An overview of the Canadian Collection of Agricultural Soil Microbes (CCASM) and an update on rhizobial taxonomy

BENEFIT

queensu.ca/microbes-for-agriculture

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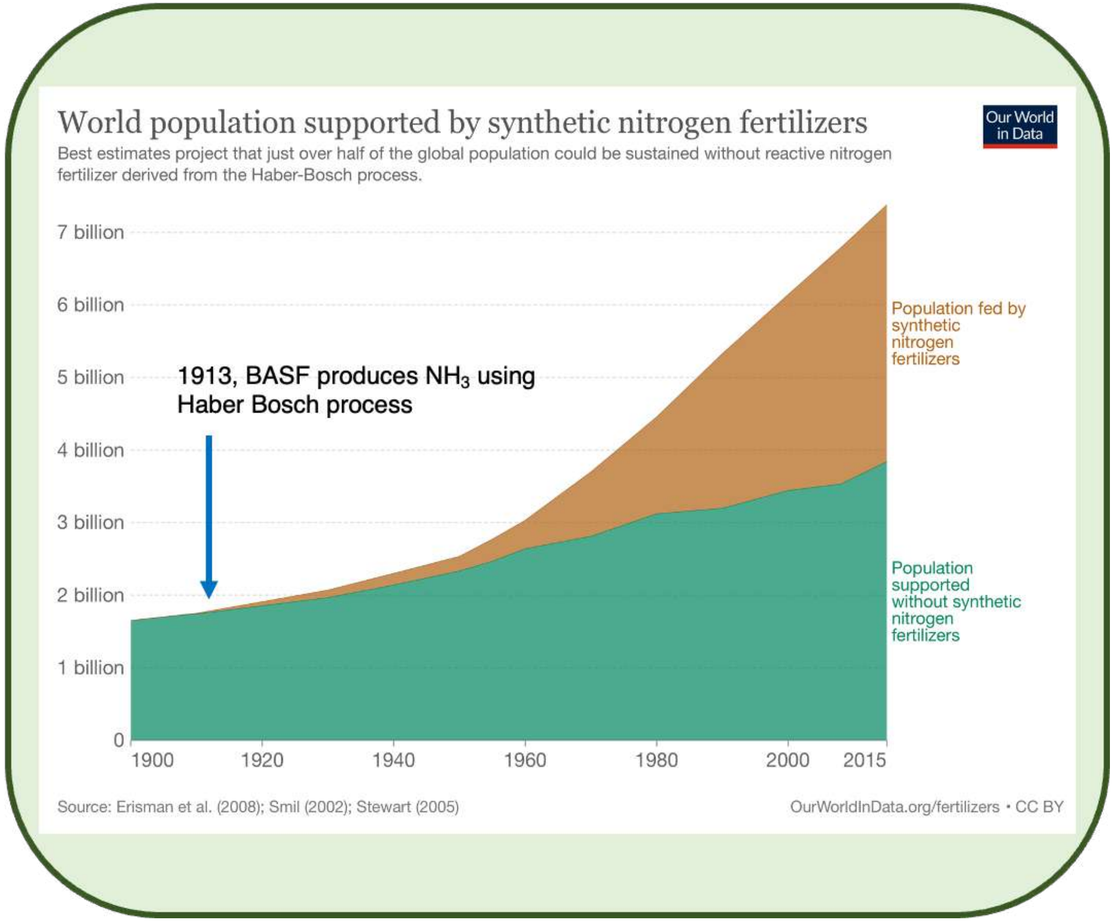
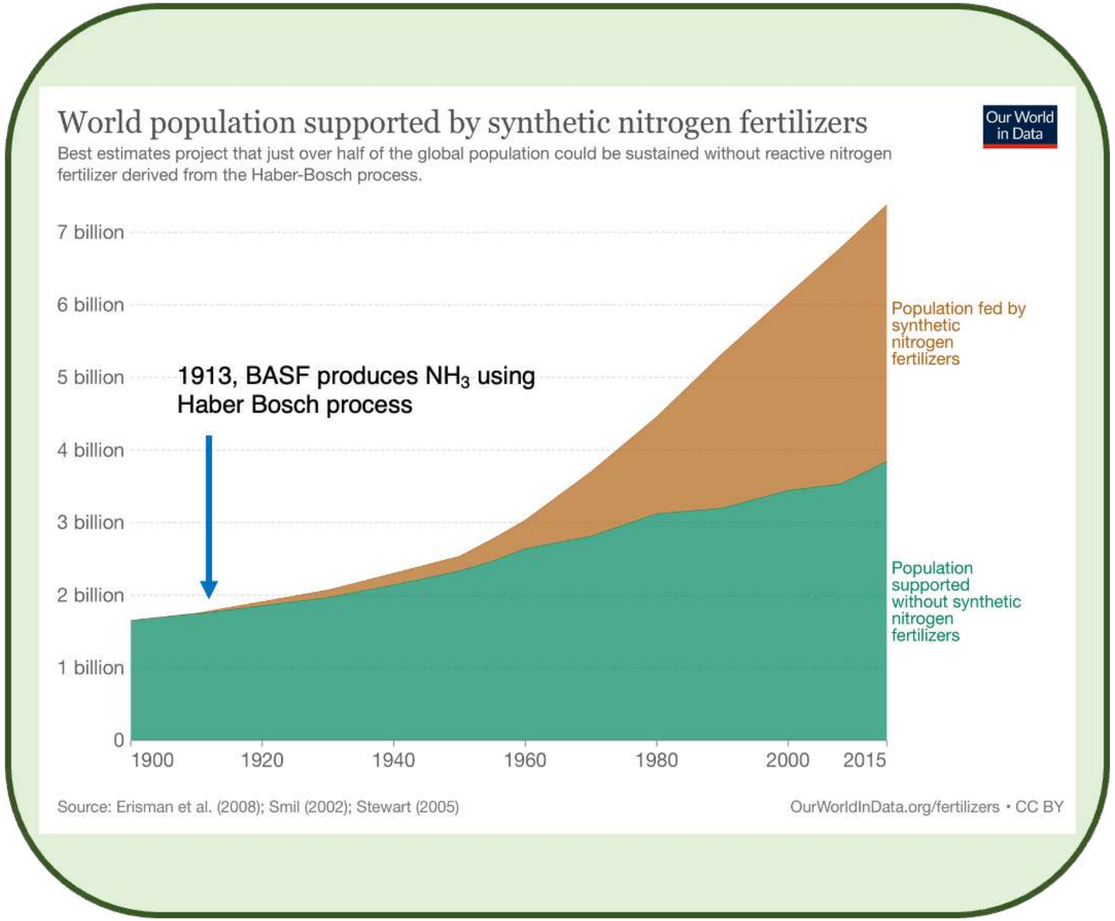
Impacts of nitrogen fertilizers

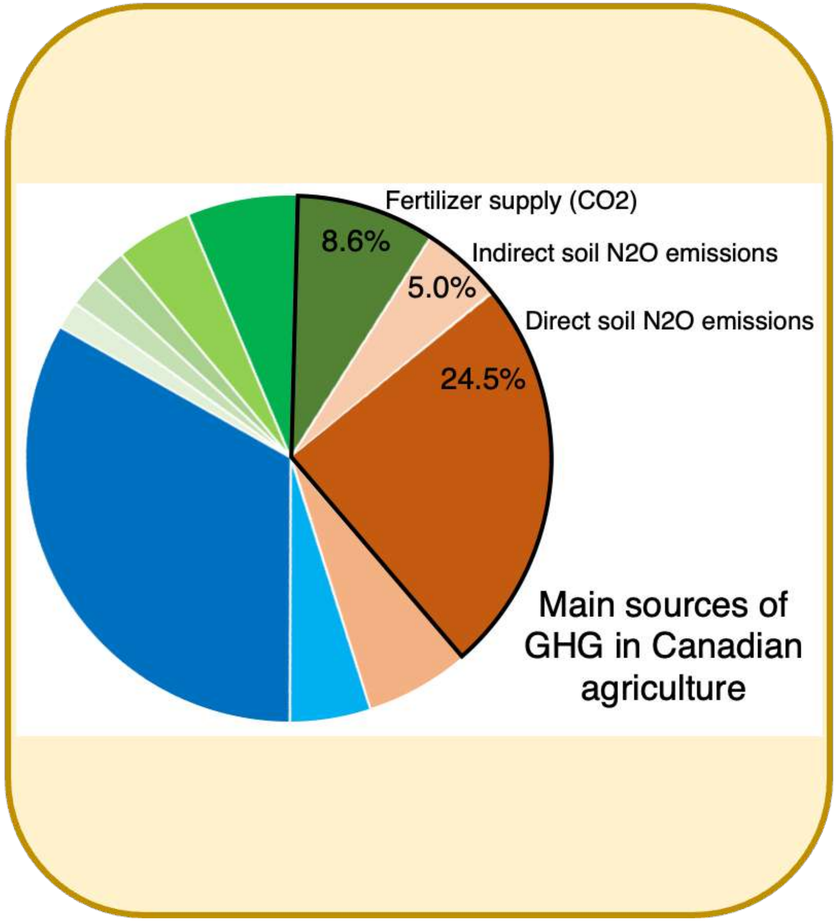
Synthetic fertilizers enhance food production



Impacts of nitrogen fertilizers

Synthetic fertilizers enhance food production, but also increase greenhouse gases





