



**Project 1:** *Optimizing Parameters for Efficacy of Biological Control Agents of FHB.*

**1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?**

- a) **Problem:** The optimization of a broth growth medium fostering dense growth of the *Bacillus* strains we use as biological control agents (BCAs) is very important to successful spray application of the BCAs. High cell density, little or no clumping or flocking of cells, and detectable high levels of lipopeptide biosurfactants are desired, to facilitate spray application of the BCAs onto grain heads. We continue to examine the use of lipid amendments (plant oils) in growth media to promote these properties in BCA broth cultures.

--**Resolution:** We are continuing to optimize composition of broth formulations that encourage the above qualities. Also, physical parameters (including shaking speed and temperature) of broth incubation are being examined to find optimal parameters. In 2010 we found that the BCAs grow better at higher rpm (> 100 rpm versus 70 rpm). At 100 rpm, the BCAs formed a more uniform suspension of cells.

- b) **Problem:** Availability of a rapid estimate of production of lipopeptide biosurfactants by the BCAs is important to our broth formulation studies. These lipopeptides are thought to be a major mechanism whereby 1BA and several other *Bacillus spp.* used as BCAs inhibit growth of *F. graminearum*, reduce FHB, and/or reduce DON levels. We hypothesize that the more biosurfactant is produced in the culture broth, the more effective the BCAs will be in deterring FHB and/or reducing DON levels after spraying BCAs onto grain heads.

**Resolution:** A simple turbidometric analysis was used to determine lipopeptide production by our BCAs. After seven days of incubation, the cultures showed lipopeptide production in this assay. Differences were observed in the lipopeptide production among the BCA strains; with 1D3 being the best producer of lipopeptides. It was observed that shaking and oil amendment had a role in biosurfactant production. The lipopeptide production in shaken cultures was many times more than static cultures. Oil amendment increased biosurfactant production; with differences observed among the strains for lipopeptide production. Additionally an oil spreading assay and emulsification assay were performed to determine lipopeptide production quantitatively. Different oils were used in the oil spreading assay to find the differences among the strains. Differences in pellicle formation and pigmentation were observed among the strains, when grown in static and shaking conditions. A qualitative droplet collapse assay demonstrated there are differences in biosurfactant production between the four BCA strains we have used; and that the lipid-amended broth results in increased amounts of biosurfactant production by the BCAs.

- c) **Problem:** A major part of the project was to quantify numbers of bacterial biocontrol agents (BCAs) (for our project, selected *Bacillus spp.*) after they are sprayed onto heads of wheat and barley. With our current use of a much better growth medium than we previously used to culture the BCAs, we need more BCA population data to better understand how BCAs including strains 1BA and 1D3 behave in the field after spray application.

**Resolution:** For the recovery of BCAs from wheat heads, the salt and temperature conditions of plating media were optimized. It was observed that the organisms could withstand 9.0 % NaCl and 47° C. The growth of the BCAs started to decline at elevated temperatures and salt concentrations (>9.0 % NaCl and > 47° C).

Our hypothesis was that the population counts of *Bacillus* strains (1BA, 1D3 and 1BAC) fluctuate over time on the sprayed wheat heads (Feekes stage 10.51). After application of BCAs on the wheat heads, sampling was done every three days for 24 days. In the 2010 biocontrol trials conducted at Brookings, SD the treatments with strains 1BA and 1D3 used the most probable number (MPN) method employing high temperature and high salt selection in the MPN growth media, while treatments with the antibiotic-resistant mutant 1BAC used rifampicin in growth media to track mutant numbers. The control plots that did not receive spray application of BCAs had very low bacterial numbers, indicating that a small number of native bacteria can tolerate the high salt and temperature conditions and/or the rifampicin antibiotic. The plots inoculated with BCAs had detectable numbers of BCAs, with highest counts being about  $1.5 \times 10^4$  CFU/g fresh weight plant mass. The population counts of BCAs on wheat heads fluctuated between the sampling days and treatments. In most of the treatments at Brookings, the vegetative cell count of BCAs fluctuated between the sampling days of different treatments, and over time in the same treatment. In the heat pasteurized MPN assay of most treatments, the endospore counts did not increase appreciably till sampling day 21. The treatment 1BA with plant oil + Chelated Mn + Induce NIS showed higher population counts in comparison to other treatments. It was clear that the BCA *Bacillus* strains that were sprayed onto heads were able to colonize and sustain detectable populations, and were not washed entirely off plant surfaces despite the excessive rainfall amounts in summer of 2010.

- d) **Problem:** There is a need for more evidence of lipopeptide genes on treated grain heads using PCR.  
**Resolution:** Different methods of DNA extractions were performed to determine the optimized protocol for extracting DNA from wheat heads. The slight modified version of the traditional organic extraction method worked best for DNA extraction. Optimization of PCR to detect lipopeptide genes on grain heads is being done.
- e) **Problem:** There is a continuing need to screen for the efficacy of our BCAs acting alone or in concert with fungicides, to control FHB and/or reduce DON levels in field plot trials.  
**Resolution:** We will conduct further field plot trials, in South Dakota and elsewhere including Langdon, ND with Scott Halley when the opportunity arises.
- f) **Problem:** A commercial formulation of one or more of our BCAs is not presently available.  
**Resolution:** We are working with the SDSU Foundation/Technology Transfer Office to make a commercial BCA product available.

**2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):**

**a) Accomplishment:**

In 2010, Briggs hard red spring wheat was planted at Brookings, SD. Trial treatments included an untreated check; the fungicide premix Prosaro; *Bacillus* strain 1BA and its mutant 1BAC cultured in different broth formulations; *Bacillus* strain 1D3 cultured in different broth formulations; a combination of *Bacillus* strain 1BA and *Bacillus* strain 1D3; and combinations of Prosaro with one or more of the *Bacillus* BCAs. Chelated manganese was added to the spray mix for some treatments. All treatments were applied at anthesis, and included Induce NIS. Plots were treated with pathogen by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field, and applying overhead mist irrigation each day for 10 days following anthesis. Following the treatments, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON). Grain yield was less than some years, probably due in large part to the excessive rainfall. Statistically significant reduction in FDK was observed for the application of 1BA, 1D3, chelated manganese, and Prosaro. Test weight was significantly greater for this treatment, too; as it was for treating with 1BA, 1D3, Prosaro, and Induce NIS; and for treating with 1D3, Prosaro, and Induce NIS. This contrasted with results from 2009 field plots where FHB incidence, FHB index, yield, and FDK were all significant for at least some of the BCA treatments. Many of these significant treatment differences in 2009 were in treatments that omitted Induce NIS. We hypothesize that inclusion of Induce NIS may not be beneficial as part of some of these BCA treatments, and want to test this hypothesis in future trials. Results from some of our BCA treatments at Langdon, ND in summer 2010 further suggest that Induce NIS may not help in promoting efficacy of some of these BCA formulations.

**Impact:** The trials demonstrated that *Bacillus* strains 1BA and 1D3 when co-applied with Prosaro can sometimes reduce DON levels in grain more than can application of Prosaro and Induce alone.

**Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.**

Murthy, N. S., and B. H. Bleakley. 2010. Populations of *Bacillus* strains applied to wheat heads for biological control of FHB: Results of Brookings, SD 2010 field plots. Abstract, pg. 91. In S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky.

Ruden, K. R., L. E. Osborne, N. Srinivasa Murthy, and B. H. Bleakley. 2010. 2010 Trial for the performance of biological control agents for the suppression of Fusarium Head Blight in South Dakota. Abstract, pg. 94. In S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky.