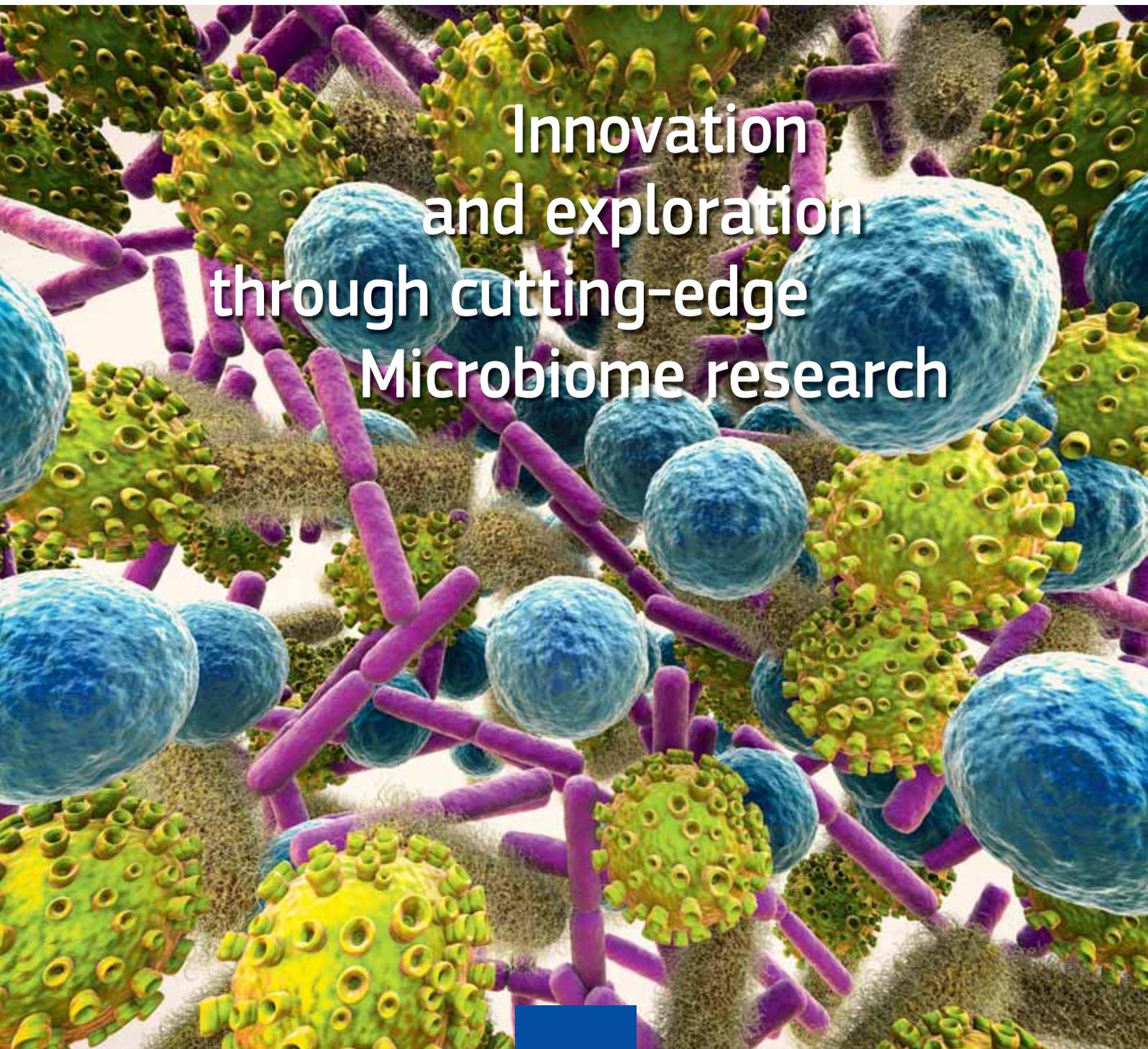




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RESULTS PACK

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 scientific 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 offers 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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Understanding the role of bacterial symbionts in ants

To learn more about how bacterial symbionts impact the social evolution of ants, researchers with the EU-funded AMIABLE project conducted a uniquely interdisciplinary study.

When it comes to digesting food and staying healthy, most animals depend on bacterial symbionts. This is particularly true for farming societies that produce their own food, such as most humans and some ants. In fact, we can learn a lot about the bacterial symbionts that humans depend on by researching fungus-growing ants. Next to humans, fungus-growing ants have realised large and highly complex animal societies – societies that depend on the services of multiple bacterial symbionts.

To learn more about the impact this dependence has on the social evolution of ants, researchers with the EU-funded AMIABLE project conducted a uniquely interdisciplinary study of microbial symbiosis.

