

Final Meeting Agroscope Research Program Microbial Bio Diversity

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Abstract Collection

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1 Meeting Program

Opening: 08:30 – 08:45 Jürg E. Frey Agroscope

Agroscope Research Program Microbial Biodiversity – An Overview

Keynote: 08:45 – 09:25 Julia Vorholt ETHZ

The leaf microbiota: disassembling and rebuilding to explore plant microbe interactions

09:25 Adithi Varadarajan

Comparative genomics of 9 *Pseudomonas* strains isolated from the native potato microbiome to explore their varying antagonistic activity against late blight

09:50 Andrea Braun Kiewnick

Insights into the apple flower microbiome: First data on effects of fungicide application and different varieties

10:15 – 10:45 Break / Coffee

10:45 Aaron Fox

BIOINVENT: A Pan-European inventory of grassland soil microbial biodiversity and its functional properties

11:10 Johanna Mayerhofer

Relation of soil properties, land use types and soil microbial communities in a nation-wide survey

11:35 Florian Gschwend

Metabarcoding for a poly-phasic soil quality assessment

12:00 – 13:00 Lunch

13.00 – 13:45 Poster Session

Keynote: 13:45 – 14:25 Shinichi Sunagawa ETHZ

Microbiome composition, activity and variation profiling: tools and applications

14:25 Christian Ahrens

Towards utilizing microbiomes – applying functional genomics approaches to move from genomes towards functions

14:50 Vincent Somerville

Studying low complex metagenomes by whole genome sequencing

15:15 – 15:45 Break / Coffee

15:45 Aline Moser

The diversity of *Lactobacillus helveticus* in dairy products

16:10 Marco Meola

Microbial diversity in cheese and dairy products

16:35 – 17:00 Discussion

2 Agroscope Research Program (ARP) Microbial Biodiversity – An Overview

Jürg E. Frey

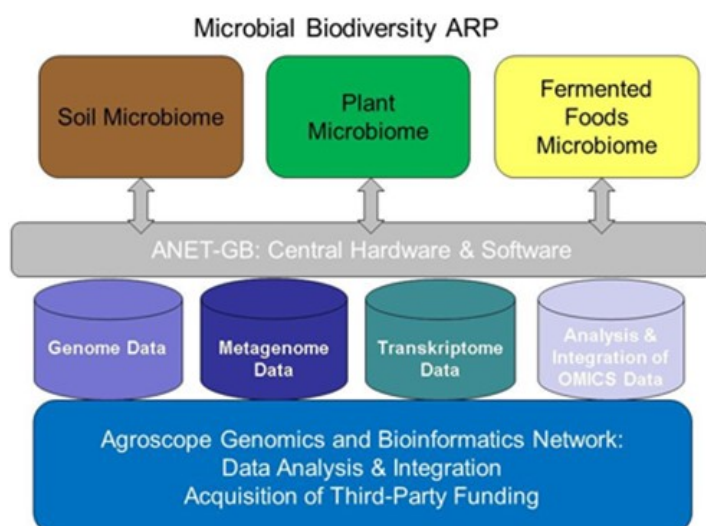
Agroscope, Molecular Diagnostics, Genomics & Bioinformatics, Wädenswil

Over the past decade, program research has become one of the cornerstones of Agroscope research. It enables to specifically promote agricultural research topics that were identified as highly relevant, that are innovative, interdisciplinary, and importantly, that further collaboration among different Agroscope research groups as well as with external researchers. The selection process for ARPs is highly competitive and requires acquisition of matching third-party funds.

The ARP Microbial Biodiversity was initiated by Elisabeth Eugster and Barbara Guggenbühl, then of the research group “Cultures, Biodiversity and Terroir” at Agroscope Liebefeld-Posieux ALP in Liebefeld. The overarching aim of this ARP is to identify and eventually further, utilize and apply possible beneficial effects of specific microbial communities for improved and sustainable agricultural and food production. This is to be achieved by studying and describing microbiomes, i.e. the totality of microorganisms in an ecosystem, as well as the functions of the most important players in three ecosystems that are highly relevant for the agriculture and food sector: soil, plant, and fermented dairy products.

These three ecosystems are studied in three Work Packages (WP). WP 1 deals with the soil microbiome, with its manifold functions in agriculture. WP 2 analyses the plant microbiome, with a main aim to identify microorganisms, which have a favorable influence on important crop plants, and which may contribute to reduce pathogen infestation. WP 3 analyses the microbiomes of fermented dairy products, which are important Swiss agricultural commodities.

