



Bluetools

Technology Catalogue



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BLUETOOLS represents an ambitious and cutting-edge project with a broad interdisciplinary approach to capitalize the value that the Blue Economy may bring. The project integrated sustainable sampling methodologies of biological material in selected aquatic, mainly marine, hotspots with potential evolutionary pressure, the development of novel techniques to properly obtain the genetic biodiversity and subsequent screening and finally, exploitation of a wide range of innovative material including novel enzymes, molecules and microorganisms. It is important to stress that BLUETOOLS has generated a tremendous amount of useful data that has been leveraged not only throughout the project lifetime, but also beyond, in the form of new research follow-up projects, know-how for transfer, and generation of innovative tools. BLUETOOLS enduring impact will definitively have contributed to strengthen Europe's capacity to address pressing societal challenges, support evidence-based policymaking, and foster sustainable innovation with long-term benefits for researchers, industry, public authorities, and society as a whole.

Dr. Pablo Domínguez de María

CEO & Founder of the white biotech consultancy firm Sustainable Momentum.
External Advisor of the BLUETOOLS project

Foreword

Project BLUETOOLS represents a large-scale effort in the development of methods for the sustainable exploration and exploitation of marine microbiomes.

In this project we have developed new bioinformatic pipelines, unconventional hosts and vectors for functional expression, novel microscale cultivation approaches, and we have expanded the range of ultrahigh-throughput screening assays available to the community to include in vitro screening, complex cascade assays and growth-based reporter strains. With these developments, new genes and enzymes have been discovered for exciting applications such as plastic degradation or crop improvement.

We sincerely hope that all these findings and developments will be useful to academic and industrial stakeholders worldwide.



> 450 Samples

> 40 Metagenomic libraries

> 500 Novel enzymes

16 Resistance genes

14 New plastic degrading enzymes

19 Publications and Pre-prints

> 50 Outreach events

Partners

14



Countries

7

European and
Innovate UK Funding

€9.0M

Project coordinator

Prof. Aurelio Hidalgo
Universidad Autónoma de Madrid

Months

48



Call identifier

HORIZON-CL6-2022-CIRCBIO-01

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Higher Education Institutions:

- Universidad Autónoma de Madrid (UAM)
- Universitat d'Alacant (UA)
- Norwegian University of Science and Technology (NTNU)
- University of Bielefeld (UNIBI)
- University of Cambridge (UCAM)
- Technion - Israel Institute of Technology (IIT)

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Research & Technology organizations:

- Instituto Nacional de Técnica Aeroespacial Esteban Terradas (INTA)
- Institut National des Sciences Appliquées de Toulouse (INSAT)
- SINTEF AS



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Small & Medium Enterprises:

- CONTACTICA SL
- European Science Communication Institute (ESCI) gGmbH
- PROZOMIX Ltd.

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Large companies:

- Servier Gyogyaszati Vegytani Kutatointezet Zartkoruen Mukodo Reszvenytarsasag (SERVIER)
- BASF SE