‘Our focus was on three types of gut bacteria abundant in fungus-growing ants that we recently discovered,’ explains project coordinator Professor Jacobus Jan Boomsma. ‘These bacteria appear to function as nutritional mutualists in this complex symbiosis that has become one of the best-studied model systems of social evolution.’



The AMIABLE project showed that identifying the mechanisms of bacterial acquisition and loss is necessary to fully understand the putative mutualistic functions of microbial gut symbionts.

Digging deeper

AMIABLE’s research builds on earlier studies conducted by the Centre for Social Evolution (CSE) at the University of Copenhagen, which shed light on the composition of bacterial symbiont communities in the guts of attine fungus-growing

ants. These studies showed that the microbial gut communities of the most highly-evolved fungus-growing ant lineage, the leaf-cutting ants, is dominated by just a few abundant types of bacteria. AMIABLE took this finding further by conducting transmission electron microscopy studies to show that each of these symbiotic bacteria has structural adaptations for life within the ant hosts, either intracellularly, extracellularly or a combination of both.

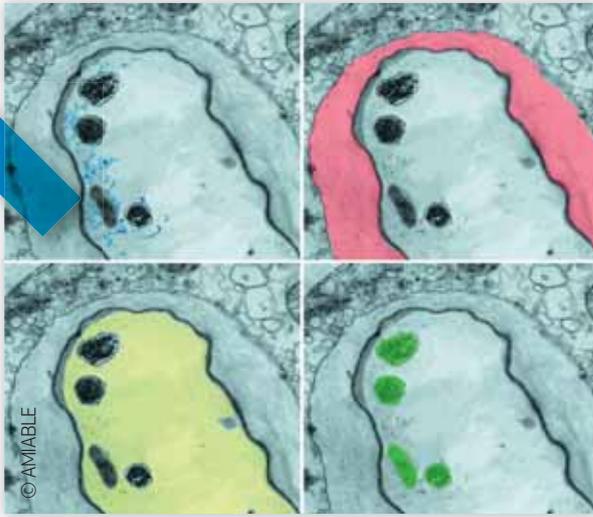
One of the project’s most interesting findings came from its analysis of dynamic changes in the presence and abundance of gut bacteria during larval development and pupation. ‘From this analysis, we could reconstruct the transmission routes of bacterial symbionts from the egg to the adult stage, both in *Acromyrmex* and *Atta* leaf-cutting ants,’ says Boomsma.

What researchers found was that the overall bacterial density remains similar across the development stages in *Acromyrmex* ants. However, the larvae and pupae of *Atta* ants have a negligible prevalence of bacteria, while adult workers often have many. This suggests that bacterial gut symbionts of *Atta* are not actively maintained before the adult stage. ‘These results show that transmission pathways for bacterial symbionts can be very different between developmental stages and between sister genera,’ explains Boomsma. ‘The AMIABLE project showed that identifying the mechanisms of bacterial acquisition and loss is necessary to fully understand the putative mutualistic functions of microbial gut symbionts.’

Numerous benefits

According to Boomsma, AMIABLE’s work may help Europe meet both its environmental and food safety goals by reducing the use of harmful pesticides. ‘As many arthropod pests and vectors of animal/human diseases live in association with bacteria, increasing our knowledge on symbiont-host interactions facilitates the future development and use of environmentally friendly insect control solutions,’ he says.

Leaf-cutting ants are major agricultural pests in neotropical America. For example, in Latin America they are responsible for



destroying millions of euros worth of coffee, cacao and sugar cane crops, which has a direct impact on European consumers. 'Being able to disrupt bacterial symbionts could result in a new approach for controlling these ants in an environmentally friendly way, thus providing economic benefits for both producers and consumers worldwide,' concludes Boomsma.

| | |
|-----------------|---|
| Project | Advanced Microscopy of Attine ant Bacterial Endosymbionts |
| Coordinated by | Copenhagen University, Denmark |
| Funded under | Horizon 2020-MSCA-IF |
| Project website | N/A |

The dangers of using wastewater for urban agriculture

Researchers with the EU-funded ARBUATEM project are raising awareness about the dangers of using wastewater for urban agriculture in low and middle-income countries.



By measuring the potential of wastewater used in urban agriculture to disseminate antibacterial resistance in LMICs, we'll be able to better protect our food chains.

Nearly 20 million hectares of arable lands around the world are irrigated with wastewaters, giving rise to 10 % of the world population's food production. Yet despite this significant use, almost nothing is known about the presence, evolution or dissemination of antibiotic resistant bacteria and genes – or their possible transmission to humans and animals via the food chain.

To fill this knowledge gap, researchers with the EU-funded ARBUATEM project set out to raise awareness about the dangers of using wastewater for urban agriculture in low and

middle-income countries (LMICs). 'The goal of the project is to thoroughly investigate the role of the environment and wastewater as a source for the emergence and spread of antimicrobial resistance in two African countries,' says project coordinator Laura Piddock. 'By measuring the potential of wastewater used in urban agriculture to disseminate antibacterial resistance in LMICs, we'll be able to better protect our food chains.'

