MICROBIOLOGY
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2018

Microbiomes Underpinning Agriculture

POSTER ABSTRACT BOOK

Rochestown Park Hotel,
Cork, Ireland
1–2 October 2018

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Poster Book Content

Poster Number: 01

Refining direct fed microbials (DFM) and silage inoculants for reduction of methane emissions from ruminants

Abstract

The agriculture industry currently produces 14% of the world's annual Greenhouse Gases (GHGs) emissions. It is estimated that ruminants are responsible for 31% of methane's contribution towards global warming - 86 million metric tonnes per annum. This is a problem for the agricultural industry, as the ability of methane to trap infrared radiation is significantly high in comparison to other GHG's.

This research focuses on the design and development of direct fed microbials (DFM) for GHG reduction strategies. Lactic acid bacteria (LAB), a natural part of the microbiota of many higher organisms (including ruminants), will be isolated, identified and screened for their anti-methanogenic properties. Many LAB produce antimicrobial peptides (bacteriocins) and as such, have the potential to inhibit methanogens. The route of application will be through DFMs and silage inoculants, where a short term impact would be experienced by the ingesting ruminants and possibly maintained due to the colonisation of the rumen by the LAB. Other possible strategies for GHG Methane reduction which may be explored include: Archaeal bacteriophaephage, chemical inhibition and alternative hydrogen sinks.

Employing various *in vitro* techniques, such as headspace Gas Chromatography, spectrophotometry, qPCR and subsequent *in vivo* studies such as F6 Tracer Technique will allow for the quantification of methane emitted.

It is hypothesised, with the reduction of methane in the rumen, excess energy would result in greater energy consumption for the ruminants, leading to greater feed efficiency. The use of LAB for methane reduction would contribute to a more competitive, sustainable and profitable global Agri-food sector.

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Poster Number: 02

Ecological selection of siderophore-producing microbial taxa in response to heavy metal contamination

Abstract

Some microbial public goods can provide both individual and community-wide benefits, and are open to exploitation by non-producing species. One such example is the production of metal-detoxifying siderophores. Here, we investigate whether conflicting selection pressures on siderophore production by heavy metals – a detoxifying effect of siderophores, and exploitation of this detoxifying effect – result in a net increase or decrease. We show that the proportion of siderophore-producing taxa increases along a natural heavy metal gradient. A causal link between metal contamination and siderophore production was subsequently demonstrated in a microcosm experiment in compost, in which we observed changes in community composition towards taxa that produce relatively more siderophores following copper contamination. We confirmed the selective benefit of siderophores by showing that taxa producing large amounts of siderophore suffered less growth inhibition in toxic copper. Our results suggest that ecological selection will favour siderophore-mediated decontamination, with important consequences for potential remediation strategies.

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Poster Number: 03

Characterisation of the regulatory interplay between the plant microbiome and root endodermal cell wall diffusion barriers

Abstract

In nature, plants live in close association with microbes. Beneficial microbes increase nutrient uptake and use in the host, improving plant growth and health. The capacity to colonize roots is one of the main factors that determines beneficial microbe's effectiveness for plant nutrition enhancement. Roots have evolved bi-directional diffusion barriers at the endodermis to control the flow of water and nutrients between the soil and xylem. These barriers are comprised of Casparian strips and suberin in endodermal cell walls. Mutations that disrupt these barriers cause significant alterations in normal mineral nutrient homeostasis. It is unknown how the plant root diffusion barriers coordinate with the plant microbiota to regulate the ion balance of the plant. Here we found that a genetic network controlling the synthesis and function of the root diffusion barriers influences the configuration of the plant microbiome. Using plant-bacterium binary association, we identify bacteria that modified the function of the root barriers. We further detect a strong phylogenetic signal in the ability of bacterial strains to modulate the root barriers function. Our work will help to define the molecular basis of the plant ion balance regulation and to use beneficial microbes to enhance plant nutrition.

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Poster Number: 04

Improved animal husbandry through inhibition of gut microbial bile salt hydrolase

Abstract

Bile salt hydrolase (BSH), a gateway enzyme for bile acid (BA) modification in the host, is a promising gut microbiome target for developing innovative, non-antibiotic growth promoters to enhance animal production and health. Compelling evidence suggests that inhibition of BSH activity could cause weight gain. We hypothesize that dietary supplementation with BSH inhibitors could adjust host metabolism and energy harvest, consequently enhancing feed efficiency and body weight gain in food animals. We have recently identified several promising BSH inhibitors. In this study, the *in vivo* efficacy of three BSH inhibitors (caffeic acid phenethylester, riboflavin, carnosic acid) were evaluated using a chicken model. The 7-day old chicks were assigned into four groups (10 birds/group) that received control or specific BSH inhibitor (25 mg/kg body weight) *via* oral gavage for 17 days. The chicks in inhibitor treatment groups consistently displayed higher body weight gain than control chicks. Metabolomic analysis demonstrated that BSH inhibitor treatment led to significantly altered BA profiles changes both locally (intestine) and in circulation indicating altered intestinal BSH activity. Consistent with this finding, RNA-Seq analysis of liver and intestinal samples showed that carnosic acid treatment led to significant differential expression of the genes involved in lipid and bile acid metabolism. Taken together, this study demonstrates the *in vivo* efficacy of the identified BSH inhibitors and supported both our hypothesis at physiological, biochemical, and molecular levels and our long-term goal of developing BSH inhibitor-based non-antibiotic growth promoters.

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Poster Number: 05

Casing microbiome reveals the absence of a natural suppressive response against bubble diseases in button mushroom crops

Abstract

The casing material required in mushroom cultivation host a rich ecological niche, including bacteria and fungi. Dry bubble (*Lecanicillium fungicola*) and wet bubble (*Mycogone perniciosa*) are resilient fungal diseases which provoke yield losses.

Two trials have been set up evaluating the native suppressive effect of blonde peat, black peat and a mixture of both by 50% each, against bubble diseases. The evolution of the casing microbiome along the crop cycle has been characterised though next generation sequencing (NGS).

Heavy production losses were recovered from the infection with dry bubble. None significant differences were detected among the different casing materials for any disease. Metagenomics analysis has detected an ubiquitous presence of *L. fungicola*, increasing its relative abundance along the crop cycle. The microbiome of the casing at the beginning of the crop cycle shows significant singularities. However, colorization of mushroom mycelium derives into an increase of the bacterial diversity while reducing the fungal diversity. Noteworthy, after colonization, casing shows a very conservative pattern along the crop cycle.

The equilibrium of casing microbiota naturally established is not sufficient to cope with a high pressure of fungal inoculum. The presence of the mushroom mycelium modifies significantly the microbiome of the casing material, which would therefore condition the fungistasis observed in raw casing.

Acknowledgement: The project leading to this report has received funding from the European Union's H2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 742966. The research was also funded by the ADER project, grant No 2016-IDD-00013.

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Poster Number: 06

Influence of gut microbes on host immune system in early life

Abstract

Increasing pressure in recent years to reduce greenhouse gas emissions from agriculture has stimulated research focused on manipulating the ruminant gut microbiome to yield profiles which generate less methane. There is some evidence that interventive treatment in the first weeks of an animal's life can shape the rumen microbiome, and these changes may persist even when intervention has ceased. The short-term nature of such a strategy would be economically and practically feasible, making it an appealing solution. However, microbes colonising the gut in the days after birth interact with and influence the host immune system's development. If 'early life programming' is to be used as a means of reducing methane emissions then it is imperative that the early life interventions do not disrupt the maturation of the host immune system. This study was concerned with identifying key periods of interaction between colonising microbes and the developing host immune system. Gastrointestinal tissue and content samples were taken from calves at intervals from day 0 to day 96 after birth. Tissue samples were H&E stained to assess organ development and immunohistochemistry was carried out to identify patterns of immune cell population in sections of tissue over time. The duration of initial inflammation in response to gut colonisation and the identity of microbes with potentially crucial roles in priming the host immune system were assessed by examining the expression profiles of host genes involved in pro- and anti-inflammatory pathways in association with gut bacterial and archaeal community profiles over time.

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Poster Number: 07

Impact of long term crop management on total soil biodiversity and function

Abstract

Unmanaged forests are sites of high biodiversity, where complex relationships between fauna, flora, and microflora are maintained due to the age and structural richness of the habitat. In montane forest, belowground biodiversity remains largely unexplored, and the effect of conversion to agriculture on biodiversity is unknown. This study investigates the impact of land use change from primary montane forest to tea and gum plantation systems on soil bacterial, fungal and animal diversity using metabarcoding. Furthermore, we link these microbiological communities to key soil physical, chemical and biological properties fundamental to soil function.

Replicate soil samples were collected in a tea estate in Kenya from 7 sites including primary forest, conventional and organic tea and gum plantations of different ages. Soils were analysed for a number of key properties, including texture, pH, CEC, nutrient status, organic matter and aggregate stability. Amplicon libraries of 16S, ITS and COI genes were analysed for all sites.

A suite of community ecology analyses were performed to test the effect of land-use change on total soil biology, and to quantify this. The analyses clearly reveal that land-use history and current land-use affect community composition for bacterial, fungal and animal kingdoms. Moreover, preliminary results show a reduction in organic matter and aggregate stability from forest to gum to tea. Further analyses will allow us to correlate community biodiversity to soil properties and to explore the overall effect of the shift in land use from forest to tea and gum plantation on total soil health.

