

# Characterization of Microorganisms from Alfalfa Seeds Inoculants

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## Abstract

Atmospheric nitrogen fixation can be accomplished by free living microorganisms or by symbiosis between legume plants and rhizobia. The symbiosis between the alfalfa plant and *Sinorhizobium meliloti* is one example of the symbiotic relationship between legume plants and rhizobia. During the symbiosis, the rhizobia fix nitrogen for the plant and the plant in return provides carbohydrates for the bacteria. In order to maximize the plant's yield in agriculture, recent technologies used include seed inoculants. Seed inoculation methods rely on coating the seeds with plant-growth promoting bacteria. Besides nitrogen-fixing bacteria, the seeds may also be coated with microorganisms aiding in mineral solubilization, biological control or nutritional requirements.

The goal of this study was to identify the different bacteria present in coated alfalfa seeds and their impact on alfalfa plant yield. Bacteria were isolated from the coated seeds and purified. Several morphologically different bacteria were isolated from the coated seeds. Their 16S rRNA genes were amplified and sequenced. Several *Bacillus* spp. were identified. Surprisingly, no rhizobial strains were found among the isolated strains. Nevertheless, we performed a plant inoculation assay with the coated seeds and nodules were observed, suggesting the presence of rhizobia in the inoculants. We also screened for the presence of nitrogen fixation (*nifH*) genes in the bacterial isolates. The results indicated that two strains contained the *nifH* genes. Another plant inoculation assay with uncoated alfalfa seeds is under investigation in order to evaluate the impact of each isolated strain on alfalfa growth.

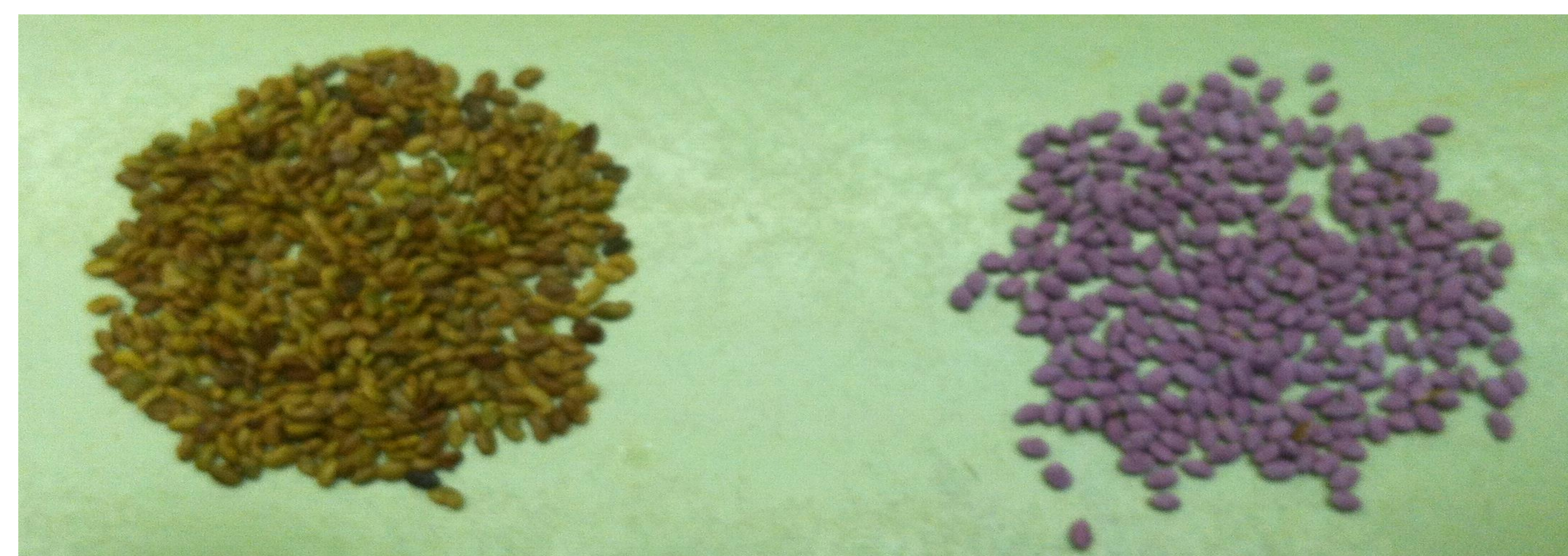
## Objectives

- Identification of the different bacteria strains present in the coated seeds
- Investigate the impact of each individual strain on alfalfa plants, particularly nitrogen fixation

