

**M1 or M2 internship proposal (2023)**

***Screening of BioSun’s proCULTIVATM endophytic microbial consortia for biostimulant and biocontrol properties in economically important crops like yellow beans, tomtato and cannabis.***

Key words: Biostimulants, biocontrols, plant growth promotion, priming, seed treatment, induced resistance, preventive, curative.

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| **The laboratory** |
| Unit | University of McGill and Bio Sun Products Inc.( [BioSun.live (biosunsolutions.com)](https://www.biosunsolutions.com/pages/innovation-pipeline)) |
| University of McGill, INRAE, and Bio Sun Products Inc. team  | **BioSun is committed to unlocking the extraordinary potential of beneficial bacteria, revitalizing depleted soils and breathing new life into agricultural landscapes. The company started a collaborative project with McGill and successfully built a bacterial bank covering a wide range of species with biostimulant and biocontrol properties.** **This project is funded by Mitacs Accelerate IT29988** "[The Next Generation Agriculture: Role of functional endophytic microbiome in Cannabis varieties against mycotoxigenic fungi and Powdery mildew.](https://www.mitacs.ca/en/projects/next-generation-agriculture-role-functional-endophytic-microbiome-cannabis-varieties)" Project duration: April 2022- March 2024. BioSun and INRAE have been working closely since two years for the screening of CelexT07 for biocontrol properties as part of the **CapZeroPhyto** initiative.  |
| **The internship** |
| Context | The world food bank estimates that feeding a population of 9.1 billion people in 2050 requires raising food production by 70% between 2005 and 2050. This resulted in heavy reliance on agrochemicals (1, 2). One of the critical issues is the rapid decrease in soil fertility. Canada's Supreme Court has ruled in favor of a national carbon tax, highlighting the urgent need for sustainable agricultural practices. To address these challenges, there is a growing emphasis on developing biostimulants that aligns with trends of the growing market. The North American Biostimulants market alone was worth USD 392.79 million in 2020 and is projected to grow at a CAGR of 11.29% (3). This market growth presents significant opportunities for innovative and sustainable biostimulant products. Accordingly, BioSun and McGill, worked together to develop proCULTIVATM that innovatively combines a consortium of probiotic bacteria to add a new eco-friendly product to BioSun’s portfolio. proCULTIVATM is a liquid plant supplement made from a consortia of *Bacillus sp*. and *Rhizobium sp*. proCULTIVA helps improve plant growth, crop yield, and protection from environmental stress. Field results from 2021-2023 showed its superior performance compared to commercial controls, enhancing yield, fruit quality, and shelf life. In frost-prone conditions, Plantiful enabled early bean commercialization. This proposal aims at conducting a deep screeningofthe beneficial microbial consortia (***proCULTIVATM***) isolated previously in the MITACS IT29988 project for biostimulant and biocontrol properties. |
| Objectives of the internship | The proposed internship is meant to provide support for the two PhD students responsible for the MITACS IT29988 which eventually target the development of microbial based solutions for both plant promotion and disease control.The objective of this internship will include the following:1. BioStimulant assays: Seed germination and viability, yield (growth chamber and greenhouse), and impact of bacteria on plant nutritional content.
2. B- Biocontrol assays: Confrontation assays against some of the mycotoxigenic fungi and other economically important phytopathogens (e.g., Botrytis spp.), to offer an alternative solution for the elimination of cannabis flowers contaminated with mycotoxins and other crop diseases.

