

Agroscope Research Programme ‘Microbial Biodiversity’

Agroscope Research Programmes (ARPs) serve to selectively promote especially relevant and promising research areas through additional resources. They take account of the constantly growing importance of interdisciplinary research, and aim to strengthen the transfer of knowledge from basic research into problem- and application-oriented research, thereby achieving concrete practical benefit for the Swiss agriculture and food sector.

Programme Description

1. BRIEF DESCRIPTION: A World Full of Microorganisms



Figure. 1: The Earth with all its ecosystems, habitats and life which is adapted to them. Photographed from Apollo 17 on 7 December 1972 (Wikipedia).

Biodiversity encompasses the global diversity of ecosystems and all of the organisms living within them; moreover, it also involves the interactions within and between these systems. Microorganisms exhibit the greatest diversity of all living creatures, and play a fundamental role in all ecosystems. Since microorganisms – as their name indicates – are microscopically small, their study and description is highly challenging. We only know precisely what functions a very few species exercise – most functions are still unknown, or little investigated.

Over the last few years, new methods of genetic analysis have become established which not only allow the description of the totality of microorganisms in various ecosystems (the so-called ‘microbiome’), but have also enabled us to decode the genetic material (genome) of microbial strains, thereby providing us with initial indications of their functions.

The aim of the ‘Microbial Biodiversity’ ARP is to study and describe the microbiomes and functions of microorganisms in three ecosystems of relevance for the agriculture and food sector. These ecosystems are dealt with in three work packages (WPs) through the interdisciplinary cooperation of researchers from different Agroscope Institutes (see Chapter 7, CONTACTS). **Work package 1** deals with the soil microbiome, with its manifold functions in agriculture. **Work package 2** analyses the plant microbiome. Plants constitute a key group of agricultural products which interact with microorganisms in highly varied ways. This work package is intended to identify microorganisms which have a favourable influence on the plants, and which e.g. reduce infestation by pathogens. **Work package 3** analyses the microbiomes of fermented dairy products which are important Swiss agricultural commodities. An additional **fourth work package** establishes an Agroscope Network for Genomics and Bioinformatics (ANET-GB) meant to safeguard and expand methodological know-how for Agroscope research. ANET-GB aims to expand skills in molecular-genetics and diagnostics in the medium-term into further areas beyond the microbial context.

2. RESEARCH ISSUE: The microbial biodiversity of agriculturally important ecosystems is a major unknown

The immense value of biodiversity has been recognised for some time now: In 2008, the Swiss Parliament was prompted to mandate the development of a ‘Swiss Biodiversity Strategy’. The strategy

was adopted by the Federal Council on 25 April 2012 [1]. Nevertheless, it is precisely the most diverse of all groups – the microorganisms – which to date have attracted little to no attention in the ‘Swiss Biodiversity Strategy’.

