed.

Artificial intelligence was applied for the prediction of enzyme activities. Genome-scale-models (GEMs) of microbial species involved in synthetic microbial consortia were continuously improved.

Yeast, *S. cerevisiae*, was engineered for the production of flavonoids as part of a microbial community.

A variety of biosensors were developed for the detection of different flavonoid precursors and flavonoids in selected microbial strains.

Enzymes were optimised via protein engineering and high-throughput screening using a robotic platform.

Variants of a lyase enzyme were improved to enable the production of an important precursor for the synthesis of new flavonoid derivatives.

Novel glycosyltransferases from the intestinal microbiome for flavonoid functionalisation were idenitified.

Gut bacterial flavonoid-modifying enzymes for synthesis of novel flavonoid derivatives were applied.

Metabolisation by human gut microbiota of flavonoids produced in the project were evaluated.

The SEVA collection was expanded and the SEVA-based SynBio tools for multiple automated genome engineering were advanced.

DOULIX has been improved covering a vast spectrum of SynBio applications. The public collection of standardised and validated DNA parts was expanded.

Collaborative efforts led to the development of a versatile and efficient Modular Cloning Kit tailored for synthetic biology, enabling broad prokaryotic host modification.

A comprehensive gene library for diverse flavonoid scaffold decoration was created facilitating natural product modification.

A versatile flavonoid C-8 hydroxylation platform was implemented, enabling high-yield production of rare, highly antioxidant flavonoids that are naturally present in scarce amounts.

Efficient glycosylation modules for both in vivo and in vitro modification of diverse flavonoids were developed and optimised.

Efficient and selective methylation modules for in vitro and in vivo methylation of glucosides, enabling more lipophilic products, were developed and optimised.

Flavonoids structural diversification in *Streptomyces albus* has generated more than 35 different compounds.

Metabolic engineering in *Streptomyces albus* led to over 450 times enhancement in production titres.

In vitro bioactivity tests on colon cancer cell lines and in vivo assays on animal models for colon cancer and ulcerative colitis have shown strong antitumour and anti-inflammatory activities for some flavonoids.

SMC fermentation was optimised from lab scale to industrial pilot scale.

Flavonoids were evaluated functionally using the *C. elegans* model.

Optimisation and scaling-up of various transformation/decoration bioprocesses of flavonoids was achieved.

The catalogue of phytochemical standards was extended, and greener and more cost-effective processes were implemented.

Efficient downstream process parameters were identified allowing to separate naringenin from biosynthetic by-products in *S. cerevisiae* fermentation broths.

Additional downstream process requirements were highlighted through sensory evaluation of microbially produced flavonoids.

Patents were applied in the field of biotechnological flavonoid decoration.

More than 80 scientific papers were published during project duration.

Animations on the project objectives, on flavonoids and synthetic biology were created.

A virtual exhibition was installed on the project website in response to COVID19 restrictions.

Three public conversations were held on the potential of metabolic engineering for providing solutions to global challenges.

The podcast series ‘Made by Microbes’ was implemented to communicate key topics of the project.

It is important that people get informed on new developments in biotechnology, in synthetic biology, because it may revolutionize our future life.

We encourage the dialogue on the rapidly advancing new fields such as synthetic biology through our podcast miniseries 'Made by Microbes' and through our 'Conversations on metabolic engineering' with different experts from various fields.

Waltraut Hoheneder, LIQUIFER Systems Group, Austria

photo: LIQUIFER

PUBLIC PANELS

20 May 2021 The conversation involves experts from Philosophy of Science, Art and Science, and Science Communication, as well as coordinators and a scientific advisor of research projects in the field of synthetic biology, moderated by Victor de Lorenzo.

Angelo Vermeulen

Biologist, ecologist, artist, TED-Fellow, co-founder of SEADS

Gabriele Gramelsberger

Philosopher, RWTH Aachen University, Faculty of Philosophy, chair of Theory of Science and Technology

Michele Catanzaro

Physicist & award-winning science journalist, Lecturer in Science Communication

Pablo I. Nikel

Coordinator of SinFonia, Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability

Lars M. Blank

Coordinator of Mix-Up, RWTH Aachen University, head of Institute of Applied Microbiology

Victor de Lorenzo

We should leave our laboratories and engage in a constructive and informal conversation with a more general public, to communicate better what we're doing, and to what extent we can deliver solutions to ongoing problems.

