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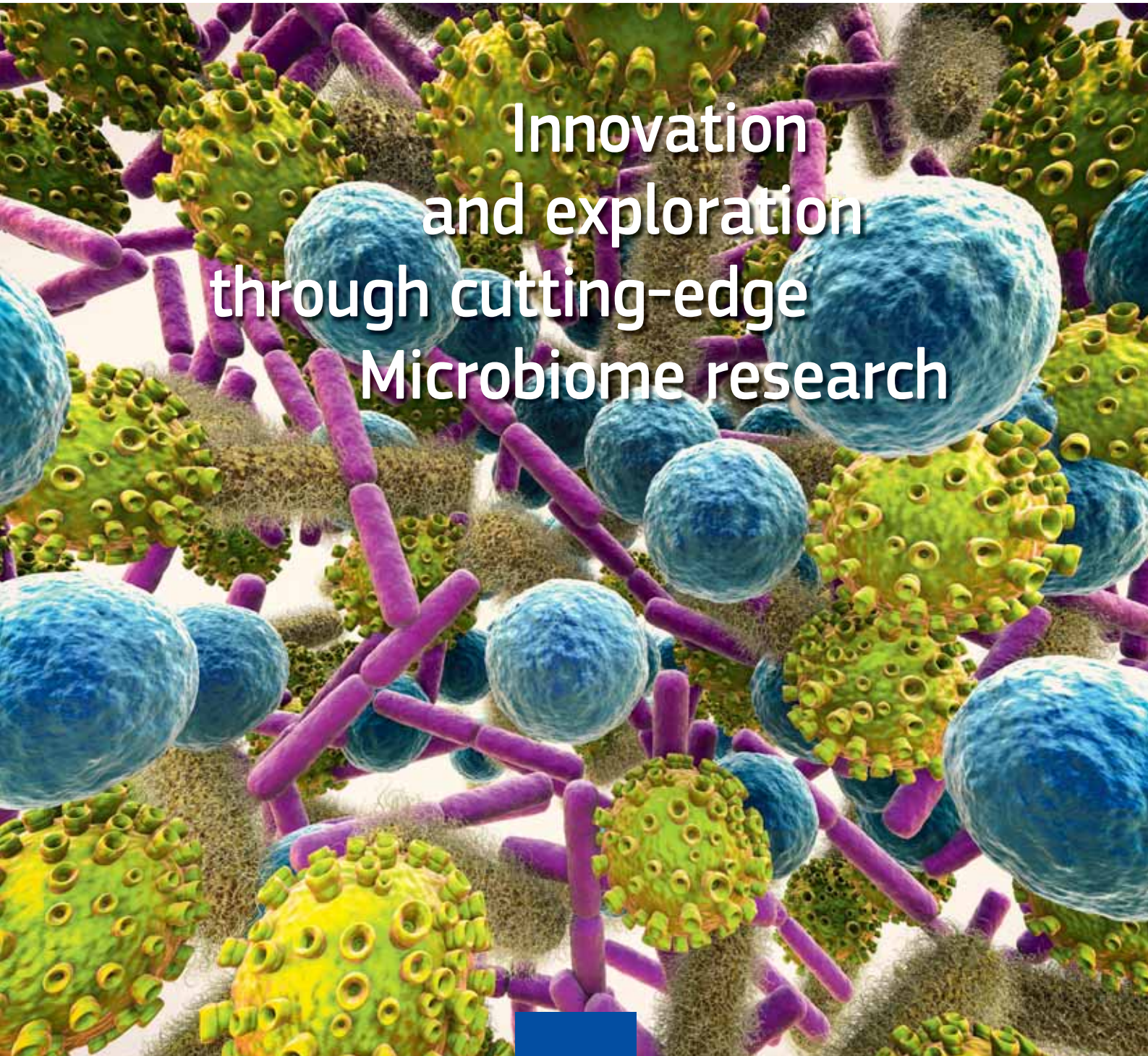

Innovation and exploration through cutting-edge Microbiome research

EDITORIAL

INNOVATION AND EXPLORATION THROUGH CUTTING-EDGE MICROBIOME RESEARCH

Everybody and everything is surrounded by microbiomes and understanding what microbiomes do, what they are, and how they interact is a new scientifi c frontier made now reachable by rapid advances in genomic mapping, robotics, and chemical analysis. What we know and understand so far is that the microbiome has essential impacts on our health and on the food we produce, on plants and animals and on ecosystems in general. Unravelling their complexity off ers huge potential for innovation and will be a major game changer in the way we manage our planet's resources to obtain our food and improve our health.

Our own human microbiome is made up of communities of symbiotic, commensal and pathogenic bacteria as well as fungi and viruses. Our own body microbiome accounts for 100 times more genes, and over 1 000 species live in our gut only. Human-hosted microbiome communities are found everywhere in our body - from our eyes, mouth and lungs to our skin, genitals and intestines.

Contributing to health and wellbeing

Some of these microorganisms have no effect on their hosts, while others like symbiotic bacteria offer a mutually beneficial relationship such as by breaking down food. Pathogens in the form of a disease-causing opportunistic microorganism are also found but in smaller numbers.

Currently there is a lack of understanding about the importance of the human gut microbiome, although it is known to affect the body's ability to extract energy from food and influence brain function. Greater insights into the gut microbiome would therefore contribute to the development of dietary interventions and other new ways to treat both chronic and acute illnesses.