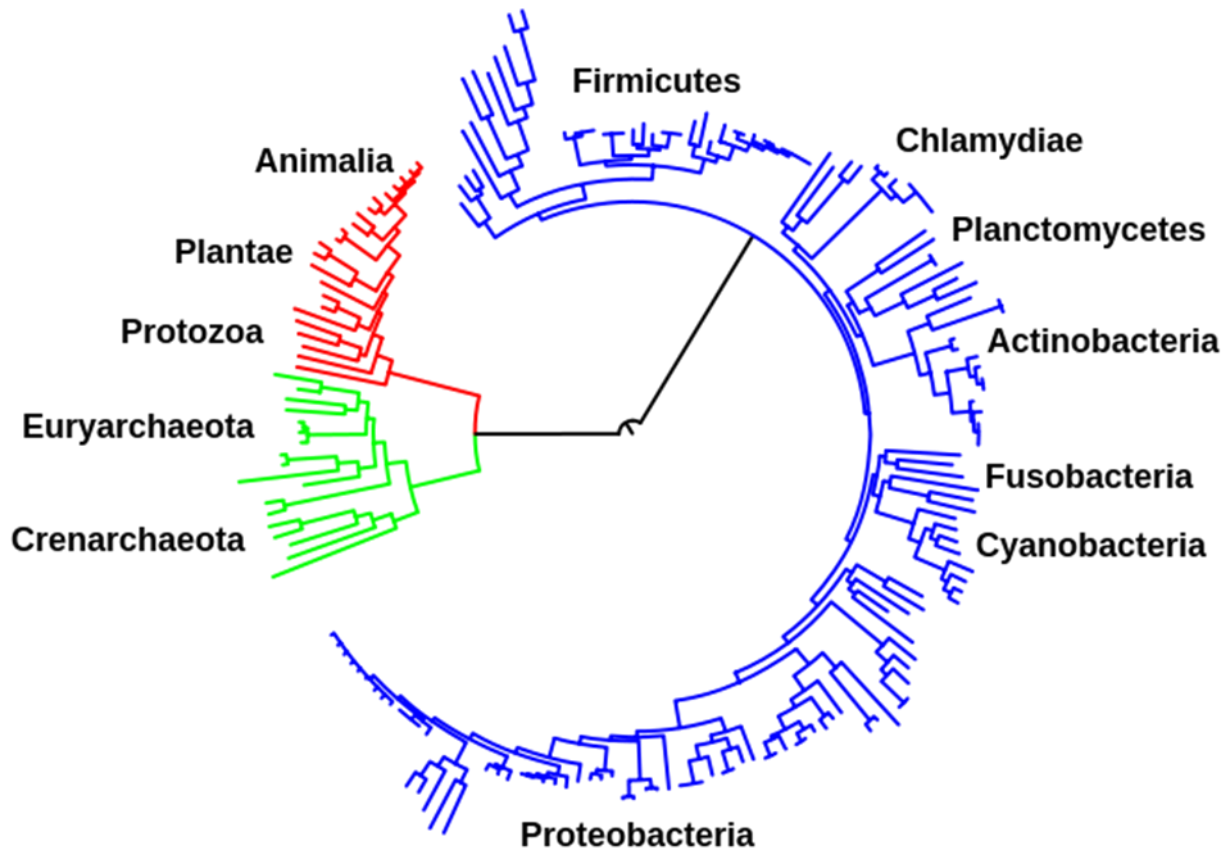


Figure 2: The Tree of Life [2]. The three domains into which all living organisms are classified are differentiated by colour: Bacteria (blue) and Archaea (green), which belong to the prokaryotes, and eukaryotes (red). This tree emphasises how the lion's share of species on earth consists of microorganisms rather than the higher organisms.

Only micrometres big and hence invisible to the naked human eye, microorganisms often escape our notice. On closer analysis, however, we realise that they are ubiquitous, and occur in high numbers. The human body, for instance, is colonised with around 10 times more bacteria than it possesses cells of its own (10^{13}), and 10 billion (10^{10}) microorganisms – around as many as there are people on the Earth – can live in a single gram of soil. Anything that occurs in such mind-bogglingly high numbers and has been present for so long on the Earth – the first microorganisms existed billions of years ago – must play a fundamental role on the planet. In fact, quite a few processes are known in which microorganisms play an important part. As primary decomposers, for example, they are responsible for the breakdown of organic material in the soil, as well as for the nutrient cycles which decisively influence plant growth. For millennia, microorganisms have been used in biotechnological processes in order to manufacture food or process raw agricultural products. The latest research findings have shown that microorganisms have a high level of biodiversity, and that we are not familiar with, let alone make use of, the vast majority of them. Exploring microbial biodiversity provides us with the requisite knowledge to make targeted use of naturally occurring microorganisms for a sustainable agricultural and food sector: for instance, specific soil fungi are already being used for the targeted and long-term control of certain insect pests in the field [3]. Plant-associated microorganisms can also be used e.g. to suppress diseases, stimulate growth, or increase yields through the mobilisation of nutrients [4]. In the cheese-manufacturing sector, it was shown that the use of a cheese smear composed of a large number of microorganisms with antagonistic activity led to an improvement in food safety (inhibition of *Listeria*) [5]. In order to achieve this long-term aim, however, we must first identify the most important players in the ecosystems of relevance for the agriculture and food sector, since changes in their composition, distribution or activity can have a decisive impact on these complex ecosystems.