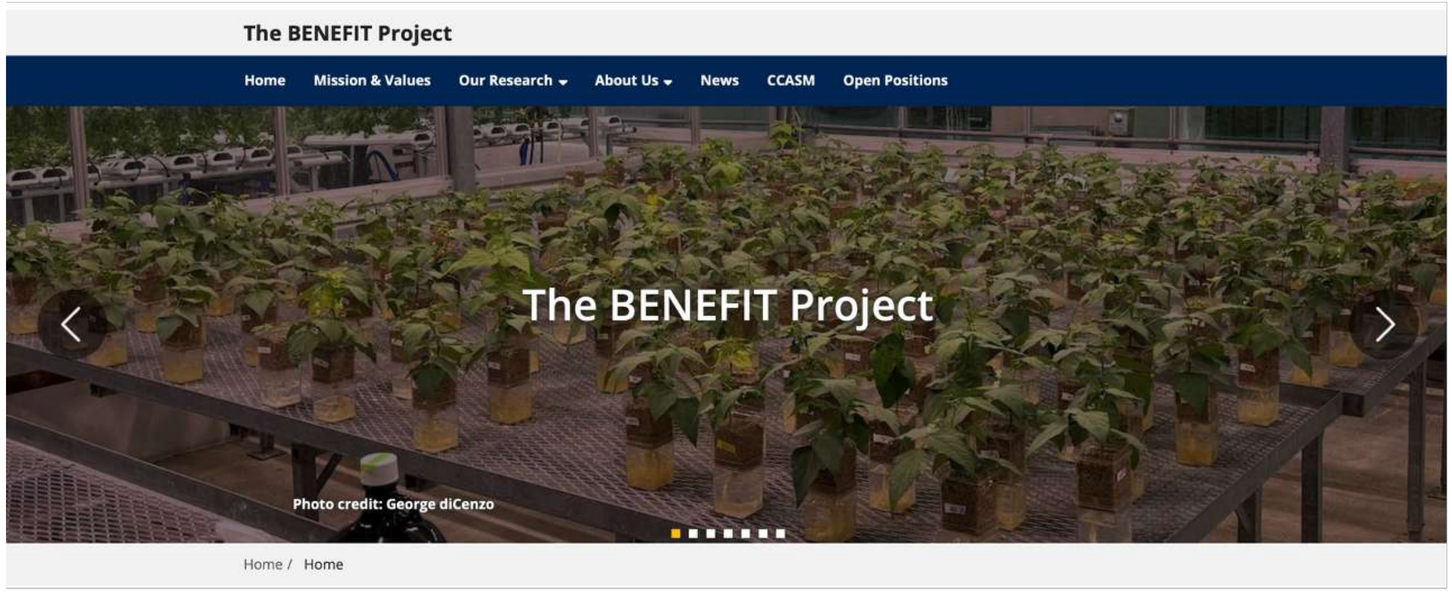
The BENEFIT Project

Bio-inoculants for the promotion of nutri**en**t **ef**ficiency and crop resiliency in Canad**i**an Agricul**t**ure

queensu.ca/microbes-for-agriculture

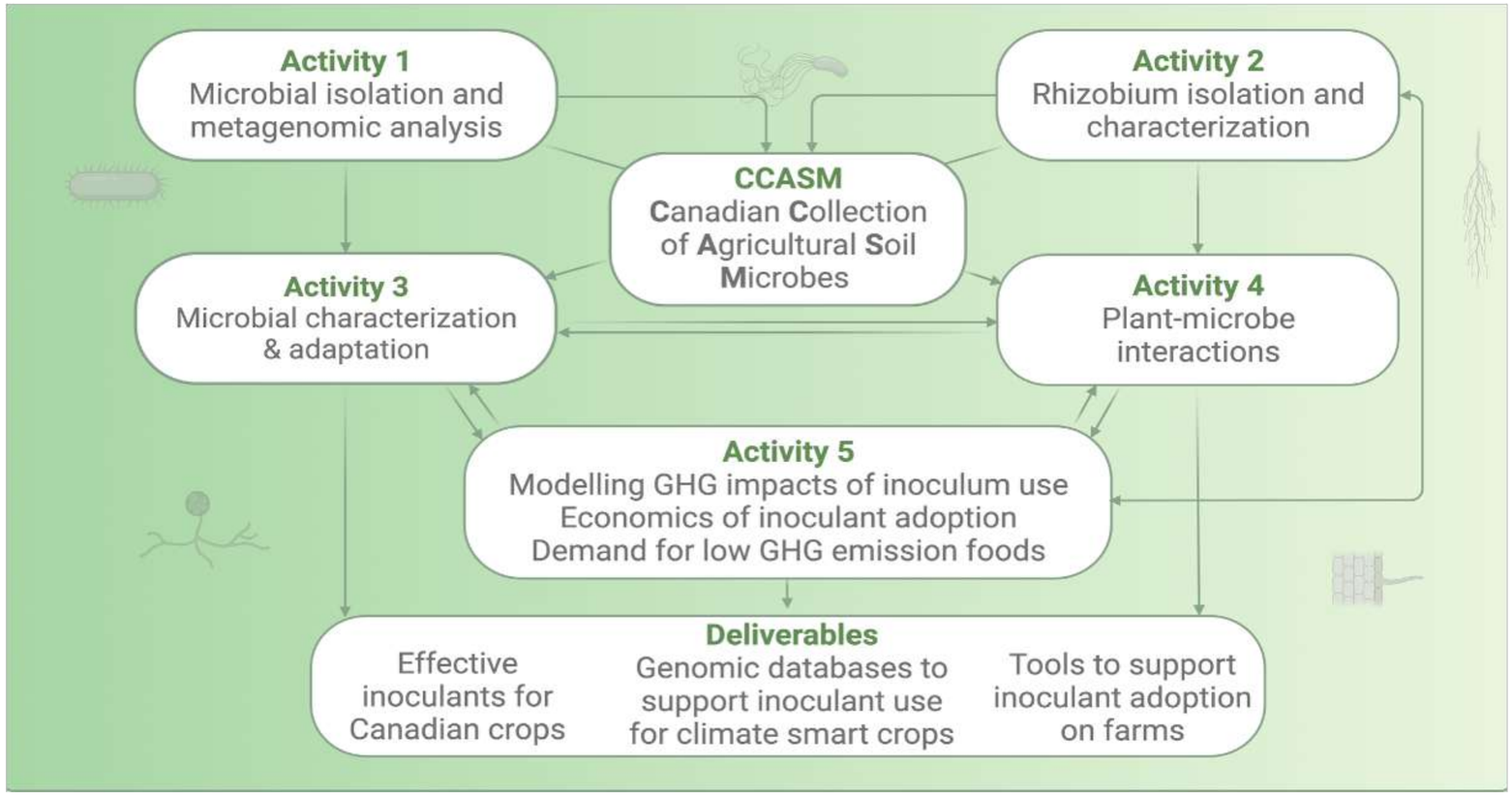
Identify and optimize microbial inoculants for Canadian crops that allow growers to maintain or increase current crop yields with reduced nutrient input

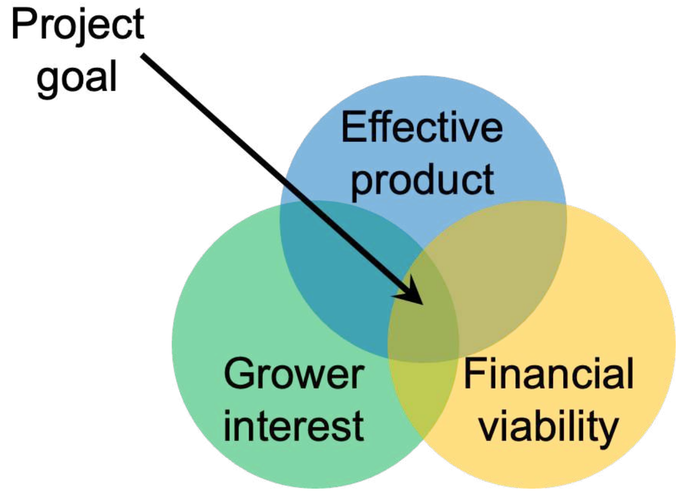
Focused on cereals (wheat, barley), brassicas (canola, kale), and legumes (bean, pea)



The BENEFIT Project

A holistic approach to the development of microbial inoculants, spanning microbe isolation and characterization; elucidation of plant responses; and the economic and environmental impact.





Developing new inoculants for Canada

Where do we start?

- First, need a bio-bank of bacterial isolates to screen for traits of interest
- Prioritize isolates from Canadian soils

A diverse library of agricultural soil bacteria

We have collected over 2,000 bacterial isolates from soils collected across Ontario and the Prairies and completed WGS of all isolates

Of ~1,100 Ontario isolates

- ≥288 species from 88 genera across 38 families in 4 phyla
- *Bacillota* (57%), *Actinomycetota* (30%), *Pseudomonadota* (12%), *Bacteroidota* (1%)
- Potentially ≥150 putatively undescribed species

Images of colonies or liquid cultures of the isolates

In collaboration with Dr. Matthew Bakker (University of Manitoba)

A collection of legume nodule isolates

We have collected and genome-sequenced over 1,200 bacterial isolates from legume nodules (common bean, pea, adzuki bean, and soybean) using soils from Ontario and Manitoba

Navy bean plants inoculated with rhizobia isolated from Ontario soils

In collaboration with Dr. Ivan Oresnik (University of Manitoba) and Dr. Rebecca Doyle (Queen's University)

A collection of bacteria from arctic soils

We have collected and genome-sequenced 99 bacterial isolates (19 species) from near Daring Lake, Northwest Territories

In collaboration with Dr. Paul Grogan (Queen's University)

Questions arising from this sampling effort

Why go through the trouble of generating our own collection?

What do we do with all of these isolates?

Why generate our own bacterial collection?

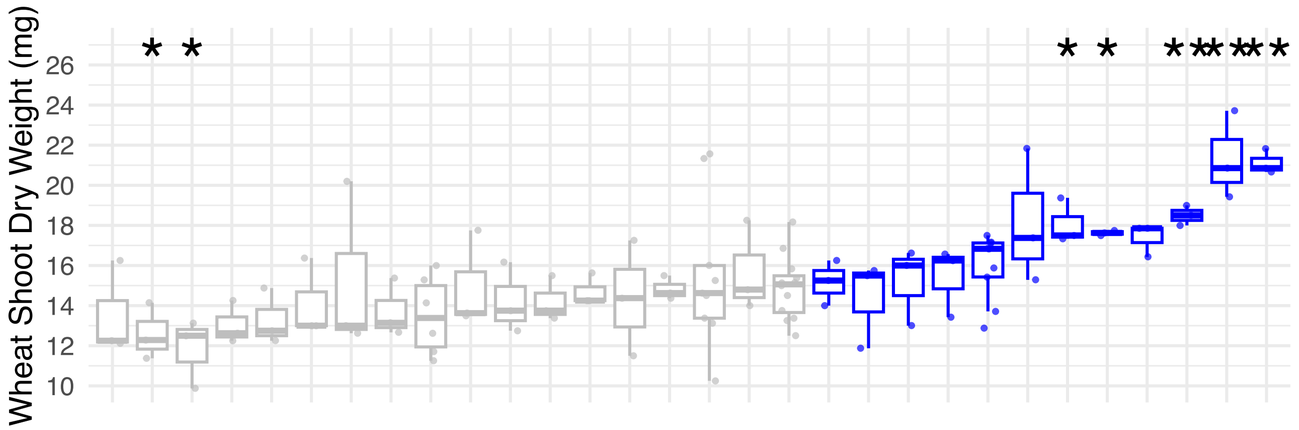
Comment from a reviewer: "How this differs or complements previous scouting work for microbial inoculants in Canadian agriculture would help the panel better understand the need."