A fourth WP involves the setting up of an Agroscope-wide expert network in genomics and bioinformatics and the corresponding infrastructure. The aim is to develop and apply state of the art methods to describe microbiomes and to functionally characterize relevant strains using functional genomics approaches. These core competences are applied in interdisciplinary collaboration with the ARP and beyond.



Internet: <http://www.agroscope.ch/mikrobielle-biodiversitaet>

During the past four years, technologies and methods have been implemented and developed in Agroscope projects and fundamental knowledge has been built that will promote and direct Agroscope research activities into the future.

3 Keynote – The leaf microbiota: disassembling and rebuilding to explore plant microbe interactions

Julia A. Vorholt

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The aerial parts of the plants, which are dominated by leaves, represent one of the largest terrestrial habitats for microorganisms. There is a growing interest to study commensal bacteria to elucidate their interactions with the plants, among each other and to learn how they withstand the hostile conditions of their habitat. A predominance of Proteobacteria, Actinobacteria and Bacteroidetes living in the phyllosphere of numerous plants has been revealed, while metagenomics and metaproteomics approaches gave insights into the general bacterial adaptation strategies to the phyllosphere. We conducted large-scale experiments to isolate *Arabidopsis thaliana* leaf bacteria as pure cultures. Individual plants as well as individual leaves were sampled at different European sites to determine their core leaf community and to establish a reference strain collection using flow cytometry and dilution series plating. After identifying approximately 3,000 isolates using a high-throughput DNA sequencing-based method we selected more than 200 representative strains belonging to 52 genera of the major phyllosphere phyla covering the majority of the culture-independent taxonomic diversity. Draft genomes of all selected isolates were generated. Recolonization experiments using synthetic communities in a gnotobiotic model system showed reproducible colonization patterns and represents a valuable starting point to identify mechanisms of community formation and function. Examination of plant responses to its microbiota revealed that the plant reacts differently to members of its natural phyllosphere microbiota. A subset of commensals increase expression of defense-related genes and thereby contribute to plant health and performance.

4 Comparative genomics of 9 *Pseudomonas* strains isolated from the native potato microbiome to explore their varying antagonistic activity against late blight

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In natural and agro-ecosystems, plants are largely colonized by diverse communities of microorganisms. Some of these microorganisms, alternatively called Plant Growth Promoting Bacteria (PGPB), show protection against various phytopathogens that otherwise suppress plant growth and negatively affect food production. Late blight, caused by the oomycete *Phytophthora infestans* remains a major threat for potato production worldwide. With the EU banning the use of copper-based fungicides, the identification of alternative biocontrol agents is urgently needed.

In this interdisciplinary collaboration, we build upon the results of Prof. Weisskopf's group who had isolated 200 bacterial strains from the phyllosphere and rhizosphere of field-grown potatoes and assessed their potential for biocontrol activity against *P. infestans* using a functional *in vitro* screening assay. The 9 most promising strains, all belonging to the genus *Pseudomonas*, were further characterized for their effects on mycelial growth, sporangia germination, and zoospore production and behavior using *in vitro* growth assays and a leaf disc assay to assess symptom development on plant tissue.

To enable the identification of the genomic determinants of their antagonistic activity, these 9 strains were subjected to whole genome sequencing. Using data from both Pacific Biosciences and Illumina we *de novo* assembled a complete genome for each strain using our modular genome assembly pipeline that integrates state of the art, open source software solutions. Genome annotation and comparative genomics analysis revealed the presence of genes coding for well-known antimicrobial and anti-oomycete compounds such as HCN, pyoverdine, pyrrolnitrin, phenazine and several cyclic lipopeptides, but not in all active strains. By correlating the *in vitro* and *in planta* phenotypic assays with the genotype, i.e. strain specific genes and orthologous genes that are shared only between the active strains, we aim to gain further clues about differences in the mechanisms of inhibitory action between active and inactive strains, first at the genome and ultimately at the transcriptome level. These findings should provide a better understanding and insights into the potential utility of microbiome-isolated bacteria, more specifically *Pseudomonas*, in fighting late blight.