Previously, there was a major impediment to the identification of the microorganisms of an ecosystem: With the classic cultivation methods, only a very small proportion (sometimes only about 1%) of the

microorganisms can be isolated and described. The overwhelming majority are deemed to be 'non-culturable', and thus for a long time eluded description. Only with the most recently developed molecular biological methods was it possible to isolate and describe the genes of the non-culturable microorganisms. These 'genetic fingerprints' have led to the discovery that microorganisms possess a heretofore unimagined biodiversity and occupy the most varied of habitats, where they play pivotal roles. In the field of human medicine, major advances have already been achieved in describing the human microbiome, and the role of the composition of the microbiome both in the development of diseases (e.g. bowel cancer, obesity, cardiovascular diseases) and in protection from diseases (e.g. by bacteria-induced diarrhoea) has been demonstrated [6]. Although initial results from various environmental systems are available, there is still a great deal of catching-up to do here. With the analytical methods available at present, however, the microbiomes of agri-food industry systems can also be investigated and the associated microbial communities determined, thereby bringing us one step closer to being able to make targeted use in agri-food sector systems of what is currently a major unknown.

3. PURPOSE AND AIMS: Investigation of the microbiomes of selected ecosystems in the agriculture and food sector

In its 'Microbial Biodiversity' research programme, Agroscope uses the latest technology to develop in-depth knowledge on microbial diversity in selected ecosystems of the agriculture and food sector. The focus here is on areas where microorganisms play a pivotal role, but where many details are still unknown. The research programme encompasses four main objectives that are each dealt with in separate work packages:

The 'Soil Microbiome' work package will research microbial biodiversity in various soils and systems that are representative for Switzerland and the agricultural sector, in order to better understand the interaction of soil type, soil use, and soil-dwelling microorganisms. The aim here is to be able to use this information for a more comprehensive assessment of, and improvement in, soil quality.



Figure 3: The DOK trial in Therwil, Basel-Landschaft canton – a cooperative venture between Agroscope and FiBL – has been comparing various conventional and organic cultivation systems since 1978. The effects of these long-term influences on the microbial communities in the soil are the subject of the current research, and are also being studied as part of the 'Microbial Biodiversity' ARP (Photo: Agroscope and FiBL).

The 'Plant Microbiome' work package studies the microbial biodiversity found on the surface of ryegrass, potato and apple plants. The impact of the natural microbiome on the protection of the plant from pests and pathogens as well as the microbiome's resistance to environmental and anthropogenic influences is analysed, with the aim of being able to selectively optimise the plant microbiome to inhibit pathogens.