Response: "…Collections of Canadian strains from other studies are not readily available and so we must first establish our own collection…"

What do we do with all these isolates?

Screen for traits relevant to bio-inoculant success

- Plant growth promotion



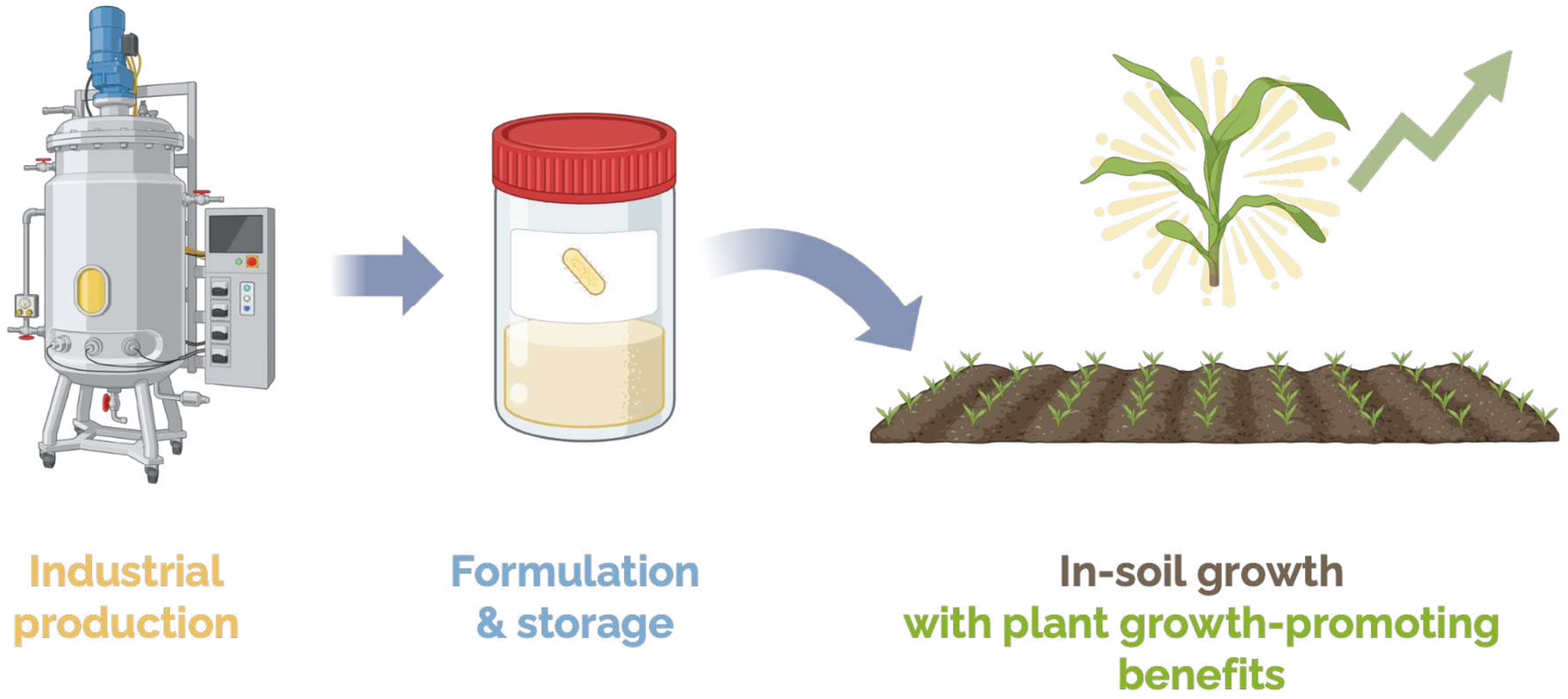
Impacts of isolates on the weight of wheat seedlings

Data from Dr. Olivia Wilkins (University of Manitoba)

What do we do with all these isolates?

Screen for traits relevant to bio-inoculant success

- Plant growth promotion
- Robust across industry pipeline (Hemara et al. 2026. ISME Communications. ycag190)



Schematics from Dr. Lauren Hemara (University of Toronto - Scarborough)

What do we do with all these isolates?

Screen for traits relevant to bio-inoculant success

- Plant growth promotion
- Robust across industry pipeline (Hemara et al. 2026. ISME Communications. ycag190)

But there are many other potential uses of the isolates!

- Examine ability to protect crops from abiotic stress
- Evaluate bio-control potential
- Screen for other traits relevant to biotechnology
- Screen for novel antimicrobial compounds
- Characterization of novel species

Thus important that the isolates be accessible for reuse by others

Canadian Collection of Agricultural Soil Microbes (CCASM)

A new culture collection designed to facilitate the sharing of bacteria (and associated genomic data) isolated from agricultural soils within Canada

Duplicated at Queen's University and the University of Manitoba (Dr. Matthew Bakker)

Stored as glycerol stocks at -80˚C in barcoded vials

CCASM

Canadian Collection of Agricultural Soil Microbes

Canadian Collection of Agricultural Soil Microbes (CCASM)

Will contain all isolates collected as part of BENEFIT

Will accept deposits from other research groups

Distribution and deposits initially limited to just Canada

A nominal fee associated with use

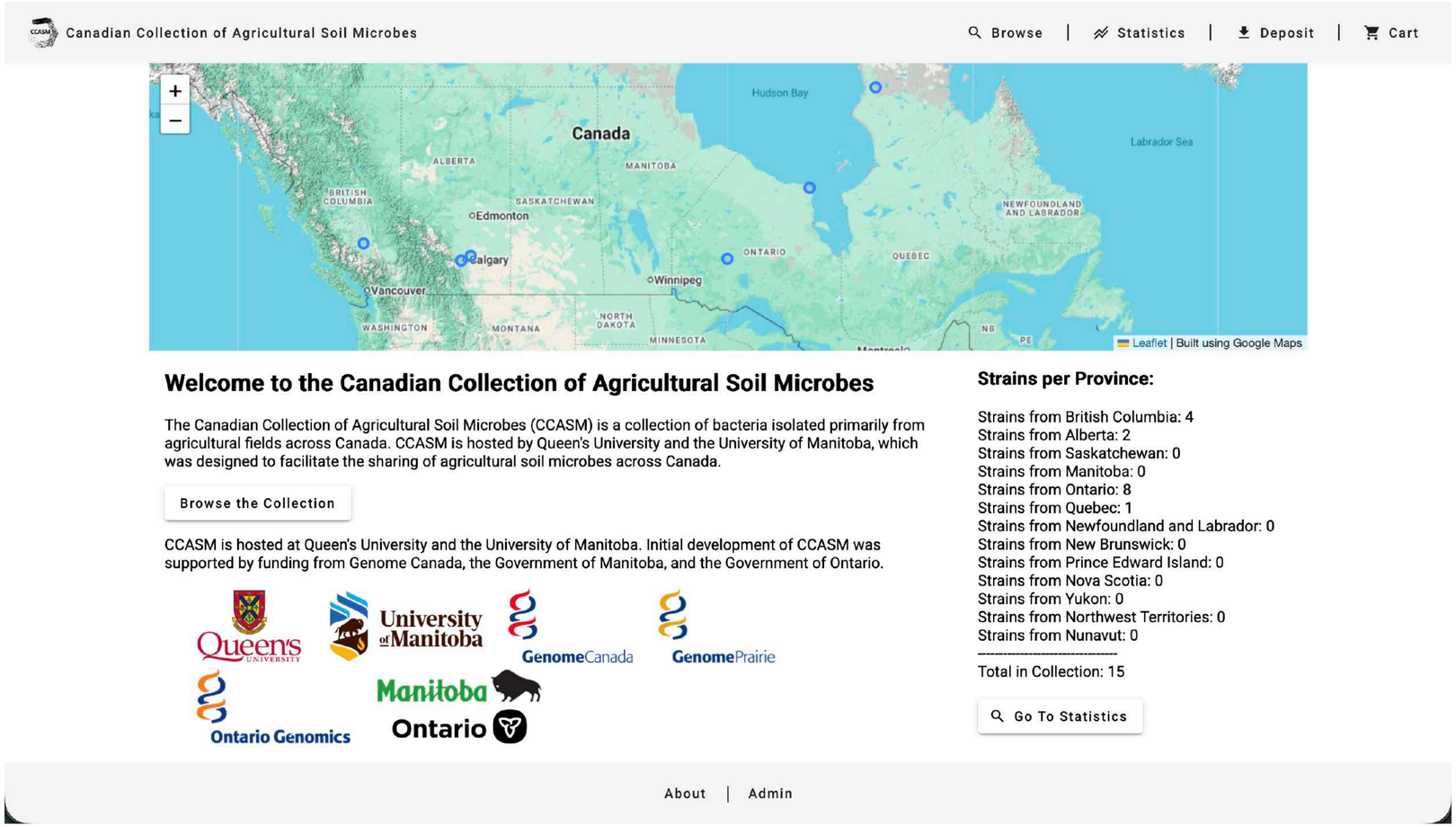
Not yet public but should be public this year

CCASM

Canadian Collection of Agricultural Soil Microbes

Canadian Collection of Agricultural Soil Microbes (CCASM)