5 Insights into the apple flower microbiome: First data on effects of fungicide application and different varieties

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The indigenous microbiome of apple flowers may have a strong influence on the occurrence of diseases such as fire blight, depending on the presence of antagonists. Therefore, we analyzed the microbiome of apple flowers in dependence of sampling time, application of pesticides or apple variety. We used a metabarcoding approach based on amplicon sequencing of 16S rRNA and ITS markers for bacterial and fungal identification, respectively. Samples were taken from flowers of fungicide treated and untreated two-year old Golden Delicious trees placed between older Boskoop background trees in an orchard in 2016 and 2017. Samples from additional apple varieties located in the same orchard were collected in 2017 only. Sequence analyses of all samples resulted in 1381 bacterial and 1797 fungal OTUs. Alpha-diversity indicated an overall low diversity of bacterial and fungal communities for both years, with higher values for Golden Delicious flowers when samples were taken at full bloom compared to petal fall. No fungicide effect was observed on bacterial and fungal community structures. However, a detailed analysis at the single OTU level of most prevalent species demonstrated a fungicide treatment effect on the bacterial species *Buchnera aphidicola* and the fungal species, *Podosphaera leucotricha*, an important apple powdery mildew pathogen. In addition, rank abundance curves revealed five bacterial and eight fungal OTUs to be dominant in the samples, including the well-known fire blight antagonists *Erwinia tasmaniensis* and *Aureobasidium pullulans*. Finally, an influence of the apple variety on the relative abundance of most dominant bacterial and fungal species was detected, indicating that apple flowers shape their own microbiome. Thus, we could demonstrate that fire blight antagonists were present in high abundance in the apple flower microbiome, which may be used to develop new measures for fire blight control.

6 BIOINVENT: A Pan-European inventory of grassland soil microbial biodiversity and its functional properties

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Comprising 58 million hectares, permanent grasslands account for 34 % of agricultural land in the European Union (EU) plus Switzerland. Currently, the Common Agricultural Policy and Habitats Directive of the EU highlight the need to promote extensively managed grasslands (PEGS). In contrast to the more conventional intensively managed grasslands, PEGS are characterized by limited or no fertilizer input and enhanced above-ground biodiversity. The capacity of PEGS to promote soil microbial diversity, however, is largely unknown, as are the interactions between above- and below-ground biodiversity. Such research gaps hinder the justification for the larger-scale adoption of these systems. The principle aim of the BIOINVENT project is to further the understanding of how PEGS influence microbial diversity and its functional potential on a European-scale. The centralizing hypotheses for the project are (i) grassland management is a stronger regulator of soil microbial diversity than agro-ecological distinctions in Europe and (ii) PEGS select for, and harbor, microbial groups, which show a stronger functional adaptation to below-ground resource limitation.

For a pan-European survey a North-South transect was established with 4 participating countries and 5 regions, i.e. Sweden (SE), Germany (DE), Switzerland (CH), Portugal (PT) and the Azores (AZ) in the summer of 2017. In each country, permanent grasslands of 3 management types were sampled; intensive, low intensive and PEGS. Furthermore, the sampling campaign of each participating country was divided into a 'favorable' agricultural region, where agricultural productivity is optimal and an 'unfavorable' region where agricultural productivity may be constrained by certain environmental/climatic conditions, with 360 sites being sampled in total. From each site, soil samples were taken to study the compositional and functional characteristics of the microbiome and the plant community composition was also recorded. A multidisciplinary approach will be employed to analyze the microbiome, with each country specializing in a technique, namely; next generation sequencing of the bacterial and fungal communities (CH), phospholipid fatty acid analysis (SE), qPCR of functional genes involved in the Nitrogen (N) and Phosphorus (P) nutrient cycles (DE) and enzymatic analyses of the N and P nutrient cycles (PT). In addition, to complement the European survey, CH is undertaking a number of more focused experiments. These aim to untangle the effects that the multiple interconnecting variables inherent to such a survey (i.e. plant community composition, soil nutrient status and discrepancies in sampling time) would have on microbial composition and function. The same interdisciplinary strategy used in the survey is also employed to examine the microbiome in these experiments. Analyses from both the survey and the focused experiment is ongoing.

The BIOINVENT project aims to further understand how the composition and functional potential of the microbiome is controlled along gradients of both management and agro-ecological gradients in Europe. The knowledge gained on how PEGs enhance these capabilities will be used to develop strategies for monitoring both their status and trends in European grasslands.

