BENEFIT Symposium & Project Oversight Committee (POC) Meeting
Queen's University, Department of Biology
1st Floor of Biosciences Complex, 116 Barrie Street, Kingston, ON, K7L 3N6
May 30 – 31st, 2026 (Saturday – Sunday)
9:00AM – 5:00PM (EDT) Hybrid

ATTENDEES

Research Team:
Tasbiha Mahveev
Ivan Oresnik
George diCenzo
Alexander Ennin
Allyson MacLean
Ana Vargas
Asher Kim
Autumn Hodgins
Derek Brewin
Emily Perry
Jangwoo Lee
Jesse Branje
Karen Quan
Ken Mugambi
Kevin MacColl
Kevin Wurtz
Kristy Moniz
Lauren Hemara
Livia Titley
Malaika Khan
Marina Cvetkovska
Matthew Bakker
Michelle Drapeau
Mohammed Adam
Mubashshir Bari
Natalia Naranjo-Robayo
Neal Scott
Nedishtha Gobin
Ni An
Nicolas Corradi
Olivia Wilkins
Oona Esme
Pranab Das
Raveena Badu
Rebecca Doyle
Rebecca Pagès
Sachinthya Attanayake
Terrence Bell
Tia Harrison
Yen Chi Dang

POC Members:
LaKisha Odom *(Chair)*
Franz Bender
Getu Hailu
Lewis Lukens

Observers:
Valar Gurusamy *(WGRF)*
Alexa Pierce *(Lallemand Plant Care)*
Nicolas Watters *(Lallemand Plant Care)*
Meghan Rains *(Lallemand Plant Care)*
Laramy Enders (*Perdue University*)
Kevin De France (*Queen's University*)
Erika Gagnon (*Queen's University*)
Nicholas Smith (*Queen's University*)
Ryan Payton (*University of Manitoba*)
Isabella Ippolito (*McMaster University*)
Narendra Singh Yadav (*D-Mark*)

Genome Centre Representatives:
Ryan McKenzie *(Genome Canada)*
Les Kondejewski *(Ontario Genomics)*
Didi Kaur *(Government of Ontario)*
Lester Young *(Genome Prairie)*
Ifeoma Okwor *(Genome Prairie)*
Jelene Pugoy *(Genome Prairie)*

PROJECT DEVELOPMENT | RESEARCH MANAGEMENT | COMMUNITY ENGAGEMENT

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AGENDA – DAY 1: May 30th		
Time:	**Item:**	**Lead:**
9:00 – 9:20AM *(20 mins)*	**Badge Pick-up** *Attendees pick-up their badges from the registration desk*	-
9:20 – 9:50AM *(30 mins)*	**Welcome and BENEFIT Overview** *Team to provide an overview of the project, expected deliverables, and status of the project.*	George diCenzo, Ivan Oresnik
9:50 – 10:50AM *(60 mins)*	**Keynote Presentation** Developing Plant-Guided Selection Approaches for Designing Beneficial Microbial Inoculants that Protect Against Insect Pests (**L.E**).	Laramy Enders
10:50 – 11:20AM *(30 mins)*	**Break**	-
11:20AM – 12:20PM *(60 mins)*	**Project Update** Dynamic Crop Rotation Decisions, Soybean Nitrogen Fixation, and Game Theory in Inoculant Markets (**M.A**). Fit for Purpose: Evaluation of Agricultural Bioinoculants Across Diverse Production Landscapes (**L.H**). Isolation of Effective Nitrogen-Fixing Rhizobia for Common Bean from Ontario Soil (**T.H**).	Mohammed Adam Laura Hemara Tia Harrison
12:20 – 1:20PM *(60 mins)*	**Lunch**	-
1:20 – 2:40PM *(80 mins)*	**Project Update** Description of Four Novel Rhizobium Species from Canadian Soils (**N.N.R**). Impacts of Enhanced Nitrogen Fertilizer on Soil Microbial Genes Associated with Plant Growth Promotion (**R.P**). A Project Update on Isolating Arbuscular Mycorrhizal Fungal Populations to Identify Crop Growth-Promoting Strains (**K.M**).	Natalia Naranjo-Robayo Rebecca Pagès Kevin MacColl Emily Perry

	Improving Bioinoculants by Exploring the Impact of Microbial Competition and Nitrogen Supplementation on Rhizobial Partner Quality (**E.P**).	
2:40 – 3:10PM *(30 mins)*	**Break**	-
3:10 – 4:30PM *(80 mins)*	**Project Update** A Multi-tiered Screening Pipeline for Identifying Plant Growth-promoting Rhizobacteria from the Canadian Collection of Agricultural Soil Microbes (**N.A**) Genome-Guided Discovery of Soil Bacteria for Sustainable Nitrogen Management in Agriculture (**R.M.B**). The 3D Genome of Gigaspora Margarita Unveils Stable Chromatin and Nucleolar Organization and Symbiont-Dependent Genome Dynamics (**K.M**). Isolation and Characterization of Competitive Bean Nodulating Rhizobia (**I.O**).	Ni An Raveena Manikku Badu Ken Mugambi Ivan Oresnik
4:30 – 4:35PM *(5 min)*	**Concluding Remarks, Meeting Adjourns**	George diCenzo, Ivan Oresnik
6:00 – 8:00PM *(120 mins)*	**Dinner -** *Curry Original, 175 Bagot St, Kingston, ON K7L 3E9*	-

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AGENDA – DAY 2: May 31st		
Time:	**Item:**	**Lead:**
9:00 – 10:20AM *(80 mins)*	**Project Update** Multi-iterative Competition Screening for Nodule Occupancy to Identify Competitive Strains for Dry Bean Inoculants (**M.B**). Modeling the Impact of Bioinoculants on Soil Biogeochemical Processes: Field Measurements and the DayCent Modeling Framework (**N.S**). Competition for Nodule Occupancy of Bradyrhizobium Strains on Soybean (Glycine Max) and Adzuki Bean (Vigna Angularis) (**E.G**). Farm and Inoculant Firm Modeling of Economic Gains from New Inoculants (**D.B**).	Mubashshir Bari Neal Scott Erika Gagnon Derek Brewin
10:20 – 10:50AM *(30 mins)*	**Break**	-
10:50AM – 12:10PM *(80 mins)*	**Project Update** Optimizing Bean Inoculants for Canadian Varieties: Enhancing Biological Nitrogen Fixation in Common Beans (**S.A**). Evaluating the Potential of Putative Root Nodule Endophytes for Co-inoculant use in Common Bean (**J.B**). Simulating Nitrogen Losses Resulting from Bio-inoculant Applications in Manitoba Dry Bean Systems Using the DayCent Model (**M.K**). Towards Effective Bio-inoculation: Expanding Host Ranges and Optimizing Watering (**J.L**).	Sachinthya Attanayake Jesse Branje Malaika Khan Jangwoo Lee
12:10 – 1:10PM *(60 mins)*	**Lunch**	-
1:10 – 2:20PM *(70 mins)*	**Poster Session** Farm-Level Trade-offs of Microbial Inoculants: Yield Gains, Input Costs, and Rotation Implications in Prairie Cropping Systems (**A.E**). A Multi-tiered Screening Pipeline for Identifying Plant Growth-promoting Rhizobacteria from the Canadian Collection of Agricultural Soil Microbes (**Y.C.D**).	Alexander Ennin Yen Chi Dang Karen Quan Kristy Moniz & Oona Esme Asher Kim

	Impacts of Prolonged Liquid Culture on Growth and Soil Performance Across Diverse Bacterial Isolates (**K.Q**). Generating a Genome Sequenced Microbial Library from Ontario Agricultural Soils (**K.M** & **O.E.**). Alleviation of Waterlogging Stress in Barley Using Novel Plant Growth-Promoting Microbes (**A.K**). Antibiotics in the Field: Consequences for Plant Health and Microbial Partnerships (**I.I.**) Battle of the Strains: Multi-Round Screening for Competitive and Effective Nitrogen Fixing Symbionts (**L.T**). Evolutionary Changes in the N-Terminus of NodZ Facilitated Nod Factor Fucosylation (**N.S**). Fit for Purpose: Evaluation of Agricultural Bioinoculants Across Diverse Production Landscapes (**L.H**). Evaluating the Potential of Putative Root Nodule Endophytes for Co-Inoculant use in Common Bean (**J.B**). Description of Four Novel Rhizobium Species from Canadian Soils (**N.N.R**).	Isabella Ippolito Livia Titley Nicholas Smith Lauren Hemara Jesse Branje Natalia Naranjo-Robayo
2:20 – 3:20PM *(60 mins)*	**Group Discussion of Progress and Next Steps**	George diCenzo, Ivan Oresnik
3:20 – 3:30PM *(10 min)*	**Concluding Remarks,** **Symposium Adjourns**	George diCenzo, Ivan Oresnik
3:30 – 4:00PM *(30 mins)*	**Break**	-
4:00 – 4:30PM *(30 mins)*	**POC Meeting: In-Camera Discussion** (POC and Observers enter Break-out Room) *Discussion on observations arising from the project proposal and Project Team presentation. (Attendees: POC Members, Observers, and Genome Centre Representatives)*	POC Chair, Genome Prairie
4:30 – 5:00PM *(30 min)*	**POC and Observers Re-Join the Main Meeting Room** **Final Discussion and Feedback**	POC Chair Genome Prairie Activity Leads

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Genome Prairie is inviting you to a scheduled Zoom meeting.