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Poster Number: 08

Soil microbial community and nematodes shift in response to application of organic and inorganic Phosphorus and Sulfur fertilizer in grassland columns

Abstract

Phosphorus (P) and sulfur (S) are vital for living organisms and are widely used as fertilizers in agro-ecosystems. However, mineable reserves of P are finite and atmospheric supply of S has drastically reduced leading to plant nutrient deficiencies. In this study, soil columns with *Lolium perenne* were setup in a greenhouse and fertilized with Urea only (control), NPKS + micronutrients (inorganic) and cattle slurry (organic) fertilizer sources. Weekly measurements of nutrient concentrations revealed that most of the nitrate in the organic treatments leached out by week four. Grass dry matter yield, shoot uptake of N, P and S were significantly higher in the inorganic treatment compared to the control and organic treatments. *L. perenne* rhizosphere of the organic treatment had significantly higher abundance of nematodes, bacterial-feeding nematodes, mycorrhizal colonization rates, cultivable heterotrophic bacteria, phosphonate- and sulfonate-utilizing bacteria, arylsulfatase activity and available P compared to the inorganic control treatments. Phosphatase activities were similar in organic and inorganic treatments but significantly higher than the control. Organic and inorganic treatments significantly shifted the bacterial, alkaline phosphatase-producing bacteria, fungal, AM fungal, and nematode community structures compared to the control. Overall, our findings suggest that although organic treatment favoured the soil microbiota and nematodes involved in P and S cycling, there was no plant growth promotion effect probably due to N limitation caused by nitrate leaching. Further analyses are underway on microbial diversity.

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Poster Number: 09

Soil microbial abundance following anaerobic digestate application

Abstract

This study evaluated microbial abundance in soils following application of different types of anaerobic digestates in a short-term glasshouse trial. The experimental design was full factorial, with four plant types: perennial ryegrass (*Lolium perenne*), white-clover (*Trifolium repens*), mixed ryegrass-clover, and bare soil; and eight fertiliser types, with three replicates. Fertilisers consisted of five types of liquid anaerobic digestates, N-control (NH_4NO_3), cattle slurry, and no fertiliser. Anaerobic digestates and cattle slurry were applied at a rate of 33 m^3 ha^{-1} in three intervals of 45 days over one growing season, with soil collected at the end. Microbial abundance was evaluated by qPCR of bacterial (16S), archaeal (16S), and fungal (18S) gene copy numbers (GCN). Factorial analyses with two fixed factors (plant type and fertiliser) were performed. For archaeal and fungal GCN, only plant type significantly affected the GCN ($p<0.01$), with bare soils having lower numbers than soils with plants. Bacterial GCN was significantly affected by plant type, fertiliser type, and their interaction ($p<0.01$). Higher bacterial GCN were found in mixed ryegrass-clover soils treated with two types of anaerobic digestates. The anaerobic digestates that showed higher bacterial GCN were from food waste (dairy) and mixed food waste + swine slurry, and had the highest available N of biofertilisers evaluated. Bacterial GCN in ryegrass, clover and bare soils were not significantly affected by the types of fertiliser used.

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Poster Number: 10

Dynamics of feed particle colonisation by anaerobic fungi

Abstract

Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of many herbivorous animals, and play an essential role in degrading food with a range of powerful hydrolytic enzymes. The dynamics of feed particle colonisation is not well-understood, so we incubated Dacron bags containing fresh grass forage in the rumen of cannulated cows in order to explore the dynamics of colonisation. The succession of fungi colonising the forage particles over a 24 hr period was explored using DNA metabarcoding (D1 region of the LSU locus). A range of anaerobic fungi were also isolated from the rumen faeces of a range of large herbivores. After roll tube culture to isolate single-zoospore cultures, sequencing of LSU was undertaken to identify the fungi to species level. We also obtained genome sequence data from several of these and other cultures, allowing genomic comparisons to be made between members of different genera. In addition to elucidating the mechanisms of feed particle degradation, such analyses also permit exploration of fungal genomes for novel enzymes and potential antimicrobial compounds.

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Poster Number: 11

The Comparative Risk of Antimicrobial Resistance Transfer from Pig, Poultry and Bovine Manure to Grassland

Abstract

The agricultural industry produces vast quantities of organic manure that is frequently recycled as organic fertiliser on grassland, returning valuable nutrients to soil. The often abundant use of veterinary antibiotics in animal production and the subsequent land application of manure from these animals can lead to an elevated amount of antibiotic resistant bacteria (ARB) in the environment. Manure is a known hotspot of antimicrobial resistance due to the presence of antibiotic residues and ARB. However, little is understood about how continuous manure application affects the environmental resistome and microbiome, and the impact of different manure types. This project aims to investigate the impact three manure types (bovine, pig and poultry) has on the survival and transfer of antimicrobial resistance over time in grassland, and the resulting impact on the soil and plant microbiome. In addition, the impact of soil type on the transfer of ARB in the environment and on the resistome will be assessed. Mesocosm experiments, combined with cultivation dependent and independent analysis of collected samples, will be used to identify temporal changes in the microbiome and resistome of grassland between the different treatments. The findings from this study can therefore allow for the identification of manure and farming practices that minimise the risk of AMR transfer to soil and grass.

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Poster Number: 12

Advances in pursuing genome closure from short sequence reads through rDNA repeat resolution and streamlining assembly

Abstract

The vast majority of bacterial genome sequencing has been performed using Illumina short reads. Because of the inherent difficulty of resolving repeated regions with short reads alone, only ~10% of sequencing projects have resulted in a closed genome. The most common repeated regions are those coding for ribosomal operons (rDNAs), which occur in a bacterial genome between 1 and 15 times, and are typically used as sequence markers to classify and identify bacteria. Here, we exploit conservation in the genomic context in which rDNAs occur across taxa to improve assembly of these regions relative to de novo sequencing and the uniqueness of their flanking regions within a genome. We describe a method, riboSeed, which constructs targeted pseudocontigs generated by iteratively assembling reads that map to a reference genome's rDNAs. These pseudocontigs are then used to more accurately assemble the newly-sequenced chromosome.

We show that this method, implemented as riboSeed, correctly bridges across adjacent contigs in bacterial genome assembly and, when used in conjunction with other genome polishing tools, can result in closure of a genome. riboSeed is a standalone package available through conda, and can be used with the BugBuilder genome assembly pipeline. By removing the main barriers to genome polishing (difficulty in installation, tool selection, and pipeline integration), researchers can easily go from raw reads to high-quality, polished assemblies.

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Poster Number: 13

Genomic Insights into the Virulence Capacity of Soil-Persistent *E. coli*

Abstract

Escherichia coli's remarkable ability to thrive both in the gut and in soil confounds many assumptions made about the bacterium's ecological niche specialization, and a growing body of research has challenged the long-held assertion that E. coli can only survive transiently outside of the gut. In order to probe the genetic mechanisms that allow for such long-term adaptation and assess any potential human-health risks associated with soil-adapted E. coli, whole-genome sequencing was performed on 153 long-term soil-adapted E. coli isolated from maritime temperate soils that had been protected from fecal contamination for periods of at least 9 years. Further understanding of these isolates will potentially lead to improved water quality diagnostics and a more complete knowledge of stress adaptation and virulence in E. coli. The isolates had previously been shown to be phenotypically diverse, possessing characteristics that may enhance their capacity to survive in the soil environment. Average nucleotide identity on aligned genomes revealed 7 distinct subgroups; while certain subgroups correlated well with the phylo-groups assigned to them by Clermont 2013 typing method, others showed more heterogeneity. We surveyed the presence or absence of ~65 virulence or virulence-related genes using BLAST. Our findings show that many virulence-associated genes (including fim, traT rfaH, EAST1, and others) are present, and may be functional in large proportion of the isolates. Future work will include a pangenome comparison of these soil-persistent isolates to a wider panel of E. coli isolates, and assessment of genome composition in relation to phenotypic characteristics of the strains.

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Poster Number: 14

Archaea in agriculture: Investigating the role of ammonia oxidising archaeon *Candidatus* Nitrosocosmicus franklandus C13 within the root associated microbiome

Abstract

Root associated microbiomes (RAMs) are complex communities with constituents spanning all three domains of life. Members of these communities provide benefits to host plants including disease suppression, stress relief and increased nutrient bioavailability; resultantly RAMs have been studied as a potential sphere for new agricultural innovation. Often studies focus on plant growth-promoting bacteria; the role of archaea is frequently overlooked as studies rarely utilize archaea-specific 16S primers and therefore fail to assess the extent of archaeal RAM colonisation. A number of studies have detected and isolated archaea from the endosphere of several plant species including *Solanum lycopersicum* and *Oryza sativa*, additionally some have begun to identify genetic features associated with plant growth promotion. We show that ammonia oxidising archaea (AOA) of the *Candidatus* genus *Nitrosocosmicus* are enriched within the rhizosphere and endosphere of *Arabidopsis thaliana* plants grown in Levington compost. Stable isotope probing experiments have confirmed that bacterial species metabolise root exudates in these compartments; fractions are now being examined to confirm if this is also the case for archaeal species. We are additionally investigating whether a model AOA (*Candidatus* Nitrosocosmicus franklandus C13) can promote the growth of *A. thaliana*. Bacterial and archaeal profiling studies as well as plant growth promotion assays are also being extended to the endosphere and rhizosphere of the important crop species *T. aestivum.* Our results suggest that specific archaeal species are abundant in the rhizosphere and endosphere of *Arabidopsis*. Understanding their contribution to host fitness may lead to novel plant growth-promoting strategies.

