Quantification of *B. japonicum* in Field Soils using a PCR Based Diagnostic Assay and the Relationship of *B. japonicum* to other Rhizobacteria found in the Field.

Year 1 Progress Report Summary

1. A PhD student, Harry Yudistira has been recruited to carry out the work.
2. A short description of the work was submitted to Pulse Beat.
3. The MPGA monies were used as matching monies to get ARDI funding $50,000 over three years. (Summary below)
4. A scientific poster on the work was presented at the annual Canadian Society of Microbiology conference that was held in St. John's Newfoundland. (poster attached)

Work has shown a steady progression. We believe that we have developed a series of quantitative PCR primers that have the ability to detect *B. japonicum* in field soils. We have demonstrated that field soils spiked with known quantities of *B. japonicum* can be accurately detected. In addition, we analyzed all soils that were provided to us by Bruce Brolley. The results suggest that a population of *B. japonicum* is not becoming established in Manitoba (see poster). An alternate explanation for the data is that; due to how the soil was sampled, the volume of soil may dilute localized populations of *B.japonicum*. To address this possibility, we have received soil samples from Dennis Lange to determine how to accurately sample. The samples have were collected at one inch depths (0-1, 1-2, 2-3, 3-4) and at varying distances from the row. It is hoped that this data will allow us to accurately determine how to sample Soybean fields to determine populations of *B. japonicum*. We hope to be able to submit a manuscript describing the assay as well the findings from our sampling within this next calander year.

ARDI Grant Summary

Legumes, including soybeans, are unique crop plants because they can derive nitrogen from a symbiotic association that occurs with the various species of Rhizobium. The end result of this association is a plant-derived structure termed the root nodule. A soybean plant that is effectively nodulated can have its entire nitrogen needs supplied through this symbiosis. A problem that is often encountered is having sufficient numbers of the correct Rhizobia species present as the seed is germinating so that effective nodules can develop in a timely manner. This is usually circumvented by the application of inocula of Rhizobia that is supplied by seed companies and applied either directly to the seed, or to the field at the time of planting. However it has been often reported that the introduced bacteria are often out competed by related bacteria that are less efficient at nitrogen fixation.

Bacteriocins are defined as narrow spectrum antibiotics that are produced by many bacteria that affect only closely related species including *B. japonicum*. *The objectives of this project are to* 1) isolate, characterize, and determine if *B. japonicum* bacteriocin producing strains can be used to produce superior inoculum strains. 2) Determine what effect bacteriocins have on the microbial community structure of a soybean field.

AE78

Real-Time PCR Based Assay for the Quantitation of *Bradyrhizobium japonicum* in Field Soils

Harry Yudistira, Barney A. Geddes, Amanat Ali* and Ivan J. Oresnik

Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

*National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan

UNIVERSITY OF MANITOBA

INTRODUCTION

Soybean is an agronomically important crop. It was first introduced into Manitoba in the early 1990's. In 2009, approximately 425,000 acres of fields were seeded with soybean with an estimated value of almost $120 million. Soybean is a legume which forms a symbiotic relationship with the bacterium *Bradyrhizobium japonicum*. This symbiosis manifests itself as nodules formed on the roots of the soybean plants. A well nodulated plant can derive all the nitrogen necessary for growth from the bacteria. A problem that is often encountered is having sufficient numbers of the correct Rhizobia species present as the seed is germinating so that effective nodules can develop in timely manner (4). This is usually circumvented by the application of inocula of Rhizobia either directly to the seed, or to the field at the time of planting (2). Currently, the method for determining the amount of *B. japonicum* in the soil is dependant on culture methods that involve a "most probable number" assay that can take up to 3 weeks to yield usable results. This assay is time consuming and lacks the ability to distinguish between the strains of bacteria (3). The objective of this research is to develop a culture independent quantitative polymerase chain reaction (qPCR) based assay to quantify *B. japonicum* in field soils. In order to achieve this, primer pairs have been designed for use with qPCR. 16S rDNA and *B. japonicum* specific genes (1) have been used as the template for creating specific primers for identification in field soils. PCR was utilized to determine the ability of these primers to specifically amplify the target genes from *B. japonicum* and not from the other Rhizobia genus. In addition, DNA samples were extracted from the field soils. qPCR was then performed with these primer pairs to confirm their ability to distinguish between *B. japonicum* and other soil bacteria.

bll5079 and *bsl6289* can be used to specifically detect *B. japonicum*

We had previously designed a primer pair (J16S) that appeared to be specific for *B. japonicum*. Although this primer pair seemed specific using lab strains, we questioned their specificity using field soil samples. More recently, a comparative genomic analysis identified a number of genes that appeared to be specific to *B. japonicum* (1). Utillizing this analysis, we designed primer pairs to 2 of these genes; *bll5079* and *bsl6289*. The data shows that these primers are specific for *B. japonicum* and can discriminate between *Bradyrhizobium* species

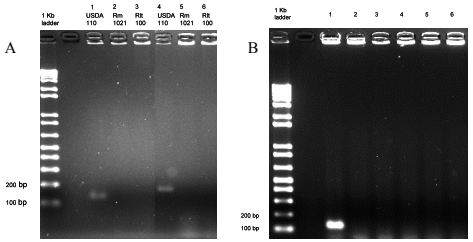

Figure 1. *bll5079* and *bsl6289* primers are specific for *B. japonicum*.