Topic: BENEFIT's POC Meeting (Kingston, ON)
Time: May 30, 2026 09:00 AM Eastern Time (US and Canada)
Every week on Sun, Sat, until May 31, 2026, 2 occurrence(s)
Please download and import the following iCalendar (.ics) files to your calendar system.
Weekly: https://us02web.zoom.us/meeting/tZIkd-2uqTgiHt36OIvdqoI1yRjOKhyFFwNp/ics?icsToken=DF5_d6vErj6O031-9gAALAAAAHOCIN1z34WBkgqPymDKTNmJGnLnUVa_fZ2mnWf43eEjlEr_Gikwg-JLPhsBckmBW8SoX7_oeehPSQdsqjAwMDAwMQ&meetingMasterEventId=Ev_R-gEbQOWmutWBSznVPA
Join Zoom Meeting
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Meeting chat link
https://us02web.zoom.us/launch/jc/86904673849

Meeting ID: 869 0467 3849
Passcode: 867574

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Join by SIP
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Join instructions
https://us02web.zoom.us/meetings/86904673849/invitations?signature=kHqPlSNhvowXFvJoQ4bEjlvXnP-X345D_mLkVdMgADg

Developing Plant-Guided Selection Approaches for Designing Beneficial Microbial Inoculants that Protect Against Insect Pests

Laramy Enders[1], Ian Kaplan[1], Elizabeth French[2]

[1]Department of Entomology, Purdue University, Lafayette, IN, U.S.A.; [2] Nutrien Ag Solutions, Loveland, CO, U.S.A.

Microbial communities are essential for combating crop damage caused by agricultural pests. Recently, it has been suggested that the natural mechanisms plants employ to manage relationships with microbes can be used to selectively engineer whole microbial communities with beneficial or protective properties. This approach, known as host-guided selection, is underdeveloped for crop plants in agricultural settings but could provide a novel route to designing effective microbial inoculants. Our goal was to naturally engineer tomato root-associated microbiomes that increase resistance to common insect pests. First, we tested several approaches for engineering insect-suppressive rhizosphere soil microbiomes that reduce damage from aphid feeding (*Macrosiphum euphorbiae*) or caterpillar defoliation (*Manduca sexta, Spodoptera exigua*) using an iterative soil microbial inoculation process. Second, using metabarcoding approaches we characterized the bacterial and fungal communities of selected soils associated with differences in aphid performance on tomato. Overall, host-guided soil microbiome selection for higher or lower insect performance was successful for aphids but not caterpillars, although effects were transient over time. There were minor shifts in relative abundance of fungal and bacteria taxa observed in insect-suppressive communities, but substantial changes in community composition occurred over time. Network analysis indicated aphid feeding was disruptive to rhizosphere microbiome assembly, resulting in lower community complexity, connectivity and keystone taxa compared to un-infested controls. Overall, our results suggest several factors are critical for successful host-guided engineering of beneficial microbiomes, particularly microbial community stability and microbe-microbe interactions, and the extent to which the crop trait of interest is microbially mediated.

Dynamic Crop Rotation Decisions, Soybean Nitrogen Fixation, and Game Theory in Inoculant Markets

Mohammed Adam, Derek Brewin

University of Manitoba, Winnipeg, MB, Canada

The global population is projected to increase from about 8.2 billion in 2024 to around 8.5 billion by 2030 and nearly 9.7 billion by 2050, despite a slowdown in global population growth (UN DESA, 2024). This means more mouths to feed, and more food production is needed, but with limited land expansion and increasing environmental costs (GHG emissions, soil degradation). This puts pressure on food production systems to meet our obligations under the Sustainable Development Goals, particularly SDGs 2 and 13; ensuring Canadians have access to sufficient, affordable and nutritious food and achieving 40% to 45% reductions in greenhouse gas emissions by 2030 and achieving net-zero greenhouse gas emissions by 2050 (Gov't of Canada, 2021). Given the limits of arable land across the globe, one of the options to respond to this is to advance technological innovation in agricultural production. As argued by Boserup (1965), increasing population necessitates innovation in agricultural production. Innovations such as microbial inoculants will enhance productivity, improve cost efficiency, and reduce environmental impact, thereby supporting both food security and sustainability. However, inoculant adoption among farmers is influenced by several factors, including the availability and effectiveness of the inoculants, farmers' crop rotation structure, risk, and other input costs. We consider farmer crop-rotation decisions and the interactions among inoculant-producing firms in a dynamic game-theoretic framework to provide insights for farmers, inoculant firms, and policymakers. We model a two-stage game in which inoculant producers choose cost and/or R&D levels that optimize inoculant effectiveness and probability of success; farmers observe the probability of success and make crop rotation, fertilizer use, and inoculant adoption decisions. We assume that firms in the inoculant industry compete primarily through cost efficiency and product effectiveness. This reflects the nature of the inoculants market in North America, an oligopolistic market structure where strategic interaction occurs through R&D and cost reduction rather than price undercutting.

Reference

Boserup, E. (1965). The conditions of agricultural growth: The economics of agrarian change under population pressure.
Government of Canada. (2021). *Moving forward together: Canada's 2030 Agenda national strategy*.
United Nations Department of Economic and Social Affairs. (2024). *World population prospects 2024 revision*. United Nations.

Fit for Purpose: Evaluation of Agricultural Bioinoculants Across Diverse Production Landscapes

Lauren Hemara[1], Kristy Moniz[2], Raveena Manikku Badu[3], Matthew Bakker[3], George diCenzo[2,3], Terrence Bell[1]

[1]Department of Physical & Environmental Sciences, University of Toronto Scarborough, Toronto, Ontario, Canada.
[2]Department of Biology, Queen's University, Kingston, Ontario, Canada.
[3]Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Conventional agriculture relies on chemical fertilizers to reliably boost plant yield, yet their environmental impacts are substantial, contributing to greenhouse gas emissions and eutrophication. Microbial bioinoculants present a promising alternative. However, many suffer from unpredictable establishment and in-field function. To overcome this uptake barrier, we must focus on their environmental compatibility - specifically, with the conditions they will be exposed to during industrial production. Current inoculant development efforts focus on desired plant growth-promoting (PGP) traits. However, a commercially viable inoculant must also be fit across highly variable production and application landscapes - including the industrial production pipeline and the diverse and fluctuating conditions in recipient fields. As part of the Genome Canada-funded BENEFIT project, we set out to evaluate microbial performance and potential trade-offs in the often-overlooked environments of the industrial pipeline: batch cultivation, storage, and in-soil application. We have developed a framework to assess microbial survival across production stages, categorizing potential inoculants as industrial pipeline generalists or single-stage specialists. Using a diverse panel of isolates from the recently-established Canadian Collection of Agricultural Soil Microbes - representing over 50 genera - we observed diverse performance outcomes across environments. Many isolates are pipeline generalists, including diverse genera with similar (or better) industrial potential to those commonly commercialized. This screening approach has demonstrated the importance of explicitly evaluating industrial production potential, given that these traits cannot be easily predicted from a microbe's identity. Future work will extend evaluations to physicochemically-diverse soils and key crops, as we aim to produce inoculants that reliably perform across production landscapes.