But the microbiome is not just a feature of the human body, it is also found within livestock, plants, soil and the oceans. All of these interact in complex ways, which science has only just begun to comprehend. Hence, the microbiome represents a vast new area of research that offers the potential for nothing less than a food and nutrition revolution.

Highlighting groundbreaking EU research

Through the FP7 and Horizon 2020 framework programmes the EU has seized upon this unique opportunity to support ambitious cutting-edge research projects, allowing it to become a truly global leader in the study of microbiomes. This CORDIS Results Pack brochure focuses on 13 such EU-funded projects that are spearheading microbiome research.

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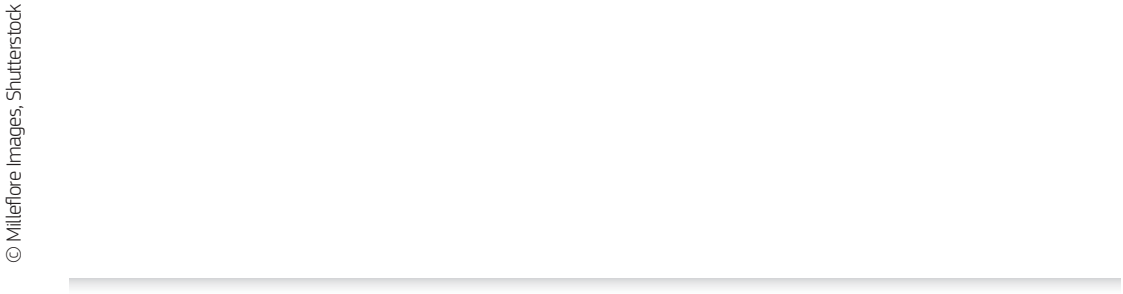

The project enlisted four universities, five research institutes and eight ingredients companies. The work focused on the effect of NPS on enhancing immune defence against pathogens, increasing the gut barrier function and reducing infectious diseases such as the common cold and influenza.

Nutrition, gut microbiota and immune responses

Uncovering the mechanism of how polysaccharide fibres interact with the gut and immune cells found in the intestine was a major research pillar according to FIBEBIOTICS' project coordinator Jurriaan Mes. Researchers developed *in vitro* screening methods to compare different NPS for their bioactivity towards gut and immune cells. Using small intestinal epithelial cells and macrophages, the project team found that NPS can activate immune-related genes such as NFkB and therefore secrete signalling proteins called chemokines to attract other immune cells to the place of action. Such an *in vitro* assay can support product quality control when change occurs in product processing and can be used to check products for their claimed bioactivity.

Dietary fibres play an important role in stimulating the growth of specific beneficial bacteria in the colon, leading to the production of short chain fatty acids that bring significant health benefits. Having thus engaged in another *in vitro* study, the project team optimised and applied a simulated model of the human digestive system, called SHIME, and investigated changes to gut microbiota and metabolites when replacing starch by different types of NPS.

Improving vaccination efficiency with NPS

Scientists conducted a pilot trial on 240 elderly subjects (40 subjects per research arm) who received food intervention for five weeks. Some of the NPS included in this study were derived from yeast, shiitake mushrooms, oats, wheat, apple and a probiotic bacterium. As Mes explains: 'To test the health effects of NPS, we just used the powder that contained purified NPS. These were put in sachets and people had to mix it with liquid and drink it. Using some of the NPS, we later produced biscuits, smoothies and cheese to see how NPS can be integrated in real fortified food products.'

The heart of the FIBEBIOTICS project was the conduction of a five-week trial where healthy elderly people received an influenza vaccination combined with a food supplement containing the different types of NPS tested. Results regarding the vaccination efficiency showed that certain NPS boosted the vaccination response, opening up promising avenues for further research.

Future impact

In addition to offering a benchmark for better understanding immune-health biomarkers, FIBEBIOTICS can help prove company health claims that their fibre ingredients can really boost the immune system, or support other health effects, and can support in including the fibre in their food products with maintained bioactivity.

	Dietary Fibers supporting Gut and Immune Function – From polysaccharide compound to health claim
	Stichting Wageningen Research, the Netherlands
	FP7-KBBE
	http://www.fibebiotics.eu/

Human microbiome: educating the immune system against fungal infections

It is now well established that the human microbiome influences host health and disease. A European project has demonstrated that the host-microbiome interaction also shapes immune tolerance to fungi.

Humans have coevolved with ubiquitous or commensal fungi (referred to as the mycobiome) occupying mucosal surfaces, the skin, lungs and the oral cavity. Emerging evidence indicates that the mycobiome dynamically interacts with the host and the microbiome, regulating immune reactivity at mucosal surfaces as well as distal sites.

However, a weakened immune system more vulnerable to microbial insults or microbial dysbiosis can divert commensal fungi to act as parasites or facilitate infection by opportunistic fungi capable of causing serious disease. This is exemplified by the occurrence of candidiasis following antibiotic use. Therefore, understanding the host/microbiota signals that determine whether a fungus is defined as a commensal or a pathogen constitutes a medical priority.

With this in mind, scientists of the EU-funded FUNMETA project went beyond traditional reductionist approaches of the past and employed a systems biology approach to investigate host–fungus interactions, focusing especially on metabolic pathways.