Understanding the resistance

The project aims to connect agriculture, the environment and health. In LMICs, urban agriculture has been developed by urban farmers to supply food to the city inhabitants. Due to water scarcity and cost, urban farmers generally use untreated wastewater irrigation.

In three different cities, researchers randomly collected wastewater samples used for urban agriculture from canals near agricultural fields. The three cities were Ouagadougou, Burkina Faso, and Ngaoundere and Yaounde in Cameroon.



© ARBUATEM

‘Using state-of-the-art metagenomic DNA sequencing and bioinformatic pipelines, we analysed the presence of bacteria and drug-resistant genes found in the wastewater,’ explains Piddock. Along with DNA sequencing, ARBUATEM researchers also used analytical chemistry, molecular biology, metagenomic approaches and computational biology to characterise antibiotic resistant bacterial community structures and antibiotic resistant genes (ARGs) in untreated wastewaters and the corresponding irrigated agricultural sites.

‘The new scientific data arising from this project will provide new information on factors that drive resistance,’ says Piddock. ‘We can then work on minimising these factors by developing strategies to prevent further spread of antibiotic resistant bacteria and ARGs worldwide.’

European leadership

Antimicrobial resistance (AMR) is a global threat that knows no borders. They disseminate very rapidly via the food chain, global travel and medical tourism. If not urgently addressed, drug resistance in LMICs will likely become a global issue, putting additional pressure on European healthcare facilities. ‘This project highlights Europe leadership in AMR research,’ says Piddock.

Although the project is ongoing, results have already been produced. ‘What we have found is that wastewaters harbour a wide

diversity of pathogenic bacteria, as well as antibiotic resistant genes,’ adds Piddock. ‘All the factors involved in antibacterial resistance were found in these wastewaters, thus revealing them to be reservoirs for the dissemination of antibiotic resistance.’ According to Piddock, the solution is water sanitation and good public health systems.

As the project moves forward, Piddock says that the challenge will be to obtain funding to build on their findings. ‘It is critical to further investigate the dynamic of antimicrobial resistance in wastewater ecosystems and to further understand to what extent the exposed human and animal populations are affected,’ she says.

At the end of the project, Piddock says that all information will fall into the public domain, where it will help scientists and decision makers improve policies for tackling antibacterial resistance.

| | |
|-----------------|---|
| Project | Antibiotic resistant bacteria and genes, associated with urban agriculture in Low and Middle Income Countries: Ecological and medical perspectives |
| Coordinated by | University of Birmingham, United Kingdom |
| Funded under | Horizon 2020-MSCA-IF |
| Project website | N/A |

Innovative approach to more efficient and eco-friendly pig and poultry production

The pig and broiler chicken industries are key contributors to the European economy, and one of the main ways to achieve sustainability is by improving feed use efficiency (FE).

An EU initiative delivered strategies and tools for the European pig and poultry sectors to feed a growing population in a resourceful and environmentally-friendly manner.

'Improving FE, while also feeding the rising global population and minimising environmental impacts, are strategic targets for the livestock industry, policymakers and scientists alike,' says Dr Niamh O'Connell, project coordinator for the EU-funded project ECO-FCE. To achieve this, a better understanding is needed of the interactions between animal genetics, gut characteristics and feed attributes.

'We took a multidisciplinary approach to better understand the biological basis of FE in pigs and broiler chickens,' explains Dr O'Connell. 'We also examined nutritional and genetic methods to optimise it.'

Using cutting-edge technologies, ECO-FCE identified key factors driving good and poor FE in pigs and broiler chickens reared across different environments. Strong environmental effects were observed, and the work suggested that the physiological mechanisms underpinning FE differed in males and females. Interesting correlations were observed between FE and feeding behaviour, gut microbiota, macronutrient transport, endocrine regulation, osmohomeostasis and mitochondrial function.

Food efficiency and reduced ecological footprint

The development of *in vitro* and *in vivo* nutritional tools to improve FE and the ecological footprint was another key focus of ECO-FCE. *In vitro* 'ileal digestion' methods proved useful for the multiple screening of exogenous enzymes and additives when used in combination with different feed ingredients. According to Dr O'Connell, this approach has potential to be used as a screening tool in future research projects.

Feeding trials demonstrated that non-soya diets support pig and broiler chicken performance to levels comparable to when soya-based diets were offered, but that the effects of enzymes were variable. Interestingly, the use of certain plant-based additives appeared to reduce ammonia emissions in pigs – an area that warrants further investigation.



