



## KEY ACHIEVEMENTS



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## Coordinator's welcome

It is with great pleasure that I present the SIMBA Key Achievements Booklet.

Funded by the European Union, the SIMBA project explored the potential of exploiting microorganisms in plants, soils, marine and food to improve food security and promote sustainable food production. Starting in November 2018 and running until October 2023, the project has achieved a lot over the past five years.

The main aim of SIMBA was to use innovative, microbial-based solutions to tackle the growing challenge of supplying food to meet global population demands within a changing climate. Now more than ever, we need to utilise innovative ideas to promote sustainable food production, and the microbiome can offer a possible alternative to intensive and unsustainable agriculture practices.

The following pages contain some of the key achievements and outcomes from the project. For more information please visit the SIMBA website ([www.simbaproject.eu](http://www.simbaproject.eu)), where the project outputs and reports are available to download.

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## Sustainable innovation of microbiome applications in the food system

### The Challenge

The threat of food insecurity is a critical global challenge, compounded by climate change and population growth. Forward-thinking solutions are needed to meet this challenge and one potential area for exploration is microbiomes, which are communities of microbes (bacteria, viruses, fungi, etc) in a certain environment. Microbiomes are known to regulate the productivity and health of major food sources across land and sea. Therefore, they can play a positive role in food production, and food and nutrition security, ultimately influencing human health. However, we lack a deep understanding of the microbiomes associated with our food systems.

and plant health;

- Deepen understanding of the effect of microbiome composition on high quality algal biomass, fish health and saline agriculture
- Delivery of 1-2 designed starter cultures originating from characterised microbiomes to be applied in food and feed products
- Understand the inter-individual differences in the function of the gut microbiota, its interaction with diet/foods and how all this affects health outcomes
- Solutions to perform better environmentally, as well as economically and socially

### Project Objectives

Focusing on crop production and aquaculture the aim of the SIMBA project was to:

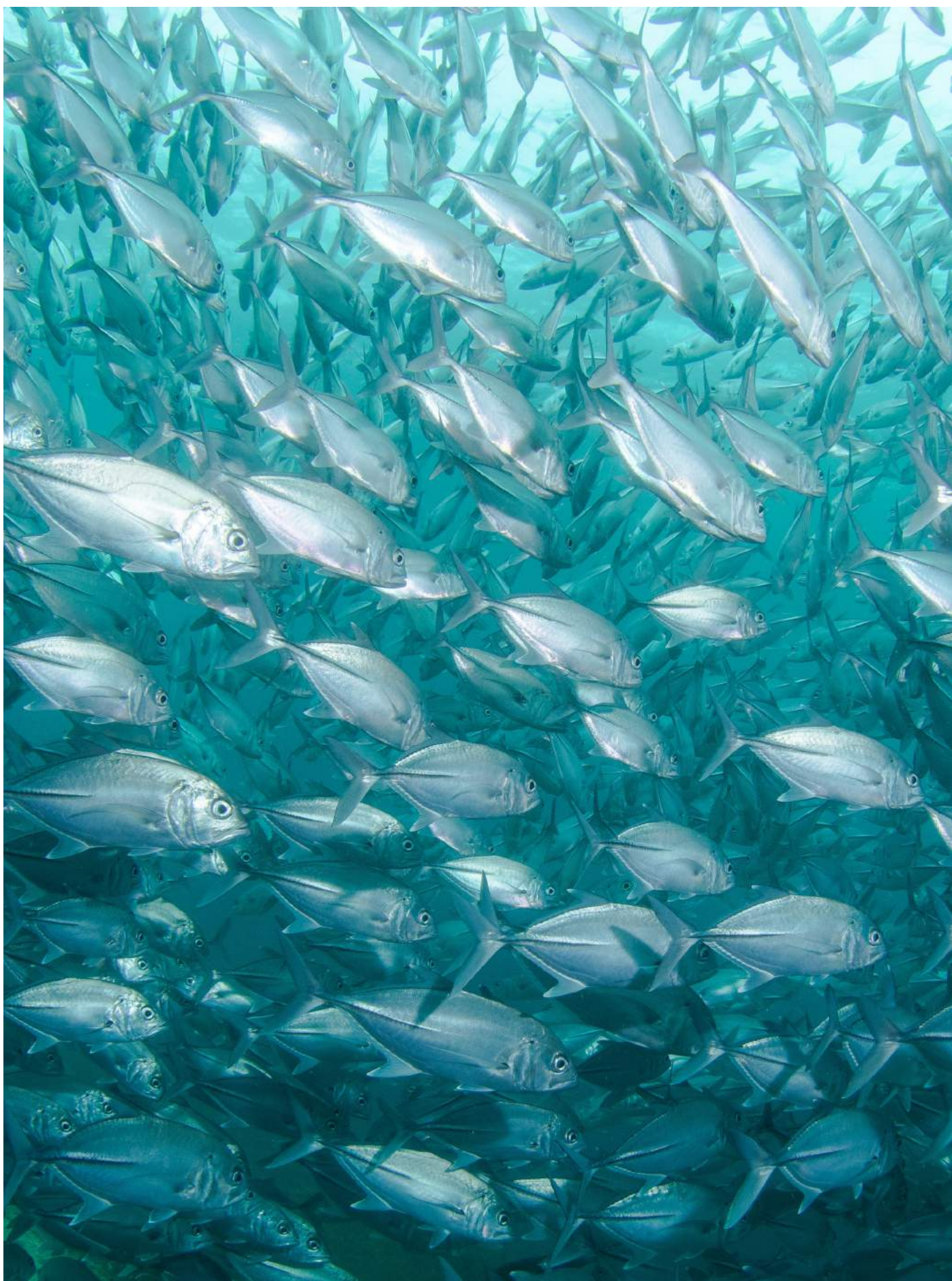
- Get a better understanding of microbiome structures and functions, related to land and sea related food chains;
- Verify the sustainability of microbial innovations of the food systems as a whole;
- Create a better EU Agro-Aqua-Food system that is resource efficient, climate resilient, sustainable and consumer centred;
- Improve the overall knowledge of microbiomes from land and sea towards the market needs in areas where applicability and readiness is not yet visible;
- Bring new and cost-effective commercial applications to the market that assist different stages and processes throughout the food chain by 2025