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Poster Number: 15

Microbiological Quality of Water Used in Production and Preparation of Fresh Produce

Abstract

Microbial contamination of fresh produce is a major public health concern, with the number of associated microbial disease outbreaks increasing in recent years. The quality of water used in production and preparation of these foodstuffs is of utmost importance to ensure quality and safety for consumers.

This study aimed to analyse the microbiological quality of water used in production of salad leaves, soft fruits and sprouted seeds in selected production facilities in Ireland, Portugal, Serbia and the United Kingdom. Water samples were collected, concentrated via dead-end ultrafiltration and DNA was extracted. The presence of generic *E. coli, E. coli* O157, *Salmonella, Cryptosporidium,* Hepatitis A and Norovirus was evaluated by qPCR. Samples were also analysed for the parameters defined in the European Union Drinking Water Directive. A total of 165 water samples and 65 food samples were analysed.

No pathogenic microorganisms were detected in the study. *E. coli* was detected in a number of samples, including untreated source water, product washing or spent irrigation water. This indicates potential contamination issues that require intervention to ensure product safety.

Further knowledge on the microbial communities present in these water samples would be of great value. Analysing the microbial composition of the samples where *E. coli* was detected could provide clues on the contamination source. Understanding the changes microbial populations undergo during the different stages of production of these foodstuffs will provide greater insight into the potential for water reutilisation. This knowledge will be beneficial for the quality and safety of fresh produce.

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Poster Number: 16

The Impact of Diet on the Rumen Microbiome and Antimicrobial Resistance (AMR)

Abstract

Changes in the rumen microbiome can have positive/negative impacts on the host. Microbial diversity and composition are important factors in contributing to an efficient microbiome. With antimicrobial resistance (AMR) now recognised as a major threat to global health, the role of the rumen as a reservoir of antimicrobial resistance is an important area.

Five rumen-cannulated cows were allocated to one of five treatments [high grass (HG), low grass (LG), LG and baled grass silage (GB), LG and pit grass silage (GP) and LG and concentrate (GC)]. Shotgun metagenomic sequencing was used to determine community composition and functionality.

Supplementation of concentrate to a pasture-based diet can reduce the abundance of archaeal species and methanogenesis pathways and urea pathways. Genes responsible for methane formation and the activity of methanogenic archaea were more pronounced in GP treatment. Genes associated with urea breakdown pathways were more pronounced in HG treatment. Significant differences in the archaeal Kingdom were also found between GC and HG diets, with higher abundances of the archaeal species found in HG. Ensiling appears to have a negative effect on functionality in terms of promoting methanogenesis, while supplementing with concentrates appears to have a positive effect. The addition of ensiled forages also seems to affect the microbiome, leading to a more diverse ecosystem. Ensiling also appears to have an effect on AMR.

This study establishes differences between antimicrobial resistance genes associated with diet. Differences in specific species and pathways were also detected suggesting diet has an impact on these particular species.

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Poster Number: 17

Exploring the relationship between soil hydraulic conductivity and the potential of the soil microbiome to transform nitrogen

Abstract

The conversion of nitrogen (N) from one form to another is carried out principally via biological processes. Nitrification, which occurs in aerobic conditions, involves the conversion of ammonia to nitrate (NO_3^-), a form which is preferred for plant uptake. Denitrification, which occurs under anaerobic conditions, involves the sequential reduction of biologically available N to gaseous forms, where it is removed from the system. During this process, nitrous oxide, a potent greenhouse gas, may be released. Both of these processes are critically important in determining N supply to crops and the environment. Saturated hydraulic conductivity (K_s) is a quantitative measure of a saturated soil's ability to transmit water when subjected to a hydraulic gradient. It has been shown previously that subsoil K_s is a good predictor of NO_3^- concentration in groundwater, suggesting it may be related to N transformation process rates. Herein we examine topsoil K_s and its correlation with nitrification and denitrification enzymatic assays and genetic potentials through the quantification of essential genes involved in N transformations (*amoA*, *nirS*, *nirK, nosZ*1 and NosZ2). We hypothesise that topsoil with low K_s may have microbial communities primed for anaerobic N transformation processes such as denitrification, while soils with high K_s may have microbial communities primed for aerobic N transformation processes such as nitrification. A predictive relationship between K_s and N transformation processes could be used to better model N balances and losses, and to inform agricultural management.

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Poster Number: 18

Coastal Plants as a Source of Halotolerant Plant Growth-Promoting Endophytic Bacteria

Abstract

Global soil salinity is rising; it is estimated that 20% of irrigated agricultural land globally is currently contaminated by high salinity levels (>40mM NaCl), and more land is contaminated each year. Plant growth promoting (PGP) endophytic bacteria have the potential to mitigate the impact this has on growth of agricultural crop plants worldwide, as they can be capable of promoting plant growth and minimising the effects of salt stress. Plants already living in saline environments, such as coastal soils, are a potential target for discovering novel PGP endophytic bacteria for agricultural application.

Halotolerant bacteria were isolated from the endosphere of plants living in saline coastal environments around Aberystwyth. *Brachypodium distachyon* BD21 inoculated with these strains were subjected to salt stresses of 0mM, 10mM and 200mM NaCl in the National Plant Phenomics Centre in Aberystwyth in order to screen for growth promotion. Traits evaluated included height, tillering and dry weight of plant and seeds. The isolates themselves were also evaluated *in vitro* to assess their halotolerance.

Isolates that promote the growth of the Brachypodium under salt stress will be further analysed as potential candidates for an agricultural inoculation, with a view to developing low-cost, sustainable crop productivity, and will demonstrate the viability of coastal plants as a source of halotolerant PGP endophytes.

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Poster Number: 19

Development of a defined matrix for plant-microbiome studies

Abstract

There is an urgent need to intensify agriculture to meet the demands of a growing population. However, this needs to be done in a sustainable manner and finding environmentally-benign alternatives to artificial fertilisers is essential in this regard. The rhizosphere contains microbes capable of forming beneficial symbioses with host plants to improve plant health and nutrient provision. Arbuscular mycorrhizal fungi (AMF) and plant-growth promoting rhizobacteria (PGPR) form a tripartite symbiosis with many crop plants, though little is known about the way AMF interact with other microbes in the mycorrhizosphere. It is proposed that AMF recruit beneficial bacteria from the soil and act as a conduit for colonisation of host plant roots. This project aims to better understand the interaction between AMF, host plants and the microbiome in grassland and arable soils in order to inform optimal management strategies under different nutrient availability conditions to promote sustainable agriculture. In order to study the interactions between microbes, and their impact on plant health, a nutrient limited compost system was devised with potential to be used as a matrix for microbial PGPR and AMF screening. Screening of a bacterial library for the ability to solubilise P revealed that P solubilising *Pseudomonas* bacteria could be phylogenetically discriminated according to plant host. The compost system was used to test the ability of these bacteria to rescue the growth of plants in soluble P deficient conditions, and determine if a host specific effect exists. Future experiments will examine the interaction between PGPR, AMF and host plants.

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Poster Number: 20

Quantitative PCR: the importance of choosing the correct taq polymerase for environmental samples

Abstract

Quantitative PCR (qPCR) uses the linearity of DNA amplification to determine absolute or relative quantities of a targeted sequence in a sample. qPCR is widely used in microbial ecology to quantify functional and phylogenetic genes in environmental samples (multi-template) but environmental samples can often pose challenges for gene quantification assays not encountered in clinical samples. There are many components in the PCR reaction mixture that may affect the efficiency of amplification. The taq polymerase is particularly important as it is the enzyme responsible for the synthesis of the DNA molecule. The performance of taq polymerase can be affected by inhibitors such as humic acids which are often found in DNA extracted from soil. Further, taq polymerase has been shown to exhibit preferences for some primer-template combinations. Optimising qPCR assays can be time consuming and costly for many laboratories, therefore, many laboratories focus on developing new assays with a single taq polymerase. As a result, the differences in taq efficiencies affect the ability to compare data across labs and between genes. In this study, we evaluate several different taq polymerases from well established companies specialising in real-time PCR on a range of different soil samples. The aim of the study was to assess the impact of the taq polymerase on the quantification over a range of functional genes and the impact inhibitors has on their efficiency. Our findings indicate the importance of choosing the best polymerase for your samples and that taq performance can be gene specific.

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Poster Number: 21

Assessing the impact of mixed species pastures on the soil microbiome and functional microbial communities involved in nitrous oxide production

Abstract

Developing agricultural tools that enable more sustainable production while limiting N_2O emissions is a major challenge for agriculture. Soil microorganisms are key drivers of denitrification and nitrification, which are the main N cycle processes responsible for N_2O production, a potent greenhouse gas that is also involved in the ozone layer depletion. Bacterial, archaeal and fungal community structure and functioning are influenced by a wide range of abiotic (pH, soil moisture, N concentrations) and biotic (plant species) factors. Ruminant urine is a large source of N_2O emissions in temperate grazed grasslands. It also represents a perturbation for soil microbial communities, modifying abiotic parameters as pH and N concentrations. A pot trial was set up to determine how N related soil microorganisms are affected by pasture forage species under urine addition. There were four treatments of commonly used pasture species 1.*Trifolium repens, 2*. *Lolium perenne*, 3. *Plantago lanceolata* and 4. a mixture of the three species. Ruminant urine was applied and soil samples were taken before and on four occasions after urine deposition. Soil pH, DOC, NH_4^+, NO_3^- and NO_2^- concentrations, as well as N_2O fluxes were measured. After DNA extraction from soil, qPCR was undertaken to provide information on N related genes abundances. In addition, a network analysis based on the Miseq sequencing data of 16S and ITS will be used to give insight into the microorganisms' interactions under two plant treatments and to assess how the soil microbial community responded to perturbation with urine.