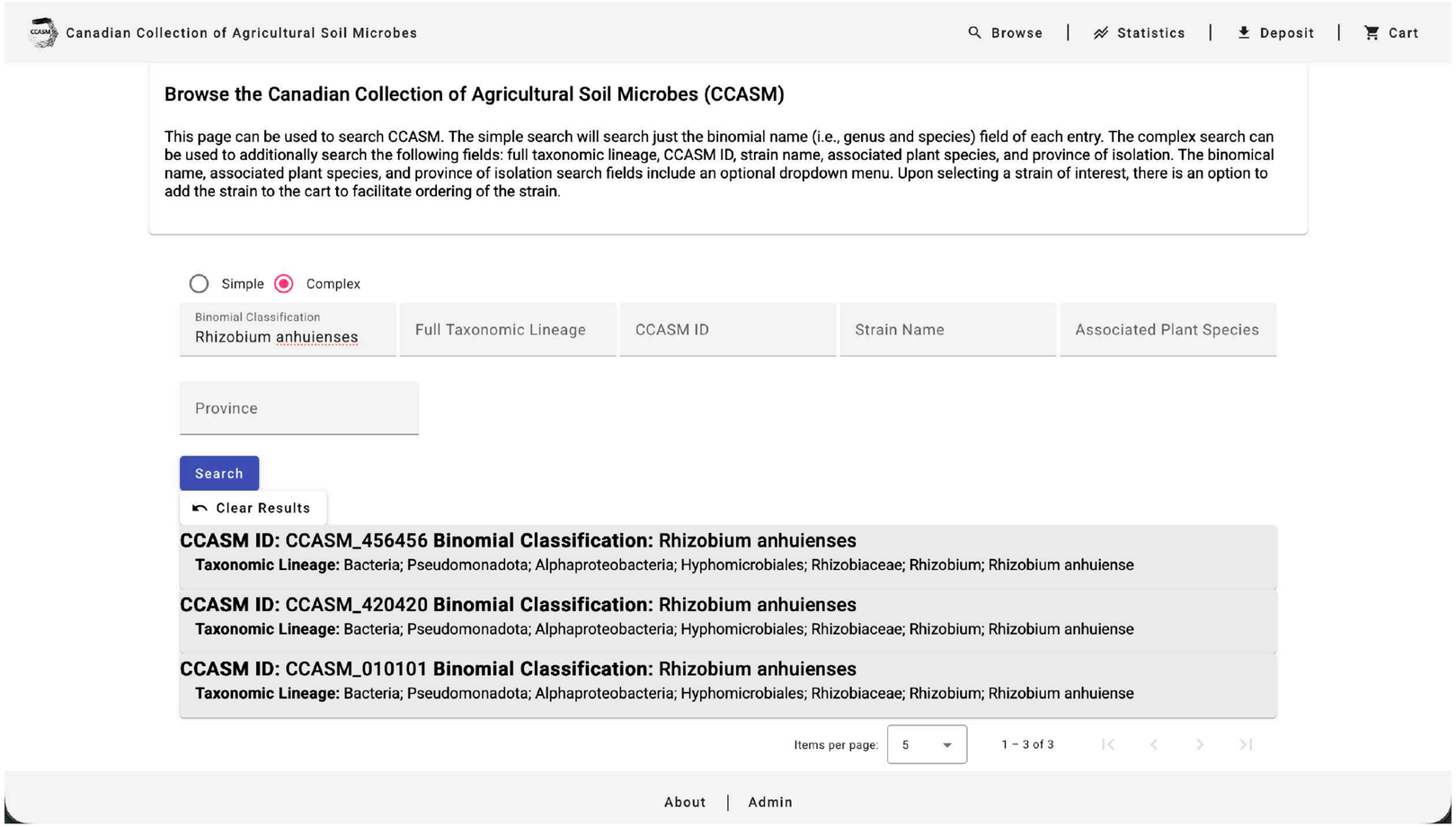
Home page provides a geographic breakdown of the source of isolates



All information in these screenshots is based on test data

Canadian Collection of Agricultural Soil Microbes (CCASM)

Can browse the collection based on various parameters



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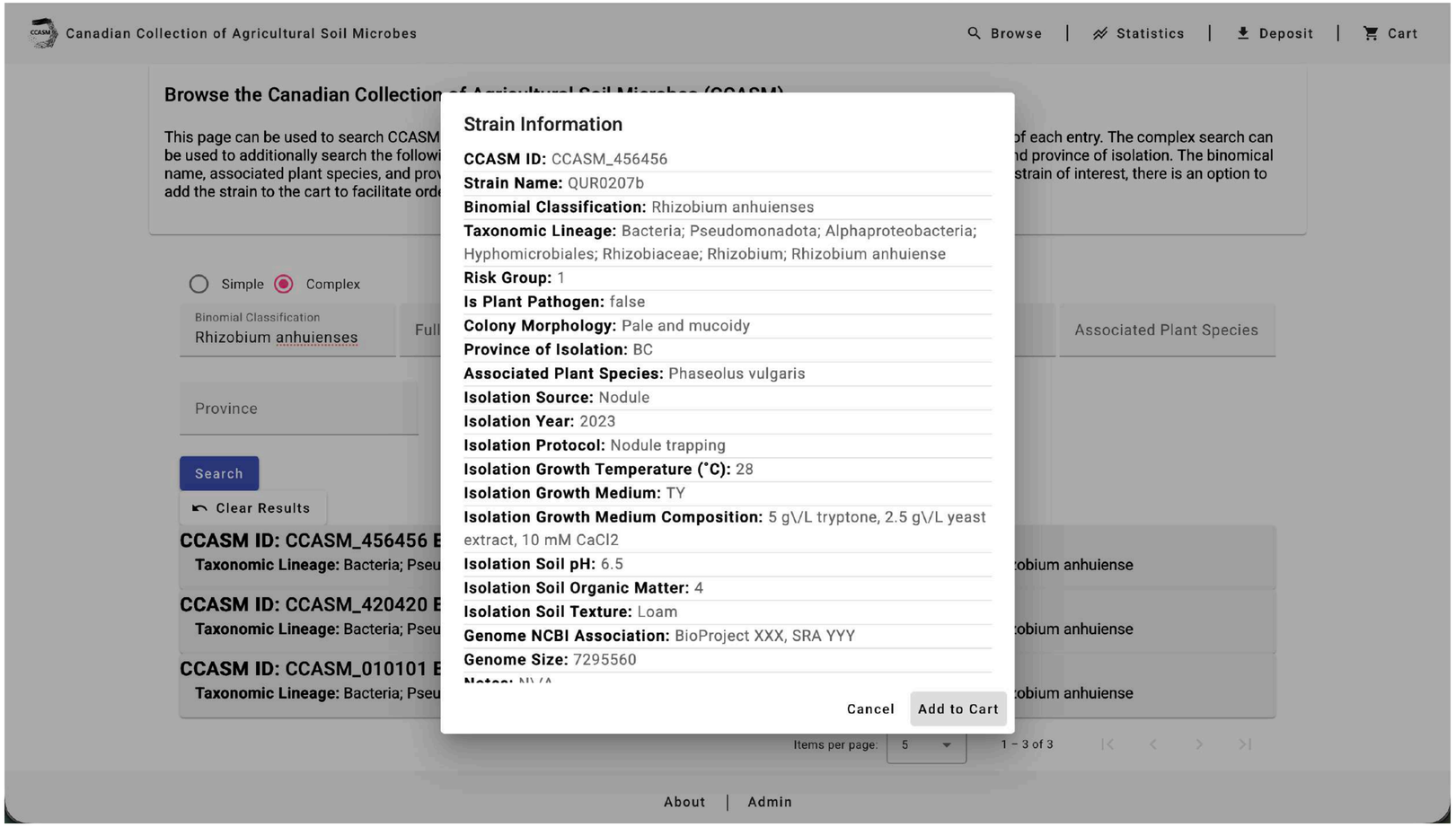
Canadian Collection of Agricultural Soil Microbes (CCASM)

Selecting a strain provides various pieces of information, including soil information (if available), genome accessions, and optionally a photo



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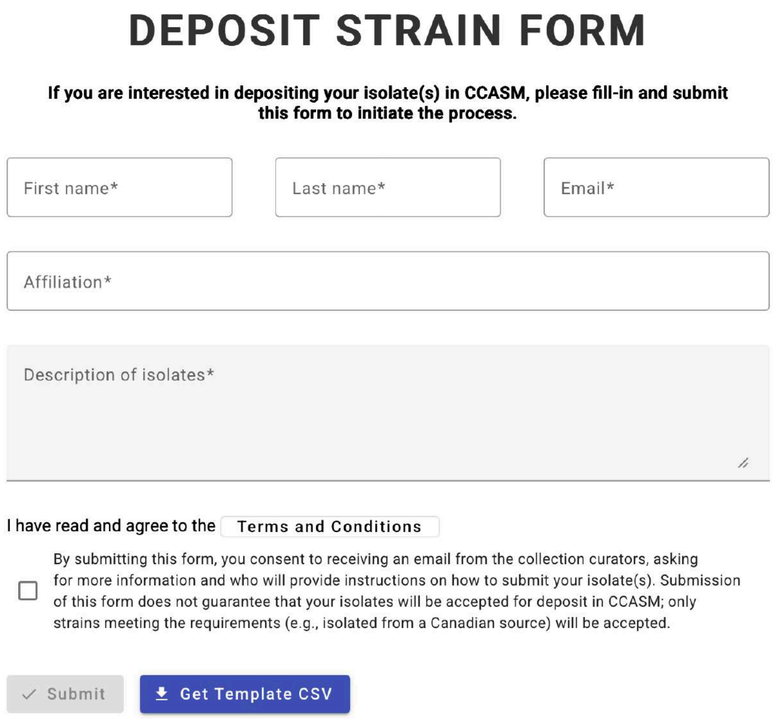
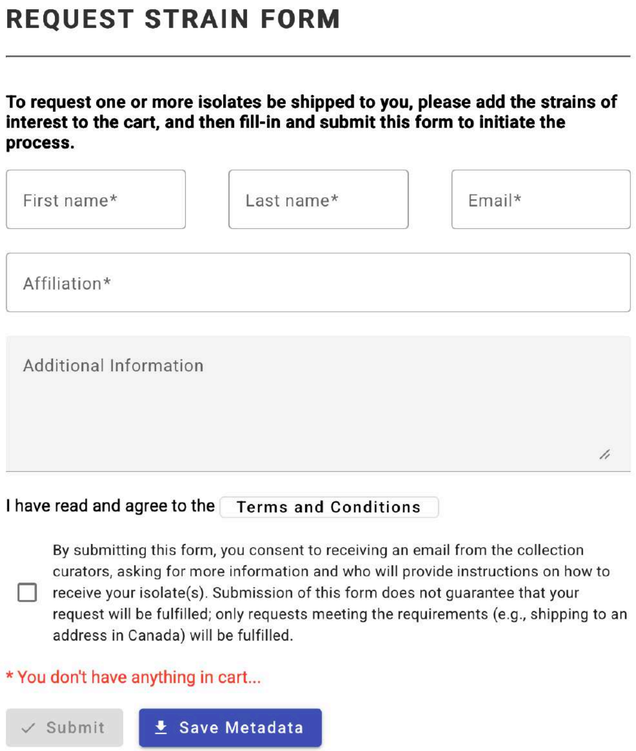
Canadian Collection of Agricultural Soil Microbes (CCASM)