7 Relation of soil properties, land use types and soil microbial communities in a nation-wide survey

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Soil monitoring programs, such as the Swiss Soil Monitoring Network (NABO), aim at assessing current and long-term soil quality in order to ensure sustainable land-use. At the same time, they provide a framework to study potential effects of spatial and environmental factors on microbial community structures in soils. Switzerland is particularly suitable for such a project because of its highly diverse landscape within a relatively small area. We sampled soil from 255 sites (with four replicates per site) on a regular grid across the entire nation covering various environmental gradients, e.g., elevation (267 – 2,741), mean annual temperature (-3 – 12°C), precipitation (19 – 466 mm) and pH (2.7 – 7.8). Sites were characterized based on plant communities, land-use types as well as climatic, geologic, and geographic factors; soils were characterized according to pH, bulk density, as well as carbon- and nitrogen-content. Soil bacterial and fungal communities were assessed using metabarcoding of genetic markers from the ribosomal operon.

In total, 28.7 million bacterial sequences were clustered into 49,280 operational taxonomic units (OTU with 97% sequence identity) and 30.5 million fungal sequences were clustered into 21,291 OTUs. The 255 sites harbored distinct bacterial and fungal communities, as shown by reclassification success revealed by canonical analysis of principal coordinates (CAP). Permutational analysis of variance (PERMANOVA) revealed that land-use type and biogeographic regions significantly affected bacterial and to a lower degree fungal communities. Environmental factors influencing the bacterial community structures included, in descending order of contribution, pH, index of indicator plants for nutrients, elevation, carbon-to-nitrogen ratio and bulk density. Only little variation of fungal communities was explained by environmental factors where the most important factor was the index of indicator plants for nutrients. Bacterial and fungal communities were significantly correlated with the plant communities present at the sites.

In summary, an inventory of bacterial and fungal communities along environmental gradients as well as among land-use types and biogeographic regions was compiled, which revealed distinctness of microbial communities at sites, land use-types, and geographic regions. Greater explanatory power of environmental factors was found for bacterial than for fungal communities.

8 Metabarcoding for a poly-phasic soil quality assessment

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Routine soil quality monitoring relies mainly on physico-chemical analyses, despite the known functional importance of microorganisms for soil ecosystems. To assess the potential of metabarcoding of soil microbial communities for long-term soil quality monitoring, we first studied the stability of soil microbial community structures over five years in soils of 30 sites of the Swiss Soil Monitoring Network (NABO), and, secondly, we compared the performance of metabarcoding and physico-chemical analyses for discriminating samples from seven fields at one location according to their exposure history to waterlogging and agricultural use. In the first study system, samples were taken from arable land, grassland, and forest sites with yearly triplicates from 10 sites per land-use type, resulting in 450 samples. Metabarcoding analyses of bacterial and fungal community structures revealed land-use type and site specificity as well as temporal stability of the soil microbial communities. Furthermore, among the 11'083 fungal OTUs, we could detect 62 OTUs assigned to species of Swiss national priority, underlining the potential of metabarcoding to support and complement conservation strategies based on morphological surveys. The design of the second study system, allowed discrimination of the three factors waterlogging, agricultural use, and field site. Metabarcoding data revealed better discrimination of exposure history compared to physico-chemical data, indicating that metabarcoding data better differentiates alterations in closely related soils. At the OTU level, a potential indicator group for waterlogging stress assigned to the bacterial genus *Anaerolinea* could be identified, which demonstrates, how metabarcoding can be used for the development of bioindicators for a targeted monitoring. Together, these results show the long-term stability and habitat sensitivity of soil microbial communities, allowing to implement metabarcoding for soil microbial biomonitoring. Metabarcoding yields data from community to single OTU level, which enables use in a poly-phasic soil quality assessment. Therefore, next-generation DNA sequencing technologies pave the way for next-generation soil quality monitoring.

9 Keynote – Microbiome composition, activity and variation profiling: tools and applications

Shinichi Sunagawa

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Microbial communities are found everywhere. The advent of next generation sequencing (NGS) and the development of computational approaches has transformed the way how we tackle questions, such as: who is there, what do they do and why? I will highlight recent trends and challenges using real-world examples of studies on human-associated and ocean microbial communities. These systems have been studied at large scale and using different strategies of metagenomic (and metatranscriptomic) data analysis to uncover the biodiversity, ecological drivers, and population structure of microbial communities.

10 Towards utilizing microbiomes – applying functional genomics approaches to move from genomes towards functions

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A central aim of the Agroscope Research Program Microbial Diversity was to build up core competences in genomics and bioinformatics in order to describe the species composition of diverse microbiomes by metagenomics and to study individual isolates with functional genomics technologies.