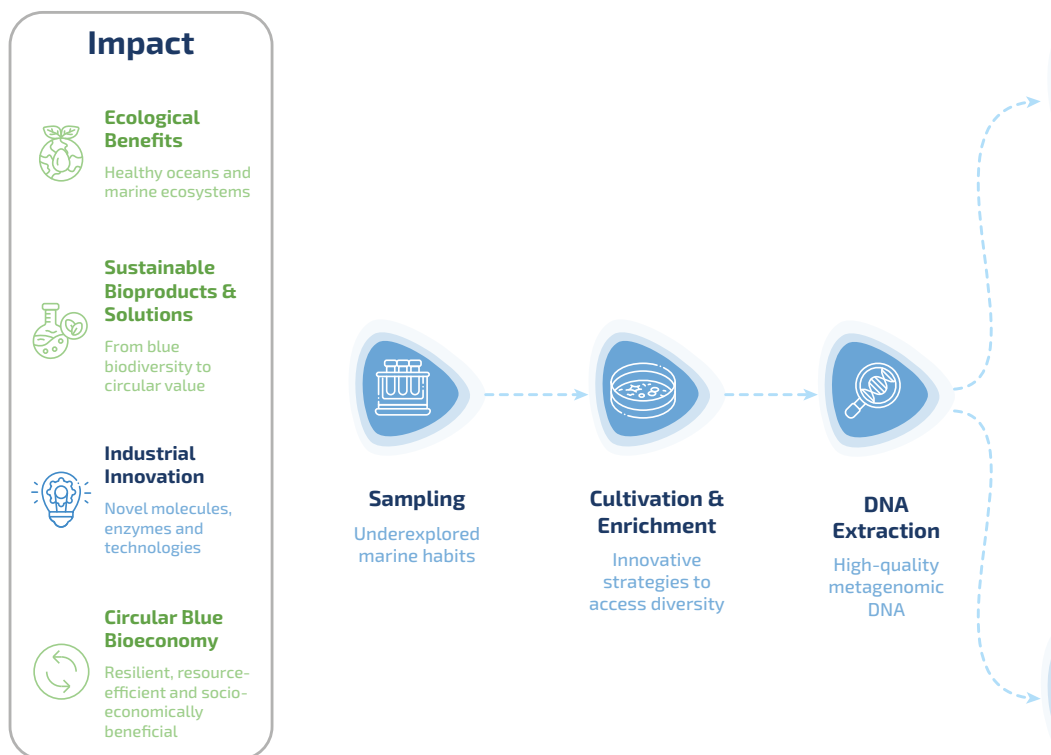
Our Approach

BLUETOOLS integrates metagenomics, advanced cultivation, machine learning and microfluidics into a unified platform to explore marine microbiomes rapidly and responsibly. Sequence- and function-based discovery can recover biological information from very small environmental samples. This reduces material collected at each site and limits disturbance of sensitive habitats while retaining access to genetic and functional diversity.

To complement direct metagenomics, BLUETOOLS has implemented reduced-volume culturomics and enrichment strategies that increase throughput while lowering consumption of samples, media, plastics and energy. Miniaturised cultivation

is combined with flow-based systems that reproduce environmental gradients, nutrient regimes and hydrodynamic conditions more realistically than static cultures. These approaches improve access to previously uncultivated microorganisms and enable communities and biofilms to be studied under controlled, ecologically meaningful conditions.

The BLUETOOLS computational pipeline processes short- and long-read metagenomic data through assembly, binning and functional annotation. Machine-learning components improve the recovery and classification of genomes and genes from complex datasets. Dedicated ML-based mining tools

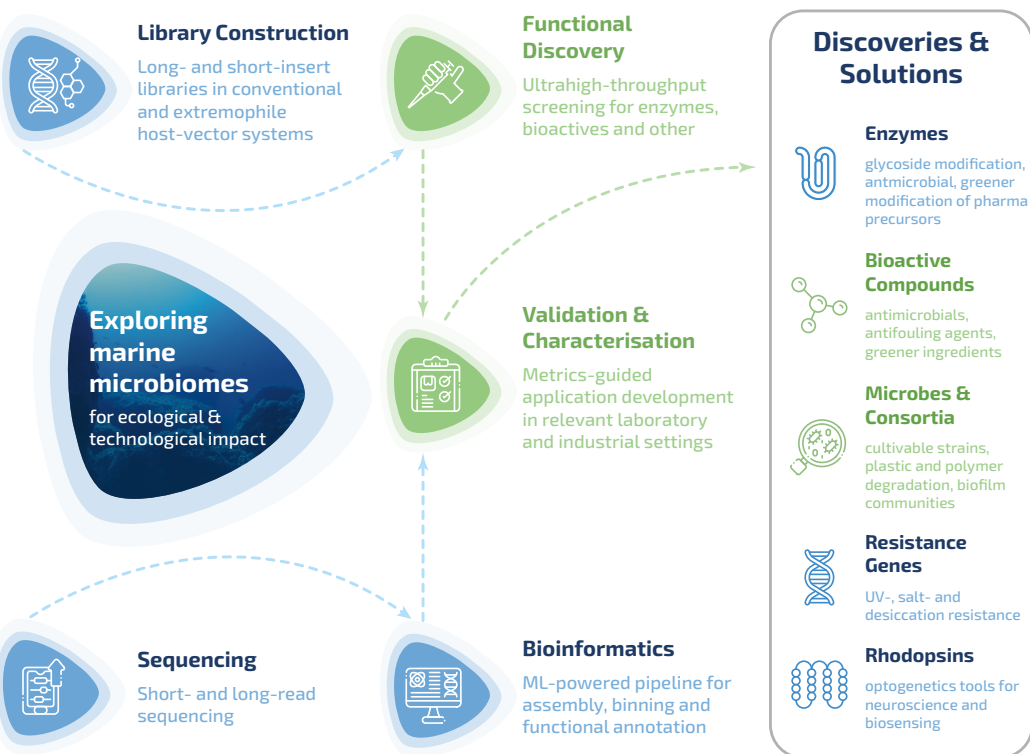


target microbial rhodopsins and enzymes involved in plastic and polymer degradation. By prioritising candidates with relevant sequence, structural or functional signatures, these tools reduce the experimental search space and increase the probability of actionable leads.