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Metabolic pathways regulate fungal immune responses

Recent data suggests that the metabolic pathways of the essential amino acid L-tryptophan (trp) crucially contribute to immune homeostasis in fungal infections by reducing inflammatory responses and inducing immune tolerance. Present in mammals and fungi, the trp metabolic pathways are required for survival. The first step in trp catabolism takes place through the kynurenine pathway and involves the dioxygenase enzymes IDO1 and TDO2. 'IDO1 is now widely recognised as a suppressor of inflammation and a regulator of mammalian immune homeostasis with significant biostatic activity on microbes' project coordinator Dr Romani explains.

Microbial and fungal stimuli activate IDO1 to lower host immunity, thus facilitating pathogen persistence. Alternatively, IDO1 may be utilised as an evasion mechanism for microbes to establish chronic infection. However, trp starvation may also be a host strategy for limiting the infectivity of trp-requiring intracellular pathogens. Symbiotic bacteria further contribute to antifungal resistance by converting dietary trp to various indole derivatives that signal downstream of the aryl hydrocarbon receptor (AhR).

AhR is a cytosolic transcription factor that is activated through binding of various ligands. It is involved in many biological processes, including development, cellular differentiation and immune responses. Signalling downstream the AhR affects regulatory T cell development and induces interleukin-22 expression, a cytokine that controls the microbial composition and contributes to immune tolerance.

FUNMETA researchers investigated the contribution of microbiota to the AhR/IDO1-mediated immune tolerance to fungi by correlating the metabolic profile with microbiota composition. Results demonstrated that the IDO1 enzyme is implicated in the interplay between trp catabolism by microbial communities, host metabolite production, and the AhR-dependent immune homeostasis at mucosal surfaces. 'Therefore, AhR has a pivotal role in connecting trp catabolism by microbial communities, the host's own pathway of trp metabolite production with the orchestration of immune responses,' Dr Romani continues.

IDO1 is now widely recognised as a suppressor of inflammation and a regulator of mammalian immune homeostasis with significant biostatic activity on microbes.

Clinical implications

An important achievement of the FUNMETA project was the identification of the bioactive indole-3-aldehyde (IAld) of microbial origin that contributed to AhR-dependent mucosal protection. This compound was patented for its therapeutic activity of protecting and maintaining mucosal integrity during fungal exposure, thus preventing a variety of fungal diseases. IAld could also be prospectively used for diagnostic purposes to monitor homeostasis and microbial symbiosis at mucosal surfaces in certain clinical settings.

Overall, the FUNMETA project underscored the importance of a steady state dialogue between the host and its microbiota to maintain local immune homeostasis. Importantly, it helped decode this dialogue serving as the basis for the future development of therapies that integrates the microbiota, metabolism and immunity. The generated findings support the concept that targeting microbial dysbiosis through a combination of antibiotics, probiotics, prebiotics, and microbial metabolites is a promising druggable pathway for infections and other human diseases.

Project	**Metabolomics of fungal diseases: a systems biology approach for biomarkers discovery and therapy**
Coordinated by	Università Degli Studi di Perugia, Italy
Funded under	FP7-IDEAS-ERC
Project website	N/A

Laying down a marker for efficient cattle production

By identifying feed efficiency biomarkers in growing cattle fed high-forage diets, EU-funded researchers have taken a significant step towards achieving greater efficiency in cattle production. Moving away from grain-based feed will also help to reduce competition between crops destined for feed and crops destined for human consumption.

Growing demand for meat and milk, fuelled by an expanding and increasingly affluent global population, is placing a significant strain on our natural resources. Livestock are a major source of greenhouse gas emissions and waste, and require significant amounts of land for growing feed crops.

'The extent to which grain is currently used in the feeding of ruminants is not sustainable,' agrees MARKEFFICIENCY project coordinator Diego Morgavi from the Institut National De La Recherche Agronomique (INRA) in France. 'A key point that is often missed is that ruminants have a capacity for digesting cellulosic substrates that we humans cannot eat and convert into energy. Naturally occurring microbes help cattle convert forage into the high levels of protein and essential micronutrients found in meat and milk. Transitioning to a foraging-based diet would actually be very efficient as you get more human-edible energy and protein from the animal than you put in.'

Reducing environmental impact of cattle

The goal of the MARKEFFICIENCY project was therefore to develop ways of encouraging this switch from grain to forage-based diets in cattle. This was achieved by exploring potential biomarkers of feed efficiency in cattle fed forage-based diets. 'These could then be used to effectively measure and rank this trait in young animals,' explains Morgavi. 'Being able to select the most efficient animals and formulate diets according to animal potential would benefit farmers economically, while at the same time help to reduce the environmental impact of ruminants.'

Transitioning to a foraging-based diet would actually be very efficient; you get more human-edible energy and protein from the animal than you put in.

The study combined multiple sample types – blood, plasma, hair, faeces and carcass – as well as numerous different analytical techniques, including near infrared spectrometry (NIRS) and mass spectrometry. These were used to identify promising biomarkers for the two primary metrics of feed efficiency – residual feed intake and feed conversion efficiency. This approach also gave Morgavi and his team an insight into the factors behind divergent feed efficiencies in growing cattle.

Many of these techniques had not been previously used for tackling feed efficiency. For example, the use of easily obtainable samples of hair for the determination of nitrogen isotopic discrimination, and the exploration of plasma as a biomarker for feed efficiency using NIRS, have not been previously explored. Similarly, the methods used to quantify vitamins B_2 and B_6 were developed specifically for this project, providing novel information that Morgavi hopes can be adopted in future research.