Can submit forms to initiate deposit or ordering of strains





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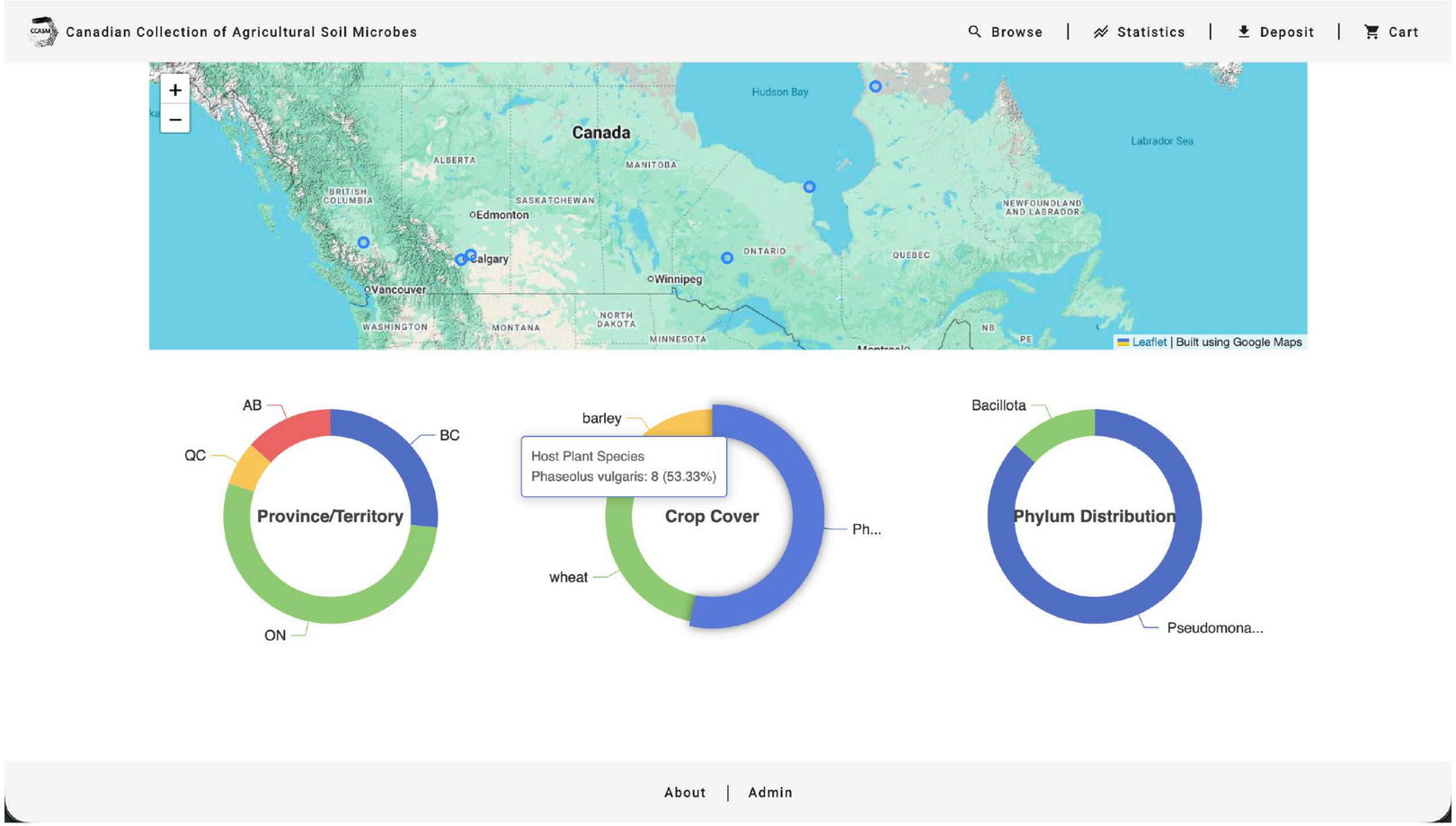
Canadian Collection of Agricultural Soil Microbes (CCASM)

Statistics page provides some high-level statistics summarizing the collection



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Canadian Collection of Agricultural Soil Microbes (CCASM)

A new culture collection designed to facilitate the sharing of bacteria (and associated genomic data) isolated from agricultural soils within Canada

Support reuse of isolates, open distribution of strains and metadata, and reduce the need for new sampling efforts

CCASM

Canadian Collection of Agricultural Soil Microbes

Introduction to rhizobia and their taxonomy

Rhizobium - legume symbioses

Legumes associate with nitrogen-fixing bacteria called rhizobia that colonize a legume organ known as a nodule

Left - rhizobia
Right - no rhizobia

Dark - rhizobia
Light - no rhizobia

Left - rhizobia
Right - no rhizobia

Images from:
interchopen.com; pioneer.com; qaseeds.com.au

Rhizobium - legume symbioses

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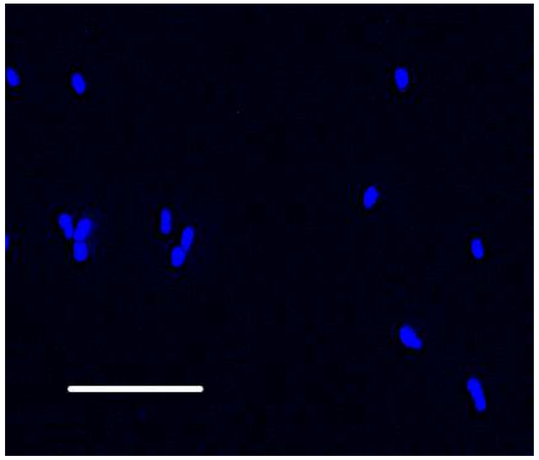
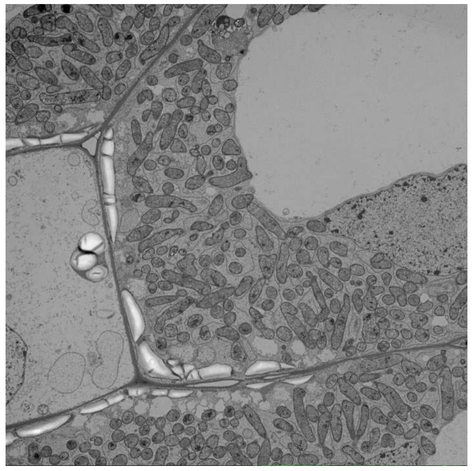
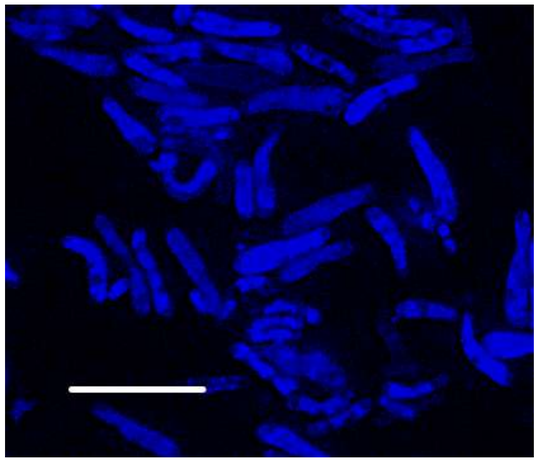
Alfalfa root nodule