Functional screening is accelerated through droplet microfluidics, in which reactions are compartmentalised in picolitre droplets. This enables ultrahigh-throughput analysis with orders-of-magnitude lower reagent, plastic and sample consumption than conventional robotic liquid handlers. Life-cycle assessment within BLUETOOLS confirmed the sustainability advantage of droplet workflows

through reductions in reagents, disposable materials, energy demand and waste.

Sustainability is treated as a design criterion rather than an endpoint. Application-specific metrics are introduced during characterisation, scale-up and process development to compare alternative routes and identify environmental trade-offs early. This metrics-guided approach steers enzyme processes, bioactive compound production, microbial solutions and other marine-derived technologies towards options combining technical performance with reduced resource use, lower emissions and minimal ecological impact.



Sample Collection

BLUETOOLS sampled understudied marine habitats, waste-impacted environments and invasive invertebrates, and reused material from prior projects to minimise new collection and reduce impact on fragile ecosystems without compromising the discovery of new and useful functions. From 458 archived

samples, the consortium has generated approximately 1.11 Tbp (1,110 Gbp) across 71 datasets. Data and metadata not constrained by IP or ABS obligations will be released progressively in trusted public repositories up to 5 years after the completion of BLUETOOLS.



"Waste"
environment



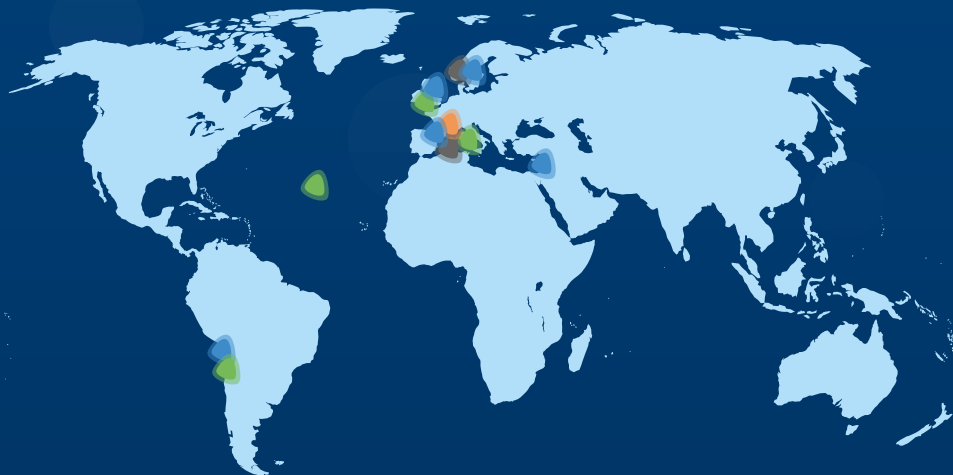
Cooperations
& collections



Extreme
environment



Aquatic
organisms



BLUETOOLS observed Access and Benefit-Sharing (ABS) regulations throughout the biodiscovery pipeline, documenting sample provenance, access permits and conditions of use. Recognising DSI as an emerging policy field, BLUETOOLS established dialogue between scientists and policymakers to analyse existing policy on ABS [10.5281/zenodo.22861775] and inform evolving policy on DSI [10.5281/zenodo.22861919].



PROZO

PROZO collected samples from UK waters including a circumnavigation of the UK during the BLUETOOLS project. These samples were sequenced and used in the discovery of novel enzymes for API manufacturing.

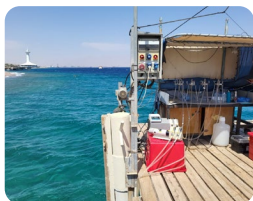
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INTA

INTA sampled microbial mats from Atacama's Aguas de Quelana (hypersaline lakes) and Tatio (>50 °C waters), plus biofilms from northern Spain's coast. UV/desiccation made them rich in resistance genes.

