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# MPSG ANNUAL EXTENSION REPORT

**PROJECT TITLE:** Development of Superior Rhizobium spp Strains to Support Biological Nitrogen Fixation in Dry Beans

**PROJECT START DATE: 1 April 2019** **PROJECT END DATE: 31 March 2019**

**DATE SUBMITTED: 4 February 2020**

## *PART 1: PRINCIPAL RESEARCHER*

**PRINCIPAL**

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|---|---|---|---|
| **NAME:** | Ivan Oresnik | **NAME:** | |
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## *PART 2: EXECUTIVE SUMMARY*

*Outline the project objectives, their relevancy to pulse and soybean farmers, and a summary of the project to date, including methods and preliminary results.*

The objectives of this project is develop Rhizobium strains which are capable of providing symbiotically fixed nitrogen to dry beans such that the amount of nitrogen fertilizer can either be greatly reduced or eliminated. The ability of growing dry beans without added nitrogen will both increase the profitability of the crop as well as reducing the ecological impact by reducing nitrogen run-off as well as reducing the amount of energy that is needed to produce nitrogen fertilizer.

Dry beans that are currently used often do not produce productive nodules with wild-type strains of Rhizobium etli or Rhizobium phaseoli. We propose that the reason for this is that during the breeding of the bean varieties the ability to interact with Rhizobium has been altered, and that by using an adaptive evolutionary approach to continually select Rhizobium that can be more efficient at nodulation and nitrogen fixation can be used to overcome this problem.

We have almost completed our proof of principle experiments showing that approach is feasible using a sequence strain of *Rhizobium etli* that no longer interacts with dry bean. We are currently sequencing these strains to determine the nature of the naturally occurring mutations. We are planning on trying some of these strains on micro-plots to determine if the results that were seen in growth chambers is reproducible in the field.

## *PART 3: PROJECT ACTIVITIES AND PRELIMINARY RESULTS*

*Outline project activities, preliminary results, any deviations from the original project and communication activities. You may include graphs/tables/pictures in the Appendix*.

The development of a superior dry bean inoculant was based on 3 broad objectives. These were; 1) Identification and characterization of candidate Rhizobium strains, 2) Adapting Rhizobium to conditions and cultivars, and 3) Field Trial**s.**

Objective 1 we wished to complete our pilot experiment using CFN42 as well as to systematically start assessing other Rhizobium strains that were in our culture collection as well as isolating Rhizobium that are native to Manitoba by using field soil and carrying out nodule trapping experiments. The idea being that native strains would probably be more robust for the climate. To date we have completed our first adaptive evolution set of experiments using lab culture collection strains. We found that carrying out the adaptive evolution protocol is reproducible (carried out 3 times). Table 1 shows the results of the characterization of a single lineage and are arranged such that strains are least evolved at the top of the table and most derived at the bottom (Table 1). To determine how effective each strain was, they were compared with bean plants that were uninoculated but fertilized with ammonium nitrate (Figure 1). We are currently waiting for sequence data to be returned so that the nature of the mutations can be determined.

Objective 2. Within the second objective we had proposed to use flow through gas exchange to monitor nitrogenase activity as a marker for finding strains that can fix nitrogen on the bean root. This should be a more sensitive assay and it should be able to detect the presence of nitrogen fixing nodules more quickly than relying on the colour of the leaves. To date we have bought the gas exchange equipment and are currently setting it up.

Objective 3-Field trials. We have developed protocols that allow us to adhere our derived strains to seeds and have determined how long they can survive on the seed coat. We have conditions that will allow us a 24 hour window, allowing us to coat the bacteria on the seed, and plant them in a small plot the following day. Our data shows that in controlled conditions, the plants derived from these seeds do form robustly crown nodulated bean plants. We anticipate testing these strains in field plots during the summer of 2020.