'This project also has real practical applications,' says Morgavi. 'For example we are located in a mountainous area of France, where cattle farmers face major challenges from land constraints. Feeding our cattle with as much forage as possible makes sense.'

Tackling methane emissions

The project also explored the possibility of modulating the microbial community in ruminants early in life, in order to reduce methane emissions. 'Every mammal acquires microbiota when it is born; in fact colonisation begins immediately after and perhaps during birth,' explains Marie Curie Fellowship recipient Sarah Meale, who worked with Morgavi at INRA on this project. 'We wanted to know what would happen if we modified this early implantation, and whether we could reduce the carbon footprint of cattle.'

While the team is cautiously optimistic that this approach might yield positive results, Morgavi stresses that it is too early to draw conclusions. 'We know that the microbial community can be modulated early in life, but these tests will now have to be repeated again and again,' he says.

Project	**Digestive and nutritional indicators of feed efficiency in cattle fed forage-based diets**
Coordinated by	Institut National de la Recherche Agronomique (INRA), France
Funded under	Horizon 2020-MSCA-IF
Project website	N/A

Culturing the uncultured marine microorganisms with innovative biotechnology

Microorganisms are running our ecosystems and could offer us bottomless novel antibiotics and alternative energy sources. Our understanding of marine microbial diversity applications is limited and the EU-funded MACUMBA project has dredged up revolutionary biotechnological methods to finally isolate and cultivate a treasure chest of uncultured marine microorganisms.

European societies face global challenges from climate change, increasing CO_2 levels, to energy and food crises that increasingly affect our everyday life. 70 % of our planet is covered in an ocean full of unlimited and hidden diverse microbes. These microorganisms provide food for higher organisms such as fish and are also responsible for the 'carbon pump' which counteracts the increase of CO_2 levels and global warming.

With 99.9 % of the total number of species remaining unknown and 90 % of oceanic biomass being microscopic, it is crucial that we start to understand how these processes work in the microbiome. Within this context, MACUMBA's (Marine microorganisms: Cultivation methods for improving their biotechnological applications) core mission has been to develop innovative techniques to isolate and culture the various marine microbial microorganisms – bacteria, archaea and eukarya. 'There has been little attention in microbiological education in isolating and culturing microorganisms and MACUMBA was among other things designed to revive these skills and the importance of keeping microorganisms in culture collections and biological resources for society,' shares MACUMBA project coordinator Professor Lucas Stal.

Using biotechnology to overcome culture changes

Being able to determine, mimic and sample the natural conditions of hidden and hitherto unknown microorganisms was a major challenge. The sea is not homogenous and entails many different environments.

To meet these challenges, MACUMBA decided to culture microorganisms as both pure and co-cultivation to find clues for new microorganisms, compounds and traits. In the past it was thought that microorganisms could be best understood as pure cultures by analysing nucleic acids (DNA and RNA).

However this thinking has now shifted as microorganisms do not live alone in nature but rather provide each other with essential growth factors. As a result, MACUMBA used a whole range of innovative techniques on marine microbiome. Optical tweezers were developed for the isolation of single cells out of a mixture of single cells as was a 'Survival Box' for collecting cyanobacteria to monitor their overall growth conditions, resilience and reaction to external cultivation strategies. 'We extracted DNA and RNA from isolated microorganisms and analysed their genome and transcriptome in order to find clues for specific traits.

There has been little attention in microbiological education in isolating and culturing microorganisms and MACUMBA was among other things designed to revive these skills and the importance of keeping microorganisms in culture collections and biological resources for society.

We also extracted DNA and RNA from co-cultures and compared these with the pure cultures to get a clue of the interactions,' underlines Professor Lucas Stal.

Preservation of these isolated organisms proved to be another major challenge and the team made up of 22 partners from 12 EU countries focused on cryo-preservation in the form of liquid nitrogen vapour (-196 °C), using various cryo-preservants (such DMSO, glycerol, methanol, and others) and various freezing protocols.

Moving forward from MACUMBA

The project officially ended in July 2016 with project partners uncovering thousands of biodiverse microorganisms such as the world's smallest aquatic bacterium and new culture methods and collections all over Europe.

Finding novel species and unravelling the mysteries of the ocean remains a huge challenge. Many more years of similar collaboration will be necessary to unlock the full potential of marine microbes as marketable products.

Nevertheless, Professor Lucas Stal who is retiring, believes that this marks the beginning of new era. MACUMBA's methods and techniques certainly now represent a significant first step towards making similar future wide-ranging research approaches that will sooner rather than later lead to pharmacological and biotechnological products.

Project	**Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications**
Coordinated by	Stichting NIOZ, Koninklijk Nederlands Instituut voor Onderzoek der Zee, the Netherlands
Funded under	FP7-KBBE
Project website	http://www.macumbaproject.eu/

Tapping microbe potential to tackle malnutrition

Ground-breaking results from an EU-funded initiative could one day lead to probiotic therapies to counter malnutrition and help strengthen scientifically proven probiotic claims on labels.

Fundamental lab work conducted by the MUTFLYGUTBACT project focused on a microbe that helps promote growth and digestive activity in both flies and mammals. The discoveries that have been made are highly relevant to the probiotic industry, which sells bacterial and yeast strains designed to promote health in humans and animals. The findings could also help address the pressing issue of global malnutrition, which affects some 150 million children worldwide.