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Poster Number: 22

The effect of liming on microbially-mediated soil organic matter nitrogen turnover

Abstract

Nitrogen (N) is a limiting nutrient in terrestrial ecosystems and one of the key nutrients for plant growth. The main store of N for plant uptake is soil organic matter (SOM). The conversion of organic N in SOM to inorganic (plant-available) N is dependent on microbial activity. It's known that, through root exudation, plant release of carbon compounds affects the rate of microbially-mediated SOM mineralisation (i.e. priming) and N release from SOM. There is limited knowledge on how plants and microbes interact during this process to mobilise N; it's unknown which microbes mediate this process, what enzymatic activities are involved, the effect of community composition, and how the community interacts with physical and chemical properties of soil determining the rate of mineralisation. Predicting when and in what form N becomes available from SOM is essential for managing nutrient balances. Farm management affects plant-microbe interactions. Liming is a management practice which increases nutrient availability, alters soil pH, and stimulates biological activity. However, the impact of soil pH changes on the mechanisms involved in priming and N mineralisation are unknown. Increased understanding of the role of microorganisms in mineralisation has the potential to enhance farm management by linking nutrient cycling with plant growth. This has benefits agronomically, reducing reliance on expensive N fertilisers, and environmentally, as N use becomes more soil specific. This project plans to assess; enzyme activity, N mineralisation potential, and combine stable isotope analysis with metagenomic approaches to examine the effect of liming on microbially-mediated SOM N turnover.

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Poster Number: 23

Porcine feed efficiency-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake

Abstract

Achieving optimal feed efficiency (FE) is important in pig production for both economic and environmental reasons. Previous research has identified FE-associated bacterial taxa within the intestinal microbiome of pigs. This study aimed to investigate whether FE-associated bacteria and selected FE-associated physiological traits were consistent across geographical locations, namely the Republic of Ireland (two batches of pigs; ROI1 and ROI2), Northern Ireland (NI), and Austria (AT), where differences in genetic, dietary and management factors were minimised. Pigs (n=369) were ranked on divergence in residual feed intake (RFI, a metric for FE) and 100 extremes were selected (50 high RFI, 50 low RFI) across geographical locations for intestinal microbiota analysis via 16S rRNA amplicon sequencing, and examination of FE-associated physiological parameters. Microbial diversity varied due to geographical location and intestinal site, but not by RFI rank, except in ROI2, where more feed efficient pigs had greater ileal and caecal diversity. Although none of the 192 RFI-associated taxonomic differences found were common to all locations/batches, *Lentisphaerae*, *Mucispirillum*, *Methanobrevibacter*, *Ruminococcaceae*, *RF16* and two uncultured taxa were more abundant within the faecal and/or caecal microbiota of low RFI pigs in two geographical locations and/or in the two ROI batches. These taxa are major contributors to carbohydrate metabolism, which was reflected in functional predictions. Faecal volatile fatty acids and salivary cortisol were the only physiological parameters that differed between RFI ranks. This study indicates that rearing environment is more influential on intestinal microbiota than FE, despite controlling genetics, diet and management strategies.

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Poster Number: 24

PARASITOLOGICAL EVALUATION OF ORGANIC FERTILIZERS

Abstract

Background - Sanitary safety of organic fertilizers used in agriculture is essential for public health protection. The aim of the study was to assess the parasitological contamination of organic fertilizers to be admitted into commercial use in Poland in 2015-2017.

Methods - In the study 355 samples (113 sludge samples from biogas plants, 75 samples produced on the basis of sewage sludge, and 167 samples other organic fertilizers) were examined, with the use of own accredited procedures.

Results - Viable eggs of parasites were found in 54 samples from biogas plants (47.8%) in 23 samples based on sewage sludge (17.3%) and 21 samples of other organic fertilizers (12.6%). Viable eggs of *Ascaris* spp., *Trichuris* spp. *Toxocara* spp. were found in 83, 33 and 24 samples, respectively. The most numerous were eggs of *Ascaris* and *Toxocara*. The most contaminated were samples from biogas plants. In these fertilizers were found 60 to 251 640 live eggs of the genera *Ascaris* and *Trichuris* in 1 kg dry mass. Organic fertilizers samples produced on the basis of sewage sludge were contaminated by eggs of *Ascaris*, *Trichuris* and *Toxocara*. Total number of parasites eggs in these samples ranged from 858 to 19 010 kg of eggs in dry mass. In other organic fertilizers were found small amounts of viable parasites eggs.

Conclusion - The obtained results confirm the necessity of the parasitological examination of the organic fertilizers before admission to trade in order to eliminate the potential risks to human and animal health.

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Poster Number: 25

Decoupling of belowground biodiversity revealed within major temperate ecosystems

Abstract

Mounting evidence suggests that microbial communities do not conform to the trends in biodiversity seen in animal and plant communities across large geographic scales. Additionally, many large-scale assessments of soil biota have not included the breadth of microbial life (i.e.bacteria, archaea, fungi, and protists) and there have been few attempts to provide a synthesis between microbial and faunal analyses.Here, we conducted an exhaustive assessment of soil communities at a national-scale over an ecologically representative productivity gradient. Environmental DNA and soil properties were extracted from 436 co-located samples collected as part of the Glastir Monitoring and Evaluation Programme, which is responsible for the assessment of Glastir; the Welsh Government's agri-environment scheme. High-throughput sequencing of the complete soil communitywas used to investigate how the combined effects of land use and soil properties influence α- and β-diversity of soil life. We found a decoupling between microbial and animal biodiversity. Land use most strongly influenced the richness of animal communities, while soil properties were the dominant drivers of microbial community richness. Microbial α-diversity followed linear relationships across a productivity gradient of land use types. Conversely, animal α-diversity showed a normal-like distribution along the productivity gradient, indicating strong influences of aboveground plant communities. Across all organisms, α- and β-diversity was most strongly influenced by pH, following established trends. Other interactions between diversity and soil properties were hard to disentangle. Our findings confirm the prominence of pH in shaping soil community and support recent challenges to the notion that microbes follow conventional macroecological paradigms.

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Poster Number: 26

Training agriculture-based anaerobic digester microbiome for improved faecal coliform removal

Abstract

Manure and slurry may contain a range of bacterial, viral and parasitic pathogens and landspreading of these organic fertilisers typically occurs without prior treatment. This common practice may pose potential human and animal health risks *via* bio-aerosol inhalation or contamination of soils, plants and water. *In-situ* treatment through farm-based anaerobic digestion (AD) of slurry co-digested with food-production wastes, such as fats, oils and grease (FOG), is multi-beneficial due to energy recovery, increased farm incomes and noxious gas reduction. AD digestate suitability for landspreading is assessed by faecal indicator bacteria (FIB) monitoring, required by EU legislation to be below 1000 CFU g^{-1}.

This work examined the survival of FIB in slurry co-digested with FOG. Three mesophilic 10L continuously-stirred suspended biomass reactors were operated for 450 days with the following initial feeding regime: four consecutive one-day feeds alternating with one three-day feed with a loading rate equivalent to three one-day feeds. FIB numbers were monitored as indicators of pathogen fate throughout the trial, as were physico-chemical parameters including pH, ammonia, volatile solids and biogas.

Results from the first 100 days exhibited a trend of increased FIB die-off during the three-day feeds, approaching and occasionally achieving the required threshold. Hence, bioreactors were fed every three days, whilst maintaining the same loading rate. After acclimation, coliform and *E. coli* die-off consistently achieved the required standard, whilst methane output also improved. These findings highlight the potential for optimisation of agriculture-based AD, both in terms of energy production and reduced pathogen load to the environment.

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Poster Number: 27

Investigating the evolutionary divergence of the *Butyrivibrio* group

Abstract

Exploring and understanding the phylogeny of the *Butyrivibrio* group is imperative if we are ever to fully understand the consortium of ruminal microbial enzymes that are responsible for the catalysis of multifaceted reactions, such as biohydrogenation.

At present, taxonomic classification of the *Butyrivibrio* group is based primarily on butyrate production. This approach has become antiquated with the development of sequencing technologies and downstream bioinformatics analysis. This study investigated the taxonomic relatedness and functional capacity of the ruminal *Butyrivibrio* group using 72 genomes. Seventy-one *Butyrivibrio* group genomes were obtained via JGI (the Hungate 1000 project), and one additional bacterial strain was sequenced by ourselves. 16S rDNA were aligned using RDP, and a phylogenetic tree created using FastTree. An additional tree was constructed using 40 gene markers. Visualisation was by Interactive Tree Of Life. Pangenome analysis was conducted using Spine/AGEnt/ClustAGE. Orthologous gene affiliations were identified using OrthAgogue. Data obtained showed that three primary clades were observed, namely the genus *Pseudobutyrivibrio*, *B. fibrisolvens*, and the remaining *Butyrivibrio* species using both 16S rDNA and 40 gene marker approaches. Pangenome analysis and orthologous gene affiliations revealed greater diversity within *Butyrivibrio* than *Pseudobutyrivibrio*. *Butyrivibrio* clades also consistently showed smaller core genome sizes in comparison to *Pseudobutyrivibrio*, indicating high levels of genomic variance. These findings suggest that the *Butyrivibrio* group are highly evolved to maintain competitiveness in the rumen and emphasises the need for further research into the biochemical capacity of the *Butyrivibrio* group.