Isolation of Effective Nitrogen-Fixing Rhizobia for Common Bean from Ontario Soil

Authors: Tia L. Harrison[1], Upkardeep S. Pandher[1], Avery Dixon[1], Oona Esme[1], Erika Gagnon[1], Natalia Naranjo-Robayo[1], Rebecca T. Doyle[2], Ivan J. Oresnik[3], George C. diCenzo[1,3]*

[1] Department of Biology, Queen's University, Kingston, Ontario, Canada; [2] Department of Biology, McMaster University, Kingston, Ontario, Canada; [3] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Common bean (*Phaseolus vulgaris*) is an economically and nutritionally important legume worldwide, including in Canada, where Ontario and Manitoba produce over 70% of national output. Although nitrogen fertilization is commonly used to increase yields, it is environmentally damaging and inefficient. Biological nitrogen fixation via rhizobia is a more sustainable alternative, but no commercial inoculants are available for Canadian common bean production. Effective inoculants must be competitive against native rhizobia, efficient at nitrogen fixation, and adapted to local soils, yet *P. vulgaris* is a promiscuous host that often forms suboptimal symbioses. In our study, we isolated 232 rhizobial strains from nodules of common bean grown with seven different Southern Ontario soils and tested their nitrogen-fixation capacity for *P. vulgaris* under greenhouse conditions. We uncovered substantial species diversity among *Rhizobium* strains from Ontario that can form associations with common bean. The composition of *Rhizobium* species associated with *P. vulgaris* varied considerably across soil types but not host cultivar in our nodule trapping experiment. Greenhouse experiments identified one novel species as having the greatest potential for use as an inoculant, though high-performing strains were also found in multiple known species of *Rhizobium*. Our work provides a foundation for developing effective, locally adapted rhizobium inoculants to improve the sustainability and productivity of Canadian common bean agriculture.

Description of Four Novel *Rhizobium* Species from Canadian Soils

Natalia Naranjo-Robayo[1], Avery Dixon[1], Tia L. Harrison[1], Oona Esme[1], Erika Gagnon[1], Emily H. L. Perry[7], Sofiyyah Oladipupo[7], J.M. Igual[4,6], Esther Menéndez[2,3,4,5], Rebecca T. Doyle[7], Ivan J. Oresnik[8], Anna Motnenko[8], George C diCenzo[1,8]

[1] Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain. [3]Instituto de Investigación en Agrobiotecnología (CIALE), Universidad de Salamanca, Salamanca, Spain. [4] Grupo de Interacción Planta-Microorganismo, USAL, Unidad Asociada al CSIC por el IRNASA, Salamanca, Spain. [5] Research Unit of Excellence "Agricultural Production and Environment" (AGRIENVIRONMENT), University of Salamanca, Salamanca, Spain. [6]CSIC – Instituto de Recursos Naturales y Agrobiologia de Salamanca (IRNASA), Salamanca, Spain. [7]Department of Biology, McMaster University, Hamilton, Ontario, Canada. [8] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

The development of effective rhizobial inoculants relies on identifying native rhizobia strains that are well adapted to local soils and environmental conditions, making them promising candidates for inoculation. In Canada, enhancing biological nitrogen fixation in legume crops is particularly important for reducing dependence on chemical fertilizers and promoting sustainable agricultural practices. Previous work isolated and genome-sequenced a large collection of hundreds of rhizobial strains nodulating major Canadian legume crops. This collection provides a valuable resource to explore the diversity and taxonomy of native populations and to identify novel lineages with potential agronomic relevance.

In this study, we used genome-based phylogeny and phylogenomic metrics to investigate native *Rhizobium* populations associated with legume hosts isolated from agricultural soils in Ontario. We analyzed a collection of hundreds of strains obtained from nodules of common bean (*Phaseolus vulgaris*), adzuki bean (*Vigna angularis*), and pea (*Pisum sativum*) cultivated from Ontario and Manitoba soils. Following dereplication of our *Rhizobium* collection using an average nucleotide identity (ANI) threshold of 99.9%, we identified 19 strains that could not be assigned to existing *Rhizobium* species based on ANI comparisons with *Rhizobium* type strains. To further analyze these 19 isolates, a core-proteome phylogeny was reconstructed and overall genomic relatedness indices, including ANI and digital DNA-DNA hybridization (dDDH), were calculated using these 19 isolates and all *Rhizobium* type strains. The results of these analyses grouped these 19 isolates into four species-level clusters that did not include the type strains of previously described species. These genomic analyses were complemented with morphological, physiological, biochemical, and chemotaxonomic characterization, including Gram staining, TEM-based cell morphology, growth under varying conditions, antibiotic susceptibility, metabolic profiling, and fatty acid analysis. Taken together, our results support the classification of our 19 isolates into four novel *Rhizobium* species.

Impacts of Enhanced Nitrogen Fertilizer on Soil microbial Genes Associated with Plant Growth Promotion.

Pagès R[1]*, Tenuta M[2], Bakker MG[1]

[1] Department of Microbiology, University of Manitoba, [2] Department of Soil Science, University of Manitoba.

With the growing global population, fertilizers are critical to meet the rising demand for food. However, nitrogen (N)-fertilizer that is not assimilated by plants, whether due to NH_3 volatilization, NO_3^- leaching, or N_2O emissions, represents a loss to farmers and contributes to environmental degradation. To mitigate N losses, urease- and nitrification enzyme inhibitors are used by some farmers to slow nitrogen transformation rates and improve plant nitrogen uptake. However, we do not fully understand how these inhibitors impact soil microbial communities. We hypothesize that nitrogen-transformation inhibitors may impact microbial functional potential.

To explore this possibility, a field-based metagenomic study was conducted at the TGAS-MAN site for the long-term monitoring of greenhouse gas dynamics from agricultural soils (Glenlea, MB). Replicated wheat plots received either untreated urea (46-0-0), or an enhanced fertilizer formulation containing urea supplemented with the urease inhibitor N-(n-butyl)thiophosphoric triamide (NBPT) and the nitrification inhibitor dicyandiamide (DCD) (SuperU, Koch Agronomic Services). DNA was extracted from both rhizosphere and bulk soil samples and sequenced (Illumina NovaSeq X) to a median read depth of $1.19\times10^8 \pm 8.65\times10^6$ reads per sample.

Data processing and analysis followed a standard pipeline of tools for quality control, trimming, de novo assembly, and functional annotation highlighting Plant Growth-Promoting Traits (PGPT), identified using the PGPg_finder workflow. Differential abundance of PGPT-associated genes was assessed using linear models in the MaAsLin2 framework.

Results indicated that soil compartment (rhizosphere vs. bulk) is the primary driver of functional variation, with gene responses to soil compartment being highly correlated across fertilizer types (Spearman's $\rho = 0.977$). Fertilizer type did impact some genes, but impact differed among soil compartments (PERMANOVA interaction between fertilizer type and soil fraction: $p = 0.001$): it altered only 34 genes in bulk soil, versus 285 in the rhizosphere. Notably, nitrification genes (*hao*) were enriched in bulk soil, while urea uptake genes trended to be found at higher frequencies in the rhizosphere.

These findings demonstrate that inhibitor effects on microorganisms harboring PGPT associated genes are amplified in the rhizosphere. This highlights the need to evaluate fertilizer additives for their ecological compatibility with host-microbiome interactions.

A Project Update on Isolating Arbuscular Mycorrhizal Fungal Populations to Identify Crop Growth-Promoting Strains

Kevin MacColl, Nicolas Corradi

Department of Biology, University of Ottawa, Ottawa, ON

The application of arbuscular mycorrhizal fungal (AMF) inoculants in agricultural fields is notoriously challenging, primarily due to difficulty in establishing viable populations at target sites, and unpredictable growth-promoting functions on target crops. These challenges stem from the widespread application of mass-produced generalist strains that are not adapted to their target sites and crop species. One solution is to isolate new AMF strains from specific agricultural fields, identify strains that thrive and boost the productivity of target crops, and produce inoculants from these new strains. This approach has its own challenges, as AMF strains are exceptionally difficult to isolate, for example, the highest number of AMF strains ever isolated in a single study only amounted to 16 new strains. Here, we will provide an update on a novel protocol developed in our lab to isolate high volumes of new AMF strains from agricultural fields. We faced a challenging yet productive year, as we had to overcome lost cultures due to pathogen invasions, mysterious declines in plant health and poor mycorrhizal colonization. Yet these were overcome through adjustments in our protocol, resulting in the isolation of >80 new AMF strains across 3 species (*Funneliformis mosseae, Racocetra fulgida, Septoglomus sp.*). Forty of these strains have advanced to a 'trap culture' stage where we boost spore production to test their efficacy as agricultural inoculants. Despite these delays, crop growth-promoting experiments and genomic sequencing are on track for this summer.