### Expected Results

- An open access central database containing microbiome data useful for EU food production;
- Up-scaled crop production: delivery of selected microbial consortia on crop yield, food quality







**Boosting Accessibility to  
Microbiome Data for  
Enhanced Usability in  
European Research and  
Development**



## Plaspline, a pipeline for plasmid analysis form metagenome

To increase and enhance accessibility to microbiome data, SIMBA developed a tool which can be used to provide a comprehensive analysis of plasmids present in a microbial community.

Plaspline is a snakemake based workflow, which aims to get a comprehensive analysis of plasmids, at both gene and plasmid community level from shot-gun sequences. A 'Snakemake workflow management system' is a tool (essentially a set of code or instructions) which can be run to generate reproducible and scalable data analysis workflows using the Python language. It applies rules based on patterns to analyse multiple datasets. It is useful for keeping track of complex workflows. The main steps are quality control, assembly, circular/linear plasmids isolation, circular/linear plasmids genome verification and classification, and plasmids-relative-genes analysis.

Plaspline improves on existing tools both in terms of performance and output. Currently, most metagenome analysis tools assess just one aspect of the genome at a time, Plaspline strategically

combines existing, state-of-the-art tools and uses them together but with novel strategies. It can collate information on circular and linear plasmids simultaneously and provide more information on the plasmid such as the plasmid type, plasmid encoded antibiotic resistance gene, and so on. Thus, Plaspline is an excellent choice for those looking to analyse plasmids.

Plaspline is available to use for free. It can be accessed on github: <https://github.com/Wanli-HE/Plaspline>, where users will find instructions on how it can be used.

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# Improvement of Plant Growth Promoting Microorganisms Field Applications Efficacy and Reproducibility

## Emergent bacterial community properties induce enhanced drought tolerance in *Arabidopsis* in vivo

As climate change makes extreme weather events more common, incidences of drought are increasing. Such stresses can negatively impact crop growth and restrict plant production. As the global population increases, it is important that this threat to food security is tackled. Any solutions we put forward to deal with this issue must be both environmentally friendly and economically viable.

There is a growing body of research examining the ability of specific soil microbes to improve stress tolerance in plants. This KO describes the development of a bacterial consortium which is able to significantly improve the survival rate of the *Arabidopsis* plant during periods of water deprivation.

This KO is a novel bacterial consortium consisting of four species (*Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans* and *Paenibacillus amylolyticus*, termed as SPMX for short) that was found to significantly improve the survival rate of *Arabidopsis* (rockcress) during periods of water deprivation.

The SPMX consortium was found to synergistically produce more biofilm biomass together than the sum of the four single-species cultures. No drought-tolerant effects were observed in *Arabidopsis* when the four single-species cultures were applied individually.