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Poster Number: 28

Linking the rumen microbiome metagenome with quality traits of the meat from cattle raised on diets to promote different growth speeds.

Abstract

Early life nutrition can have long lasting impacts on the development of the rumen microbiome and its functionality. Understanding the rumen microbiome and factors that affect its development may allow novel techniques to improve the nutrients gained by the animal, therefore improving meat quality. Weaned cows were raised on diets to promote different growth speeds; concentrates for rapid growth, abundant high quality forage grazing for medium growth, and limited poor nutritional forage grazing to achieve slow growth. Data were collected relating to the quality of meat at slaughter. Full metagenome shotgun sequencing was performed on rumen samples obtained following slaughter. Metagenomic analysis was performed on the sequences using MG-RAST to discover microbial populations and their metabolic capability. Analysis showed that meat from concentrate based diets was less appealing in terms of taste and texture and was less healthy in terms of fatty acid proportions, principally the proportions of n-3 to n-6 fatty acids. Metagenomic analysis highlighted differences in bacterial populations and their metabolic capability which could explain some of the differences observed in the meat analysis. This indicates that more research needs to be done to further explore the links between the rumen microbiome and its effect on the animal. With greater knowledge into early life nutrition on the development of the rumen it may be possible to manipulate the microbiome to have positive effects on the animal to achieve better meat, from less input producing less waste.

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Poster Number: 29

Distribution and population structure of endobacteria inarbuscular mycorrhizal fungi at North Atlantic dunes

Abstract

Arbuscular mycorrhizal fungi (AMF, Glomeromycotina), form symbioses with majority of land plants, provisioning them with vital mineral nutrients in exchange for photosynthesis-derived carbon. Consequently, AMF are of rising interest in sustainable agriculture as alternatives to non-renewable mineral fertilizers. AMF themselves can harbour vertically transmitted endosymbiotic bacteria '*Candidatus* Glomeribacter gigasporarum' (*Ca*Gg) and '*Candidatus* Moeniiplasma glomeromycotorum' (*Ca*Mg). *Ca*Gg is a nonessential mutualist of AMF, whereas the lifestyle of *Ca*Mg is unknown. To start unraveling the interactions between AMF and their endosymbionts in nature, we examined diversity and distribution of AMF-associated endobacteria in North Atlantic dunes. Of nearly 500 foredune AMF isolates surveyed, 94% were classified as Gigasporaceae. 2% of all AMF spores harboured *Ca*Gg, and 88% contained *Ca*Mg. *Ca*Gg was found only in the Gigasporaceae, whereas *Ca*Mg was present in Gigasporaceae, Acaulosporaceae, and Diversisporaceae. Incidence of *Ca*Gg across AMF was not affected by any of the environmental parameters measured, whereas distribution of *Ca*Mg in one of the hosts was impacted by plant density. *Ca*Mg populations associated with AMF individuals displayed high levels of genetic diversity but no evidence of gene flow, suggesting that host physical proximity is not sufficient to facilitate horizontal transmission of *Ca*Mg. Lastly, in addition to a novel lineage of *Ca*Gg, we discovered *Burkholderia*-related bacteria previously not known to associate with Glomeromycotina, and likely living inside AMF. They are closely related to free-living *Burkholderia* and endobacteria of other Mucoromycota fungi. Collectively, we conducted the first ecological study of AMF-associated endobacteria and assessed their diversity and population structure.

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Poster Number: 30

Pseudomonas syringae in lichen-associated microbiomes

Abstract

Although generally described as bipartite mutualistic associations of a fungus and an alga or cyanobacterium, lichens are also known to harbour species-specific communities of endolichenic bacteria, typically dominated by Proteobacteria. Among lichen-associated bacteria, revealed by both metagenomics and culture-based studies are several known plant pathogens, including the well-known pathogen, *Pseudomonas syringe.*

Analysis of 30,033 bacterial contigs, from a partial shotgun metagenome of the microbiome associated with *Peltigera membranacea,* yielded multiple hits on several genes likely to play a role in plant pathogenicity. Among plant virulence genes found in the metagenome are several homologs of the *virB/D4* type IV secretion system from diverse bacteria, including *P. syringae*.

In our culture collection, stored at the Unviersity of Akureyri, more than 700 strains have been isolated from various lichen thalli. Using a PCR-based method, with primers targeting *P. syringae*, we have confirmed that *P. syringae* has been isolated from lichen thalli from Icelandic heathland. A total of 15 strains have been assigned into 5 different phylogroups of *P. syringae*. Furthermore, selected strains have been PCR-screened for the type III secretion system components and other virulence factors, using primers for *avrE* and *hrcC*. The genes were found in majority of the selected strains, suggesting the type III secretion system to be present.

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Poster Number: 31

Soil microbial community responses to a combined slurry and flooding event

Abstract

In order to understand how climate shifts will affect soil ecosystem services, significant research has been undertaken into the stability of soil microbial communities. However, biological pulse disturbances and disturbance combinations require further investigation. Thus, we investigated soil microbiome responses to a compounded disturbance consisting of slurry application preceding an extreme precipitation event. We hypothesised that the influx of nutrients and biomass from slurry would alter resistance and/or resilience to flooding. To test this, both microbial community composition and functional potential were monitored. Slurry application resulted in transiently increased rates of basal respiration, litter decomposition and altered carbon utilisation profiles (CUP). Upon flooding, slurry improved microbiome resistance to waterlogging, considering basal respiration and litter decomposition. Flooding reduced *in-situ* nitrification in both slurry-amended and non-amended soils, as evidenced by significant NH_4 accumulation. However, despite 21 days of waterlogging, potential nitrification rates for slurry-amended soils remained at least double that of the non-slurry controls. Once moisture content had returned to that of the unflooded controls, resilience was observed for all measured assays. Recovery of *in-situ* nitrification was observed by depletion of accumulated NH_4 to similar levels as unflooded controls, with NH_4 consumption occurring more rapidly in slurry-amended *vs* non-amended soils. Together, this suggests slurry amendment conferred a benefit to the nitrifying community in the face of flooding. Despite evident resilience, CUPs and community structure of microcosms subjected to flooding remained distinct to unflooded microcosms throughout the recovery period, suggesting an alternative stable state of the disturbed microbial community.

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Poster Number: 32

Characterizacion of bacterial communities of baby spinach and surrounding soil irrigated with water treated with chlorine dioxide in a commercial open field

Abstract

Background: The use of water disinfection treatments for irrigation water is an intervention strategy available for growers to reduce microbial risk during primary production. Chlorine dioxide (ClO_2) has been proven to be an efficient treatment. However, in most of the cases, the suitability of ClO_2 to treat irrigation water has only been tested against artificially inoculated pathogenic bacteria without taking into account the potential effects on the bacterial community of the crops.

Aim: To evaluated the impact of $ClO_{2,}$ as a water disinfection treatment for irrigation water constantly applied during the growing cycle of baby spinach, on the bacterial community of the crop and the surrounding soil.

Methods: Two trials were performed in commercial production open fields of baby spinach. In each trial there were two plots, one irrigated with untreated irrigation water (IW) and a second one where irrigation water was treated with ClO_2 (IW- ClO_2). The bacterial community of samples was evaluated using Illumina Miseq® Illumina sequencing platforms and multivariate statistical analysis.

Results: ClO_2 treated irrigation water showed significantly different bacterial community structure and composition than untreated water (ANOSIM= 0.01). The obtained sequences revealed that disinfection with ClO_2 significantly decreased the relative abundance of *Pseudomonaceae* (14%) and *Enterobacteriaceae* (19%) of baby spinach, when compared with the product irrigated with untreated water. We also observed that the phyllosphere bacterial community of baby spinach was similar to soil bacteria communities rather than that of irrigation water.

Conclusion: The use of ClO_2 as a disinfection treatment for irrigation water in a baby spinach open field did not cause significant changes in the baby spinach and soil microbiota.

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Poster Number: 33

Investigation of effects of plant biostimulants on symbiotic microorganisms in cereals

Abstract

Plant biostimulants can enhance crop growth performance by influencing plant metabolism. This can result in yield increase, earlier harvest or improvement of crop qualitative parameters. However, little is known about the interaction of biostimulants with symbiotic microorganisms, which significantly contribute to proper functioning of a majority of plants. This work aims to investigate the effects of a new biostimulant based on biorefined waste products on symbiotic microorganisms and their interactions with plants. A first screening of possible effects of foliar biostimulant treatments on plant symbiotic microorganisms was carried out on wheat and barley in a greenhouse experiment and covered early stages of plant development. A subsequent experiment with wheat and barley has been set up to verify the effects of plant biostimulants under field conditions. Plant leaves and roots were sampled at two time points after the biostimulant application to measure plant growth parameters and root colonization by symbiotic microorganisms. Biostimulants increased leaf/root dry weight, leaf chlorophyll content or plant height depending on crop species and a type of biostimulating product. Preliminary results may suggest possible effects of biostimulant treatments on the plant colonization by selected symbiotic microorganisms. Further analyses should elucidate whether the observed changes are accompanied or mediated by a change of root-inhabiting microbial communities.