A complete and accurately annotated genome sequence provides the basis to study genome rearrangements and evolution over time and to identify strain-specific sequences for diagnostic purposes. Moreover, it represents the optimal basis to carry out functional genomics studies relying on condition-specific gene or protein expression data. Therefore, we first established a modular pipeline for *de novo* genome assembly and continuously extended its capabilities and applications. Furthermore, a proteogenomics solution for improved genome annotation was developed with funding from the Swiss National Science Foundation.

We closely collaborate with several experimental groups that have established functional assays enabling them to isolate individual, functionally relevant strains involved e.g. in biocontrol, biostimulation, or antibiotics resistance from complex microbiomes. Such interdisciplinary collaborations are essential for Agroscope's aim to bring microbiome research into applied practice. We subsequently *de novo* assembled complete genomes of such strains using the latest Next Generation Sequencing (NGS) technologies and assembly algorithms. Applying comparative genomics approaches allows us to place the isolated bacterial or fungal strains into the context of publicly available genome sequences, while using transcriptomics we aim to identify the genes responsible e.g. for the protective effect of an isolated strain against a plant pathogen and to subsequently unravel the mechanisms of action.

In this talk, I will provide a synopsis of our work over the past four years and highlight some of the lessons learnt. Going forward, we will continue to develop bioinformatics capabilities to analyze and integrate additional functional genomics data types including proteomics, metabolomics and transposon mutagenesis data.

11 Studying low complex metagenomes by whole genome sequencing

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The soil, plant and food microbiomes that were studied in the ARP Microbial Biodiversity differ substantially in terms of their complexity: the soil microbiome represents by far the most complex, while the natural whey starter cultures (NWCs) that are used in cheese making represented the least complex microbiome. Previous studies, mostly based on amplicon-based metagenomics, concur that the microbial composition in NWCs is indeed of rather low complexity. Three species are generally pre-dominating: *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii*.

Using two NWCs from Swiss Gruyère producers as a pilot study, we subjected them to whole genome metagenomics sequencing on the Pacific Biosciences Sequel system. For one NWC, we achieved the complete assembly of all dominant genomes, including those of plasmids and phages, while the second NWC was more complex. We further show that the complexity of the samples was even lower than implied by previous studies. These findings were corroborated by a 16S rRNA based amplicon survey. In addition, we could uncover biologically relevant insights by linking the plasmids to their respective host genomes and by matching the CRISPR repeats of the phage with its prokaryotic target. These results could only be achieved by employing long reads that were able to span intragenomic as well as intergenomic repeats.

Here, we show the feasibility of *de novo* assembly of complete genomes based on shotgun metagenomics sequencing data (3rd generation NGS long reads) from of low complex NWCs, which has not been explored in depth so far.

12 The diversity of *Lactobacillus helveticus* in dairy products

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Lactobacillus helveticus is known for its highly proteolytic activity and certain strains exhibit a high rate of autolysis. Both characteristics beneficially influence cheese ripening, for example by decreasing the ripening time and by improving the flavor development. These biotechnologically important characteristics are strain specific and *L. helveticus* strains isolated from the same ecological niche can exhibit a high degree of phenotypic heterogeneity.

The aim of the present study was to analyze the diversity and population dynamics of *L. helveticus* in dairy environments and thereby gain insights into the ecological mechanisms of intra-species diversity. In order to achieve these goals, a culture-independent typing method was developed. The amplicon sequencing-based sequence typing method targets the *slpH* locus that encodes a putative surface layer protein and showed high level of genetic heterogeneity. High-throughput sequencing of the *slpH* gene and the detection of single-base DNA sequence variations differentiated between 30 different sequence types out of 78 different isolates. The sequence typing method was used to assess the diversity of *L. helveticus* in natural whey cultures (NWCs) collected from different Swiss Gruyère cheese factories. In addition, the population dynamics of *L. helveticus* was monitored during six months of cheese ripening in Swiss Gruyère-type cheese. The study showed that three to four *L. helveticus* sequence types (STs) occurred in NWCs and that these STs were also found in cheese manufactured with the respective NWC. Although the relative abundance of STs fluctuated during cheese ripening, most of the STs persisted during the entire ripening time. The results showed that NWCs harbor a wide diversity of *L. helveticus* strains that form a stable co-existence over time. Furthermore, the developed method proved to be a suitable tool for tracking the population composition of *L. helveticus* in dairy products. The method can be used in future studies to track individual strains and possibly identify associations of desired as well as undesired cheese properties with certain strains or strain compositions.