Improving Bioinoculants by Exploring the Impact of Microbial Competition and Nitrogen Supplementation on Rhizobial Partner Quality

Emily H. L. Perry[1], Sofiyyah A. Oladipupo[1], Oona Esme[2], Upkardeep S. Pandher[2], Ivan J. Oresnik[3], George C. diCenzo[2,3], Rebecca T. Doyle[1]

[1] McMaster University, Department of Biology, Hamilton, Ontario, L8S 4K1, Canada; [2] Department of Biology, Queen's University, Kingston, Ontario, Canada; [3] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Chemical nitrogen (N) fertilizers are economically and environmentally costly, yet essential for ensuring high crop yields. Biofertilizers are biological agents, including bacteria, that provide both a cost effective and environmentally sustainable alternative to chemical fertilizers. However, quality control is critical for promoting adoption of this alternative fertilization method. Rhizobia, a common biofertilizer, are soil-dwelling microbes that form mutualistic relationships with legumes (e.g. beans, peas, clovers) in which they fix N for their hosts in return for legume-fixed carbon. Unfortunately, their quality (i.e. N-fixing efficiency) is impaired by certain environmental factors including soil microbial cell density and diversity. Lower-quality native rhizobia (i.e., standing population of rhizobia), which are frequently less effective nitrogen-fixers, often outcompete rhizobial inoculants for nodule occupancy leading to less plant-growth promotion than expected. Additionally, chemical N fertilizers, which are often applied alongside biofertilizers, provide a cheaper source of N to legumes, allowing legumes to forego partnership with rhizobia. Microbial competition and N supplementation are often overlooked in lab settings which tend to perform experiments in sterile and N-free soil, ultimately resulting in biofertilizers that underperform in the field compared to the lab. Our experiment explores (1) the impact of microbial competition among rhizobial species and strains to identify "elite" strains that are both high-quality and competitive and (2) the impact of N-supplementation on conferred host benefit. To do this, (1) 286 rhizobial isolates from Southern Ontario pea farms were collected and sequenced, (2) 100 of the 286 rhizobial isolates were singly inoculated onto green pea plants to measure single strain quality, and (3) 54 of the 100 isolates whose quality were previously characterized were co-inoculated onto green pea plants to assess population-level quality and strain competitive fitness. These 54 isolates were divided into 9 subgroups each containing 18 isolates, where each of the 54 isolates appeared in exactly 3 subgroups, to maximize genetic diversity within the subgroups (average ANI <96%, max ANI <99.9%). Nodule occupancy was then assessed by collecting and pooling nodules from the same treatment, extracting DNA, and sending them in for metagenomic sequencing. These experiments will allow us to identify elite strains that can undergo further testing, serving as promising candidates for commercial inoculants. The relationship between single partner quality and population partner quality will also be analyzed.

A Multi-tiered Screening Pipeline for Identifying Plant Growth-Promoting Rhizobacteria from the Canadian Collection of Agricultural Soil Microbes

Ni An[1], Chi Dang[1], Olivia Wilkins[1]

[1] Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

Excessive reliance on chemical nitrogen fertilizers poses significant environmental and economic challenges to modern agriculture. Plant growth-promoting rhizobacteria (PGPR) offer a promising biological alternative; however, scalable and reliable screening pipelines for identifying effective strains remain limited. This study presents a multi-tiered screening framework to evaluate 136 bacterial isolates sourced from the Canadian Collection of Agricultural Soil Microbes (CCASM) for their potential to enhance crop growth and nutrient uptake. All 136 isolates were subjected to comprehensive biochemical characterization, including assays for phosphate solubilization, potassium solubilization, indole-3-acetic acid (IAA) production, and siderophore production, to establish a functional profile for each strain. In parallel, plant growth promotion was assessed using high-throughput paper roll assays, followed by two rounds of soil-based consortia experiments—first with 80 strains, then with 56 strains—using randomized consortium assembly. Top-performing strains from the paper roll assays were further evaluated in a greenhouse system to assess efficacy at later developmental stages. Transcriptomic profiling of four top-performing strains across distinct genera is underway to investigate the molecular mechanisms underlying plant–microbe interactions. This research establishes a scalable pipeline for PGPR identification and delivers a well-characterized strain list from the CCASM collection.

Genome-Guided Discovery of Soil Bacteria for Sustainable Nitrogen Management in Agriculture

Raveena Manikku Badu[1], Anna Motnenko[1], George C. diCenzo[1,2], Ivan Oresnik[1], and Matthew G. Bakker[1]

[1] *Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada*
[2] *Department of Microbiology, Queen's University, Kingston, Ontario Canada*

Inefficient nitrogen fertilizer uptake by crops has a major negative impact on the environment. Enhancing nutrient availability through microbial management could reduce dependence on synthetic fertilizers and mitigate emissions of greenhouse gases like nitrous oxide. Also, we can utilize microbes as bioinoculants to promote plant growth. To support this goal, the Canadian Collection of Agricultural Soil Microbes (CCASM) is being established, including whole-genome data for hundreds of bacterial strains. Microbial isolates were obtained from agricultural soils, deliberately picking strains based on distinct colony morphologies and phenotypic traits. The collection currently includes 1,034 isolates from the Prairies, of which we have whole genome sequenced 768 isolates. Of these, 744 genome sequences are of high-quality, and are distributed among the phyla Actinomycetota (348), Bacillota (207), Pseudomonadota (165), and Bacteroidota (24). Current screens on the annotated high quality whole genomes for the ability to produce 1-aminocyclopropane-1-carboxylate deaminase (ACCD) protein, responsible for plant growth promotion during salt stress conditions, has shown 153 isolates from across 14 genera to be strong candidates for possession of this trait. We have also identified 8 isolates from across 5 different genera that have genomic potential to fix nitrogen. Out of the 8 candidate isolates for nitrogen fixation, 5 isolates are novel species according to the Genome Taxonomy Database classification. We will be continuing to explore this microbial culture collection for additional traits related to plant growth and fertilizer uptake.

The 3D Genome of *Gigaspora margarita* Unveils Stable Chromatin and Nucleolar Organization and Symbiont-Dependent Genome Dynamics

Ken Mugambi[1], Jordana Oliveira[1], Franco Magurno[2], Alessandra Salvioli di Fossalunga[3], Mara Novero[3], Luisa Lanfranco[3], Stefano Ghignone[5], Gökalp Yildirir[1], Yan Wang[4], Paola Bonfante[3], Nicolas Corradi[1]

[1]Department of Biology, University of Ottawa, Ottawa, ON, K1N 6N5, Canada
[2]Institute of Biology, Biotechnology and Environmental Protection, University of Silesia, Jagiellońska 28, Katowice, 40-032, Poland
[3]Department of Life Sciences and Systems Biology, University of Torino, 10125, Torino, Italy
[4]Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON, M1C 1A4, Canada
[5]Institute for Sustainable Plant Protection - Turin Unit, Consiglio Nazionale delle Ricerche, 10125, Torino, Italy

Arbuscular mycorrhizal fungi (AMF) are widespread plant symbionts that enhance nutrient acquisition and influence ecosystem productivity. Previous chromosome-level assemblies of the model species *Rhizophagus irregularis* revealed a two-compartment genome architecture (active A and repressed B chromatin compartments), yet its conservation across evolutionarily distant AMF lineages remains unresolved. Here, we present a chromosome-scale and 3D genome assembly of *Gigaspora margarita* isolate BEG34—the largest and most repeat-rich AMF genome to date—alongside that of its obligate endobacterium, *Candidatus* Glomerobacter gigasporarum (*Ca*Gg), using PacBio HiFi and Hi-C sequencing. The *G. margarita* genome comprises 43 chromosomes (792 Mb) organized into A/B compartments and Topologically Associating Domains, structures that are conserved across two AMF orders and remain stable irrespective of the presence of endobacteria in germinating spores. We uncover 21 divergent rDNA operons distributed across six chromosomes and show that these physically interact, suggesting conserved nucleolar organization. We also reveal that the *Ca*Gg genome is tripartite and mobilome-rich, encoding prophages, an orphan CRISPR array, and complete pathways for many novel and essential cofactors, including heme, which may enhance host bioenergetics. We also find that the endobacterium's presence modulates transposable elements expression in *G. margarita*. These findings reveal conserved principles of chromatin architecture in AMF symbionts and highlight the tight molecular interplay between fungal hosts and their endosymbionts, offering new insights into genome evolution and symbiotic adaptation.