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Poster Number: 34

A meta-analysis of the bovine ruminal and gastrointestinal microbiota

Abstract

The bovine ruminal and gastrointestinal (GI) tract microbiota are important contributors to the health and production of cattle around the world. In the last 5 to 10 years, a large number of studies have been published that characterize the bovine microbiota using high-throughput sequencing of the 16S rRNA gene. In addition to study-specific factors, researchers often utilize different DNA extraction methods and sequence different hypervariable regions from the 16S rRNA gene. However, it would be valuable to know which factors are most strongly shaping the bovine microbiota and the commonalities in microbial composition that are shared by all bovine microbiota-related studies. Therefore, we identified and downloaded publicly available 16S rRNA gene datasets from more than 50 studies that focused on the rumen and/or GI microbiota of cattle (*Bos taurus*). Among rumen samples, 60% of the samples shared bacterial OTUs identified as *Prevotella*, *Ruminococcus*, and *Saccharofermentans*. OTUs classified as *Alistipes*, *Bacteroides*, *Escherichia*, *Parabacteroides*, *Rikenellaceae* RC9 gut group, *Roseburia*, *Ruminococcus*, and *Saccharofermentans* were shared by 60% of the fecal samples. The archaeal OTUs *Candidatus* Methanomethylophilus and *Methanobrevibacter* were identified in 75% of rumen samples and *Methanobrevibacter*, *Methanocorpusculum*, and *Methanomicrobium* OTUs were shared among all fecal samples analzyed. As expected, there was a strong study effect ($R^2 = 0.28$; $P < 0.001$) but sample type also influences the bovine microbial community ($R^2 = 0.12$; $P < 0.001$). This work demonstrates that despite significant inter-study variability, several taxa in the ruminal and GI microbiota are shared among the majority of cattle.

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Poster Number: 35

Naturally present lactic acid bacteria as a biocontrol agent against *Listeria monocytogenes* in the mushroom production environment

Abstract

Listeria monocytogenes is a growing concern for the mushroom industry, as studies have shown that this pathogen can be found in mushroom production facilities, which therefore poses a risk of product contamination. Thus, it is important to take proactive steps to maintain the industry's reputation for food safety by exploring novel biocontrol agents to provide enhanced assurance of product quality and safety. Anti-listerial bacteria were isolated from different types of mushroom growth substrates and were identified using 16s rRNA sequencing, while colony MALDI-TOF mass spectrometry was used to identify the bacteriocins produced. Competitive exclusion activity of *L. lactis* strain Ca55 was then tested in mixed-biofilm conditions with *L. monocytogenes* on stainless steel coupons for 72h. *L. lactis* subsp. *lactis* strains with anti-listerial activity were found to be Nisin Z producers and naturally present in the mushroom production environment. Growth of *L. lactis* subsp. *lactis* using mixed-biofilm conditions with *L. monocytogenes* on stainless steel resulted in a significant ($p<0.05$) 4-log reduction of *L. monocytogenes* cell numbers. This study found that the *L. lactis* subsp. *lactis* strains isolated from mushroom growth substrates have potential for application as biocontrol agents against *L. monocytogenes,* while also raising the possibility of other bacteriocin-producing LAB to be present in mushroom growth substrate which can be utilised as biocontrol agents. The use of shotgun metagenomic sequencing on mushroom growth substrate during the production cycle will yield information about the growth dynamics of lactic acid bacteria present, along with presence of known or novel bacteriocin genes.

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Poster Number: 36

Gut and whole-body microbiota of the honey bee separate thriving and non-thriving hives

Abstract

The recent worldwide decline of honey bee colonies is a major ecological problem which also threatens pollinated crop production. Several interacting stressors such as environmental pressures and pathogens are suspected. Recently, the gut microbiota has emerged as a critical factor affecting bee health and fitness. We profiled the bacterial communities associated with the gut and whole body of worker bees to assess whether non-thriving colonies could be separated from thriving hives based on their microbial signature. The microbiota of thriving colonies was characterized by higher diversity and higher relative abundance of bacterial taxa involved in sugar degradation that were previously associated with healthy bees. In contrast, the microbiota of non-thriving bees was depleted in health-associated species, and bacterial taxa associated with disease states and pollen degradation were present in higher abundance compared to thriving colonies. Gut and whole body microbiota shared a similar dominant core but their comparison showed differences in composition and relative abundance. More differences between gut and whole body were observed in non-thriving bees, suggesting that microbiota associated with other bee organs might also be altered. Thus, microbiota profiling could be used as a diagnostic tool in beekeeping practices to predict hive health and guide hive management.

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Poster Number: 37

Bacterial communities associated with the surface of fresh sweet pepper (*Capsicum annuum*)

Abstract

Fresh produce vegetables are inhabited by different bacterial species, some of which are antagonistic to microbes that cause postharvest losses. However, no comprehensive assessment of the diversity and composition of bacteria inhabiting surfaces of fresh pepper plants grown under different conditions have been conducted. In this study, 16S RNA amplicon sequencing was used to reveal bacterial communities inhabiting the surfaces of red and green pepper from plants (fungicides-treated and non-fungicides treated) grown under hydroponic and open field conditions. Results revealed that pepper fruit surfaces were dominated by the bacterial phylum *Proteobacteria, Firmicutes, Actinobacteria* and *Bacteroidetes.* The majority of the bacterial operation taxonomic units (OTUs; species level) were shared between the two habitats, two treatments and the two pepper types. The bacterial genera classified as antagonists (e.g., *Acinetobacter, Agrobacterium, Arthrobacter, Bacillus, Burkolderia, Curtbacterium, Enterococcus, Flavobacterium, Lactobacillus, Methylobacterium, Microbacterium, Novosphingobium, Pseudomonas, Sphingomonas and Weissella*) were the most abundant. These results suggest that peppers are exposed to substantially different bacterial communities with antagonistic activities independent of growing conditions, sample treatment and sample type.

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Poster Number: 38

Management-induced differences in the bacterial community composition of permanent grasslands are temporally stable over a growing season

Abstract

Agricultural management is a strong driver of microbiome composition in permanent grassland agro-ecosystems. It is not currently clear, however, how temporally stable such management-induced differences are over the course of a growing season. 3 permanent grassland management types were used in this study; intensive, low intensive and extensive, with a sown grassland-to-maize rotation used as a control. 10 separate fields of each management type were selected from the region of Zurich, Switzerland from which soil samples for microbiome analysis were taken seven times over the 2017 growing season (April to October). The bacterial 16S rRNA gene was PCR amplified from each sample and an amplicon-based Illumina Miseq sequence analysis was conducted. Agricultural management had a highly significant effect on bacterial community composition ($P < 0.001$), with each of the four management types harboring significantly distinct bacterial communities. No significant difference in the bacterial community was seen between the seven sampling events in either the intensive, low intensive or extensively managed permanent grasslands ($P > 0.05$). There were, however, significant differences seen between the first two samplings and the later sampling events in the maize rotation ($P < 0.05$). Such changes were associated with the ploughing of the sown grassland. Further work will examine the fungal community composition in the same samples. These results highlight the stability of management-induced differences in bacterial community composition in permanent grasslands. They will inform a larger European study investigating the influence of both agro-climatic region and management type on the microbiome composition in such systems.

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Poster Number: 39

The BioLighthouse: Reusable Software Design for Bioinformatics

Abstract

Advances in next generation sequencing has accelerated the field of microbiology by accessing the wealth of information available about microbiomes. Unfortunately, these experiments are among the least reproducible in terms of bioinformatics. Software tools are often poorly documented, under maintained, and commonly have arcane dependencies requiring a significant time investment to configure them correctly. This work proposes a software development pattern, the BioLighthouse. This is an approach that supports the reuse of bioinformatics tools by integrating software development and the formalized description of the dependencies needed for each tool. The BioLighthouse provides a mechanism for developers to deploy their tools, knowing they will be configured and executed in an intended way. Specifically, in the same way the developer use the tools themselves. The BioLighthouse pattern is focused on ensuring that the tools can run agnostic of specific hardware constraints and thus empowers a user to deploy the tools onto computing resources that are not maintained by the original tool developer (e.g. cloud or on-premise). To demonstrate the utility of the BioLighthouse, a microbial profiling pipeline was implemented for high-throughput processing of microbial profiling datasets. The pipeline not only performs primer removal, trimming, merging and contaminant removal before producing the abundance matrix but the specification of the pipeline enables it to be deployed onto new hardware in an agnostic fashion. This example pipeline makes use of the DADA2 R package which is based on amplicon sequence variants meaning the true sequence is used rather than clustering sequences into operational taxonomic units.

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Poster Number: 40

Identifying microbial communities using genetic algorithms

Abstract

Understanding the dynamics of how microbiome interacts with a host organism has the potential to enable novel mechanisms to positively impact agriculture. Host–microbiome dynamics involve both host-microbe and microbe-microbe interactions. As microbiome studies begin to be connected with large experiments, as in common for adequately powered agricultural studies, there is a growing challenge of how to deal with high dimensional microbiome data. We introduce a method based on genetic algorithm (GA) to identify the microbes as groups (consortium) that were differentially associated with crop seeds.

Inspired by basic evolutionary concepts of crossover,mutation and selection, GAs is typically used to find good solutions to optimization problems. We implemented a GA based selection method using Pyevolve, an open source framework written in python. GAs randomly generates microbial consortia (group of consortiums) from the set of microbes in the microbiome. Each consortium is evaluated using a fitness function and undergoes crossover (swapping of microbes between consortium) and mutation (replacement of microbe within consortium with another one chosen randomly) and select consortium with highest score.