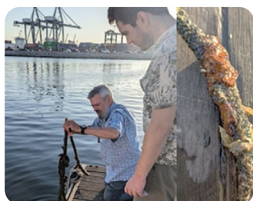
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IIT

IIT collected water from the Red Sea, Mediterranean Sea and Dead Sea, lakes across Israel, and from Antarctic seasonal sea-ice to prepare meta-omics libraries for discovering new microbial rhodopsins.

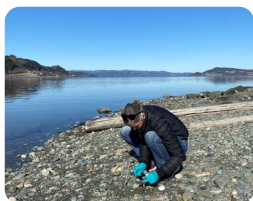
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UA

UA has an extensive sample collection (biomass, bacterial/viral cultures, DNA, plasmid/fosmid metagenomes) from wastewaters to seawater, Posidonia-degrading matter, sediments and extreme environments.

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SINTEF

SINTEF sampled marine environments from the Trondheim fjord, i.e. intertidal and brackish water zones and marine sediments, to discover enzymes/molecules with antimicrobial and antifouling activities.

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UAM

UAM collected samples from contrasting marine and aquatic habitats, spanning river and marine sediments, hot springs, and glacier runoff waters and sediments, to capture broad microbial diversity.

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Cultivation of environmental microorganisms is challenging and to increase access to the biodiversity for discovery activities, procedures to aid cultivability, and/or, integrate cultivation directly with functional screening represent promising tools to facilitate exploration of the marine microbial biodiversity. BLUETOOLS partners have established and applied complementary strategies, to support the screening for BLUETOOLS targets, such as specific enzymes, antimicrobial and antifouling candidates, as well as for the study of microbial communities themselves.

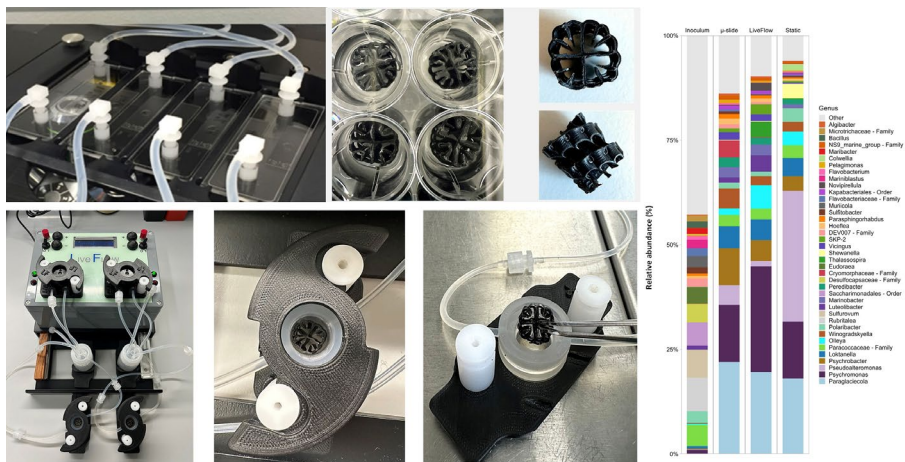
Artificial Supports and Flow Cultivation

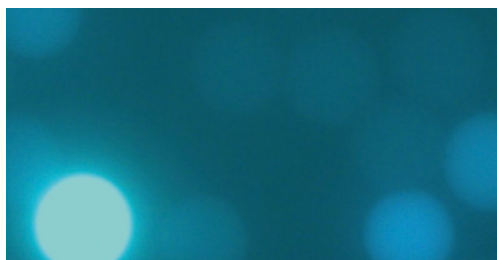
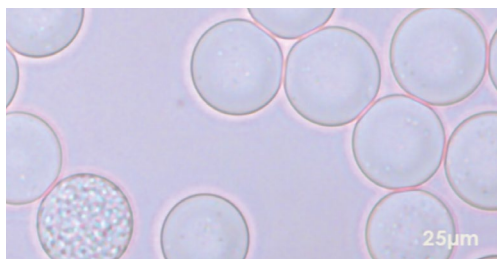
As marine biodiversity is rarely cultivated, SINTEF has established biofilm cultivation protocols applying biocarriers as artificial 3D support. Marine-originating biofilm cultivation is performed under nature simulated conditions, presenting low nutrient and fluid-over-surface-flow to trigger biofilm formation and facilitate growth of the natural microbiome *ex situ*.

as well as enrich the culture for biofilm-forming taxa. The cultivated biodiversity is then explored in bioprospecting for facilitated biodiscovery for antimicrobial and antifouling targets.