Isolation and Characterization of Competitive Bean Nodulating Rhizobia

Anna Motnenko,[1] Ishani Patuwatha Withanage,[1] Zeel Patel,[1] Mubashshir Bari,[1] Kristen MacMillan[2], George C. diCenzo[1,3] and Ivan J. Oresnik[1,3]

[1]Dept of Microbiology, University of Manitoba, Winnipeg, MB, Canada, [2]Dept of Plant Science, University of Manitoba, [3]Dept of Biology, Queen's University at Kingston, Kingston, ON, Canada

Dry beans (*Phaseolus vulgaris*) are vital for global nutrition. In Canada, they are grown mainly in Ontario and Manitoba. Since Canada lacks a viable *Rhizobium* inoculant for dry beans, growers rely on fertilizer. This study aims to isolate *Rhizobium* strains with strong nitrogen fixation and competitiveness for nodules to develop candidate inoculums.

Manitoba Pulse and Soybean Growers provided soil and plant samples. About 200 *Rhizobium* isolates were obtained either directly from bean nodule samples or by using soil to inoculate sterilized bean seedlings. Approximately 200 *Rhizobium* isolates were collected, tested for nitrogen fixation, and had their genomes sequenced. Those in the 90th percentile for nitrogen fixation were further tested for nodule occupancy competition.

The data show that the isolates belong to eight recognized species. These are *R. anhiense* (11%), *R. croatiense* (26%), *R. hidalgonense* (16%), *R indicum* (1%), *R. lagurerreae* (1%), *R. leguminosarum* (22%), *R. redzipovicii* (0.5%), and *R. sophoriradicis* (8%). Approximately 16% of the isolates could not be classified at the species level and may represent 4 new species.

To conduct nodule occupancy experiments, isolates were inoculated onto nitrogen-deficient bean seedlings. After 3 weeks, 10 crown nodules were harvested, crushed, and used to inoculate new plants, repeated twice. Ultimately, 20 nodules from three replicates were isolated, genome-sequenced, and compared. Results showed that the nodules mainly contained two isolates: *R. sophoriradicis,* present in all replicates and comprising 53% of the total nodules, and *R. croatiense*, found in 2 of 3 replicates and comprising 35% of the total nodules.
These two isolates were later tested under field conditions. The results indicate that the strains were highly competitive in the field, constituting about 80% of the tested nodules. At harvest, it was observed that seed weight for one of the strains was similar to that of plants grown with a nitrogen amendment of 70 lbs per acre.

Multi-iterative Competition Screening for Nodule Occupancy to Identify Competitive Strains for Dry Bean Inoculants

Mubashshir Bari,[1] Anna Motnenko[1], Ishani Patuwatha Withanage[1], Zeel Patel[1], Kristen P. MacMillan[2], George C diCenzo[1,3], and Ivan J Oresnik [1,3]

[1] Dept. of Microbiology, University of Manitoba, Winnipeg, MB, Canada
[2] Soybean and Pulse Agronomy Lab, Dept of Plant Science, University of Manitoba, MB, Canada
[3] Dept of Biology, Queen's University at Kingston, Kingston, ON, Canada

Legumes typically obtain much of their nitrogen through a symbiotic relationship with rhizobia bacteria that can fix atmospheric nitrogen and supply it to the plant. While most legumes have a commercially available *Rhizobium* inoculant that is either applied to seeds or added during planting, an inoculant for dry bean (*P. vulgaris*) is not readily available. To address the lack of a viable inoculant for dry beans, a collection of 168 rhizobia capable of nodulating navy beans was assembled from across Manitoba. From this collection, 15 strains representing the top 90th percentile were chosen based on greenhouse trials to identify those with superior symbiotic nitrogen-fixation traits.

In this study, we developed and refined a multi-iteration, competition-based screening method to identify the most competitive strains for nodule occupancy. Equal numbers of each of the 15 selected strains were combined into a consortium and used to inoculate bean seedlings. Three weeks after inoculation, 20 crown nodules were pooled, surface-sterilized, and the rhizobia were isolated for use in inoculating new plant sets. This process was repeated over three consecutive iterations. In the final round, rhizobia from 20 crown nodules per replicate were isolated, a single colony from each nodule was purified, and the whole genome was sequenced. To determine which of the original strains persisted through the selection process, average nucleotide identity (ANI) analysis was used. Across six independent competition experiments involving 120 crown nodules, an isolate of *Rhizobium sophoriradicis* (UMR 279) and an isolate of *Rhizobium croatiense* (UMR 297) showed the highest cumulative frequency, with UMR 297 appearing in 5 out of 6 replicates and occupying 41% of all nodules tested, while UMR 279 also appeared in 5 out of 6 replicates and accounted for 31% of all nodules tested. These strains are strong candidates for further characterization and potential development as inoculants for dry beans.

Modeling the Impact of Bioinoculants on Soil Biogeochemical Processes: Integrating Field and Lab Measurements into the DayCent Modeling Framework

Neal A. Scott, Malaika Khan, Noah Monteith

Dept. of Geography and Planning, Queen's University, Kingston, ON

Soil bioinoculants can stimulate crop growth through a variety of mechanisms. They might modify the nitrogen cycle, increasing levels of available nitrogen. Some bioinoculants can increase available phosphorus by enhancing phosphorus mineral solubility. Bioinoculants might stimulate plant growth by enhancing plant hormone production, or stimulating root growth. All of these mechanisms can lead to cascading changes in biogeochemical cycling processes in agricultural systems, including changes to the nitrogen cycle that might have adverse impacts on the environment. While extensive field trials and sampling can provide insights into the overall system changes, simulation models such as DayCent provide a tool to evaluate not only mechanisms by which bioinoculants stimulate crop growth, but also how these changes influence cropping system performance.

To apply the DayCent model to the cropping systems being evaluated in the BENEFIT project, information from other Activities is required. Soil analyses in Activity 1 help to initialize key soil carbon and nitrogen pools in the soil, and other soil properties, providing realistic starting conditions for model simulations. DayCent can also simulate the impact of drought on crop growth, and information from Activity 3 will provide insights into the ability of bioinoculants to withstand drought and still provide benefits to crops. Greenhouse trials in Activity 4 provide insights into the mechanisms by which bioinoculants might enhance crop growth. This information can be used to modify key model parameters such as root:shoot ratios. Field trials that are part of Activity 4 will provide key information that can be used to test and verify the model and any parameter changes. In Activity 5, once parameterized and performing adequately, we can use DayCent to assess the impact of bioinoculants on greenhouse gas production and nitrate leaching, as well as the impact of weather variability on the bioinoculant activity. These results can further feed into life cycle modeling that is also part of Activity 5. Overall, DayCent provides a useful framework for integrating results across the project and projecting the environmental impact of bioinoculants.

Competition for Nodule Occupancy of Bradyrhizobium Strains on Soybean (*Glycine max*) and Adzuki Bean (*Vigna angularis*)

Erika Gagnon[1], Tia L Harrison[1], Autumn Hodgins[1], Oona Esme[1], Ariane Mayrand Nicol[1], Upkardeep Singh Pandher[1], Natalia Naranjo Robayo[1], Livia Titley[1], George C diCenzo[1,2].

[1] Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Legumes are climate-smart crops that are also major Canadian exported goods. Unlike most crops, legumes can interact with rhizobia, a group of nitrogen-fixing soil bacteria. During this mutualistic relationship, rhizobia live within structures on the roots of legumes called nodules, where they convert atmospheric nitrogen into forms that are bioavailable to legumes. Inoculating legumes with commercial, highly effective strains of rhizobia allow for increased yields while reducing reliance on synthetic nitrogen fertilizers. *Bradyrhizobium* is a genus of rhizobia whose members can interact with various legume crops, including *Glycine max* (soybean) and *Vigna angularis* (adzuki bean or Japanese red bean). One challenge in inoculum development is that soils host highly diverse microbial communities, including many rhizobia native to the region. As a result, commercial inoculant rhizobia must compete with the native rhizobia for access to nodules on legume crops. *Bradyrhizobium* inoculants must therefore be both highly effective nitrogen fixers and highly competitive for nodule occupancy. Here, we generated a collection of 57 genome-sequenced *Bradyrhizobium* strains that were isolated from soybean plants cultivated in an Ontario agricultural field. To identify the relative competitiveness for nodule occupancy of the 57 isolates, we inoculated a pool of all 57 isolates onto soybean and adzuki bean plants, and we will use high throughput sequencing to characterize the relative proportion of each isolate within nodules. We additionally inoculated the isolates using three different approaches to evaluate how the relative competitiveness of the isolates is influenced by the inoculation method. These results will allow us to identify the most competitive isolates for further characterization as potential candidates for commercialization as novel inoculants for soybean and/or adzuki bean.

Farm and Inoculant Firm Modeling of Economic Gains from New Inoculants.