If we can provide clinical evidence that this strain helps to buffer the effects of malnutrition in infants this would be very significant.

Understanding benefits of microbes

The starting point of this project was an attempt to understand in a broad sense the influence of microorganisms on the physiology of animals. Do microbes shape the physiology of animals, and if so, how?

While previous research has successfully established a link between intestinal microbes and digestive efficiency, characterising this impact on animal physiology has remained somewhat elusive. In order to address this, the MUTFLYGUTBACT project focused on a specific microbe in the gut of the common fruit fly.

'We wanted to characterise the impact of *Lactobacillus plantarum* in flies and then characterise the mechanisms underlying this effect,' explains project coordinator François Leulier from the Centre National de la Recherche Scientifique (CNRS) in France. 'This microbe is very efficient in promoting growth during postnatal development and in promoting digestive activity by allowing the animal to extract more nutrients from its diet.'

Interactions between *Lactobacillus plantarum* and digestive enzymes were analysed by reducing the amount of

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proteins given to the flies. Leulier and his team noticed that adding the bacteria effectively buffered the deleterious effect of undernutrition on fly development and postnatal growth.

'The next step was to translate these results to a mammalian model,' says Leulier. 'We used germ-free mice to show that adding the microbes has the same positive buffer effect against malnutrition. We are currently working with conventional animal models (i.e. not germ-free) and treating them daily with a strain of *Lactobacillus plantarum*. We are moving slowly but surely towards clinical trials in humans.'

Moving towards clinical trials

This fundamental research into the effects of *Lactobacillus plantarum* could be highly significant for industry as well as organisations involved in the promotion of global health. 'We are confident that this microbe has real clinical potential,' says Leulier. 'If we can provide clinical evidence that this strain helps to buffer the effects of malnutrition in infants this would be very significant.'

Leulier and his team are currently planning to apply for future grants and focus on the translational aspects of their findings. While much of this work is still at the pre-clinical stage, whilst patents related to some of the discoveries made through this project have already been submitted.

A key sector likely to benefit from this continued research is the probiotics industry, which could use these findings to support health claims more robustly in the future. Leulier points out that many companies have collections of bacterial strains in freezers, and could benefit greatly if they were able to conclusively prove a real functional health benefit.

'There is a big shift towards studying specific strains like *Lactobacillus plantarum* strains in order to more clearly establish clinical benefits,' says Leulier. 'We believe that our results will help to support some of the health claims that companies put forward. For me, one of the most satisfying things about this project has been starting from a fundamental question and then using a simple fly model to begin identifying bacterial strains that could be used to promote human health.'

Project	**Host-intestinal bacteria mutualism: 'Learning on the fly'**
Coordinated by	Centre National de la Recherche Scientifique (CNRS)
Funded under	FP7-ERC-IDEAS
Project website	N/A

Better biomarkers for health and nutrition

Measuring phenotypic flexibility – how humans rapidly alter their phenotype to cope with a changing environment – could prove a vital tool for creating the basis for a new generation of biomarkers to measure health. An EU-funded project used cutting-edge technologies to determine the role of biomarkers as a guide for better health, based on methods that go beyond measuring homeostasis concentration.

Diet, foods and food components are prime environmental factors affecting the transcriptome, proteome and metabolome. This life-long interaction largely defines an individual's health or disease state. The body's adaptive capacity to alterations in dietary conditions is called phenotypic flexibility and is key to the maintenance of overall homeostasis and consequently, health and healthy ageing.

Given that health can be considered as the ability to adapt to daily stressors, new biomarkers are needed to quantify the body's ability to adequately absorb a variety of perturbations and regain homeostasis. Consequently, biomarkers should not quantify homeostasis but rather the stress-response curve after such a perturbation, namely it is important to quantify the ability to adapt.

The EU-funded NUTRITECH project used cutting-edge technologies such as genomics, transcriptomics, proteomics, metabolomics and laser scanning cytometry to measure homeostasis and phenotypic flexibility.

Extending the concept of metabolic flexibility

To date nutrition research has largely been focused on determining the effects of nutrient and non-nutrient food components on gene and protein expression and metabolic outcomes. NUTRITECH built on the foundations of conventional nutrition research using cutting-edge analytical methods to comprehensively evaluate the diet-health relationship.

Researchers quantified the effect of diet on phenotypic flexibility, based on metabolic flexibility. NUTRITECH though extended the notion of flexibility to all underlying physiological processes and cell and genetic mechanisms involved in absorbing metabolic challenges and that are essential to maintaining optimal metabolic and inflammatory health. 'Biomarkers thus report on the mechanisms that retain optimal stress responses after a metabolic/caloric challenge,' says project coordinator Ben van Ommen.

The aim was to show that using caloric restriction as an intervention should demonstrate a change in phenotypic flexibility. Based on this, the researchers conducted an extensive dietary intervention study on 72 volunteers that aimed to reduce the food intake by 20 % over a 12-week period. Phenotypic flexibility was measured by applying a dietary challenge containing high levels of carbohydrates, fat and proteins. The response profiles of numerous classical and novel biomarkers were then assessed over a period of several hours after the challenge was consumed.

Biomarkers report on the mechanisms that retain optimal stress responses after a metabolic/caloric challenge.