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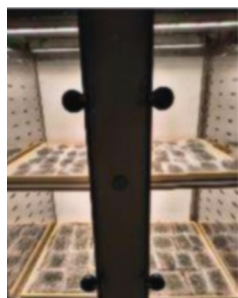
Droplet Cultivation

INSAT implemented a unique label-free microfluidic-culturomics workflow, based on encapsulation of single cells in nutrient-rich picolitre droplets and growth monitoring by detection of autofluorescence naturally produced by living microbes. Applied to several marine microbiomes, this workflow allowed identification of rare bacteria able to specifically utilize glycosides of plant, algae and microbial origins.



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Mesocosms

Mesocosms provide controlled experimental systems to study the degradation of *Posidonia oceanica* (or any other complex material) and the associated microbial communities under different environmental conditions. Seagrass is incubated over time to follow microbial succession and changes during degradation are monitored, including also emitted gases and plant polymer composition. This approach enables the cultivation and recovery of environmental microorganisms under conditions that mimic natural degradation processes.



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Computational Pipelines

To thoroughly investigate and understand the large amount of sequencing data from the marine microbiomes, different ML-based computational pipelines were developed to process hundreds of terabytes (TB) of data generated by the BLUETOOLS partners. These pipelines not only include most of the standard analyses for metagenomics sequencing data, but also tailored modules to tackle specific challenges in endeavour of discovering novel Rhodopsins and enzymes.

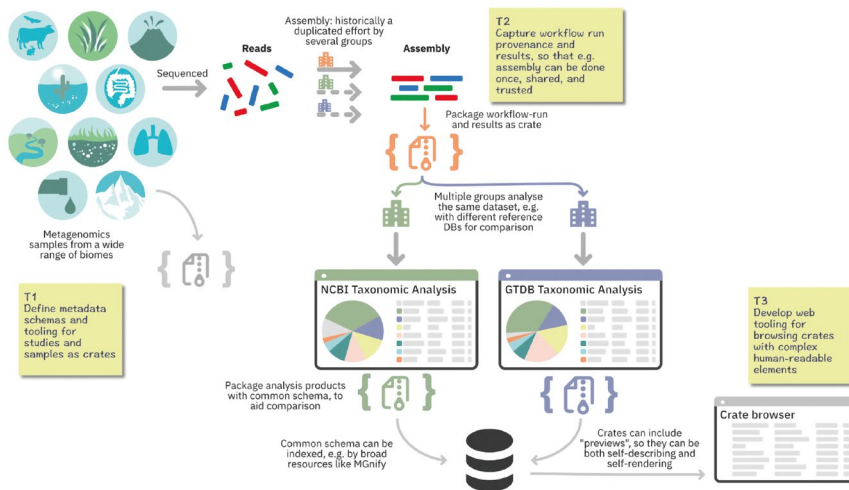
Metagenomics-Toolkit

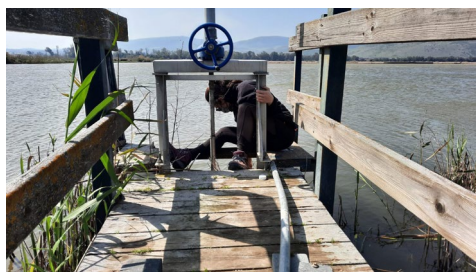
The Metagenomics-Toolkit (MG-tk) offers standard analytic modules such as quality control, assembly, binning, and annotation, along with unique features including plasmid identification, recovery of unassembled microbial community members, and discovery of microbial interdependencies through dereplication, co-occurrence, and genome-scale metabolic modeling. Additionally, the Metagenomics-Toolkit includes a machine

learning-optimized assembly step that adjusts peak RAM usage to match actual requirements. It also integrates a web-browser that allows our partners to visualize and explore their data interactively.



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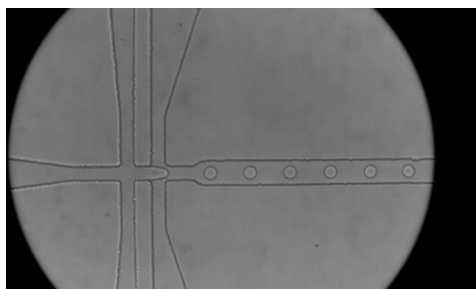
ML-based Pipeline for Rhodopsins

Red- and infrared- (IR) absorbing microbial rhodopsins are highly valued as optogenetic tools to study neural circuits, thanks to the ability of longer wavelengths to penetrate deeper into tissue. A machine learning-based pipeline for the discovery of novel rhodopsins was used to search various metagenomics and metatranscriptomics datasets for the presence of predicted red- and IR-absorbing rhodopsin channels.