Derek Gerald Brewin[1], Alexander Ennin[1], Mohammed Adam[1], Syed Irfan Shah[1] and Jenai Buchanon[1,2]

[1] Department of Agribusiness and Agricultural Economics, University of Manitoba, Winnipeg, Manitoba, Canada; [2] Ducks Unlimited, Stonewall, Manitoba, Canada

We explore the conditions most likely to affect the adoption of inoculants by farmers in Manitoba considering recently observed interactions with other inputs and in the face of changing crop and input prices. Corner solutions of zero nitrogen fertilizer and only inoculant application or and a higher than current nitrogen fertilizer application rates can be generated by the same inoculant fertilizer interaction depending on prevailing prices. We also explore recent inoculant trait acquisitions and current available product categories for the major crops in Manitoba and assess the impacts for changing seeded areas to support the modeling of inoculant demand for future input manufacturing.

Optimizing Bean Inoculants for Canadian Varieties: Enhancing Biological Nitrogen Fixation in Common Beans

S.U. Attanayake[1], K. E. Bett[1], I. J. Oreznik[2], G. C. diCenzo[3], and A. Vargas[1]

[1]Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; [2]Department of Biology, University of Manitoba, Winnipeg, Manitoba, Canada; [3]Department of Biology, Queen's University, Kingston, Ontario, Canada

Common bean (*Phaseolus vulgaris*) is an important food and protein crop but is generally less efficient in biological nitrogen fixation (BNF) compared to other legumes. In Canada, variable performance of bean inoculants has reduced grower confidence and limited adoption, increasing reliance on synthetic nitrogen fertilizers. Identifying effective, crop-specific *Rhizobium* strains and responsive bean genotypes is therefore essential for improving nitrogen use efficiency and supporting sustainable soil and crop management. This study evaluated the BNF potential of a 177-genotype Northern Bean Diversity Panel inoculated with two *Rhizobium* strains isolated in Canada (Rh258 and Rh71). Plants were grown in a sterilized vermiculite–sand medium under controlled conditions and assessed for chlorophyll status using normalized difference vegetation index (NDVI), nodulation, and biomass traits. Both inoculants significantly improved plant performance relative to uninoculated controls, with clear treatment effects across NDVI, nodulation, and shoot and root biomass. Inoculation enhanced canopy vigor and nodulation capacity, while nitrogen-fertilized controls promoted biomass accumulation but suppressed nodulation traits. Trait-specific differences between the two inoculants were observed, indicating functional specialization rather than uniform responses across phenotypes. Substantial genotypic variation and genotype-specific responsiveness to inoculation were detected, highlighting the importance of host–microbe interactions in BNF efficiency. Notably, older released varieties and landraces exhibited stronger and more consistent responsiveness to inoculation than many modern lines, suggesting that BNF responsiveness may have been unintentionally reduced during historical bean breeding under high-input systems. Findings support re-integrating BNF responsiveness into breeding programs and developing genotype-specific inoculant strategies for sustainable, low-input production systems.

Evaluating the Potential of Putative Root Nodule Endophytes for Co-Inoculant use in Common Bean

Jesse Branje, Tia L Harrison, George C diCenzo

Department of Biology, Queen's University, Kingston, Ontario, Canada

The use of rhizobia bio-inoculants is a critical tool for farmers to both cut costs and reduce the environmental impact of their operations. Biological nitrogen fixation (BNF) via the rhizobia-legume root nodule symbiosis reduces the need for synthetic nitrogen fertilizers. Although inoculation is effective for several leguminous crops such as soybean and lentils, *Phaseolus vulgaris* or common bean, forms notoriously poor symbiotic relationships with rhizobia resulting in low BNF activity. Few common bean inoculants are available in Canada and in many parts of the country, the recommendation for farmers is still to solely apply synthetic fertilizers rather than to inoculate with rhizobia. Co-inoculation of legumes with rhizobia and root nodule endophytes, which are non-rhizobial bacteria that colonize the nodules of a variety of legume plants, shows promise as a way to improve yields compared to inoculation with only rhizobia, due to potential plant growth promoting properties. Here, we investigated whether 11 non-rhizobial bacteria, originally isolated during common bean nodule trapping experiments, could colonize common bean nodules when co-inoculated with an effective *Rhizobium* strain, and the impact of co-inoculation on common bean growth. These strains are represented by four species of bacteria: *Lysinibacillus fusiformis* (5 isolates), *Pseudomonas monteilii* (3 isolates), *Pseudomonas tohonis* (2 isolates), and *Brevibacillus agri* (1 isolate). To confirm nodule colonization, we attempted to re-isolate these strains on LB agar from crushed surface sterilized nodules of mature navy bean plants that had been co-inoculated with the strain of interest and a rhizobial partner. The results indicated that only *P. monteilii* was able to be re-isolated though in very low abundance and not consistently across replicates, suggesting that most of these strains are not true nodule endophytes or that colonization is context dependent and emergent rather than obligate. Amplicon sequencing of the 16s rRNA gene from nodule DNA extracts was used as a culture-independent method of detection, with preliminary analysis of these data supporting the culture-dependent results. Preliminary measurements of shoot dry weight and chlorophyll content via NDVI of plants suggested that these non-rhizobial isolates did not have a statistically significant impact on common bean growth. Over the course of three growth trials, there were effects on growth that appear to be biologically significant. However, the trends were not consistent across trials with particular strains sometimes showing a positive effect while other times having a negative net impact on growth suggesting that the observed relationship is dynamic and outcome may be decided by variables that have not been accounted for in these experiments.

Simulating Nitrogen Losses Resulting from Bio-inoculant Applications in Manitoba Dry Bean Systems Using the DayCent Model

Malaika Khan[1], Neal A. Scott[1]

[1] Department of Geography and Planning, Queen's University, Kingston, Ontario, Canada

Synthetic nitrogen (N) fertilizer is a primary driver of nitrous oxide (N_2O) emissions and other nitrogen losses in agricultural systems. Biological nitrogen fixation (BNF) through rhizobial bioinoculants offers a pathway to reduce fertilizer dependence and associated nitrogen losses. Dry bean (*Phaseolus vulgaris L.*) production in Manitoba presents a compelling case study, as native rhizobia are known to contribute to BNF in these systems, and commercial inoculants may further enhance fixation rates, potentially displacing synthetic N inputs and reducing N_2O fluxes. However, the net environmental benefit of bioinoculant application across realistic management and climate scenarios remains unquantified. This study applies the DayCent biogeochemical model to simulate soil N dynamics and N_2O emissions in Manitoba dry bean systems over a range of fertilizer application rates. The model simulates key nitrogen-cycling processes, including mineralization, nitrification, denitrification, volatilization, and leaching, as well as carbon dynamics that influence microbial activity and soil respiration. Simulations will investigate how replacing fertilizer inputs with rhizobial inoculants influences patterns of N_2O emissions, soil nitrogen availability, nitrogen losses into the environment, and plant nitrogen uptake. We will also evaluate the long-term impacts of inoculants under different IPCC Emissions Scenarios. By isolating the contribution of bio-inoculants to N cycling and N losses across a range of N management scenarios, this work aims to quantify the GHG mitigation potential of microbial inoculants and identify the management conditions that optimize their benefit.

Towards Effective Bio inoculation: Expanding Host Ranges and Optimizing Watering

Jangwoo Lee[1], Lauren M. Hemara[2], Kristy Moniz[3], Ivan J. Oresnik[4], George C. diCenzo[3,4], Terrence H. Bell[2], and Rebecca T. Doyle[1]

[1] Department of Biology, McMaster University, Hamilton, Ontario, Canada
[2] Department of Physical and Environmental Sciences, University of Toronto Scarborough, Toronto, Ontario, Canada
[3] Department of Biology, Queen's University, Kingston, Ontario, Canada
[4] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Bioinoculation is an emerging strategy to support sustainable agriculture by using plant growth-promoting bacteria to enhance crop yields while reducing reliance on conventional chemical fertilizers. Bioinoculants, however, typically exhibit limited host ranges, being effective on a single crop species or cultivar. As a result, industrial stakeholders must maintain multiple product lines to target different crops, leading to increased production costs. In Benefit Act 3, we address this limitation through successive evolution, enhancing host adaptability by passaging strains across multiple crop species. Another challenge is the limited understanding of how environmental conditions (e.g., watering regimes) influence plant-inoculant interactions. Water stress – a common condition in real-world farming systems – can reduce crop yields by directly constraining plant physiological processes and, in some cases, indirectly by decreasing the persistence of bioinoculants. In Benefit Act 3, we investigated this issue through a microcosm experiment, incorporating watering regime as an experimental factor.