The underlying mechanisms were found to be associated with enhanced leaf chlorophyll content, enhancing endogenous abscisic acid (ABA) signalling and alleviating water stress by SPMX. SPMX abundance was promoted in the plant root area during drought compared to normal conditions. The data also show that SPMX stabilised the bacterial community composition during drought, and enhanced the abundance of *Actinobacteria*, which jointly conferred an increased drought tolerance of plants.



Yang, N., Nesme, J., Røder, H.L. et al. Emergent bacterial community properties induce enhanced drought tolerance in *Arabidopsis*. *npj Biofilms Microbiomes* 7, 82 (2021). <https://doi.org/10.1038/s41522-021-00253-0>

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## Identification of candidate plant growth-promoting microbes and bioactive compounds to formulate microbial consortia inoculants

There is a well-recognised need to define and adopt more sustainable and environmentally friendly agriculture practices, including natural strategies that reduce the application of chemicals in agriculture. The use of beneficial microorganisms combined in consortia is a promising means to improve crop yield and quality and represents a reliable and eco-friendly solution that may respond to the challenges for modern agriculture.

A growing body of evidence demonstrates the potential of various microbes to enhance plant productivity in cropping systems although their successful field application depends on several biotic and abiotic factors.

This Knowledge Output (KO) concerns the development of novel multifunctional microbial synthetic consortia that can be used in combination with suitable bioactive compounds to improve crop yield and quality for the crops maize, wheat, potato and tomato.

Many Plant Growth-Promoting Microorganisms (PGPM) associated with maize, wheat, potato and tomato have already been identified in several previous studies and data are published in peer-reviewed scientific publications. This KO is novel in that it builds upon this knowledge, in combination with existing commercial formulations, to identify specific plant growth-promoting strains that would potentially be able to co-exist (being compatible) when brought together in a new, synthetic microbial consortium.

Most approaches for plant growth promotion imply the use of single-strain inoculants as biofertilisers, while only few consider microbial consortia products, i.e., the combination of two or more microbial species. Compared to single-species inoculation, multi-species inoculum frequently

increases plant growth and yield, and improves the availability of minerals and nutrients, providing the plants with more balanced nutrition.

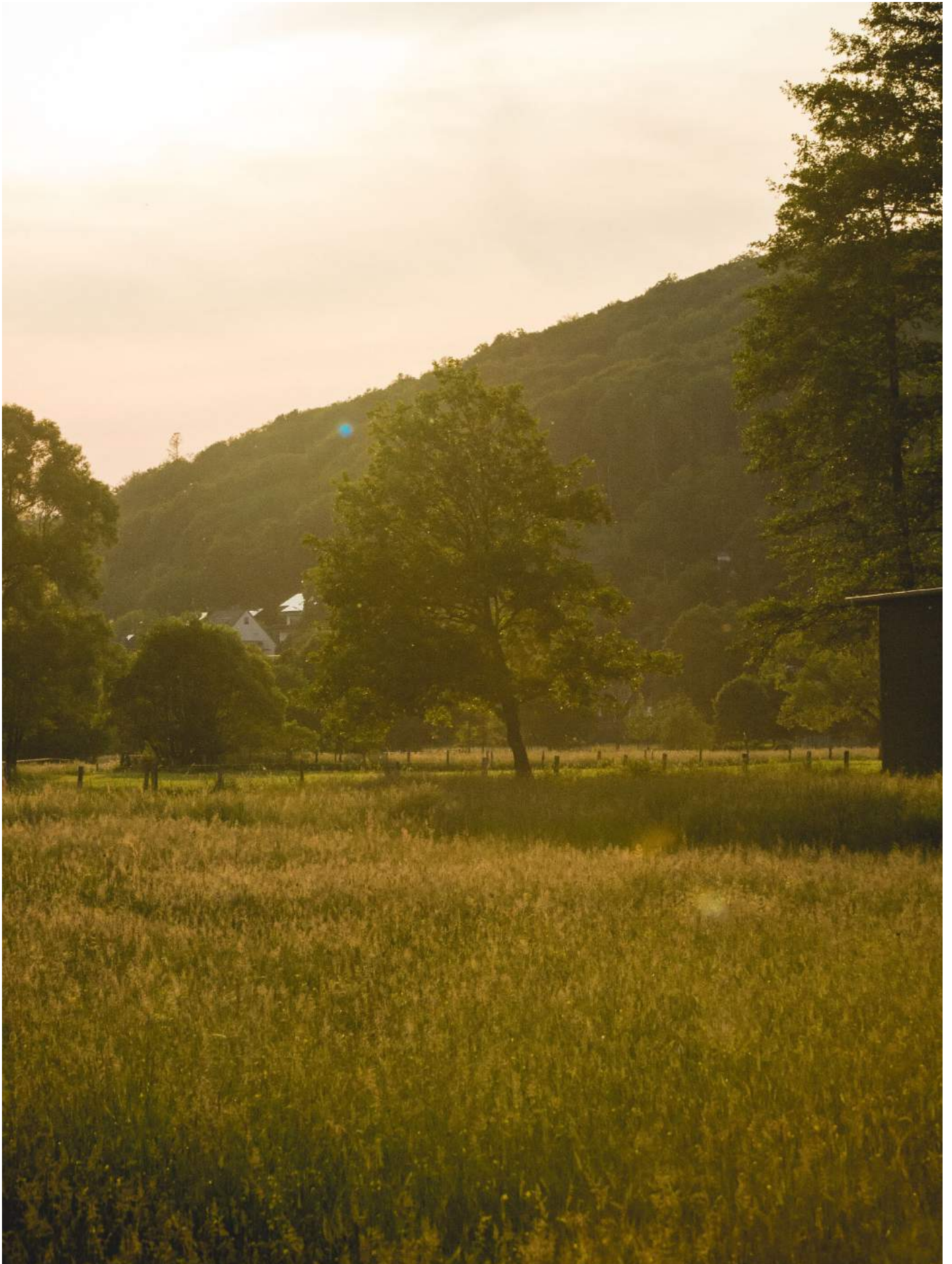
A total of 23 PGPM were identified as having potential for effective use in synthetic microbial consortia. To guarantee the development of compatible microbial consortia, selected PGP strains were preliminarily screened in vitro for their ability to coexist.