Juan Nogales

We are now for the first time probably in the position that we can move biotechnological processes outside of our labs. I am completely sure that in a few years the people, the normal people, get to see biotech in the house, in the car, in their normal life.

Gabriele Gramelsberger

For everybody else than you as metabolic engineers, it is very difficult to grasp this new technology and to understand it, and what you do not understand you're afraid of. If it becomes more tangible and understandable, I think, then a lot of problems are gone because you see the value of it, but you also can judge it and evaluated what is okay and what is not okay.

Lars Blank

Maybe we pick a topic, a product, where really our state-of-the-art has ethical issues, has really a carbon dioxide problem. And that would be meat production. I would be happy if some of the traditional manufacturing would stop, especially the one which produces a lot of CO_2 and other emissions.

Ralf Takors

We are all aware of the climate changes. And we are all aware that we have to do something against it. And one measure to do something is to activate carbon dioxide, for instance, by microbial activity, and then let the organisms produce something of purpose from this. Metabolic engineering is a key tool to prevent the further use of fossil resources and the release of carbon dioxide.

Full conversation

conversation on **metabolic** engineering

Manuel Porcar

Coordinator of BioRoboost, University of Valencia, Instituto Cavanilles de Biodiversidad y Biología Evolutiva

Ralf Takors

SynBio4Flav's Scientific Advisory Committee, University of Stuttgart, head of the Institute of Biochemical Engineering

Juan Nogales

Coordinator of SynBio4Flav, Spanish National Research Council CSIC, head of Systems Biotechnology Group

Victor de Lorenzo

Research Professor, Spanish National Research Council, head of Molecular Environmental Microbiology Laboratory

Manuel Porcar

I think there is something we shouldn't fall in, that is describing that we scientists, we have the solutions that we can save the world. What we have to say is that we are pretty sure that we have tools that work. And these tools may be part of the solution, providing solutions for the society to choose which are the best solutions to be used in each case.

Michele Catanzaro

What I can bring to the table is that I have been covering science for a long time. I have seen a lot of discussions, happy discussions, but also controversies around the scientific topics. And maybe I can bring in some ideas both for the public and for scientists about how to nurture healthy conversations around these topics. The attention on these topics is starting to build up now.

Angelo Vermeulen

I am here as an artist, a practicing artist, but I am also here as a biologist, because that's actually my original training. My particular interests are: How do we co-create from a transdisciplinary perspective? How do we bring people together from totally different perspectives, different fields, different backgrounds, and tap into that collective intelligence and make it operational?

Pablo Ivan Nikel

Science and art are by no means two completely separate and isolated things from each other but are actually very complimentary. Metabolic engineering has got a lot of this composing factor, of getting things that in principle are different or come from different sources and harmonise them in a way that makes sense from a biological point of view. This is what we do when we engineer new pathways or new properties into a microbe.

conversation on
metabolic engineering

towards a new partnership with nature

13 May 2022

Salón de Actos del Real Jardín Botánico
Plaza de Murillo 2
28014 Madrid

A panel of experts discusses the role of synthetic biology in a world facing the challenge of global change, moderated by Michele Catanzaro.

Michele Catanzaro
When I was preparing this conversation and having a preparatory chat with the speakers, I heard really mind-boggling stories, ranging from plastic eating bacteria in the sea or to microbes that produce electricity out of your pee, to bacteria that retain water in the desert. Can all this potential be harnessed to tackle some of the major environmental challenges that we are facing, and what are the ethical, the economic and the technological challenges?

Rachel Armstrong
One of the biggest causes of anthropogenic change is the built environment, which currently contributes 40% of the world's carbon footprint. Kind of thinking strategically into what microbially powered infrastructure for homes and cities could be, really raises a lot of different kinds of opportunities. I want to introduce a number of different technologies that exist today that are in prototype or pilot phase of development.

Ricard Solé
If we believe in a linear world, where things are happening continuously, we can always think that, well, someday when things go really wrong, we will go back. And that doesn't work. Complex systems do not work like that. There are tipping points, there are points of no return. That happened in the past, and we see them happening now.