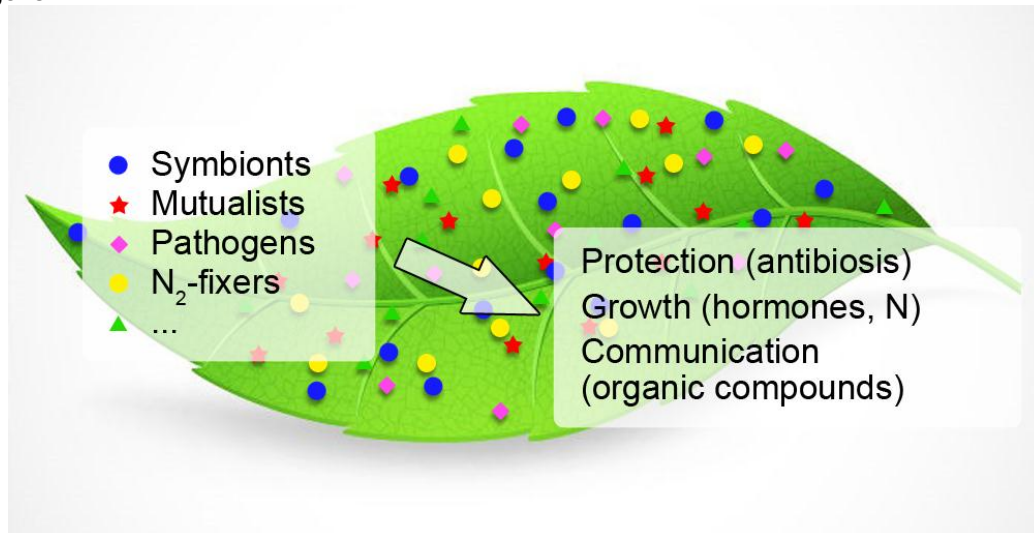


Figure 4: Plant-associated microorganisms and their functions in both plant and ecosystem (modified according to [7]).

The 'Microbiome of Fermented Foods' work package analyses the biodiversity represented in the Agroscope strain collection (Liebefeld, Wädenswil) and in the 'raw-milk cheese' ecosystem. It also investigates the microbial and biochemical cheese-ripening processes, as well as the interaction of the various bacteria species within the different types of raw-milk cheeses. The aim here is to be able to select appropriate bacteria for the manufacture of fermented dairy products so that milk processors can produce high-quality raw-milk cheeses at reduced cost and with less faulty fermentation, and consumers can be offered safe fermented dairy products with a wide range of flavours.

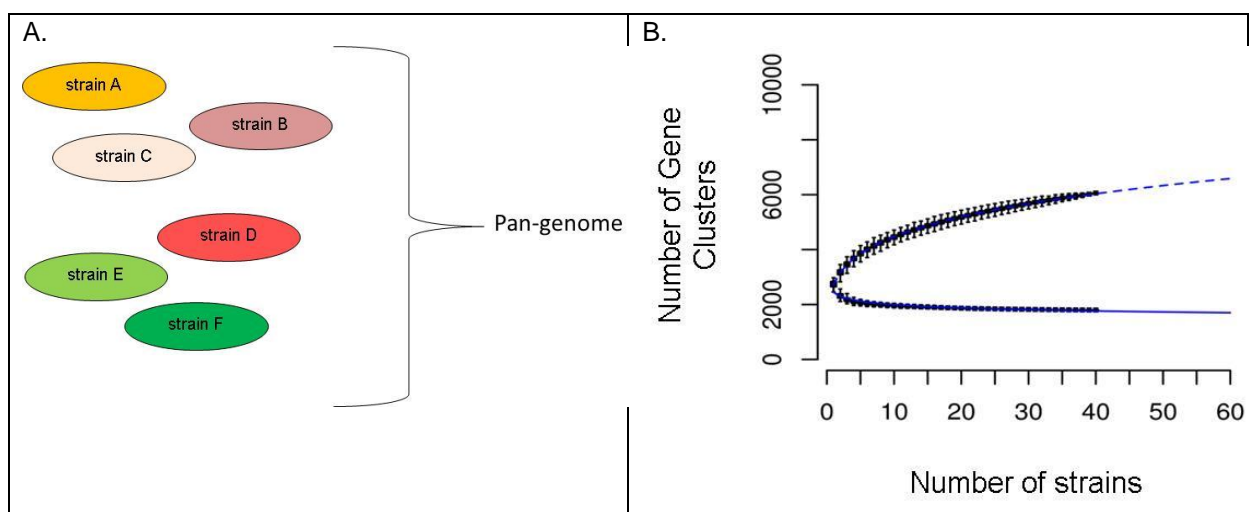


Figure 5. The *Lactobacillus casei* pangenome. A hypothesis says that bacteria distribute all of their genes over many strains (Figure 5A), and that genes are thus gained and lost over generations. The sequencing of different *L. casei* strains from the Agroscope strain collection has brought ever more new genes to light (Figure 5B). The dashed line shows the 'pangenome', i.e. the sum of all genes identified with each additional genome sequencing of an *L. casei* strain. The solid line represents the number of genes present in all strains. The studies confirm that the sum of all genes of this species is indeed distributed across individual strains. The importance of this genomic diversity in lactic acid bacteria for fermented foods is examined in detail in the present ARP.