Based on this, three novel synthetic assemblages of PGPM with different functions were developed using a bottom-up approach, common to all four crops, using compatible bacteria with or without the addition of a fungal biocontrol agent.

In addition, a set of bio-active compounds was tested for compatibility in vitro with each single microorganism composing the selected consortia, with the eventual aim to develop an effective combination of the products based on both microbial and non-microbial biostimulants (BS).

The findings indicate that bio-active compounds can enhance the growth of beneficial microorganisms composing the selected microbial consortia, suggesting that signals produced by these PGPMs can act synergistically with the organic compounds to enhance plant growth and productivity.

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## **Marine Microbiomes for Sustainable High Quality Food Production**



## Growth characteristics of *Ulva ohnoi* and selected microalgae (including several different (yellow-) green algae, diatoms, red algae and cyanobacteria) in both marine and freshwater environments

Micro- and macroalgal food-based products are experiencing a large increase in demand and are foreseen to be key sources of proteins and other beneficial compounds in the future. The algae can provide a nutritious, reliable, and sustainable food source that can be available in large amounts for both human consumption and feed stock for cattle and other aquaculture applications. This KO concerns the growth characteristics of a selection of microalgal species including Cyanobacteria and the macroalga *Ulva ohnoi*, which are biomasses that potentially could be used in the development of nutritious food in the future. *U. ohnoi* in particular was selected for its versatility, multitude of (potential) applications (food, feed, valuable extracts), ease of cultivation and high potential for upscaling of the production.

*U. ohnoi*, the selected *Ulva* species, is omnipresent in the warmer waters of the Spanish Atlantic and Mediterranean and most likely also in other parts of the Mediterranean and warmer waters in the rest of the world. It is relatively easy to isolate and maintain in cultivation. *Ulva ohnoi* can be considered as an excellent candidate for floating cage cultivation in southern Europe, as results show that it has a broad temperature and salinity range for growth, as has also been confirmed by other experiments. Furthermore, it appears that this local variety does not sporulate, which is the often the cause of large biomass loss in mass cultures of other *Ulva* species. This might be a specific property of this particular variety, as sporulation has been observed in this species in populations from Japan (Hiraoka et al. 2003) and India (Prabhu et al. 2019). Indoor production in PBRs shows great potential for the continuous, year-round production of *Ulva* biomass.