A. PCR using the *bll5079* primers (lane 1-3) or the *bsl6289* primers (lane 4-6). Templates used are; lanes 1 and 4 *B. japonicum* USDA 110; lanes 2 and 5, *Sinorhizobium meliloti* (Rm 1021); lane 3 and 6 *Rhizobium leguminosarum bv trifolii* (Rlt 100).

B. PCR using *bll5079* primers. Templates used were: *Bradyrhizobium sp.* TAL-102, SR-3, SR-5, SR-6, SR-8, and SR-9 (lane 1-6 respectively)

Quantitation and recovery of *B. japonicum* from field soils

To test our ability to quantitate *B. japonicum* in field soil, a sample of soil was spiked with a known quantity of *B. japonicum*. The data clearly shows that following extraction we were able to account for 75 ± 25% (n=3) of the added *B. japonicum* cells. We estimate that we can detect between 50-100 *B. japonicum* cells/assay.

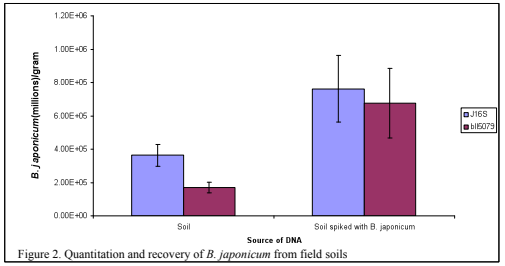

Figure 2. Quantitation and recovery of *B. japonicum* from field soils

Primer pairs have different efficiencies

The ultimate goal of this work is to define an assay that can be used to quantitate *B. japonicum* in field soils. To test our primers, 24 soils from Southern Manitoba with different cropping histories were tested (Table 1).

Initial trials were carried out to determine which primer pair would give the greatest specificity. The data show that in every case the bsl6289 primer pair appeared to be most specific (Figure 3).

Table 1. List of the soil samples used in DNA extraction

Soil	Number of years planted with soybean
Schmidt virgin	0
Rob Park	0
SW 15-3-3-W virgin	0
NE 13-2-2W virgin	0
RRR virgin	0
SW 35-1-2 W virgin	0
SW 36-2-2 W virgin	0
UM virgin	0
Schmidt 1	1
NW 36-2-2-E	1
C 1	1
Roscos 1	1
Rutherford 1	1
Byron 2	2
SW 19-1-2 E 2	2
Roscos 2	2
Rutherford 2	2
MMM 3	3
SE 7-2-2-E	3
BBBCCC 3	3
Rutherford 3	3
St. Norbert 4	4
NE 5-2-2 E 4	4
SSS6	6

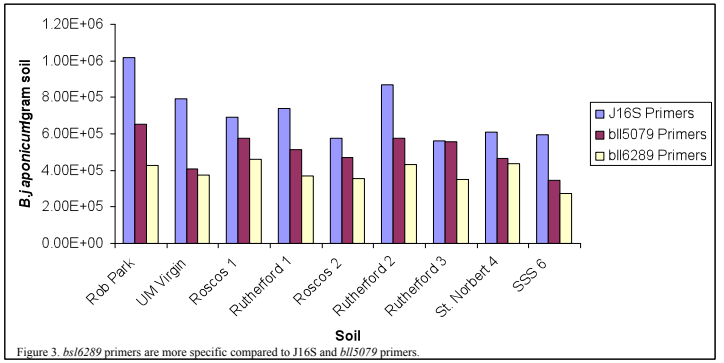

Figure 3. *bsl6289* primers are more specific compared to J16S and *bll5079* primers.

B. japonicum populations have not become endemic in Southern Manitoba field soils

Soybean has been grown in Southern Manitoba for about 20 years. Utilizing soils collected by Manitoba Agriculture that had known cropping histories (Table 1), the soil DNA was extracted and assayed using our designed primers. The data show that there does not appear to be a correlation between the number of *B. japonicum* determined to be present within the soil and the number of years soybean has been grown in each particular location (Figure 4).

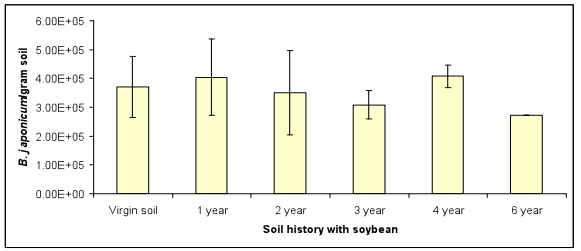

Figure 4. Number of *B. japonicum* in field soil compared to the number of years soybean was grown at each location

ACKNOWLEDGEMENTS

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