The 'Genomics and Bioinformatics' work package involves the setting up of an Agroscope-wide expert network and the corresponding infrastructure. The aim is to make available to Agroscope projects the necessary infrastructure for data processing, and to be able to provide optimal support for planning, execution and data analysis within the framework of collaborative projects. In addition, novel approaches for the analysis and integration of data are developed.

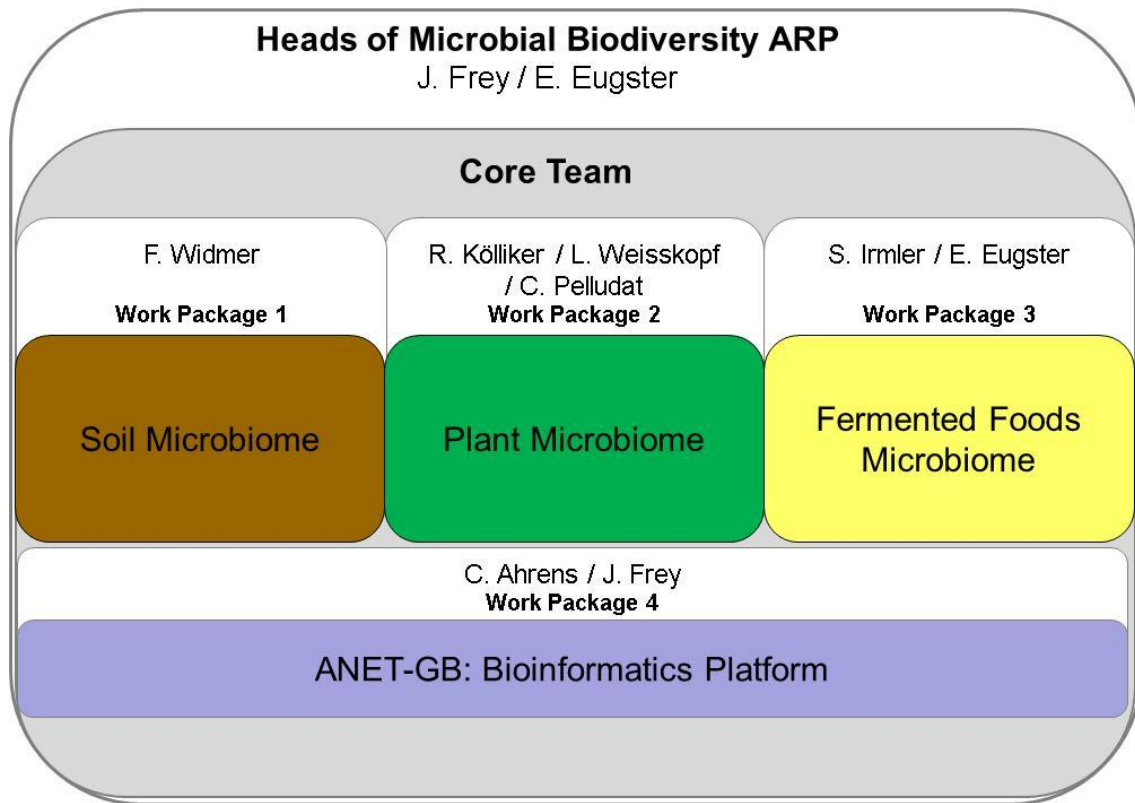


Figure 6: Organisational structure of the 'Microbial Biodiversity' ARP

4. METHODS: Genome sequencing – the method of choice

Thanks to rapid technological development over the past few years, diagnostic molecular biology is today capable of decoding entire genomes or identifying the microorganisms of an ecosystem in next to no time and at ever-lower costs. NGS (Next-Generation Sequencing) methods for decoding masses of genetic information enable us to determine the sequence of the individual building blocks of a DNA strand. This brings the major advance to research of being able to characterise microbial communities in the selected soil, plant and fermented-food systems at the genetic (DNA) level via genome sequencing (DNA-seq), as well as being able to identify and quantify the most important genes at the level of the active, expressed gene via transcriptome sequencing (RNA-seq). These methods are also used to decode the complexity of microbial communities (metagenomic sequencing), to describe their activities in the most varied ecosystems, and to recognise possible changes. This is the basis of being able to recognise, make targeted use of, and promote or control specific functions of microorganisms in different systems of the agriculture and food sector.

5. OUTLINE OF PROGRAMME CONTENT: The following stages are planned

The tasks of the Agroscope Research Programme 'Microbial Biodiversity' will focus initially on the establishment of NGS technology and on the analytical processes necessary for evaluating the generated data (from programme launch to autumn 2015 approx.). Sampling in the selected systems and the isolation of the DNA/RNA to be studied will also be a top priority in the first stage.