Victor de Lorenzo
The traditional concern of big sectors of the public is what happens when you put a new microbe in the environment, it will destroy the community, it will propagate like crazy, will spread this and that. Well, for us interested in bioremediation, the challenge is just the opposite, namely, how to put something into an environment that survives and works, because they disappear very quickly.

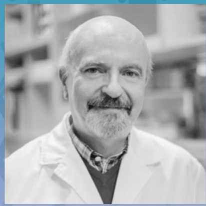
Ricard Solé
You take something that is already in the community that has co-evolved with the biodiversity you have there, our conjecture is cyanobacteria, and you make a little modification, like, it will secrete a molecule that retains a little more moisture or improves the quality of the soil. And this little difference in real systems that are highly nonlinear, can make the tipping point be pushed away.

Ricard Solé

ICREA Research Professor at Pompeu Fabra University, Barcelona and head of the Complex Systems Lab

Rachel Armstrong

Professor of Regenerative Architecture, Arts & Design at KU Leuven, Belgium and Senior TED Fellow



Victor de Lorenzo

Research Professor, Spanish National Research Council, head of Molecular Environmental Microbiology Laboratory

Michele Catanzaro

Physicist & award-winning science journalist, Lecturer in Science Communication

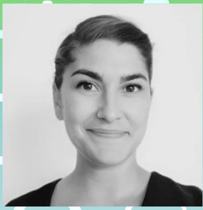
Michele Catanzaro
Bacteria do not only inherit their genes, but they interchange genes horizontally throughout their life.

Ricard Solé
Horizontal gene transfer is really part of the resilience of nature, the mixing of the genetic and the cellular levels. Adaptation is coming from that.

Víctor de Lorenzo
I will say that horizontal gene transfer is kind of the internet of microbial communities, the way they transfer information. And as a matter of fact, in my laboratory and other laboratories, we think this is a phenomenon that could be leveraged to really spread information, beneficial information from an environmental point of view, through our wider bacterial or microbial community. Rather than bringing a new member, engineered or not, into the action theatre, you may want to perhaps spread in the existing community a new information, a new activity. And in my opinion, this can be a big breakthrough in terms of biotechnological and bioremediation technologies.

Rachel Armstrong
Once you have a substrate for design that's living, it has an agency, it wants to do stuff all by itself, without you. And so essentially, you're not really in a situation of complete control, you're setting up a negotiation, and how you go about that, I think, is really critical to what the outcomes are. I think synthetic biology is helping us learn some of the communications tools that we need in order to have what actually in critical theories are called 'a parliament of things'. In a kind of natural environment, agents negotiate stuff on their own terms. And microbes are really good at not just dealing with their own species, they can talk to other species. So, that degree of communication through persuasion and intervention, and this kind of negotiated marketplace of transactions, I think, is really, really interesting. Because it's no longer about command and control.

Ricard Solé
Our microbiome, and particularly the gut microbiome, has brought a lot of interesting insights. A huge amount of medical research on bringing new microorganisms into the gut to actually correct diseases, is giving us a lot of insight, because the microbiome is nothing but an ecosystem, a really complex one, with a lot of similarities with an ecosystem like the rainforest.



Ana Merino
Senior Regulatory Scientist at Atova Regulatory Consulting, Barcelona

Francesco Visioli
Professor of Human Nutrition, University of Padova, Department of Molecular Medicine

Maya Bendifallah
Chief Scientific Officer, Co-founder of Nutropy, Paris/Evry

Michele Catanzaro
Freelance Journalist and Lecturer in Science Journalism, Barcelona

Michele Catanzaro

Culture is a word that is very polysemic, very open, it can refer to culture in the common understanding of the word, but it can also refer to agriculture, microbial culture, what is cultured, what is cultivated? And we willingly kept it open, because we want this conversation to be as open as possible. It's an exercise of projection and exercise of imagining how the future of food will be. What will be considered as cultured or cultivated in ten or fifty years from now, maybe very different from what we think now.

Maya Bendifallah

Nutropy is a company based in France, located just outside of Paris. We are producing milk ingredients through precision fermentation in order to enable dairy companies to decarbonise their cheese production. We're using microorganisms that we engineer to produce milk ingredients that enable us to actually make cheese. For example, the caseins make cheese melt and stretch, and they give us the cheese experience that we're looking for in this next generation of cheese alternatives. We are a b2b company. We are in direct contact with dairy companies. Now, they really see a lot of promise in what we are doing, in order to offer a larger variety of products to their consumers. They want to diversify. It's a business strategy, to reduce their risks.