Using an epiphytic seed microbiome data that contains wheat and canola samples, a consortium of 250 microbes was identified. Of the results obtained by GA, 84% were consistent with previous reports of research into this microbiome. This work demonstrate that GA can be applied to any agricultural settings where communities of microbes are being studied and may lead to new insights into the microbial interactions, which in turn may help to improve the sustainability of agricultural production.

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Poster Number: 41

Soil microbial communities in highland and lowland grasslands under different management intensity

Abstract

In the BIOINVENT project, the soil microbial communities in permanent grassland systems are investigated along a pan-European gradient. In this particular study, we compare the soil microbial community of grasslands in two different agro-ecological areas (highlands and lowlands) in two different countries (Germany and Switzerland). A gradient of management intensity was sampled, from intensive grasslands with high nutrient inputs and utilization frequency, to grasslands with an intermediate and extensive management intensity. It was analysed 12 replicates per management intensity and area, which adds up to 144 samples. The PLFA and NLFA (phospholipid-neutral fatty acid) analysis was used to estimate the total and the specific biomass of the soil microbial groups as well as the microbial community structure. A marked impact of the agro-ecological gradient was detected, the microbial community structure varied depending on the altitude, while the management intensity had a smaller impact, in each country. We observed that in the lowlands, the microbial community structure of the extensive grasslands was different compared to the grasslands under other management intensities. The effect was, however, not clear in the highland samples. Despite these differences in the microbial community structure, the total and bacterial biomass was not significantly different under different management intensity or in different agro-ecological regions. However, the fungal biomass – both saprotrophic and mycorrhizal – is generally higher in the extensive grasslands than in the other management intensities. We therefore conclude that, the extensive grasslands provide a distinct habitat for fungal colonization; but this work will feed into the larger survey.

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Poster Number: 42

Plant protection from a distance

Abstract

Soil microbiome is essential to maintain plant growth, health and protection against biotic and abiotic stresses. The phenomenon called soil suppressiveness, where plants show significantly reduced disease symptoms even if the pathogen and favourable conditions are present is strongly related to the microbial activity and secondary metabolites production. Among various modes of action of soil bacteria, one of the remarkable but less studied is the production of Volatile Organic Compounds (VOCs). Due to their physicochemical properties, VOCs diffuse easily through gas- and water-filled pores in soil and rhizosphere environments, allowing long-distance interactions. Many bacterial VOCs were found to possess strong antimicrobial activity. We have studied the volatile-mediated antimicrobial activity of tomato rhizosphere isolate *Pseudomonas donghuensis* P482 against fungal and oomycete plant pathogens (*R. solani*, *V. dhaliae* and *F. culmorum* and *P. ultimum*). In the VOCs blend produced by *Pseudomonas* P482, we identified several compounds with sulfur-containing compounds as dominant. We observed clear fungistatic effect caused by the VOCs produced by P482 as well as by the pure compounds S-methyl thioacetate and dimethyl disulfide that were identified in the blend.

However, can we correlate fungistasis with soil suppressiveness? Our recent findings based on screening 28 soils from the Netherlands and Germany indicate that soil suppressiveness and strong soil fungistasis does not always correlate with each other. At present we are exploring the mechanisms involved in both soil fungistasis and suppressiveness.

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Poster Number: 43

Bacterial community diversity of Uruguayan soils from different soil map units with contrasting content of phosphorus and other edaphic properties

Abstract

Soil microorganisms play key roles in the phosphorus (P) cycle, mediating the availability of this plant nutrient. Uruguay imports near to 100% of the P needed for agricultural activity; therefore, the search of more efficient production systems in the use of P is a challenge. Despite the high total P content in Uruguayan soils (150-700 ppm), the available P is relatively low (<10ppm). Soil pH ranges from 5.1 to 7. The objectives of this study were: (1) to characterize the structural diversity and composition of the microbial communities in ten sampling sites, representative of five soil map units from Uruguay, and (2) to explore their relationship to soil physical and chemical properties. Taxonomical analysis was performed with QIIME pipeline and the statistical analysis were performed with Vegan and Phyloseq R packages. A total of 4547 OTUs were obtained. The soil bacterial community richness and the Shannon index values showed significant differences among soil map units. Beta diversity analysis using NMDS ordination of Bray-Curtis distances showed five clusters corresponding with the five soils map unit and a PERMANOVA test indicate that this cluster are significative (R^2=0.7099). The Canonical Component Analysis (CCA) allowed to narrow down to four the physicochemical soil properties that explained the bacterial diversity. The taxonomical bacterial community structures were best explained by a combination of pH, N, K and clay content. Overall, our results suggest that soil physicochemical properties are among the driving forces of bacterial community diversity and composition.

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Poster Number: 44

A High Affinity Iron Transporter PiFtr from Root Endophytic Fungus *Piriformospora indica*: A promising Resource for Iron Enrichment

Abstract

Due to its insoluble form in the soil, Fe is a limiting factor for plant growth and development. Over two billion people including pregnant women and preschool children suffer from micronutrient deficiencies such as iron, zinc and vitamin A, because their plant-based diets are not a sufficiently rich source of these essential elements. Membrane transporters of fungal partner are required for this transfer of nutrients from the soil to the plant. We have functionally characterized a high affinity iron transporter gene (*PiFtr*) from a root fungus *Piriformospora indica*. *PiFtr* shows 40% similarity with Ftr1 from ascomycetes. Phylogenetic, homology and kinetics analysis indicates that *PiFtr* is a high affinity iron transporter. *PiFtr* has been expressed in *S. cerevisiae* where it complements the iron-uptake minus phenotype of an Ftr1mutant strain which suggests that *PiFtr* is a functional homologue of *S. cerevisiae* iron transporter. To understand the physiological role of *PiFtr*, knockdown (KD) transformants of the gene were prepared using electroporation and RNA interference. KD transformants transported a significantly lower amount of Fe to the colonized plant than WT *P. indica*. Higher amounts of Fe were found in plants colonized with WT *P. indica* than that of non-colonized and plants colonized with KD-*PiFtr*-*P. indica*. Characterization of *P. indica PiFtr*, will serve as a model system to study molecular mechanism of indirect uptake of Fe by plants to improve food and biomass production, as well as to develop Fe-rich crops for improved human nutrition.

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Poster Number: 45

Rhizospheric and endophytic bacteria as potential suppressors of plant pathogens

Abstract

Rhizospheric and endophytic bacteria have been reported not only to enhance plant growth but also to suppress bacterial pathogens. The objective of the present work was to isolate microorganisms from a rhizosphere and plant tissue and to test them for plant growth promotion features and activity against plant pathogens.

Endophytic bacteria were isolated on nutrient agar from the rhizosphere of herbaceous and greenhouse plants and directly from plant tissues, using classic microbiological methods. Several important bacterial characteristics, such as cyanide, siderophores, lipase, protease, cellulase, ammonia, IAA production or phosphate solubilizing were tested. Isolates that demonstrated that features, were identified by sequencing of 16S RNA and BIOLOG system. The direct influence of selected isolates on five common plant pathogens (*Pectobacterium atrosepticum*, *Clavibacter michiganensis ssp. michiganensis*, *Xanthomnas campestris* pv. *pelargonii*, *Pseudomonas syringae* pv. t*omato*, *Pectobacterium carotovorum ssp. carotovorum*) was tested on Petri dishes. In total, 214 colonies were isolated and tested under laboratory conditions. Isolates which were the most antagonistic for plant pathogenic bacteria belonged to genera *Arthrobacter, Bacillus, Geobacillus, Pseudomonas, Rautella, Serratia, Sphingobacterium, Stenotrophomonas and Virgibacillus*. It has been shown that beneficial microorganisms can replace chemical agents in combating or reducing the occurrence of phytopathogenic bacteria.

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Poster Number: 46

Changes in Plant and Soil Microbial Taxonomic and Phylogenetic Diversity Along Gradients of Land Management Intensity in São Miguel Island (Azores)

Abstract

The natural vegetation in many areas of the world has been replaced by pastures and monocultures, leading to profound changes in the soil and plant communities. Therefore, a more sustainable management is desirable for pastureland worldwide. The Azores are ideal to model those changes, with habitats ranging from semi-natural to completely artificial found at relatively small distances. Furthermore, more than 50% of the land use in the Azores corresponds to pastureland, with agroindustry being a relevant socio-economic sector. Additionally, 30% of land use is dedicated to the production of wood, with the monoculture of *Cryptomeria japonica*. Three hypotheses will be tested along gradients of land management intensity in São Miguel (i) soil microbial diversity is strongly affected by land management intensity; (ii) composition and diversity of endophytic microorganisms will differ along a gradient of management intensity; and (iii) plant and microbial phylogenetic diversities are strongly affected by land management intensity. Three levels of management intensity will be tested for grasslands: semi-natural pasture, intermediate intensity pasture, high intensity pasture (corn rotation); and three levels for forests: production, exotic and natural. So far, two sampling seasons have been conducted (Winter 2018, Spring 2018), in a total of 72 samples. Molecular methods will be used in order to assess soil microbial diversity and the composition and diversity of endophytic organisms. Numerical ecology, maximum likelihood and Bayesian methods will be used to analyse the results.