Although data from field studies already indicated that *U. ohnoi* had a wide salinity and temperature tolerance range, this has now also been confirmed in controlled laboratory experiments. Furthermore, the complete lack of sporulation of this variety is

a novel and important observation, as sporulation is often a major loss factor in *Ulva* cultivation and is on the other hand also a determining factor in choosing the cultivation method. Floating cages have (to our knowledge) not been attempted before as a means of cultivation and are a labour extensive way to grow this non-sporulating variety in particular. Cultivation of macroalgae in photobioreactors is not entirely novel, however there are very few published studies thus far and only one with *Ulva*. This study is the probably the first confirming the possibility of year-long production under semi-controlled condition (in PBRs) of this method.



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## Protocol for cryopreservation and functional reconstitution of complex microbial communities

Although microorganisms are often described and used as a single species, growing in liquid media that are often easily preserved and revived with standard methods, most species only function in consortium with other species and in a structured three-dimensional organisation. These complex microbial communities are essential as starter cultures in dairy production, food fermentation, sustainable agri- and aquaculture and for scientific research processes.

Long term preservation of these complex communities (biobanking) is essential to maintain composition, function and especially their beneficial traits for which they were selected.

Protocols for the preservation and functional regeneration of complex communities with several hundreds of species, did not exist before.

This KO describes a novel laboratory protocol for the long term cryopreservation of a photosynthesis driven microbial mat as model system of a complex three dimensional structured microbial community. This protocol not only describes the freezing and revival procedure itself in the presence of different cryoprotective agents (anti-freeze compounds: Methanol, Dimethyl Sulfoxide (DMSO), and Glycerol), but also tests the effect of these compounds on the ability to regenerate a functional microbial mat.

This protocol was tested and subjected to statistical analysis that showed that for these microbial mats, Methanol gave the best results and generated microbial communities similar to the natural communities.

DMSO and especially Glycerol, although being excellent cryopreservative agents, had a strong negative effect on the reconstitution of the microbial community and should therefore be omitted. This negative effect was due to the fact that DMSO and Glycerol can be used as substrate by aerobic bacteria resulting in a fast depletion of oxygen and ultimately anoxia that destroyed the community.

An interesting detail is that for the microbial mat model system, absence of a cryopreservative agent also gave good results, probably because of its natural production of polymeric substances. For other complex communities that do not produce these polymeric substances, methanol is the best cryopreservative agent.



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## **Microbes to Produce Healthy and Nutritional Food and Feed**



## New microbial consortia for enhanced fermentation protocol and improved nutritional value of pulses

As it becomes increasingly harder to feed growing populations, interest in plant-based foods as well as fermentation has increased. This growing interest is due to a multitude of factors, including environmental concerns, health consciousness, nutritional needs, sensory issues and safety. In particular, fermentation is recognised as a sustainable processing technique that can improve the nutrient content of food by reducing the antinutrient content and producing bioactive compounds in plant-based products.

SIMBA has aimed to enhance the fermentation process by applying microbial technologies to help produce easily used, plant-based foods with improved nutrient content for people.

SIMBA researchers have designed four new microbial starter mixtures to be used for the fermentation process – two consisting of lactic acid bacteria and other two of lactic acid and propionic acid bacteria. These mixtures have been assembled based on preliminary tests conducted with individual microbial strains from the microbe collections of SIMBA partners Luke and NMBU.

The most promising strains were added to starter mixtures and have been used in the development of new product 'prototypes' based on pulses and cereals. Through fermentation, levels of antinutrients such as oligosaccharides that cause stomach discomfort, have been reduced.

The levels of vicine and convicine (causing favism for people with deficiency of the enzyme G6PD) have been partly or totally degraded. Furthermore, with the help of the assembled microbial mixtures, bioactive compounds such as vitamin B12 could be produced in the plant-based products in Luke's laboratory scale experiments.

The potential impact of these results include: new processing options and microbial starters for food industry, new nutritionally enhanced plant-based products and increasing variety of sustainably produced plant-based products to the market to meet the consumer needs.

Four new product prototypes have been developed using these microbial starters, including a fresh porridge-type faba bean-oat product, cookies made of faba-bean oat, a pea-based yoghurt-type snack product and lentil-based fresh cheese-like product.



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## Identification of microalgae strains suitable for growth in agricultural residues

As it becomes increasingly challenging to feed growing populations, the need to increase circularity and waste valorisation in food productions systems is heightened. Algae biotechnology is an expanding field of research that looks at how we can grow and apply algae and its derivatives for use in the food and pharmaceutical industries. It represents a promising option for utilising the side-streams generated by the potato starch industry. The side-streams from this industry contains nutrients and organic carbon which could be used as a growth medium for algae. The cultivation of algae using the side-streams generated from potato starch production would enable the creation of new value chains from potato residues and improve circularity of potato starch industry.