Ana Merino

Precision fermentation has a history, in pharma and other industries. This technology uses the genetically modified microorganism as a processing aid, as a tool to produce a substance, or an ingredient that is equivalent to the naturally occurring counterpart. The modified microgoranism is not present in the final food. I think we will see precision fermentation in the short future in Europe. We already have applications submitted to EFSA. It is a centralised and very thorough process by EFSA. Before a product is placed on the market it needs to go through these really detailed evaluations by experts.

Francesco Visioli

It is a sustainability issue. The population is growing. When people become more affluent, they eat more animal protein. We cannot sustain it. It's not me, not Greta Thunberg, who says that. You do, we do arithmetic. We cannot exploit the planet to grow animal protein, we need to find alternative ways. We are always talking about circular economy. We can use waste products as a substrate to feed the microbes.

Maya Bendifallah

Animals are not as efficient as plants or as microbes to produce protein. An animal will need between 6 to 12 plant calories to produce one animal calorie. So, there's a lot of loss.

conversation on
future food cultures

Experts from the fields of human nutrition, precision fermentation, alternative proteins, regulatory consulting discuss how the progress in scientific research and biotechnological developments can be translated into a change within food cultures, moderated by Michele Catanzaro.

5 May 2023

Centre Jean Bosco
14 rue Roger Radisson
69005 Lyon

Michele Catanzaro
Talking about these topics or communicating these topics, there is this typical example, which is insulin, which is not in food, it's in health.

Francesco Visioli
It was the first recombinant drug. Before that, insulin was extracted from pigs, from the pancreas of pigs. If you asked people now, would you prefer insulin from a pig or recombinant? They will say, recombinant, after many years. So maybe in the future, if you tell them: Would you prefer to kill a cow and have a steak or cultured meat? Maybe now, they will say, no, no, no. I go the natural way. But thirty years from now, who knows? Or twenty or fifteen? Or cheese, the same thing. Things sometimes accelerate extremely rapidly.

Ana Merino
People are not going to be forced to eat something that they don't want to. I think we just need to make delicious and more sustainable food. Not everyone will choose to eat them, but some of them will, or at least try. You don't need to do a complete shift on the diet. I think the whole idea of the healthier future is coexistence. And that doesn't mean that the traditional dairy farmers are not going to be able to make a living. Because at the end, all these raw materials are needed anyway.

Maya Bendifallah
In the United States, we have egg proteins produced by microorganisms, we have meat proteins produced by microorganisms, we have dairy protein produced by microorganisms that are on the market. I have had ice cream made by a filamentous fungi. I bought it at my local grocery store. It was at a similar price as vegan or other ice creams.

Ana Merino
One of the limitations now is scaling up. The more people start eating these products and ingredients, the more capacity we need. There are many models. It can be decentralised, have more regional, tiny bioreactors for food sovereignty, or centralised.

Francesco Visioli
I published 1994 on Oleuropein, and back then everyone was laughing at me. The word nutraceuticals did not exist. And everyone was: ha, ha you are studying flavonoids, you are studying polyphenols? Then we got the European project, I think in 1995 on olive mill wastewater. When you produce olive oil, you throw away an enormous amount of polyphenols, enormous tons. So, I think when I hear things like this, that's the future! We should not be scared; we should not laugh at anyone who does different things.

PODCAST
MADE BY MICROBES

In our podcast miniseries “Made by Microbes” we explore new ways of microbial production. Engaging microbes in production processes has been practiced for thousands of years to make beer, wine, cheese, yogurt or bread. The range of microbial products is widening with advances in life sciences that enable microbes to produce substances that are naturally produced by animals or plants. New ways of microbial production hold the potential to revolutionise the pharmaceutical and agro-food sector, and to achieve circularity, where the output of one process works as the input for another. Microorganisms are involved in virtually all stages of natural cycles. Learning from microbial life strategies has fuelled the advances in life sciences.

The boundaries between science, engineering and computational technologies are increasingly blurring. Automation and modelling, as well as the development of standards and platforms are indispensable for research advances.