The second stage involves the identification and determination of the frequency and spread of individual microorganisms or genes, their influencing by changes in the environment, and the resultant impact on the ecosystems in question (autumn 2015 to approx. autumn 2017). The third stage of the research programme (beginning of 2016 to end of MikBioDiv ARP in mid-2018) involves the implementation of the knowledge acquired in initial applied trials, e.g. by investigating whether individual microorganisms or combinations of microorganisms provide suitable protection against microbial pests, or can bring about an improvement in food quality.

6. EXPECTED RESULTS: A series of further questions...

The data obtained in the 'Microbial Biodiversity' research programme will form the basis of our being able to use, promote and optimise naturally occurring microorganisms in a targeted fashion for a natural, sustainable agricultural sector, as well as for high-quality, safe agricultural products. The findings on the biodiversity of the selected ecosystems will serve both Agroscope and other interested partners as a basis for exploring microbial biodiversity in further systems, such as e.g. in animal and plant research, entomology or nematology. With this research, Agroscope directly combines cutting-edge technology with the requirements of a modern, sustainable and environmentally friendly agricultural sector.

7. CONTACTS: The most important contact persons

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IPS = Institute for Plant-Production Sciences; IFS = Institute for Food Sciences; ISS = Institute for Sustainability Sciences.

8. REFERENCES

[1] www.sib.admin.ch/de/biodiversitaetskonvention/nationale-umsetzung/nationale-biodiversitaetsstrategie/index.html

[2] Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P (2006). "Toward Automatic Reconstruction of a Highly Resolved Tree of Life", *Science*, 311: 1283-1287.
DOI:10.1126/science.1123061

[3] Enkerli J, Widmer F, Keller S (2004). Long-term persistence of *Beauveria brogniartii* strains applied as biocontrol agents against European cockchafer larvae in Switzerland. *Biological Control* 29:115-123

[4] Berg G, Grube M, Schlöter M, Smalla K. Unraveling the plant microbiome: looking back and future perspectives. 2014. *Frontiers in Microbiology*, 5:148.

[5] Roth E. Control of *Listeria* contamination on the surface of semi-hard cheeses by natural smear ecosystems and protective cultures, ETH Dissertation No. 18644, Zurich 2009

[6] Cho I & Blaser MJ. The human microbiome: at the interface of health and disease. 2012. *Nature Reviews Genetics*, 13:260-270.

[7] Penuelas J & Terradas J. The foliar microbiome. 2014. *Trends in Plant Science*, 19:278-280.