As part of this research, the Norwegian Culture Collection of Algae (NORCCA) was screened to identify microalgal strains that can grow in the potato juice (a side-stream from potato starch production). This is the first time the NORCCA has been used to identify potential microalgae strains to be cultivated under these conditions. The strains selected for screening belong to microalgae species that are commonly used in algae biotechnology. After identifying several strains that could grow in potato juice diluted in water (e.g. 1% potato juice solution), the strains were tested further to determine the optimal light intensity for growth and optimal formulation (dilution) of potato juice. The screening and optimisation experiments revealed two strains of microalgae (*Chlorella* sp. and *Chlorococcum* sp.) that show good growth and biomass production rates under optimised conditions. These strains were then grown in 3L flat-panel photobioreactors to verify that scaled-up cultivation of microalgae in potato juice is possible. Here, microalgae showed similar growth rates and reached similar biomass concentrations in 1% potato juice compared to standard algal growth medium. Obtained Microalgal biomass was harvested, freeze dried and biocharacterised for various bioproducts of interest, namely amino acids, fatty acids and carotenoids.

The comprehensive biochemical analysis demonstrated that chemical profiles were mostly similar between biomass grown in potato juice and standard algal growth medium. This work adds to the growing scientific literature demonstrating that microalgae biotechnology can provide a good solution for treatment of agricultural side-streams through new value creation. It is the first time work has been carried out to optimise growth conditions for microalgal strains in potato juice.



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## Optimising fermentation parameters to improve fermentation and quality of end product

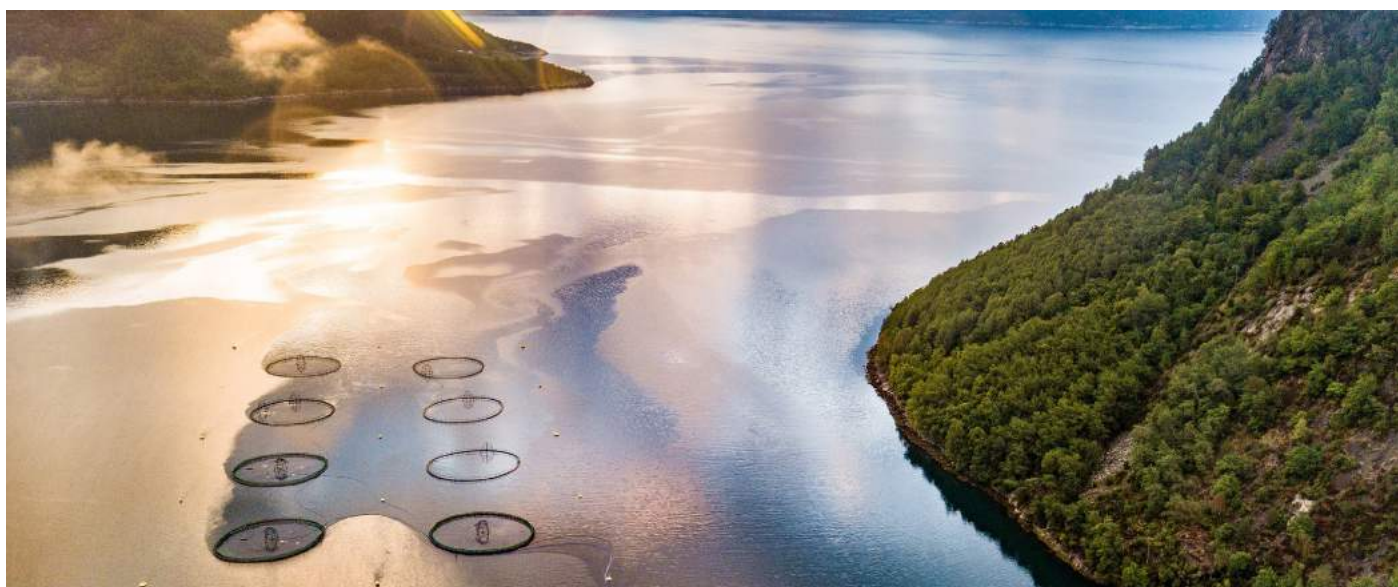
For the development of fermented food and feed products with desired composition and health promoting characteristics, there is a need to develop effective starter cultures. The right starter cultures can produce fermented products with improved digestibility and beneficial compounds, like vitamins, peptides and exopolysaccharides, which improve the nutritional value. The SIMBA project aims to establish novel microbial strains and consortia for optimised fermentation processes for different food products. Exogenously added enzymes together with the novel microbial strains have shown to further enhance the fermentation process.