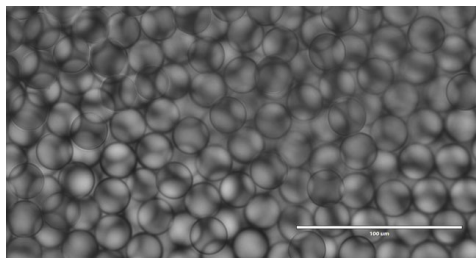
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ML-based Pipeline for Plastic Degrading Enzymes

UCAM has screened natural metagenomic DNA libraries for general esterases and then re-screened these esterases to identify a subpopulation of plastic degrading enzymes – and identified new PET-hydrolysing enzymes (PETases). These enzymes with low sequence homology to known PETases were subjected to deep mutational scanning (DMS) that reports on which amino acids can be exchanged and combined with others. Such ground truth data describing 'fitness landscape' informs predictive ML that yields high performance PETases for recycling and bioremediation.



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Ultrahigh-throughput Screening

Conventional plate-based screening is limited by low throughput, high reagent use and poor access to rare activities in metagenomic libraries. BLUETOOLS closes this gap with modular droplet microfluidics and fluorescence-activated droplet sorting (FADS), enabling millions of compartmentalised reactions to be screened rapidly, at orders-of-magnitude lower cost and with substantially less material consumption and waste generation

Modular Assay Design

BLUETOOLS shows droplet microfluidics adaptability across assays of varying complexity: single fluorogenic reactions to cascades (KREDs, CAZymes), and cell reporters (deoxyribosyl transferase, quorum quenching, antimicrobials). A shared droplet/FADS platform accommodates diverse targets, identifying hundreds of novel enzymes for glycoside breakdown from marine bacteria, plants and algae, plus lytic enzymes against gram-positive/negative bacteria.



Bluetools

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Scatter Sorter

Library screening campaigns must be direct, i.e. interrogate every candidate enzyme for the desired transformation. "You get what you screen for" is the first rule. We achieved this by directly detecting the breakdown of plastic nanoparticles via light scattering, discovering plastic-degrading enzymes. Replacing indirect proxy assays (small molecules instead of polymers) enables faster, more successful discovery of enzymes for recycling and bioremediation.



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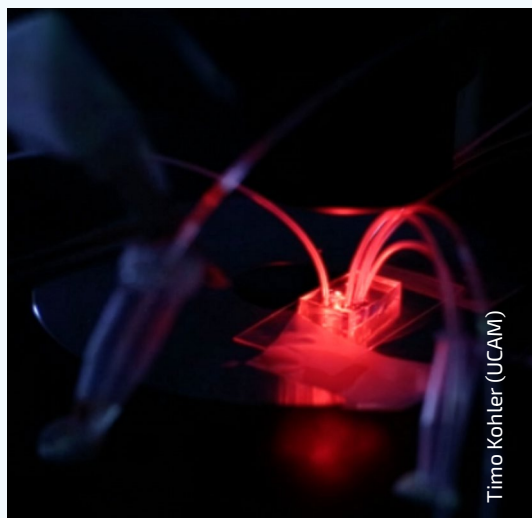
Mini-Metagenomics

Mini-metagenomics uses ultrahigh-throughput microfluidic droplet sorting to enrich microbial subpopulations before sequencing. Single cells are encapsulated in droplets, screened by digital PCR and sorted on-chip, reducing community complexity and revealing low-abundance taxa and genetic diversity missed by conventional metagenomics or culture-based approaches.



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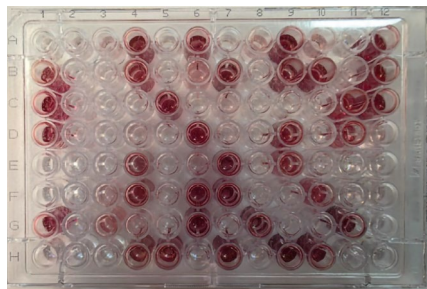


Timo Kohler (UCAM)

4

Enzyme Panels

Prozomix's Biocatalysis Toolkit comprises the largest, most diverse collection of free-to-screen biocatalysts. These enzymes, all with global freedom-to-operate, support a number of biocatalytic processes worldwide. Underpinning this large collection of novel enzymes are the extensive metagenomic libraries, all collected and processed in compliance with the Nagoya Protocol.