The industry-ready models and tools will assist various actors in understanding, measuring and managing the impact of management decisions on FCE and environmental impacts.

Specific symbiotics for *in ovo* use in broilers were also developed. They showed very promising results in terms of effects on performance-related parameters in later life.

Genome wide association studies and genomic selection approaches helped to provide links between genomic variation and FE variation, and related growth, carcass and meat quality traits. Techniques were applied to identify relevant molecular routes for the efficient transfer of nutrients to animal tissue. Such efforts led to the development of biomarkers that indicate the ability of both animals to efficiently convert nutrients.

Practical feed innovations with beneficial effects on pigs and chickens

Several hands-on tools and models were developed for policymakers, scientists, industry and the general public. A user-friendly, searchable web tool enables stakeholders to access ECO-FCE research findings on factors affecting FE in pigs and chickens. 'ECO-FCE was a large and complex project, and hopefully this tool makes it easier to quickly access findings of interest,' says Dr O'Connell.

Two calculators for pig and poultry systems were created to compute the output of nitrogen, phosphorus and greenhouse gases from the single input of performance data and dietary information. 'They represent useful tools for comparing the ecological impact of animal performance when using different diets,' she adds.



Lastly, at a genetic level, ECO FCE identified biomarkers of divergent FE and developed novel modelling methodologies to assist the breeding industries in better selecting for this trait.

'ECO-FCE introduced solutions to greatly advance knowledge of FCE for pigs and chickens,' concludes Dr O'Connell. 'The industry-ready models and tools will assist various actors in understanding, measuring and managing the impact of management decisions on FCE and environmental impacts.'

| | |
|-----------------|---|
| Project | A whole-systems approach to optimising feed efficiency and reducing the ecological footprint of monogastrics |
| Coordinated by | Queen's University Belfast, United Kingdom |
| Funded under | FP7-KBBE |
| Project website | http://www.eco-fce.eu/ |

Dietary fibres to boost the immune system

Understanding the way food ingredients interact with the body to improve health is vital to creating effective products. EU-funded scientists developed new methods to study the effects of dietary fibres on the human immune system that will help industry gain scientific proof of the claimed health benefits of their (newly) developed products.

Fibre-rich food is considered beneficial for human health as high intake of certain dietary fibres has already been linked to lowering blood cholesterol and glucose levels. In addition to the diverse wellness benefits, studies have shown that non-digestible polysaccharides (NPS) can also help maintain a healthy immune system.

Although the European Food Safety Authority (EFSA) has recognised the former health effects by approving claims for some specific fibres, further sound scientific evidence based on effects on healthy individuals is needed before approving claims that polysaccharide fibres can also boost the immune system.

Using the non-digestible polysaccharides (NPS) we produced biscuits, smoothies and cheese to see how NPS can be integrated in real fortified food products.

Researchers from industry and institutes collaborated in the EU-funded FIBEBIOTICS project in an effort to develop standardised practices, validate biomarkers and conduct clinical trials to help create products that are able to pass the EFSA assessment.





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 NFκB 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.

| | |
|-----------------|---|
| Project | Dietary Fibers supporting Gut and Immune Function – From polysaccharide compound to health claim |
| Coordinated by | Stichting Wageningen Research, the Netherlands |
| Funded under | FP7-KBBE |
| Project website | 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.

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.



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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.

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| 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

feed efficiency using NIRS, have not been previously explored. Similarly, the methods used to quantify vitamins B₂ and B₆ 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.'

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.

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.'

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

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.

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|-----------------|---|
| 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 CO₂ 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 CO₂ 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.



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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.

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|------------------------|---|
| 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.

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

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

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 post-natal 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



© Vincent Moncorge

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 post-natal 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.’

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| 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.

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.nutrittech.nl/nutrittech |

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

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.

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.



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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 competitiveness 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.

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|------------------------|---|
| 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.

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|-----------------|--|
| 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 new novel 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.

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| 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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RESULTS PACK

Published by

The Community Research and Development Information Service (CORDIS)
managed by the Publications Office of the European Union
2, rue Mercier
2985 Luxembourg
LUXEMBOURG

cordis@publications.europa.eu

Editorial coordination

Melinda KURZNE OPOCZKY

The research*eu Results Packs are published by the Community Research and Development Information Service (CORDIS) and managed by the Publications Office of the European Union. Content is prepared using information featured on the CORDIS website, as well as original material collected specifically for this publication.

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ISBN 978-92-78-41498-6 (printed version)

ISBN 978-92-78-41499-3 (ePUB)

ISBN 978-92-78-41500-6 (PDF)

ISSN 2529-3265 (printed version)

ISSN 2529-2919 (ePUB)

ISSN 2529-2919 (PDF)

doi:10.2830/051647 (printed version)

doi:10.2830/010428 (ePUB)

doi:10.2830/311977 (PDF)

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