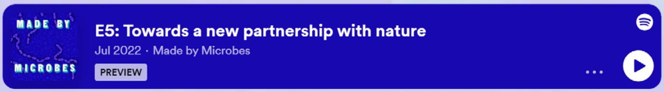
We invite people whose daily work is deeply rooted in microbial research to share their insights and discuss the expected impact of biotechnology on our everyday life.

Juan Nogales, head of the Systems Biotechnology Group at CSIC in Spain and Waltraut Hoheneder, design researcher from Liquifer in Austria engage in a discussion on the breathtaking advances in microbial research.

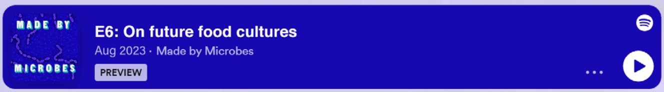
Tobias Goris, a microbiologist from the German Institute of Human Nutrition, and the biochemist Jarosław Popłoński from the Wroclaw University of Environmental and Life Sciences in Poland, discuss their research on food and nutrition.

PhD Students Yannik Brack from Greifswald University in Germany, and Cheewin Kittikunapong from Chalmers University of Technology in Sweden talk about the role of robots and computer modeling in their everyday work.

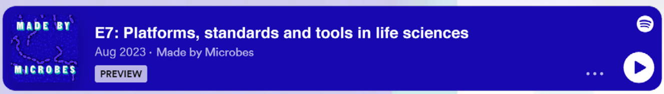
Iryna Biliaeva, responsible for Open Innovation Management at ADM Biopolis, Spain and Felipe Lombó, coordinating the BIONUC group at the University of Oviedo, Spain, talk about research cooperation between academia and industry with a focus on health.



Ricard Solé, Rachel Armstrong, Víctor de Lorenzo and Michele Catanzaro discuss the potential of synthetic biology to provide solutions to major environmental challenges.



A conversation between Ana Merino, Francesco Visioli, Maya Bendifallah, moderated by Michele Catanzaro, on their visions and engagement for a next generation of food driven by biotechnological innovations.



René de Vaumas, owner of Extrasynthese and Davide de Lucrezia, owner of Explora Biotech talk about the importance to develop platforms, standards and tools for emerging life sciences such as synthetic biology.

photo: Bruno Stubenrauch

CONSORTIUM PARTNERS

AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (CSIC)

Madrid, Spain

The Spanish National Research Council CSIC is Spain's largest public research institution, and ranks third among Europe's largest research organisations. CSIC is supported by the Spanish Ministry of Economy, Industry and Competitiveness, through the State Secretariat for Research, Development and Innovation. CSIC is a major player in the development of the European research area and therefore a significant contributor to the European integration process.

CHALMERS TEKNISKA HÖGSKOLA AB (CHALMERS)

Göteborg, Sweden

Chalmers University of Technology is a foundation university situated in Göteborg on the west coast of Sweden. Education and research are focused within life sciences, materials science, information technology, micro-and nanotechnology, environmental sciences, and energy. Chalmers has a strong tradition for collaborating with industry, enabling an extensive infrastructure for supporting the commercialization of research projects.

DEUTSCHES INSTITUT FÜR ERNÄHRUNGSFORSCHUNG POTSDAM REHBRÜCKE (DIFE) Nuthetal, Germany

The German Institute of Human Nutrition Potsdam-Rehbruecke is an independent research institute and member of the Leibniz Association, with the mission to conduct experimental and clinical research in the field of nutrition and health. The aim of this research is to understand the molecular basis of nutrition-dependent diseases and to develop new strategies for prevention, treatment, and nutritional recommendations. DIfE researchers focus on three main research areas: (i) the role of nutritional habits and factors in the development of obesity and its complications, (ii) the role of nutrition in healthy aging, and (iii) the biological basis of food choice and nutritional behaviour.

UNIWERSYTET PRZYRODNICZY WE WROCŁAWIU (WU)

Wrocław, Poland

The Wrocław University of Environmental and Life Sciences is one of the biggest agricultural universities in Poland and is at the forefront of research in the area of agriculture, food sciences, veterinary, food economy, environmental protection, and engineering. WU's organisational structure is divided into five faculties, among which two faculties; the Faculty of Veterinary Medicine and the Faculty of Biotechnology and Food Sciences are recognised as the best in Poland. WU is also a part of the EUGreen transnational Alliance of nine European universities aiming to be Gateway to Sustainability.