Scientists obtained important insight into the flexibility status of the intestines, pancreas, liver, certain muscles and adipose tissue by applying a standard glucose tolerance test and quantifying the response of insulin, and hundreds of other metabolites and proteins.

The project also focused on the maintenance of DNA integrity to oxidative stress, developing new methods to measure the maintenance of genome integrity, which examined how well participants can safeguard the DNA in the genome. A laser scanning cytometry protocol for scoring micronuclei was also created to identify damage to chromosomes and the mitotic spindle apparatus.

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Personalised nutrition approach

Volunteers appeared to respond differently to the same treatment due to differences in genetic background, body composition and eating patterns. For example, magnetic resonance imaging data showed that fat content and distribution differentiated amongst male and female participants. This allowed NUTRITECH to accurately measure how people react differently to the same dietary changes using a biochemical map of homeostasis. 'Results suggest that it is important to get up close and personal when substantiating health effects,' says Mr Ommen.

The phenotypic flexibility biomarkers have raised considerable interest in the food industry. NUTRITECH also gave rise to the launch of another project that involved five renowned food companies. Both projects aim to exploit the outcomes of the NUTRITECH intervention study by adopting a more personalised nutrition approach in an upcoming intervention study.

Project	**Application of new technologies and methods in nutrition research – the example of phenotypic flexibility**
Coordinated by	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO), the Netherlands
Funded under	FP7-KBBE
Project website	http://www.nutritech.nl/nutritech

New drugs from the ocean depths

Despite containing huge potential for the harvesting of unique chemical compounds, the world's oceans remain under-explored. The EU-funded PHARMASEA project sets out to release some of these tremendous bioresources by tackling barriers to their exploitation.

The world's oceans are home to more living organisms, especially microorganisms, than any other environment on the planet. However, successful exploitation of this exceptional resource has been limited, despite its enormous potential for harvesting unique biological compounds with benefits for all of humanity.

The purpose of the project 'Increasing value and flow in the marine biodiscovery pipeline' (PHARMASEA) is to discover new marine bacteria and novel compounds. In addition, the consortium will improve the effectiveness of the biodiscovery pipeline, thereby making bioresources attractive to industry.

Project partners will achieve their aim by processing genetic material from in-house collections of microbial strains, while seeking out new strains from extreme marine environments. These will be used to develop new products for the pharmaceutical, food and nutrition, and cosmetic sectors. The main disease areas to be addressed are microbial infection, diseases of the central nervous system and inflammation.

We chose deep seas and the polar regions especially, believing they generate unique biodiversity giving rise to unusually biologically active chemistry. This has proven to be an accurate hypothesis.

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By sampling some of the deepest parts of the ocean floor, PHARMASEA hopes to utilise microorganisms that are new to science. Deep ocean trenches are 'islands of diversity' with a unique chemistry and where evolution may have developed differently. Because so little of this extreme environment has been explored, PHARMASEA is breaking new ground.

The final goal of this exciting initiative is to produce two compounds from newly discovered microorganisms on a large scale. These compounds will be taken forward to pre-clinical evaluation for treating infection or diseases of the central nervous system. Other outputs will include a validated model biodiscovery pipeline, new chemometric tools, data mining techniques and data sets.

Furthermore, PHARMASEA will develop solutions to improving access to marine bioresources in different habitats and jurisdictions, and equitable sharing of the benefits from their exploitation. A toolbox will also be created to navigate legal aspects in order to simplify and speed up the marine biodiscovery process.

PHARMASEA is not just expected to have a major impact on scientific progress, it will also influence policy and enhance economic performance. It will also strengthen the competiveness of the European Research Area (ERA) by increasing technological know-how through interdisciplinary and international cooperation, thereby contributing to Europe's knowledge-based economy.

Project	**Increasing Value and Flow in the Marine Biodiscovery Pipeline**
Coordinated by	University of Leuven, Belgium
Funded under	FP7-KBBE
Project website	http://www.pharma-sea.eu/

Symbiosis-specific peptides – A double-edged sword to serve humanity

A steadily growing global population has brought with it concerns about coping with food security and curbing the rising incidence of drug resistance in bacteria. EU researchers took inspiration from the mutualistic symbiotic process between the Medicago genus of flowering plants and nitrogen-fixing bacteria to find solutions.

Large-scale biological nitrogen fixation would mitigate the effects of the indiscriminate use of fertilisers. In addition, the ability to manipulate bacterial cell differentiation could be used to develop novel antibiotics without running the risk drug-resistant bacterial strains emerging.

The EU-funded SYM-BIOTICS project studied the biological nitrogen fixation process to find eco-friendly solutions for increasing agricultural production and developing novel antibiotics. Biological nitrogen fixation in the Inverted Repeat-Lacking Clade (IRLC) legumes involves nodule-specific cysteine-rich (NCR) peptides that modify the properties of endosymbiont rhizobium bacteria. This host-directed differentiation of bacteria is a multistep process that culminates in the development of large polyploidy non-cultivable nitrogen-fixing bacteroids.

NCR peptides – a win-win for us

Researchers developed cutting-edge protocols and studied the role of polyploidy and irreversible differentiation in nitrogen-fixing

bacteroids to determine the role of NCRs in this process. In 10 selected IRLC legumes, they identified the NCR gene repertoire varying from a few to hundreds of genes and found a correlation between bacteroid morphology and the complexity of the NCR peptide families.