Throughout the BLUETOOLS project, Prozomix has further expanded a number of its enzyme panels, including IREDs, KREDs, NDTs and transaminases, through the discovery of hundreds of novel enzymes from marine biodiversity. These enzymes, provided to partners throughout the project, now make up a portion of Prozomix's Biocatalysis Toolkit. This toolkit contains over 8000 wild-type enzymes derived from metagenomic samples in compliance with the Nagoya Protocol. Prozomix's Toolkit model permits risk-free screening of enzymes, given that screening materials are

provided free of charge (screening plates and 100 mg samples). All Prozomix enzymes can be provided at any scale, at fixed commoditised prices, from Prozomix's manufacturing site in the North of England.



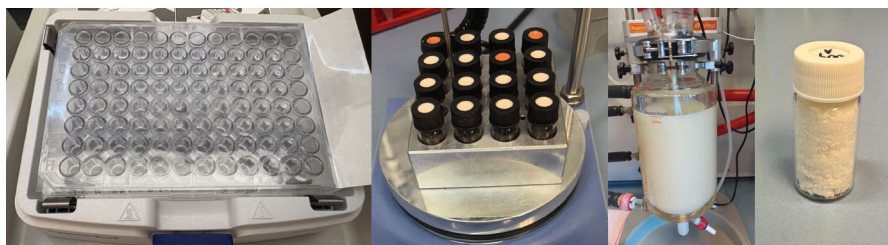
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Biocatalysis for Pharma

Biocatalysis has become a transformative tool in chemical process development in the pharma industry. The growing complexity of small molecule drug candidates requires exceptional selectivity and tunability of catalysts. Enzymes perfectly meet these requirements while operating under mild reaction conditions ultimately leading to shorter reaction routes, less purification steps and simpler reaction setup. Modern, often AI-assisted bioinformatic tools make mining and tailoring of enzymes more accessible to organic chemists.



Screening



Optimization



Scale-up

Although biocatalysis has pronounced advantages over classic organic chemistry, limitations lie in substrate specificity of enzymes and their tolerance to industrial conditions. Enzymes derived from specific metagenomes can help overcome these issues. Therefore, SERVIER has screened hundreds of enzymes mined by other BLUETOOLS partners for synthetic organic chemistry applications in pharmaceutical projects. Panels provided by Prozomix, UAM and UA were either screened against a set of pharmaceutically relevant substrates for initial profiling or tested directly on intermediates having been under chemical development. Next, operational conditions and/or protein sequence of selected enzymes were optimized followed by chemical synthesis.

For example, novel variants of nucleoside 2'- deoxyribosyltransferases displaying unprecedented activity on modified sugars (building blocks for e.g., antisense oligonucleotides) have been identified. Imine reductases and ketoreductases have been employed for the gram-scale synthesis of valuable intermediates, while novel transaminases with exceptional activity and selectivity have been obtained.

SERVIER
moved by you

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Antimicrofouling Solutions

Under natural conditions (environments, infections) microbes generally present themselves as biofilm communities rather than in planktonic growth. The biofilm is built by interspecies microbes combined with their extracellular polymeric substances (EPSs), functioning as "buffer" towards external effects, incl. antibiotic or disinfectant treatments, usually requiring higher compound dosages on biofilms. To combat biofilms, strategies acting onto the extracellular matrix can be very effective, e.g. enzymes rupturing the EPS or hindering attachment/biofilm formation, or molecules able to target the microbes of the biofilm.



SINTEF has explored sequence datasets from marine environmental samples as well as from cultivated biofilm in discovery activities for antifouling solutions, targeting enzymes for EPS degradation as well as antimicrobial peptides (AMPs). A number of enzyme candidates has been identified, subcloned and produced and are evaluated for the antibiofilm-establishment functions, presenting a few positive candidates to be further explored. Additionally, a set of AMPs have been synthesised and evaluated for their antimicrobial effects on different microbial strains in planktonic growth as well as for their potential in hindering biofilm formation, of which especially 1 AMP show promising features presenting interference with biofilm formation of the fish pathogen *Yersinia ruckeri*.