UNIVERSITÄT GREIFSWALD (UG)

Greifswald, Germany

Greifswald University was founded in 1456 and is one of the oldest academic institutions in Germany. Today, it has become a modern, moderate-sized university in five faculties. The university has established several programs to ensure gender balance, child care for university employees and a Welcome Center for foreign students. Proteomics and Protein Technologies in Infection Biology, Environmental Microbiology and Biotechnology is one of the five focus areas of research at UG.

UNIVERSIDAD DE OVIEDO (UNIOVI)

Oviedo, Spain

The University of Oviedo is a public institution of higher education and research in the Principality of Asturias, Spain. It offers a full range of undergraduate degrees adapted to the European Higher Education Area (EHEA), and postgraduate degree programmes in collaboration with national and international universities and more than 250 companies. The University of Oviedo undertakes 80% of the R&D and Innovation activities carried out in Asturias region, and has cutting-edge services and facilities to facilitate the transfer of knowledge to the business world.

EXTRASYNTHESE SAS (EXSY)

Genay, France

EXTRASYNTHESE, established for 30 years near Lyon (France), is a leading manufacturer and distributor of phytochemical reference materials worldwide. It has developed a great expertise in extraction, purification, synthesis, and analysis of natural substances. Through internal research, collaboration with academic partners, and participation in collaborative projects, EXTRASYNTHESE continuously expands its know-how and catalogue of products.

ADM BIOPOLIS (BIO)

Paterna Valencia, Spain

ADM Biopolis, is a leading biotechnology company, based in Valencia, Spain, active in new product development and applications. Created in October 2003, ADM Biopolis is owned by the Spanish National Research Council (CSIC) and by ADM, one of the world's largest nutrition companies. ADM Biopolis core competences are the isolation, selection, design, characterization, validation, and production of microorganisms and cellular metabolites with applications in agri-food, feed, pharmaceutical, and chemical industries. This includes the design of microbial strains and metabolites by strain and bioprocess development, followed by fermentation and downstream process optimization, and production at the industrial scale.

EXPLORA BIOTECH SRL (EXP)

Mestre Venezia, Italy

EXPLORA S.r.l. is a privately-owned SME, founded in 2006. Its mission is the realization of enabling technologies in several areas of biological engineering. A strong multidisciplinary and constructivist approach at different biological scales is at the core of our vision. The outcome is a coherent and comprehensive biological platform aimed at providing end-to-end solutions to unravel the inherent complexity of biological systems by exploiting the paradigm of synthetic biology: abstraction, decoupling, and standardization. This paradigm in EXPLORA is currently conveyed within DOULIX, a dedicated business unit devoted to the design and the realization of the first integrated toolkit for synthetic biology.

SYMRISE AG (SYM)

Holzminden, Germany

Symrise is a global supplier of fragrances, flavorings, cosmetic active ingredients, and raw materials, as well as functional ingredients. Its clients include manufacturers of perfumes, cosmetics, food and beverages, the pharmaceutical industry and producers of nutritional supplements and pet food. Its sales of approximately € 4.6 billion in the 2022 fiscal year make Symrise a leading global provider. Headquartered in Holzminden, Germany, the Group is represented in more than 100 locations in Europe, Africa, the Middle East, Asia, the United States and Latin America. Symrise works with its clients to develop new ideas and market-ready concepts for products that form an indispensable part of everyday life. Economic success and corporate responsibility are inextricably linked as part of this process.

LIQUIFER SYSTEMS GROUP GMBH (LSG)

Vienna, Austria

LIQUIFER Systems Group is a SME and was established in 2005 with the objective of creating a multidisciplinary task force that can design and develop architectural and engineering systems for terrestrial and space applications. LSG has gained experience in this area through contracts with the European Space Agency (ESA), the European Framework Programme, the Austrian Science Fund, and the European Industry. LSG combines a wide range of expertise within one team and covers fields from architecture and engineering, biomimetics through to human factors, and science (geology and biology). The composition of the company provides a unique environment for innovative research and product development.

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This booklet was prepared as a part of the dissemination activities in the SynBio4Flav project.

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