In this Benefit symposium, we will present the most up-to-date results from experiments where three crop cultivars (canola, barley, and pea) were inoculated with 25 bacterial strains isolated from Ontario soils. We additionally tested the effects of inoculation under two watering regimes (high vs. low). In short, we identified a significant effect of soil moisture on crop growth, with increases ranging from 3.8% to 168.6% relative to drought conditions. Although smaller in magnitude, inoculation effects were also observed, mostly for canola under high soil moisture, where inoculation increased growth by 37.8% (95% confidence interval: 2.3-85.5%). To investigate how different crop species selected different bacteria, and how the selected bacteria exhibited the observed effects, we are examining rhizospheric microbial communities using 16S rRNA gene amplicon sequencing and bacterial isolation followed by genomic sequencing.

The soils from this initial study are currently being used to guide an evolution study, where soils are directly passaged across three plant growth cycles. For each cultivar, we are testing two crop-rotation schemes: a non-rotated control whereby the same cultivar is planted in each cycle, and a rotation treatment following a pea-barley-canola sequence. After completing three passage cycles (expected by the end of June), we will conduct a confirmation experiment to evaluate the host adaptability of the passaged inoculant strains across all three cultivars.
Overall, in Benefit Act 3, we propose effective bioinoculation strategies that enhance host adaptability and optimize watering regimes, thereby promoting sustainable agriculture.

Farm-Level Trade-offs of Microbial Inoculants: Yield Gains, Input Costs, and Rotation Implications in Prairie Cropping Systems

Alexander Ennin

Department of Agribusiness and Agricultural Economics, University of Manitoba

Microbial inoculants are increasingly promoted as a means to improve nutrient-use efficiency and enhance crop productivity in Canadian agriculture. However, evidence from field trials and applied agronomy suggests that yield responses are highly variable across crops, environments, and management conditions, creating uncertainty regarding their economic value at the farm level.
This study evaluates the economic trade-offs associated with microbial inoculants by synthesizing evidence on yield effects relative to inoculant costs across major Prairie cropping systems, including canola, wheat, and pulse crops. A general evidence matrix, summarizing field and experimental evidence across crops, is developed to categorize outcomes by various scenarios including: (i) yield gains without changes in fertilizer use, (ii) partial substitution between fertilizer inputs and biological inoculants, and (iii) instances with no measurable agronomic benefit, resulting in a net cost to producers. These outcomes highlight the asymmetric nature of adoption decisions, where potential gains are uncertain while costs are incurred with certainty.

Building on this framework, the analysis will be extended to incorporate regional variability by updating gains-versus-cost relationships across Manitoba's crop risk areas using data from Manitoba Ag. Services Corporation's Regional Analysis tools MASC, nd.). Furthermore, the study will integrate these results into a farm-level economic model of crop rotations, following Sakulanda (2018), to compare profitability under scenarios with and without inoculant use. By linking agronomic variability with economic decision-making, this research contributes to understanding when microbial inoculants generate farm-level value and how these conditions influence adoption under uncertainty.

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Impacts of Prolonged Liquid Culture on Growth and Soil Performance Across Diverse Bacterial Isolates

Karen Quan, Lauren Hemara, Sarah Farhan, Terrence H Bell.

Department of Physical and Environmental Sciences, University of Toronto, Scarborough, Ontario, Canada

Microbial inoculants are widely used in agriculture to promote plant growth, increase stress tolerance, and improve soil health. The global demand for agricultural bioinoculants has been rising, driven by the need for sustainable farming practices. To produce probiotics in high quantities for commercialization, microbes are incubated for prolonged periods in liquid culture, commonly tryptic soy broth (TSB). However, formulation is challenging because a controlled laboratory environment imposes selective pressures different from variable conditions of soil. Thus, microbial adaptation to media can be unreliable when applied to the field and may not be beneficial after soil reintroduction. This discrepancy on microbial performance remains an unknown barrier to effective application across diverse soils. Moreover, there is a growing need to include diverse isolates in experimental evolution work, as most studies have been done on a few model organisms such as *E. coli*. This study investigates how prolonged growth in TSB affects the in-soil performance of diverse bacterial isolates. A total of 14 isolates from 7 genera will be cultured over 45 days and passaged every 72 hours. Here, we used 2 commercial isolates to compare fitness observations between naturally occurring soil isolates and isolates that are commercially sold as industry standards. 16S rRNA gene sequencing was performed using Sanger sequencing of full-length 16S rRNA genes to characterize all 14 isolates and verify taxonomic identify. At defined timepoints, in-culture growth will be quantified via optical density and colony-forming units, while in-soil survival, proliferation, metabolic activity, and drought-cycle stress resilience will be assessed using biotically cleared microcosms. This research characterizes the temporal dynamics of in-soil fitness loss and the variation in resilience across diverse isolates. In addition, revealing evolutionary potential and constraints can be used to address a gap in microbial inoculant development and reduce chemical input dependency.

Generating a Genome Sequenced Microbial Library from Ontario Agricultural Soils

Kristy Moniz[1], Oona Esme[1], Matthew G. Bakker[2], Ivan J. Oresnik[1,2], George C. diCenzo[1,2]

[1] Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Department of Microbiology, Queen's University, Kingston, Ontario, Canada.

The Canadian agriculture system accounts for 7% (149.2 billion dollars) of Canada's gross GDP. This includes food production, jobs, beverage and food processing among other things. With a population of over 41 million people, over 16 million of them in Ontario, Canada's farming and agricultural industries are more than just an economic staple, but a major need to keep our populations healthy and fed. Farmland accounts for roughly 6.2% of Canada's total area. Our goal with this project was to collect soil bacteria from southern Ontario crop fields and cultivate a library of beneficial soil bacteria. With this collection, we aim to screen for novel native bacteria that can be used to promote crop growth without increasing chemical nutrient inputs. The combination of natural microbes can be used to reduce the need for chemical fertilizers, supporting crops growth and the health of the soil.

To increase the diversity of microbes recovered, seven sites were sampled from southern Ontario, including two canola, two wheat, and two barley agricultural fields, as well as one garlic mustard site. Three differing treatments were used for each soil sample, followed by dilutions that were plated on 7 different medias. Three separate time points were used as well, to create a probable range of diversity in the selection. Approximately 1900 samples were collected, purified, frozen and then streaked and overnight cultures grown for nanopore sequencing

To date, 1383 samples have been sequenced, of those, 988 samples met our quality standards to be added to the CCASM library. Roughly sixty different genera were found with many having multiple species identified, including a novel *Ensifer* species. Others include: Eighteen *Streptomyces* species, four *Staphylococcus* spp., seven *Rhodococcus* spp., nine *Pseudomonas* spp., nine *Paenibacillus* spp., and 15 different *Bacillus* spp. to name a few.

Alleviation of Waterlogging Stress in Barley Using Novel Plant Growth-Promoting Microbes

Asher Kim[1], Nedishtha Gobin[1], Michelle Drapeau[1], Allyson MacLean[1], Marina Cvetkovska[1]

[1] Department of Biology, University of Ottawa, Ontario, Canada

Despite an increasing demand for more productive agriculture, abiotic stresses such as drought, cold, salinity, and heat remain major limiters of crop yields. While chemical fertilizers can greatly improve crop growth and stress resilience, they often have unwanted effects on the environment, including eutrophication and polluted drinking water. A sustainable alternative to chemical fertilizers are plant growth-promoting microbes (PGPM), microorganisms that associate with plant roots or their immediate surroundings and can improve plant growth and resistance to stress. PGPM are being examined as natural biofertilizers, due to their ability to support plant growth by improving nutrient availability and uptake, enhancing soil health, reducing the effects of abiotic and biotic stress, or promoting the biosynthesis of phytohormones.

Barley (*Hordeum vulgare*), a major Canadian crop, often faces waterlogging conditions due to excessive rainfall and snowmelt early in the growing season, but the mitigation of this stress using PGPM has not yet been explored. The goal of this project is to determine any beneficial effects of PGPM on barley growth under waterlogging stress.
T
o study the plant growth-promoting and stress-mitigation potential of the microbes, barley seedlings will be divided into four treatment groups: 1) No waterlogging (control); 2) No waterlogging in the presence of microbes; 3) Waterlogging stress; or 4) Waterlogging stress in the presence of microbes. Barley growth and stress resilience will be quantified by measuring chlorophyll content, biomass, plant morphology, light-harvesting and gas exchange. Finally, barley will be left to recover from the waterlogging, and yield will be measured.