This KO concerns the development of novel raw plant fermentation protocols which produce high volumes of lactic acid without adding glucose as a substrate for bacterial growth. Typically, glucose is added to kick-start the fermentation process in plants, and previous fermentation experiments have shown that this results in conversion of only the added glucose into lactic acid (by lactic acid bacteria). However, this is not ideal as the fibre that is present in plant cell walls should be utilised as well and be converted into lactic acid too (and other substances). In addition, once the cell walls are broken down, other cell components will also

become available to the fermentative bacteria to be converted into bioactive components. This KO is an innovative process that is effective in breaking down plant cell walls and converting several cell components into potent bioactive components, without the need to add glucose for the microorganisms to grow and ferment the substrates.

The innovation in this method is that by using certain selected lactic acid bacteria under specific circumstances, there is no need for addition of glucose for the microorganisms to grow and ferment the substrates, while producing better results in terms of utilising more parts of the plant cells. Also, depending on the microorganisms used, the produced amount of lactic acid without glucose addition was more than that produced when using glucose as the exogenously added energy source for the microorganisms.

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## **Sustainability Assessment and Potential Uptake of New Technologies/Products**

## Effective intervention strategies for the uptake of microbial innovations in arable food production

Microbiomes are known to regulate the productivity and health of major food sources across the land and sea. They can play a positive role in crop production and in food and nutrition security. As our understanding of microbiomes deepens and innovative microbiome-based technologies are developed for use in crop production, it is important to understand how these new microbiome-based technologies can indeed be taken up and used by the stakeholders chiefly responsible for food production – farmers.

This KO, concerns identification of the most effective intervention strategies that will lead to successful adoption of microbial applications by arable farmers.

There are many methodologies which can be used to identify such strategies and the Behaviour Change Wheel is one such method.

At the core of the Behaviour Change Wheel is the COM-B model (Capability, Opportunity, Motivation-Behaviour). This model suggests that behaviour is part of an interacting system involving these three components. Interventions need to change one or more of these components to bring about real behaviour change.

The study behind the KO result showed that capability and opportunity are the most important drivers for farmers to adopt microbial applications.

1. Capability means that farmers want to know how microbes work and how they can integrate the products into their farming operations.
2. Opportunity means that farmers need to be provided with support from their surroundings, such as advisors and peers.

This was the first time that the Behaviour Change Wheel method with its COM-B model has been applied to investigate uptake of innovation in the agricultural sector. In previous research, it was used to analyse hygiene and health related behaviours

The KO itself (intervention strategies for microbiome applications) is novel because it

provides concrete insights on how to support the uptake of microbial applications in the countries under investigation.



Tensi, A.F., Ang, F. and van der Fels-Klerx, H.J., 2022. Behavioural drivers and barriers for adopting microbial applications in arable farms: Evidence from the Netherlands and Germany. *Technological Forecasting and Social Change*, 182, p.121825.

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## Insights into consumers' attitude and perceived risks towards food products obtained using microbial applications

Global commercial food systems are often characterised by unsustainable and unhealthy production and consumption practices. Enhancing the uptake of innovations, such as more environmentally friendly fertilisers based on plant beneficial microbes, is required to accelerate the transition towards sustainable and healthy food systems. However, previous research from the literature found that consumer attitudes towards such innovations are not always positive. Investments in novel agri-food technologies by farmers and other value chain actors affects their competitiveness, profitability and survival. Thus, they must make prudent adoption decisions, by thoroughly understanding consumers' preferences and their willingness to pay (WTP) for sustainable and healthy food products that are produced using new agri-food technologies, such as novel microbiome-based applications. From previous research it was also found that the willingness of consumers to buy new food products including those with microbe-enhanced production, depends on consumers' psychological factors such as their food choice motives, as well as product and socio-demographic characteristics.

This KO concerns insights into consumers' attitude, including Willingness to Pay (WTP), and perceived risks towards food products that are produced with microbial applications during the plant ingredient growing phase. The KO also provides information on the effects of psychological and socio-demographic factors on the WTP.

Using data collected through online surveys from 291 consumers, primarily from Germany, Italy and the Netherlands, consumers' WTP was assessed for three food products (wheat bread, potatoes and tomato sauce) produced with microbial applications. Results show that most respondents have a (very) positive attitude towards microbial applications in food production, and most of them are willing to pay premiums for food products that are produced with microbial applications.