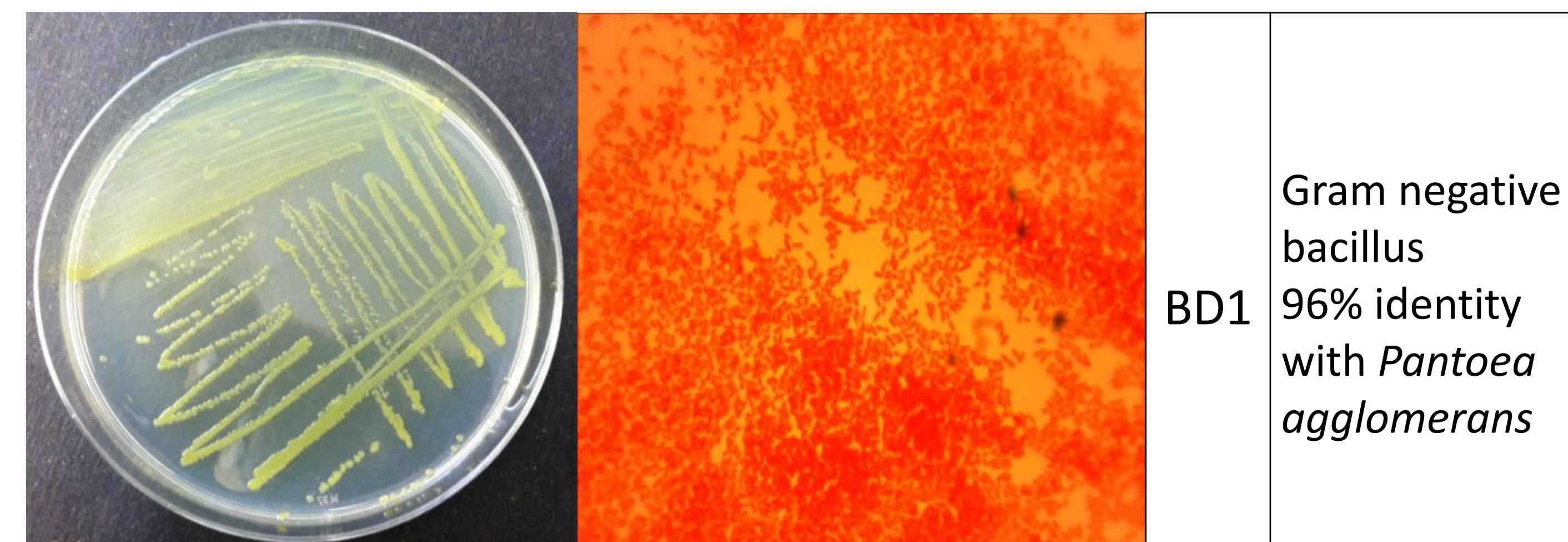
## Materials

Uncoated alfalfa seeds

Coated alfalfa seeds

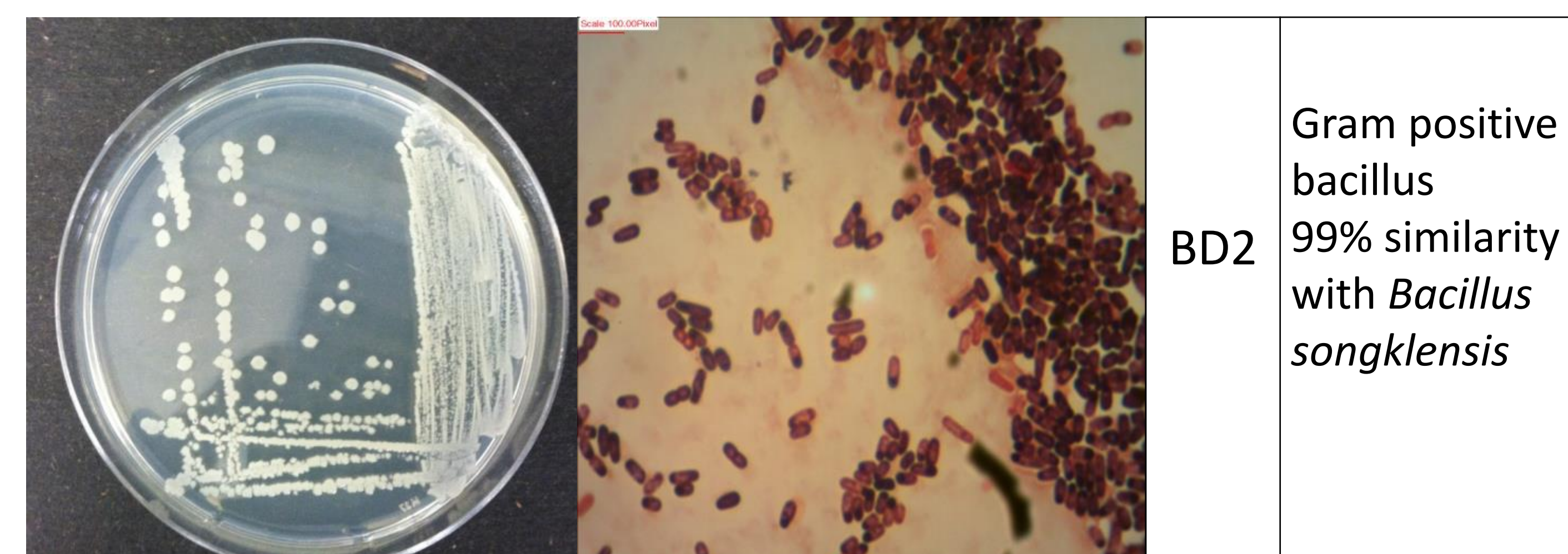