Battle of the Strains: Multi-Round Screening for Competitive and Effective Nitrogen Fixing Symbionts

Livia M Titley[1], Tia L Harrison[1], Rebecca T Doyle[2], Ivan J Oresnik[3], George C diCenzo[1,3]

[1] Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Department of Biology, McMaster University, Hamilton, Ontario, Canada. [3] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

Rhizobium inoculants offer an efficient and environmentally friendly alternative to synthetic nitrogen fertilizers by performing biological nitrogen fixation in a symbiotic relationship with legume plants. While there are various inoculants available for legume plants around the world, there are currently no rhizobial inoculants optimized for use on common beans (*Phaseolus vulgaris*) in southern Ontario, which is a major area of common bean production in Canada. In previous work, a collection of 304 *Rhizobium* strains capable of nodulating bean were isolated from southern Ontario soils. This collection was made up of 12 species. Here, we competed these *Rhizobium* isolates on two cultivars of common bean (navy bean and kidney bean), to identify candidates for development into inoculants and to evaluate cultivar specificity. Using a six-round, tier-style competition assay we selected for strains that are both highly competitive for nodule occupancy and highly effective at fixing nitrogen. The 304 isolates were randomly assigned to 32 groups (9 or 10 isolates per group), and each pool was used to inoculate one plant per host genotype. In a second replication, the same strains were randomly reassigned into 32 new groups (with different strain combinations) and again applied to both host genotypes. Our experimental design accounts for variation in strain-strain interactions and host genotype effects. In each round, 10 large and pink nodules (indicative of effective N-fixation) were selected, pooled, and crushed. The isolated pools of rhizobia from two plants of the same cultivar were then combined and used to inoculate plants in the next round, gradually enriching for highly competitive and efficient strains, repeating this process until only a single plant per replicate remained. At the end of the final round of the competition, rhizobia were isolated, whole genome sequenced, and archived from 7 to 10 nodules per plant. The recovered "winning" strains were then clustered with the initial 304 strains using dRep, with each cluster based on an average nucleotide identity threshold of 99.98%. Based on our preliminary analyses to date, the winning strains come from 6 of the 114 clusters. Interestingly, we observed strong host specificity: all isolates from kidney bean plants belonged to *Rhizobium anhuiense*, whereas all isolates from navy bean plants belonged to *Rhizobium sophoriradicis* or *Rhizobium croatiense*. In future work, single and mixed inoculation experiments will be performed to confirm whether the winning isolates are both competitive and efficient nitrogen fixers.

Antibiotics in the Field: Consequences for Plant Health and Microbial Partnerships

Isabella Ippolito[1], Andrea Braccio[1], Emily Perry[1], Sofiyyah A. Oladipupo[1], Oona Esme[2], Upkardeep S. Pandher[2], Ivan J. Oresnik[3], George C. diCenzo[2,3], Rebecca T. Doyle[1]

[1] Department of Biology, McMaster University, Hamilton, Ontario, Canada; [2] Department of Biology, Queen's University, Kingston, Ontario, Canada; [3] Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Plant-microbe interactions are well known to be important for agriculture, improving crop yields via pathogen suppression and enhancing abiotic stress tolerance. One keystone interaction in agriculture involves the symbiosis between legumes and rhizobia. Although considered a mutualism, the fitness outcomes of the legume-rhizobium symbiosis are influenced by the surrounding environment. Anthropogenic contaminants such as antibiotics that enter the environment can create selection pressures for rhizobia as well as the surrounding soil microbiome. It is not well known how exposure to antibiotics indirectly impacts plant growth via functional and taxonomic shifts in the soil microbiome. This study investigates both the direct and indirect impacts that antibiotics have on crop growth. We combine experimental evolution, cross-inoculation experiments, and sequencing of both the microbiome (16S rRNA profiling) and rhizobia (whole genome sequencing) to determine if antibiotic exposure impacts community composition and function, plant growth, and the rate of transmission of antibiotic resistance genes.

To determine which antibiotics and which antibiotic concentrations to use in our study, we conducted a minimum inhibitory concentration (MIC) test by individually exposing all 25 members (10 rhizobia and 15 non-rhizobia) of our synthetic community (SynCom) to three antibiotics commonly detected in agricultural soils: ciprofloxacin (Cip), sulfamethazine, and chlortetracycline (Ctc). While all strains were resistant to sulfamethazine, strains varied in their ability to grow at Cip and Ctc concentrations of 0.03 and 0.25 mg/L, respectively, and thus, we used these antibiotics in downstream experiments. We next conducted two pilot experiments using green pea (*Pisum sativum*) to i) determine the proportion and density of the rhizobium population relative to the non-rhizobia in the starting inoculum that would provide sufficient nodulation for passaging, as well as ii) the upper limit of antibiotic concentrations that would allow plants to grow and form sufficient nodules. We found that nodule number decreased as rhizobium proportion increased, a trend accompanied by greater aboveground plant biomass per nodule, suggesting higher nodule occupancy of high-quality strains despite lower total nodules formed. We additionally found that Cip at intermediate concentrations (0.3 mg/L) delayed germination relative to controls unexposed to antibiotics. Finally, we conducted amplicon sequencing of the 16S rRNA gene V4 region and used the whole genomes of each SynCom member (N = 25 total) to map reads and determine strain frequencies across the different treatment conditions. Overall, the results from these pilots and our full experimental evolution study allow us to disentangle both the direct and microbially-mediated effects of antibiotics in agricultural systems, while also revealing the genomic mechanisms underlying resistance with relevance beyond agriculture.

Evolutionary Changes in the *N*-terminus of NodZ Facilitated Nod Factor Fucosylation

Nicholas Smith,[1,2] Graeme Howe,[2] George C. diCenzo[1]

[1]Department of Biology, Queen's University, Kingston, Ontario, Canada
[2]Department of Chemistry, Queen's University, Kingston, Ontario, Canada

Prior to symbiotic nitrogen fixation, rhizobia produce oligosaccharide signaling molecules called Nod factors. Legumes recognize Nod factors via specialized receptors that discriminate between different Nod factor structures, helping dictate which rhizobia are compatible partners. Nod factors are comprised of a 3- to 5-unit *N*-acetylglucosamine core called a chitoligosaccharide (CO); however, some rhizobia express host-specific Nod proteins that further decorate this core, thereby altering their host ranges. For example, NodZ is a glycosyltransferase that installs a fucose sugar onto Nod factors, a modification required for efficient host recognition by some legume species.

Despite its role in host specificity, it is unknown how the NodZ family diverged from other fucosyltransferases to fulfill a biological role in Nod factor fucosylation. Moreover, the biochemical role of the NodZ *N*-terminal domain is entirely uncharacterized. We hypothesized that mutations accumulated in the *N*-terminal domain of the ancestral NodZ to imbue the ability for efficient CO fucosylation, connecting these two knowledge gaps. To test this hypothesis, we reconstructed the evolutionary history of NodZ and its closest relatives using ancestral sequence reconstruction and "resurrected" three proteins representing ancestral states of the NodZ family. After expressing and purifying the ancestral proteins, only the most recent ancestor was capable of fucosylating COs, suggesting that the earlier ancestors were not true NodZ proteins. However, domain swapping of the *N*-terminal domain could rescue the activity in older ancestors, suggesting that changes in this domain were key to the evolution of this function.

Next, we expressed and purified the NodZ protein from *Mesorhizobium japonicum* R7A as an extant reference. Kinetic analysis showed that the enzyme's affinity for COs was highly dependent on CO chain length, processing tetra/pentameric COs much more efficiently than di/trimeric ones. This corroborates other findings to suggest that the enzyme has evolved a preference for CO chains that are the same length as Nod factors. To gain a better understanding of how the *N*-terminal domain affected this preference, we used *in silico* co-modeling to predict CO-binding residues in the protein's *N*-terminus. Site-directed mutagenesis of these residues revealed two motifs that have significant roles in binding COs. Of particular interest, a single tryptophan residue was critical for CO_5 fucosylation but had no effect on CO_2 fucosylation. These motifs were highly conserved in NodZ proteins but not their relatives, implicating them in the evolutionary journey of this important family of glycosyltransferases.

Together, these results begin to reveal how the evolution of a specialized *N*-terminal module enabled the emergence of NodZ as a Nod factor-modifying enzyme. They also pave the way for engineering NodZ variants that selectively fucosylate oligosaccharides of defined lengths, opening new avenues by which to reprogram symbiotic signaling.