Moreover, INSAT employed both sequence-based metagenomics and

microfluidics-based functional screening to identify innovative antifouling solutions. These approaches led to the discovery of marine bacterial isolates and a novel halophilic lysozyme, both of them showing strong potential as antimicrobial agents. Additionally, EPS-degrading isolates were identified within biofilm-associated microbiota. These isolates produce a range of carbohydrate-active enzymes capable of breaking down structurally this complex polysaccharide, making them promising candidates for anti-biofilm applications.

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TOULOUSE

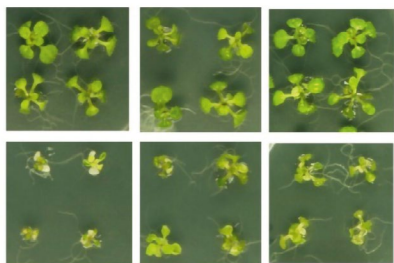
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7 Resistance Genes

Crops worldwide are exposed to high doses of UV radiation and suffer from severe droughts, expected to worsen with Climate Change in the coming years. Hence, we investigated environmental samples from extreme environments by functional metagenomics to comprehend how microorganisms thrive in these environments, with the aim of finding genetic material to improve crop resistance.



INTA used functional metagenomics on four libraries, finding new genes conferring UV and desiccation resistance, some previously not annotated. Candidate genes are being transferred into *Marchantia polymorpha* to test if their expression boosts resistance to UV and desiccation in the plant.



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8 Sustainability Consultancy

CTA is an R+D consultancy with wide experience in preparation, coordination and exploitation of European R&D&I projects. Specialised in sustainability methodologies, CTA provides expertise in the application of LCSA (LCA, LCC and S-LCA), as well as feasibility, process scale-up and modelling studies.



Within BLUETOOLS, CTA has carried out the first environmental assessment of different high-throughput screening methodologies. CTA has also evaluated the impacts of new processes developed in the consortium for the production of active pharmaceutical intermediates, providing recommendations to improve their sustainability and technical feasibility.

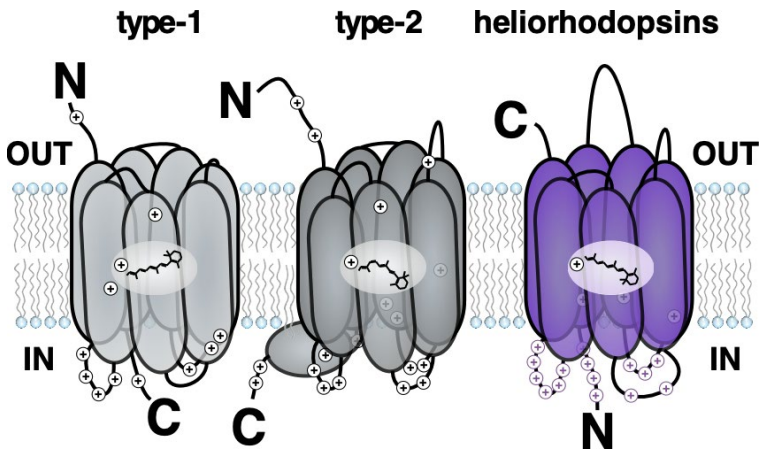


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Rhodopsins

Rhodopsin pigments are integral membrane proteins that bind retinal chromophores and are used by microorganisms to capture or sense sunlight. The use of rhodopsin pumps and channels facilitates light-activated signal firing or silencing in neurons for the study of neuronal activities in the brain (i.e., optogenetics). IIT has been instrumental to these discoveries, e.g. the discovery of the first very fast desensitizing channelrhodopsin, and new red-absorbing rhodopsins for deeper brain penetration.



Rhodopsins, photoreceptive proteins containing a retinal chromophore, are found in animals and various microorganisms. Both animal rhodopsins (type-2) and microbial rhodopsins (regular type-1 and heliorhodopsins) are membrane proteins that utilize a covalently bound retinal moiety to capture light. Animal rhodopsins belong to a specialized subfamily among the G protein-coupled receptors (GPCRs) and are used in light sensing. Microbial rhodopsins, on the other hand, represent a protein family of their own, with a much wider spectrum of functions. The diversity of microbial rhodopsins remains a field of active investigation

and is guaranteed to surprise rhodopsin researchers for generations to come. One particularly unexpected discovery has been the finding of heliorhodopsins. Heliorhodopsins represent a new kind of microbial rhodopsins which was discovered using functional metagenomics. Heliorhodopsins have less than 15% identity to regular type-1 rhodopsins and possesses an inverted membrane orientation compared to both other type-1 and type-2 rhodopsins.



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