Out of over 700 NCR peptides in Medicago truncatula, scientists identified the presence of around 150 peptides in these bacteroids, indicating their high stability. Several of these peptides, such as NCR247 and NCR169 proved to be critical for successful symbiosis, indicating their unique, irreplaceable functions.

'The 700 NCRs arise from gene duplication events with some peptides having redundant functions while others are essential. Among these, several are specific for Medicago species like NCR169, while others are major players in the bacterial differential process,' Prof. Eva Kondorosi, the principal investigator of this project explains.


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Production of 800 plant peptides in a single symbiotic cell is a miracle. These peptides represent a gold mine of novel biological activities, which provoke an amazing differentiation of endosymbionts involving a similar mechanism of differentiation as their eukaryotic host cell.

An interesting development is that some of these NCR peptides, more specifically the cationic ones, have antimicrobial properties. Prof. Kondorosi points out: 'For example, NCR247 inhibits bacterial cell division and its interaction with many bacterial proteins alters the bacterium's physiology with multiple mechanisms. This peptide *in vitro* effectively kills many pathogenic bacteria and fungi without cytotoxicity to human cells. In addition, attacking the microorganisms using many targets and pathways reduces the risk of resistance developing. These properties are thus ideal for the development of new antibiotics.'

Scientists tested around 40 synthetic NCR peptides on Gram-negative and Gram-positive bacteria, as well as fungi, with good results. Besides being non-toxic to human and animal cell lines, their performance was comparable to the commercially available antifungal amphotericin B. Furthermore, their multi-targeted mechanism of action minimises the emergence of drug-resistance.

Plant resources, a boon for mankind

This unprecedented study of how host organisms such as plants utilise NCR peptides to manipulate and modify microbes has provided novel insights with wide-ranging applications. Besides improving nitrogen fixation, this information could be used to develop resources for wastewater treatment, hydrogen production and environmental remediation.

'We have excellent candidates for NCR peptide based antibiotics. However, our work is not ended. Due to the cost of chemical peptide synthesis, we need to reduce the size of peptides. Testing the combined action of antimicrobial peptides could prove to be the key to decreasing their minimal inhibitory concentration level,' Prof. Kondorosi concludes.

Finally, a patent has been filed regarding the antimicrobial effects of NCR peptides. Thanks to the efforts of SYMBIOTICS affordable and effective peptide-based antibiotics could now be available sooner than before.

Project	**Dual exploitation of natural plant strategies in agriculture and public health: enhancing nitrogen-fixation and surmounting microbial infections**
Coordinated by	Magyar Tudományos Akadémia Szegedi Biológiai Kutatóközpont, Hungary Prof. Eva Kondorosi
Funded under	FP7-IDEAS-ERC
Project website	N/A

Understanding human immune responses

Researchers with the EU-funded SYSBIOFUN project have set out to describe the factors that influence our immune responses to fungal and bacterial infections.

Infections have shaped the human immune system, with genetic variability contributing to differing levels of susceptibility to infections. For example, fungi such as *Candida albicans* are ubiquitous colonisers of human skin and mucosa. Fungal pathogens like this invade the host when the host's defence is diminished, and the combination of fungal and bacterial colonisation modulates mucosal and systemic immune responses.

Despite its commonality, little is known about the complex interaction between fungal and bacterial colonisation, or about how this interaction affects the host genome and its immune system. The EU-funded SYSBIOFUN project aimed to describe the host and environmental factors that influence immune responses in general and, in particular, antifungal host defence. To achieve this, project researchers first set out to describe normal human immune responses in healthy volunteers and, second, to identify the defects that lead to infection in patients.

Never before has a study approached the topic of anti-fungal immune responses in such a comprehensive way.

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An extensive study

The SYSBIOFUN project is one of the very few – and most extensive – studies on the system biology of human immune responses. 'Never before has a study approached the topic of anti-fungal immune responses in such a comprehensive way,' says project coordinator Mihai Netea. 'By recruiting large groups of healthy volunteers and patients and using deep immunological phenotyping, we successfully identified completely new aspects of antifungal immunity.'

Specifically, the project used a systems biology approach to describe several crucial aspects that characterise immune responses in antifungal immunity. This included, for example, a description of the role of both the genetic and non-genetic factors that influence human host responses. The project also identified the role that microbiome has on influencing human immune responses and novel susceptibility factors for fungal infections.

Laying the groundwork for new therapies

The project successfully mapped the landscape of interaction between the fungal colonisation, bacterial flora (the microbiome) and the genetic and immunological make-up of the host. 'Our breakthrough was the identification of the variability of host factors and the pinpointing of the importance of gender and age in treating patients with infections – all of which are essential to understanding how an individual will react to a disease,' says Netea. 'This information allows us to identify who is more likely to develop a severe fungal infection and in which patients these infections will be most severe.' Netea adds that researchers now have a clear translational and clinical basis from which they can propose new approaches to therapy.

Based on the results obtained in the SYSBIOFUN project, researchers now intend to develop personalised immunotherapy for treating severe infections, meaning treating each patient according to their unique background and particularities, for which a proof-of-principle clinical trial is already underway. Furthermore, a new public-private partnership with the EU has been launched to assist with developing new pharmacological agents for treating sepsis patients.