Objective B.1: Validate the antagonistic potential of top bacterial endophytes previously isolated against the pathogens *Aspergillus spp*., *Penicillium spp*., *Rhizoctonia solani*, *Fusarium oxysporum, and Botrytis cinerea*. *in vitro.*Rationale: Recent literature (4) indicates that plant genotype has a significant impact on microbial endophytes composition and activity and the difference in the microbial community may result in the differences in the resistance to disease. The previous results on the isolated bacteria showed their ability to produce metabolites such as Indol Acetic Acid, Abscisic Acid, Kinetin and Jasmonic Acid. Objective B.1.1: Dual confrontation assay under *in vitro* conditionsThe experiment for the dual confrontation assay will be performed at three separate time points and in triplicates for each microorganism. Reduction in moytoxigenic fungal growth will be measured as percent of inhibition ratios and using the formula: (C – T)/C Å~ 100, where C is the average radial of control growth and T is bacterial treatment radius. The diffusion agar assay experiment consists of a basal layer of agar mixed with 106 conidia of pathogen/mL then 10-μL (OD600, ∼0.1) of potential endophytes will be spotted on the lawn. The plates will be incubated at 24°C and examined after 24- 48 h for zones of inhibition (halo formation). The top bacterial strains that exhibited the largest inhibition zone will be retained for validation in vivo experiments.Objective B.1.2. Burkholder agar diffusion assay.Cell-free supernatant of bacteria will be grown on LB broth for 4 days and it will be obtained by centrifugation at 16 000g for 10 min and immediately freeze-dried. One gram of freeze-dried powder will be dissolved in 5 mL of sterile distilled water. In the petri plates a disc of 5 mm of the pathogen will be placed in the center of the petri plate. Likewise, 25 mm away from the disc a hole will be done to add 150 μL of the bacteria culture. There will be three replicates per test fungus. Plates will be incubated at room temperature, and measurement of inhibition of radial growth will be recorded every 24 h. The percentage of inhibition ratios will be calculated using the same formula described above.  |
| Date and duration of the internship | Start date: TBDDuration: 3 months maximum |
| Location | McGill University - Macdonald Campus 21111 Lakeshore Rd, Sainte-Anne-de-Bellevue, Quebec H9X 3V9 |
| Expected activities of the trainee | The trainee will: work with the McGill, BioSun, and INRAE team, and:- Carry out laboratory experiments (seed germination).- Carry outgrowth assessment experiments (growth chamber and greenhouse)- Perform confrontation (antibiosis)assays. - Analyse and interpret the results.- Write bi-weekly reports and a final report. |
| Practical conditions | * McGill to accommodate the intern to work in the lab and conduct the tests (covering the research costs). The project is funded by BioSun (MITACS IT29988).
* BioSun will provide guidance and support to guarantee proper skill transfer
* INRAE contribution to ….
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| **General information** |
| Profile required | * Student M2 or M1 in agronomy or plant ecology or chemistry
* Interest in plant experiments and soil functioning
* Motivation for experimental and laboratory work (phytotron experiments, analyses)
* Interest in teamwork (other trainees, PhD students, technicians and researchers)
* Knowledge and mastery of office automation tools, if possible use of R (or Rstudio)
* Organisation, rigour, autonomy
* Writing skills. Need to read scientific literature in English
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| Contacts and supervisory staff for the internship | For more information and to apply: send a CV and a letter of motivation to the following e-mail addressesClaude DOUSSAN : claude.doussan@inrae.frAnnette BERARD : annette.berard@inrae.fr |

References :

1. Cynthia L. Curl & Meredith Spivak & Rachel Phinney & Luke Montrose. 2020. Synthetic pesticides and health in vulnerable populations; Agricultural workers. Current Environmental Health Reports 7:13-29.
2. Stephenson, B., Gill, G., Randall, J., & Wilson, J. 2003. Biosecurity approaches to surveillance and European Biostimulants Industry Council (EBIC response for new plant pest species. New Zealand Plant Protection, 56, 5-9.
3. European Biostimulants Industry Council (EBIC). Provide Fertile Ground for EU’s Continued Leadership in the Biostimulants Industry: EBIC Urges Policy Makers to Adopt the Draft Fertilizing Products Regulation; EBIC: Antwerp, Belgium, 2018.
4. Upreti, Reshmi, and Pious Thomas. "Root-associated bacterial endophytes from Ralstonia solanacearum resistant and susceptible tomato cultivars and their pathogen antagonistic effects." Frontiers in microbiology 6 (2015): 255.