Free-living *S. meliloti*

TEM image of a nodule cross-section

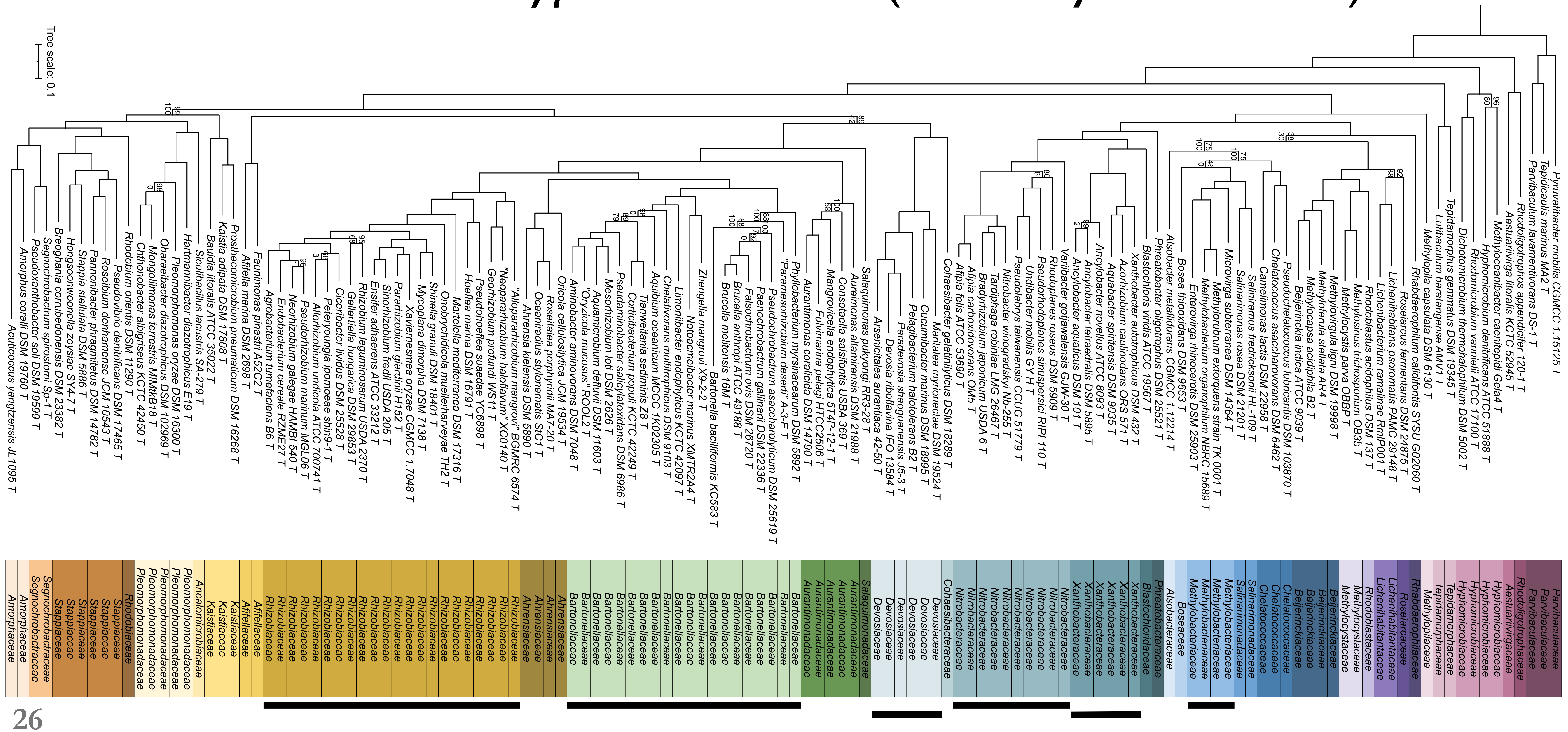




S. meliloti bacteroids

Alpha-rhizobia

A polyphyletic group of nitrogen-fixing symbionts of legumes, spread across six families of the order *Hyphomicrobiales* (formerly *Rhizobiales*)



Genome-based phylogeny of the bacterial order *Hyphomicrobiales*

diCenzo et al. 2024. IJSEM

Alpha-rhizobia

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A recent article claimed that *Sphingomonas sediminicola* DSM 18106^T (order *Sphingomonadales*) is also a rhizobium

However, our data is inconsistent with this claim

R. johnstonii 3841^T *S. sediminicola* DSM 18106^T Uninoculated

diCenzo et al. 2025. Can J Microbiol

Alpha-rhizobia

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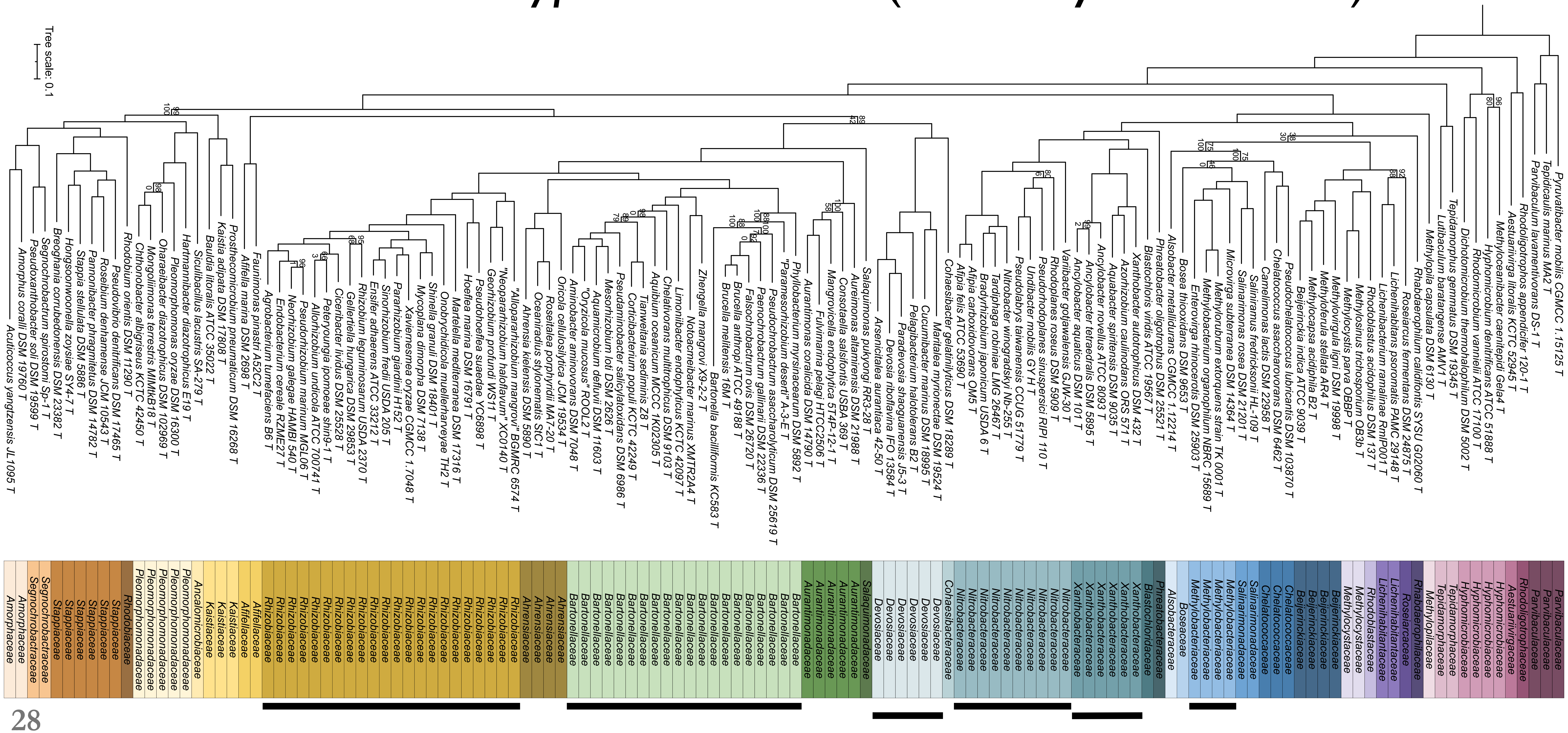

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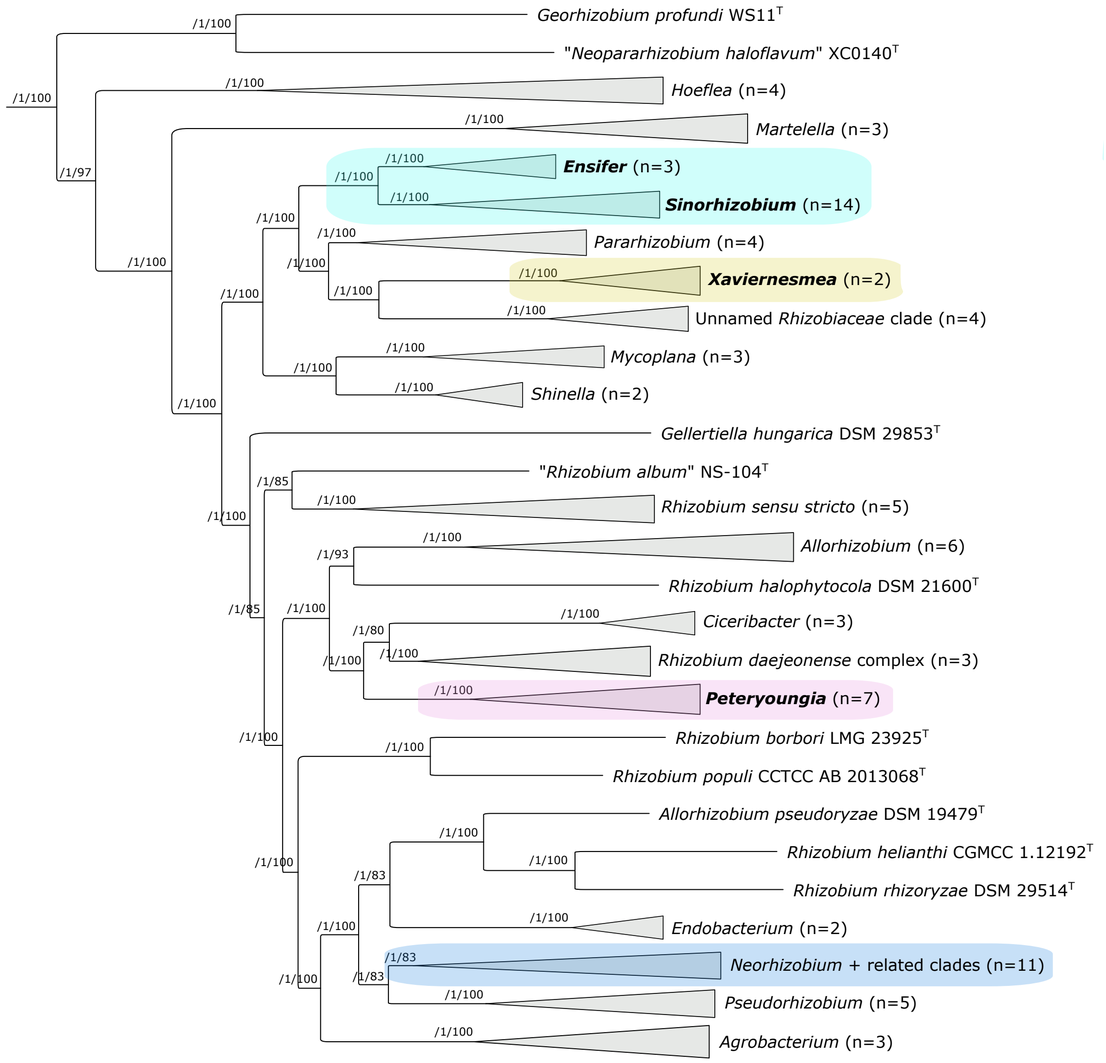
The family *Rhizobiaceae*

Now encompasses ~280 species across nearly 50 genera

Includes many plant symbiotic bacteria (rhizobia) and pathogens (agrobacteria)