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Poster Number: 47

THE EFFECT OF SOIL pH AND PHOSPHORUS INTERACTIONS ON FUNCTIONAL MICROBIAL COMMUNITIES INVOLVED IN NITROGEN CYCLING

Abstract

Nitrogen applied to agricultural soils in excess can cause environmental pollution when lost to water or air. Reducing emissions of potent greenhouse gas nitrous oxide (N_2O) is of critical importance towards climate change mitigation and sustainable agricultural systems. Nitrogen availability can stimulate microbial communities to carry out denitrification, an anaerobic respiratory pathway in which N_2O is an intermediate product. An understanding of soil, climatic and edaphic factors impacting microbial community structure and activity is essential for predicting and mitigating N_2O production. pH is known to strongly impact microbial community structure and has a direct effect on denitrifying communities as *NosZ*, the enzyme catalysing N_2O reduction, is pH sensitive. We thus would expect that microbial communities in acidic soils have a decreased capacity to mitigate N_2O emissions. This effect of pH is likely to interact with other management factors, such as phosphorus (P) application, through impacts on chemical nutrient availability and direct effects on microbial composition. The link between N_2O emissions and microbes dictated by soil pH; and the interacting role P availability plays in this relationship are not fully understood. In this project the impact of soil pH on the potential of the community to denitrify and the functional microbial community were analysed by potential denitrification assays and qPCR analysis of denitrification genes across a pH gradient and under a range of P application rates in two soil types. Understanding the link between the microbial communities and N_2O production can be applied in agricultural management to reduce emissions from fields.

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Poster Number: 48

Rhizosphere microbial communities and winter wheat (*Triticum aestivum*) yield

Abstract

The rhizosphere is a narrow and complex soil environment associated with living plant roots and colonised by symbiotic and free-living microorganisms. We hypothesise that microbial diversity and activity within the rhizosphere contributes to plant development and fitness, which could potentially be adapted to enhance commercial crop productivity and nutritional value.

Here we propose a study to identify, quantify and relate the functional activities of microbial communities in the rhizosphere of field grown wheat (*Triticum aestivum*) with the protein amounts of wheat. We have developed and implemented protocols for in-the-field rhizosphere soil collection and nucleic acid preservation, and followed co-extraction of DNA and RNA for both metagenetic and metatranscriptomic analyses. Determination of the protein content of wheat was based on measurement of total nitrogen using the Kjeldahl analysis method.

Based on protein yield, samples were split into two non-overlapping groups (p-value <0.05 for t.test between mean protein abundance) which were accordingly named low- and high-yield groups. Statistical analysis indicated no meaningful correlation between protein amounts, soil attributes or plant weight. We therefore speculate that the protein distribution at the time of sampling may be associated with the rhizosphere microbial composition and activity, which will be elucidated upon completion of sequencing analysis.

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Poster Number: 49

Variation in dairy cow manure and consequences for the efficacy of a foot bathing disinfectant

Abstract

Digital dermatitis is a polybacterial disease causing painful lesions on the heels of dairy cows, controlled on farms by disinfectant foot baths (which become contaminated with manure). This pilot work examined the impact of abiotic variation in manure from animals housed or at pasture on disinfectant efficacy.

Dairy cow manure (n=5 Housed; n=5 Pasture) was sterilised by autoclaving and dry matter content was determined. Quantitative suspension tests were carried out with each manure using *Staphylococcus epidermidis* and *Escherichia coli K12,* contact time 3, temperature 20C. Sterile manure (20% v/v) was added to the disinfectant (a trial product containing chlorocresol and triamine, Kilco Ltd, UK) before testing. Number of bacteria in suspension was determined before and after disinfectant contact and log reductions calculated. Data were analysed using Mann-Whitney U tests and Spearman's Rank correlation.

Manure dry matter content ranged from 12.5%-20.5%. Log reductions for controls (no manure) were >7.0 for each bacteria species. When tested with *E. coli K12* there was a non-significant trend for smaller log reduction with Housed than Pastured manure (median±IQR: Housed 4.39±0.98; Pasture 5.47±2.37; p=0.56) and a negative relationship between dry matter and log reduction (r_s=-0.790, p=0.007). There was no difference in log reduction between Housed and Pasture with *S. epidermidis* (Housed 5.83±2.61; Pasture 6.43±1.66; p=0.548) and no correlation with dry matter (r_s=-0.354, p=0.316). This pilot study suggests interactions between production system, manure composition, bacterial species used for testing and disinfectant efficacy, which could have implications for digital dermatitis control.

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Poster Number: 50

The effect of Actigen®-Pak supplementation on short chain fatty acids in the broiler cecum

Abstract

The primary focus of this research was to assess the effect of dietary supplementation with Actigen®-pak on short chain fatty acid (SCFA) concentrations in the broiler cecum. SCFAs are produced in the intestine by the metabolic activity of the microbiota as catabolic end-products from the fermentation of undigested dietary components. SCFAs are cited as having numerous positive effects on broiler health and performance. Actigen®-pak supplementation is known to alter cecal bacterial populations in the broiler.

In this study, the ability of Actigen®-pak to alter SCFA concentrations in 35-day old broiler cecal content was assessed using gas chromatography. Cecal content was collected from control and Actigen®-pak supplemented birds from three separate commercial broiler trials, conducted at two different sites. SCFAs were extracted from 174 cecal samples, quantified using GC-FID and the results were analysed for significant differences using independent t-tests.

Actigen®-pak supplementation did not significantly affect acetate or propionate but did significantly influence butyrate concentrations in all three trials. The results showed a 19% increase in butyrate concentration in Actigen®-pak supplemented birds when compared to the control diet in trial 1 (P=0.036). Butyrate was increased by ~10% in Actigen®-pak supplemented birds in trial 2 (P=0.024) and trial 3 (P=0.014) when compared to the control diet.

The results of this study suggest that dietary supplementation with Actigen®-pak has a positive effect on cecal butyrate concentrations.

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Poster Number: 51

Biostimulant mediated improvement of soil microbial activity

Abstract

Soil microbial community structure is a critical determinant of soil quality, nutrient cycling and organic matter turnover. The importance of soil microbial activity has gained a greater appreciation in recent years, with research clearly identifying direct links to crop productivity, yield potential and the plant's ability to adapt to abiotic and biotic challenges.

The present study investigated the effects of a biostimulant on soil microbial activity, which was quantified using colorimetric enzyme and substrate utilisation assays. A randomised block design was used to investigate treatment effects in boxes for both high and low organic matter soils. Soil treatment consisted of the biostimulant SOIL-SET™ Aid (at 1& 2 L/Ha) with samples taken from the rhizosphere of barley plants 10 weeks post-treatment.

Results indicated that SOIL-SET™ Aid treatment, particularly in high organic matter soil, corresponded to a significant increase in soil microbial activity, typically within a 10-17% range depending on soil type and treatment rate ($p \leq 0.05$). Community level physiological profiles were assessed based on carbon substrate utilisation; the results of which indicated improvements in metabolic activity and substrate usage patterns for SOIL-SET™ Aid treated samples. Differences in average well colour development and substrate functional diversity were supported with principal component analysis, which indicated clear differential clustering of treatment groups based on carbon utilisation patterns.

Findings from the present study emphasise the potential benefits of incorporating biostimulants into a crop management system. Increased metabolic activity is an important factor for soil quality; catalysing nutrient cycling and organic matter turnover.

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Poster Number: 52

Investigating the impact of fungicides and micronutrients on septoria tritici blotch and associated wheat phylloplane microbiome

Abstract

The development and spread of fungicide resistance in the major pathogens of wheat crops is increasing the necessity to investigate alternative control strategies. Amongst these the applications of micronutrients in combination with fungicides is increasingly promoted. Whilst such combinations may positively contribute in maintaining disease control and thus crop productivity, almost nothing is known about the wider impacts of such treatments on the wheat-pathogen-phylloplane interactions. Understanding these interactions is becoming increasingly important as the plant microbiome plays a vital role in plant metabolic processes as well as is essential for proper functioning of immune system, including the defense against fungi and other pathogens. Whether it is possible to manipulate these interactions to enhance disease control and productivity remains to be determined. To this end, the focus of this study is to analyze the phylloplane microbiome of wheat in response to fungicides and micronutrient applications. Following targeted disease control trials conducted in 2014-2017 the micronutrients Sulphur and Boron were selected and applied in combination with an azole/SDHI fungicide treatment to winter wheat in 2018 at three locations in Carlow, Cork and Meath. Following applications the upper leaf layers were sampled, from which the microbiome will be analyzed. Combined with Zymoseptoria tritici diversity analysis from the same samples and its transcriptome analysis to both micronutrients, it is anticipated that the above study will provide increased knowledge on how to maximize disease control programs of winter wheat.

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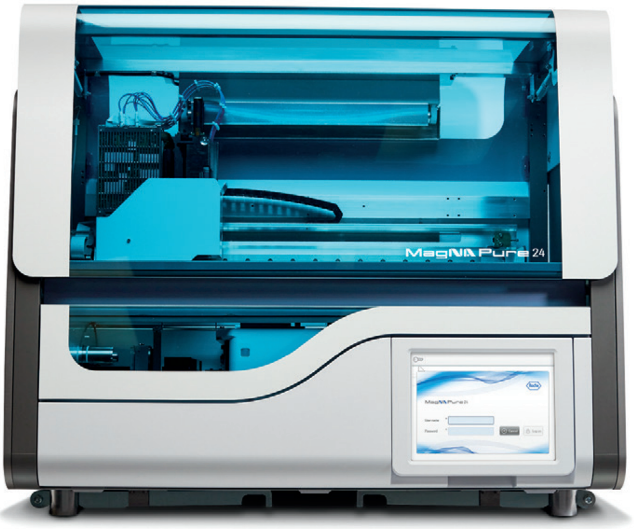

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