The amount of their WTP increases with the level of reductions in chemical use due to microbial

applications. The results also indicate that promotion-oriented consumers are willing to pay more premiums, whereas prevention-oriented consumers are less likely to pay premiums. Other things being constant, environmentally concerned consumers are also more likely to be willing to pay premiums, whereas health concerned consumers are not.

What is novel about this research is that it allows for the characterisation of consumer profiles for identifying consumer segments for microbial innovations. The insights this research gives us on consumer WTP and attitudes towards microbial-enhanced food are essential for designing marketing strategies around microbiome-based food products.



Ali, B.M., Ang, F. and Van Der Fels-Klerx, H.J., 2021. Consumer willingness to pay for plant-based foods produced using microbial applications to replace synthetic chemical inputs. *Plos one*, 16(12), p.e0260488

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## Effective and economical novel strategy to incentivise farmers to adopt microbial applications in times of crises (i.e. COVID-19)

Sustainable innovations, such as microbial applications, have the potential to simultaneously tackle the sustainability and productivity challenges that agriculture faces. This KO concerns a novel strategy that is effective in incentivising farmers to adopt microbial applications in times of crises, such as COVID-19.

The intention to adopt microbial innovations is viewed in conjunction with the subjects' perception of COVID-19 and their risk preferences. To this end, 96 Dutch arable farmers participated in an online experiment in July 2020 in which half of them watched an informational nudging video. All subjects played two lottery games, based on Holt and Laury's Multiple Price List (MPL) and an adjusted payoff-varying MPL, to elicit the subjects' individual probability weighting function and risk preferences. A positive nudging effect of the informational video on the intended innovation adoption was proven statistically. Considering the relatively low production costs of informational nudging materials, it can be concluded that this strategy is effective and economically meaningful.

Previous research suggests that extreme exogenous events, like the COVID-19 outbreak,

may increase farmers' risk aversion, discouraging essential investments for future profitability. In this research it was found that the coronavirus outbreak in spring 2020 had no statistically significant impact on the risk attitude of Dutch farmers a few months later. Likewise, risk attitudes did not influence technology uptake. This KO provides a recommendation on how to effectively nudge farmers towards the uptake of sustainable innovations and contribute to the puzzle of variable risk preferences.

The use of short informational videos as a strategy to 'nudge' farmers towards the adoption of microbial innovations, within the context of extreme events/times of uncertainty (such as Covid-19), is the novel element of this research and would be an effective strategy for future use in similar contexts.

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## Overview of SIMBA publications, deliverables, media and clustering activities

### Publications

Over the course of the project over 20 open access publications have been produced. SIMBA partners have also published book chapters and have contributed to many different conferences and events across Europe. The full list of SIMBA publications can be accessed here: [simbaproject.eu/results/publications/](http://simbaproject.eu/results/publications/)

SIMBA's open access publications and data are also available on Zenodo here: [www.zenodo.org/communities/simba](http://www.zenodo.org/communities/simba)

### Public Deliverables

The following reports related to SIMBA results are available on the project website:

- D1.3: Open beta platform opened
- D1.5: Publicly accessible database platform and web interface released
- D2.11: Practical manual for stakeholders
- D3.1–D3.20 (except D3.8)
- D5.8: Report on diet microbiota interactions
- D6.9: Release of the marketing plan
- D6.10: Producing a database containing genome information (gene prediction, annotation and species identification) of approximately 200 to 250 bacterial isolates and approximately 50 mobilome samples
- D7.5: Report on the economic impacts
- D7.6: Report on the overall sustainability

### Media

A number of communication tools were produced throughout the project such as:

- SIMBA Project Website
- SIMBA Video
- Project Newsletters
- Project Factsheet

- Work Package Factsheets
- SIMBA Seminar Series

SIMBA also maintained an active presence on Twitter using the handle @SIMBAproject\_EU

### #Microbiomes4Life Cluster

As part of a Horizon Results Booster, SIMBA joined forces with sister projects MicrobiomeSupport, MASTER, CIRCLES, and HoloFood to raise awareness of the importance of microbiomes in the food system.

Through the development of joint communication materials and the use of the hashtag #Microbiomes4Life across social media, this was a great opportunity for SIMBA to expand its reach and communicate results to new stakeholders across research, industry and policy.

More information can be found here: [simbaproject.eu/about/microbiomes4life/](http://simbaproject.eu/about/microbiomes4life/)