Historically, this family contained many taxonomic inconsistencies

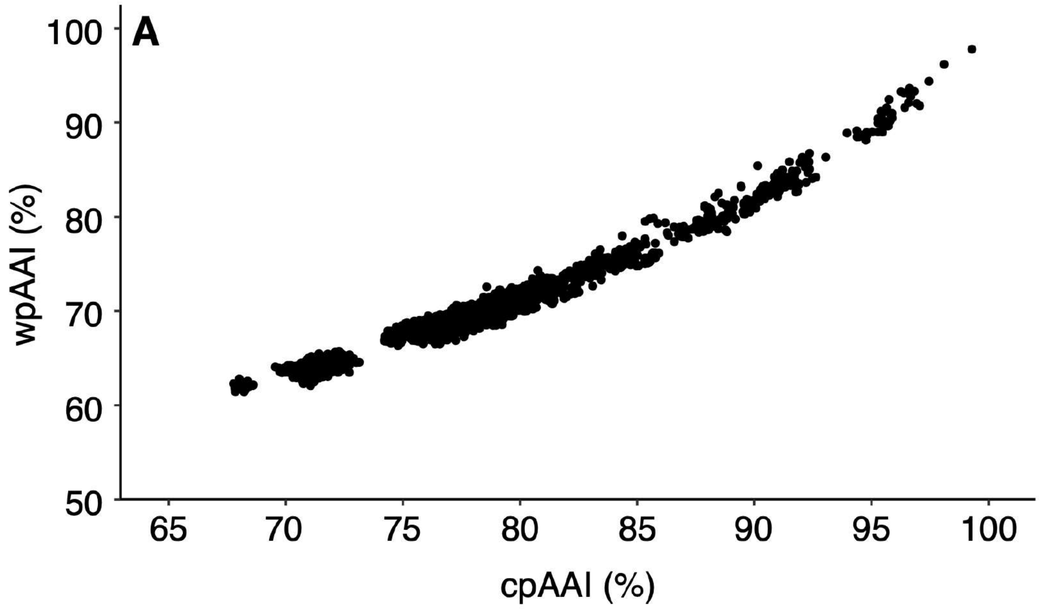


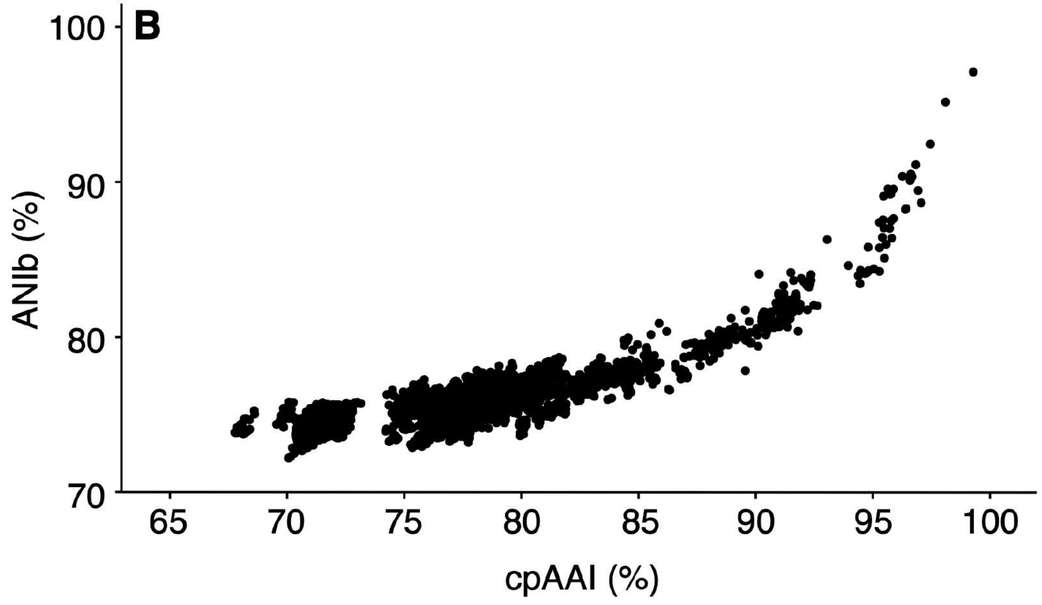
Kuzmanovic et al. 2022. IJSEM

Defining genus boundaries in the family *Rhizobiaceae*

Identified a core set of 170 single-copy, non-recombining genes across 94 representative type strains

Used for phylogenomics and to identify a core-proteome AAI of ~86.0% (~86.5%) as a proposed, flexible threshold for genus demarcation





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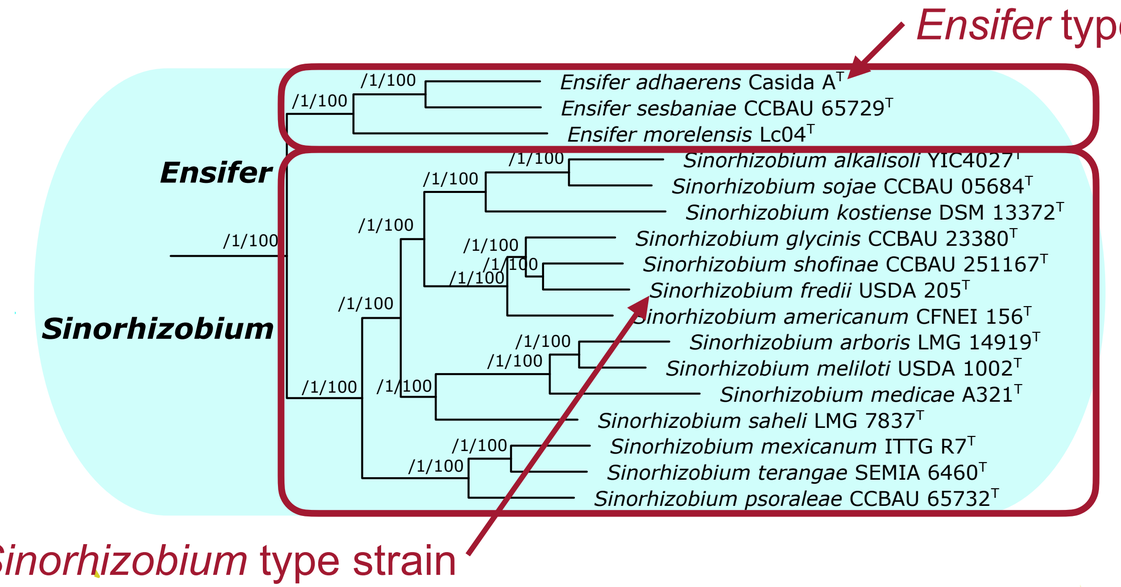

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But many species missing from this initial analysis

Kuzmanovic et al. 2022. IJSEM

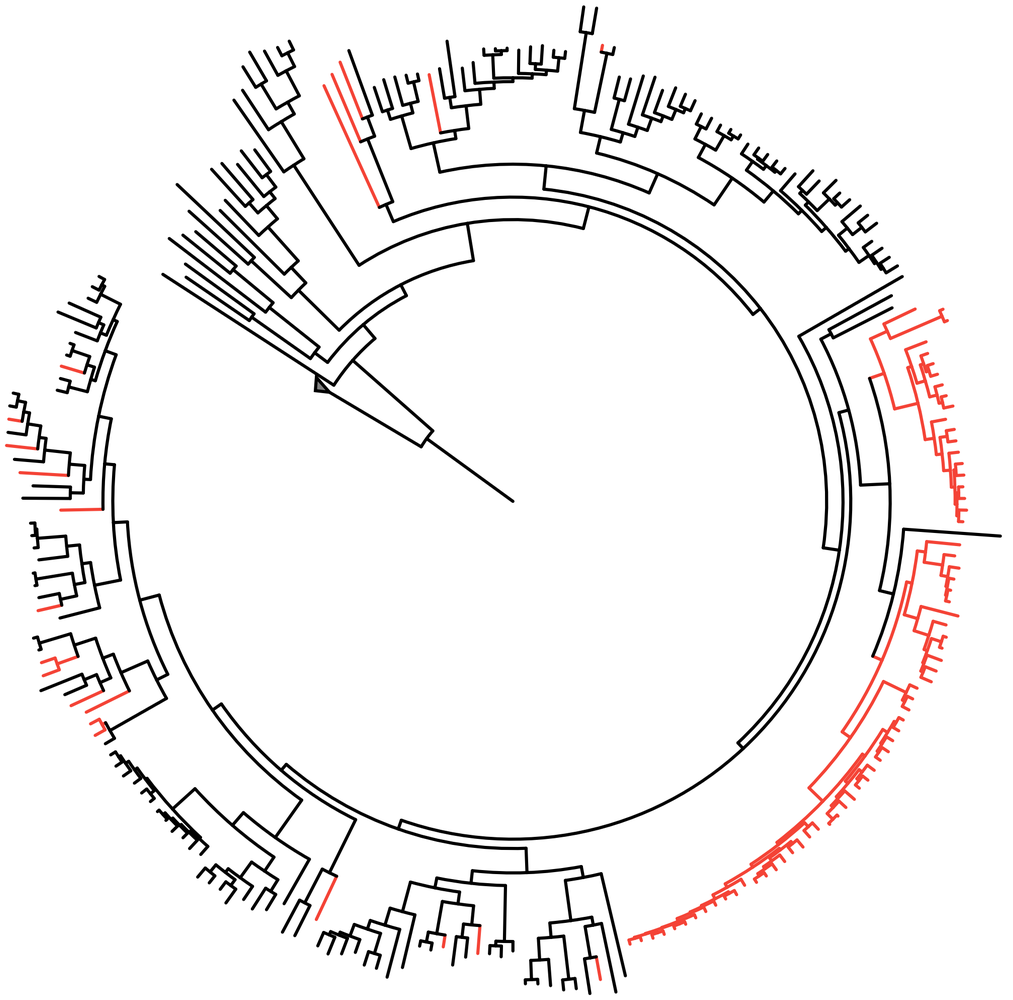
Further revisions to the taxonomy of the family *Rhizobiaceae*

Performed whole genome sequencing of 14 *Rhizobiaceae* type strains lacking publicly available genome sequences

Analyzed 242 *Rhizobiaceae* type strains (up from 94)

Dozens of *Rhizobium* species were clearly distinct from the genus *Rhizobium* and thus required reclassification