13 Microbial diversity in cheese and dairy products

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Cheese is a dairy product, whose flavor and, texture is strongly influenced by microbial activity during ripening. Despite the long cheese manufacturing tradition, little is known about the microbial diversity present in Swiss cheeses and its dynamics during ripening. In this study, a culture-independent approach was used to study the bacteria present during cheese manufacture and ripening in many different Swiss cheese types.

To fully appreciate the overall microbial diversity present in various types of Swiss cheese, we applied amplicon-based next-generation sequencing (NGS) targeting the ubiquitous and highly conserved gold marker gene encoding the 16S ribosomal RNA (16S). An optimized bioinformatics pipeline, together with the manually curated 16S DAIRYdb developed in collaboration with INRA, allowed us to significantly improve accuracy and speed of the bioinformatics analysis.

We present the microbial community structures detected in different types of Swiss cheese (*i.e.*, Emmentaler, Gruyere, Raclette) and milk. Different cheese types showed distinct microbial community structures as expected. Surprisingly, the community structures varied significantly between samples of the same cheese type. Moreover, the microbial community structure between the amplicon sequencing results of 16S rRNA (cDNA) and 16S rDNA (genomic) of the same samples differed clearly.

Overall, biodiversity in most cheese types appeared to be low (<20 different species) due to the dominance of few phylogenetic related bacterial species. However, resolution at strain level using oligotyping or shotgun metagenomics showed that biodiversity is underestimated at species level.

Further technological improvements are needed to increase resolution of microbial communities in cheeses to strain level. Quality improvements of long read sequencers, such as Nanopore Sequencing, might allow to target longer fragments of marker genes, such as the complete ribosomal operon (*rnn*). The length of about 5kb of the *rnn* would allow to increase the resolution and so to distinguish different strain or genotypes within a species. Only after achieving that level of resolution it might be possible to reveal real microbial community dynamics in cheese and dairy products and their impact on their quality.

14 Posters

Genetic diversity of *Lactobacillus helveticus* strains from the Swiss Agroscope culture collection

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Exploring the diversity of *Lactobacillus helveticus* in dairy products – a culture-independent approach

Moser A^{1,3}, Wüthrich D², Meile L³, Irmeler S¹

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Pseudomonas orientalis F9: from apple to soil - due to genome sequencing

Zengerer V¹, Schmid M², Bieri M¹, Müller DC¹, Remus-Emsermann MNP^{3,4}, Ahrens CH² and Pelludat C¹

¹Agroscope, Phytopathology and Zoology in Fruit and Vegetable Production, Wädenswil; ²Agroscope, Molecular Diagnostics, Genomics & Bioinformatics & SIB Swiss Institute of Bioinformatics, Wädenswil; ³School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; ⁴Biomolecular Interaction Centre, University of Canterbury, Christchurch, New Zealand

Bioinformatics & Proteogenomics Group – From Genome to Function

Omasits U^{1,2}, Schmid M^{1,2}, Varadarajan AR^{1,2}, Somerville V^{1,2}, Bourqui M^{1,2}, Wicki A^{1,2}, Lutz S^{1,2} & Ahrens CH^{1,2}

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An integrative proteogenomics strategy to identify the entire protein coding potential of prokaryotic genomes

Omasits U^{1,2}, Varadarajan AR^{1,2}, Schmid M^{1,2}, Götze S^{3,4}, Bourqui M^{1,2}, Dehio C⁵, Frey JE¹, Robinson M², Wollscheid B^{3,4}, Ahrens CH^{1,2}

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From isolation of naturally occurring yeasts that antagonise soilborne plant pathogens to genome assembly and mechanism of action studies

Schneeberger K^{1,2}, Hilber-Bodmer M³, Schmid M^{1,2}, Ortiz-Merino RA⁴, Butler G⁴, Wolfe KH⁴, Ahrens CH^{1,2}, Freimoser FM³

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Towards linking the variable antagonistic activity of *Pseudomonas* strains against late blight with the underlying genotype - a comparative genomics study

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Utilizing natural whey starter cultures as proof of principle for studying low complex metagenomes by whole genome sequencing

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Diversity of *Streptococcus thermophilus* in Gruyère model cheeses

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Active but undetected, detected but inactive; DNA- and RNA-based amplicon sequencing analyses reveal divergent pictures of the bacterial communities in cheese

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Correlation between biogenic amine contents and 16S rRNA gene amplicon-based sequencing data in Tilsit cheeses

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***Metarhizium brunneum* was effectively used for biocontrol of *Agriotes* spp. in pot experiments: an ideal setup to study non-target effects on soil microorganisms**

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