Project	**The interaction landscape between microbial colonization and functional genome of the host: a systems biology approach in fungal infections**
Coordinated by	Stichting Katholieke Universiteit, the Netherlands
Funded under	FP7-IDEAS-ERC
Project website	N/A

Analysing microbial influence in colorectal cancer

EU-funded researchers have applied novel analytical methods to shed new light on how microbes found in the mouth and gut might influence the onset of colorectal cancer. These advances could eventually lead to more effective diagnostic tools.

While the human microbiome plays an essential role in modulating human health and disease, the actual biological mechanisms behind this are often unclear. The EU-funded TRANSVIVOME project has confirmed that co-colonisation of many bacterial species in both the mouth and gut could have implications for understanding the pathology of certain diseases including colorectal cancer (CRC).

'The association between gut bacteria and CRC development has been reported in several large scale studies, which have often found oral microbes enriched in patient stool samples,'

This discovery opens up the possibility of developing newnovel types of diagnostic colorectal cancer tests.

explains TRANSVIVOME project coordinator Dr Peer Bork from the European Molecular Biology Laboratory in Heidelberg, Germany. 'This discovery opens up the possibility of developing new novel types of diagnostic CRC tests.'

To date however, scientists have been unclear about the underlying biology; it is not known for example if these bacteria originate from the mouth of the patient, or already exist in the gut, but bloom in disease or else come from the environment.

Analytical breakthroughs

This was the starting point for the TRANSVIVOME project. Bork and his team set about developing and testing new methods to better understand how bacterial transmission occurs; whether oral strains are implicated in CRC development; and whether these bacteria are alive.

A key achievement was developing a culture-independent method capable of determining whether bacteria are alive in samples. Using DNA and RNA data, the project found that it was possible to pin-point which bacteria species were present in any given sample, and if they were expressing these genes.

'Since RNA degrades very fast (within hours) it can be inferred that if the RNA of a species is detected, then it is alive when the sample was taken,' explains Bork. 'This is a significant methodological advancement as only living cells have the potential to transmit and colonise different environments.'

The project also shone a light on the extent to which bacterial transmission between the mouth and gut occurs. Fine scale differences in the DNA sequences of over 1 700 bacterial species were determined from oral and gut samples isolated from people across the globe (Fiji, China, France, America, Luxembourg and Germany). 'To our surprise and against common knowledge, we found that a large number of the bacteria in the mouth can indeed colonise the gut,' says Bork. 'Other species found to be in both the mouth and gut appeared to have distinct versions (strains) that are specialised to each body site.'

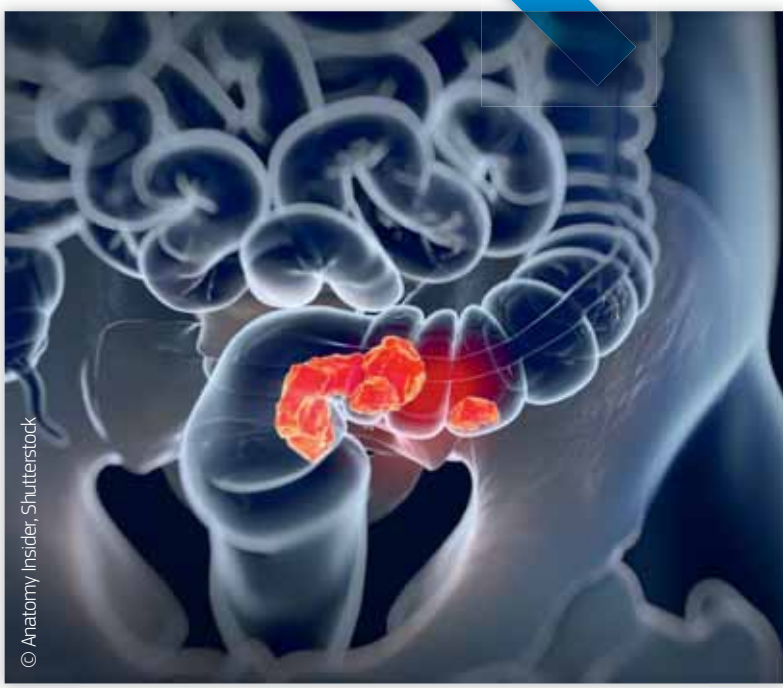
Future diagnostic potential

Analytical methods pioneered and tested during the TRANSVIVOME project could eventually lead to the development of viable diagnostic tools for CRC and other diseases. The ability to distinguish between living bacteria, which are, and are not, transmitted between the oral cavity and the gut, has the potential to improve disease control studies on stool samples considerably.

'With these methods, we can also now test whether specific strains of a given bacterial species can "leak" from the mouth to the gut,' explains Bork. 'If this is the case, there are new possibilities for developing CRC risk assessments. Determining which drivers of CRC development originate from the patient's mouth may even allow for therapeutics to be targeted at this community.'

In all, the project provides a strong starting point for follow-up studies on diagnosing disease. 'Although we were unable to collect a large enough cohort of CRC and case control samples during this project's timespan, we have since launched another project in order to obtain the data we need to further develop CRC diagnostic markers,' adds Bork.

Project	**Microbial biogeography of the gastrointestinal tract: Towards a better understanding of the drivers of oral and colorectal cancer development**
Coordinated by	European Molecular Biology Laboratory, Germany
Funded under	Horizon 2020-MSCA-IF
Project website	N/A



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