Proposed 8 novel genera and 32 novel combinations



Phylogeny of 242 *Rhizobiaceae* species type strains

Naranjo-Robayo et al. 2026. IJSEM

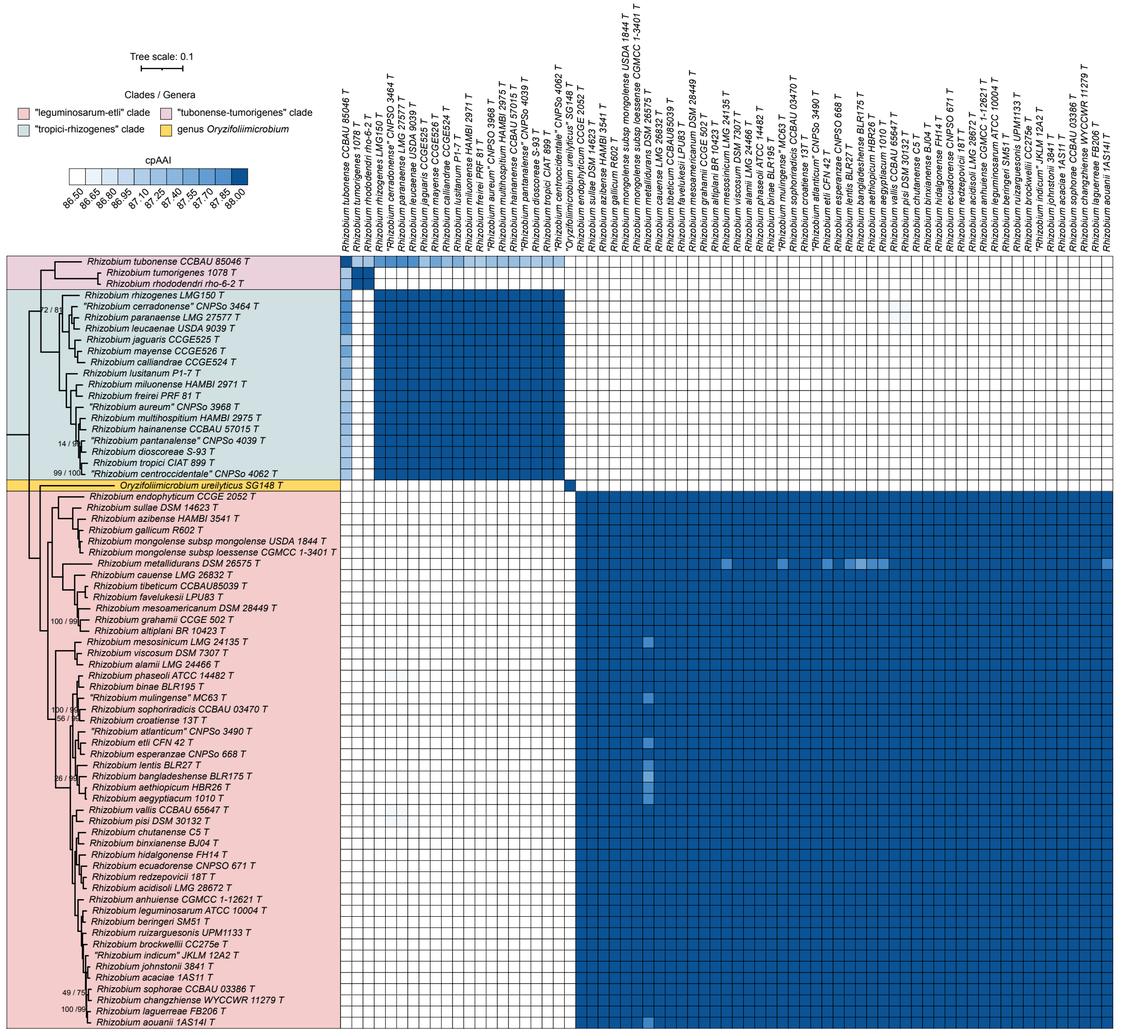
Further revisions to the taxonomy of the family *Rhizobiaceae*

Rhizobium can be split into three clades

Most within-clade cpAAI comparisons are > 88% while most between-clade cpAAI comparisons are < 86.5%

Rhizobium is paraphyletic due to a recently proposed genus, *Oryzifoliimicrobium*

Thus, the three core *Rhizobium* clades are proposed to represent distinct genera (*Rhizobium*, *Martinezella* gen. nov., and *Ariminella* gen. nov.)



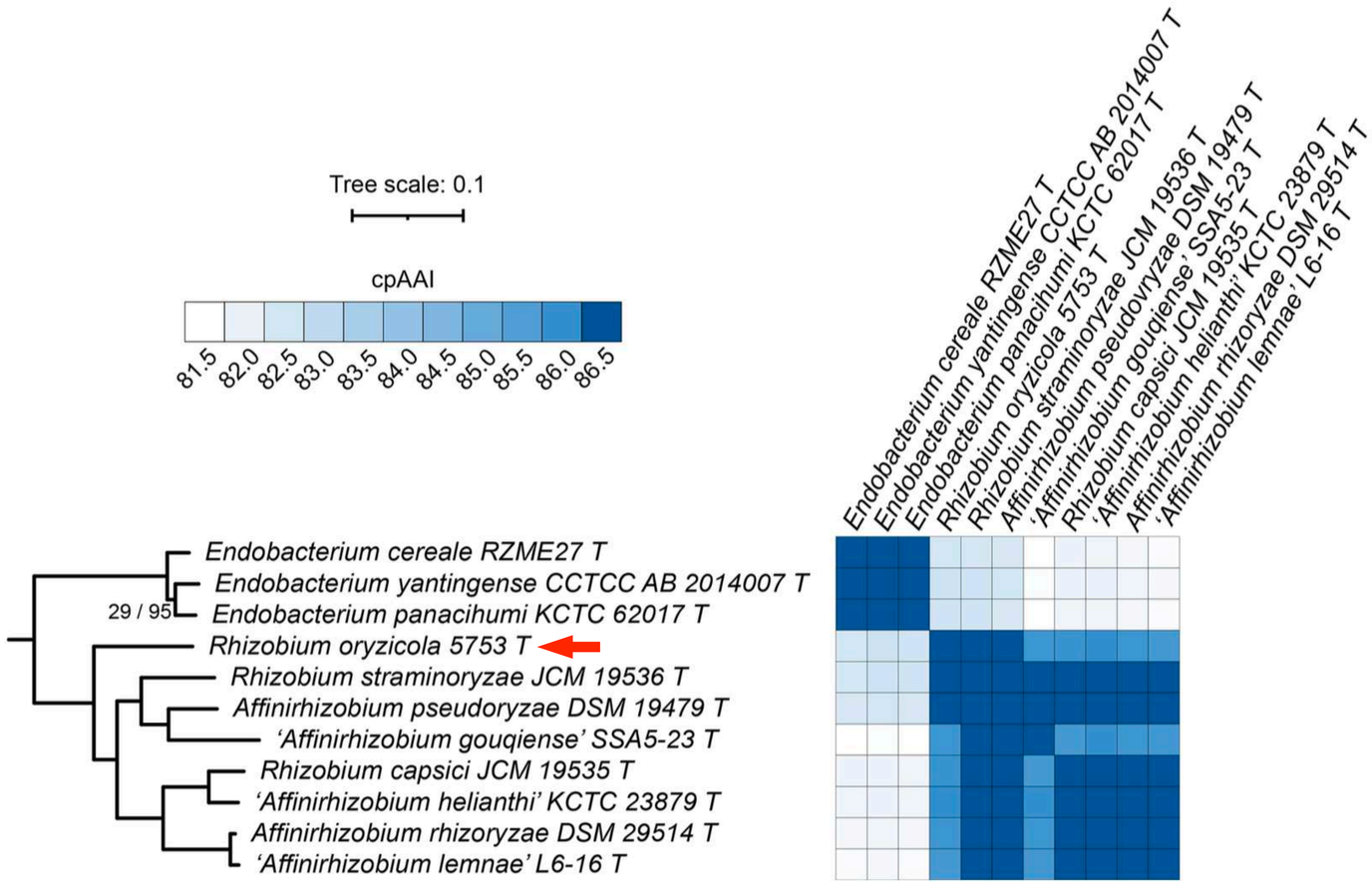
Phylogeny and cpAAI of *Rhizobium* and related genera

Naranjo-Robayo et al. 2026. IJSEM

Next steps in *Rhizobiaceae* taxonomy

Sequence additional *Rhizobiaceae* type strains and check taxonomic assignments

Use SeqCode to validate novel combinations where needed



The people who actually do the research

Current Lab Members

Dr. Tia Harrison (TMU)
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Dr. Upkar Pandher
Oona Esme
Kristy Moniz
Ameneh Nourozian
John Robinson
Mia Rondinelli
Gaia Rota
Nicholas Smith
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Dr. Neal Scott

Dr. Rebecca Doyle

Dr. Ana Vargas

Dr. Jill Hobbs

Dr. Yvonne Lawley

Dr. Olivia Wilkins

Dr. Nicholas Corradi

Dr. Marina Cvetkovska

Dr. Allyson MacLean

Dr. Sabine Liebenehm

Dr. Yang Yang

Culture collections (in no particular order)

DSMZ (Germany) and Dr. Katharina Huber-Fischer

LMG (Belgium)

JCM (Japan)

KCTC (South Korea)

NBRC (Japan)

CCUG (Sweden)

HAMBI (Finland)

USDA (USA)

Funders and Partners

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GenomeCanada

GenomePrairie

Ontario Genomics

Manitoba

Ontario

Industry

Hensall CO-OP

EARTH MICROBIAL ENGINEERING MICROBIOMES FOR AGRICULTURE

VIVE CROP PROTECTION

fieldless

Grower Organizations

MANITOBA CROP ALLIANCE

Sask Wheat DEVELOPMENT COMMISSION

WGRF Advancing Agriculture through Research

MANITOBA Pulse Soybean GROWERS

ONTARIO Bean GROWERS

GRAIN FARMERS OF ONTARIO

Research Institutions

University of Manitoba

Queen's University

UNIVERSITY OF TORONTO SCARBOROUGH

uOttawa

UNIVERSITY OF SASKATCHEWAN

McMaster University

Other

Kingston Area Seed System Initiative KASSI seedsgrowfood.org

LEGUME RHIZOBIUM SCIENCES

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