Setbacks

This project has had a few setbacks. The first, the set up of the fund did not occur until mid-July 2019. The second, personal that were recruited to work on the project chose other options. Originally Gaganpreet Kaur was recruited as a PhD student to work on this project. She was in place and started work prior to awarding of the grant. Her career goals changed when she got married. She opted to complete a MSc and graduated in December 2019. MacLean Kohlmeier had agreed to work on this project as a pdf once he completed his PhD. While writing, MacLean had an opportunity to carry out a pdf at the Centre for Rhizobium Research in Western Australlia. Since MacLean carried out his PhD work in my lab, it was in his best interest to experience research in another lab that aligns well with his own interests. MacLean did work on the project until Jan 31. Going forward, the underspent salary money is being used to hire a research technician until mid July (approx. 6 months). I have recruited a new pdf to start on July 13. He (Justin Hawkins) is currently completing a 2 year pdf in Marburg Germany. He has the expertise and the experience to work with Rhizobium. In addition, I have recruited a new graduate student that will start on this project on May 1, 2020.

## *APPENDIX*

**Table 1.** Symbiotic nitrogen fixation ability of lineage 2 strains.

| Strains | Lineage | ARA (nmol/min/mg) | NFW (mg/plant) | NDW (mg/plant) | SDW (mg/plant) |
|---|---|---|---|---|---|
| **UIC** | 2 | - | - | - | 300±19 |
| **CFN42** | 2 | 39±20 | 17±2 | 4±3 | 333±37 |
| **RE106** | 2 | 112±29 | 637±43 | 91±7 | 724±19 |
| **RE108** | 2 | 208±65 | 235±6 | 27±11 | 500±38 |
| **RE110** | 2 | 317±63 | 277±20 | 41±8 | 400±19 |
| **RE113** | 2 | 251±67 | 266±9 | 29±7 | 467±39 |
| **RE116** | 2 | 416±16 | 278±13 | 55±9 | 700±59 |
| **RE121** | 2 | 288±36 | 379±29 | 60±8 | 833±56 |
| **RE124** | 2 | 325±79 | 277±4 | 28±10 | 767±50 |
| **RE127** | 2 | 172±32 | 248±42 | 21±7 | 767±48 |

ARA, acetylene reduction activity; NFW, Nodule Fresh Weight; NDW, Nodule Dry Weight; SDW, Shoot Dry Weight

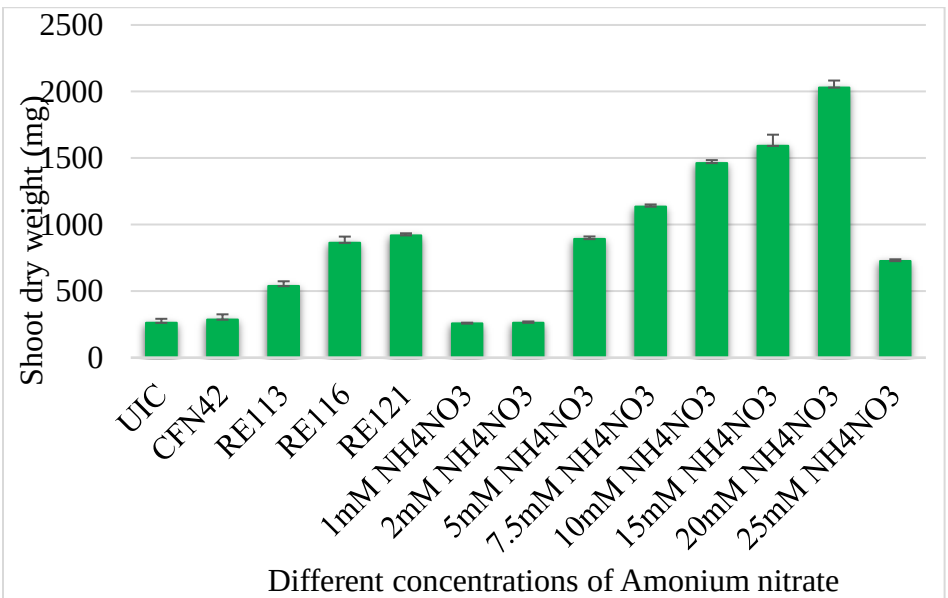


**Figure 1.** Ammonium nitrate experiment using different concentrations of $NH_4NO_3$ along with derived Rhizobium strains, *R. etli* CFN42 and uninoculated (UIC) plants. Error bars represent standard deviation (n=3).