## Results



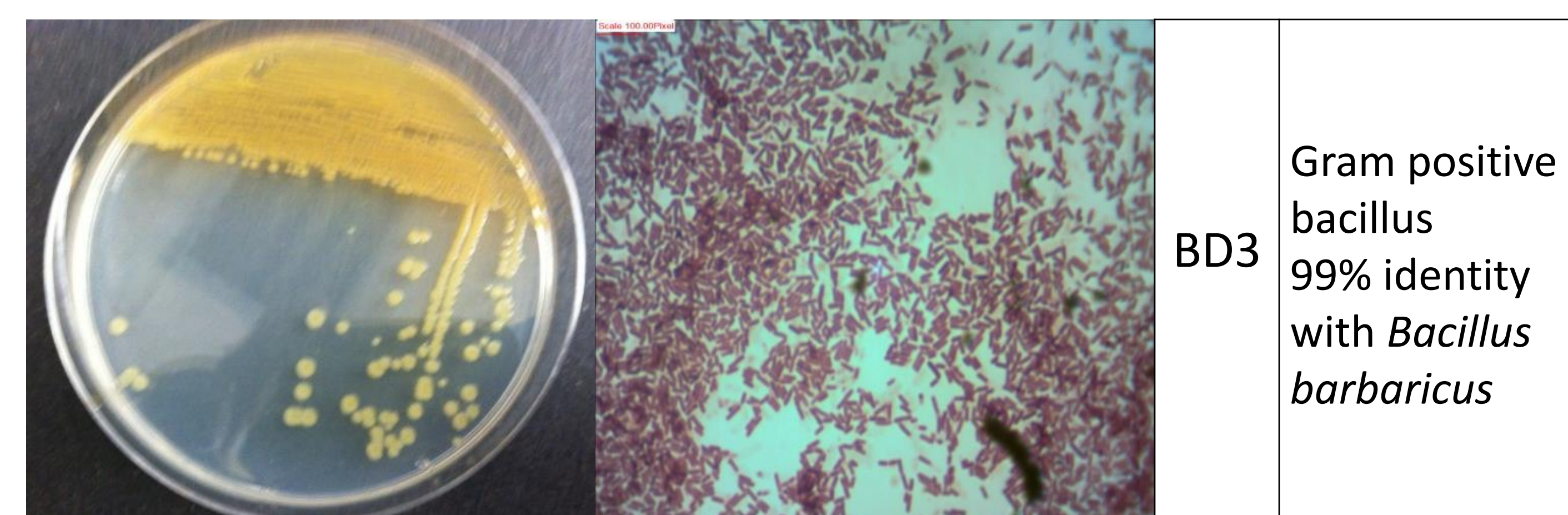
BD1

Gram negative bacillus  
96% identity with *Pantoea agglomerans*



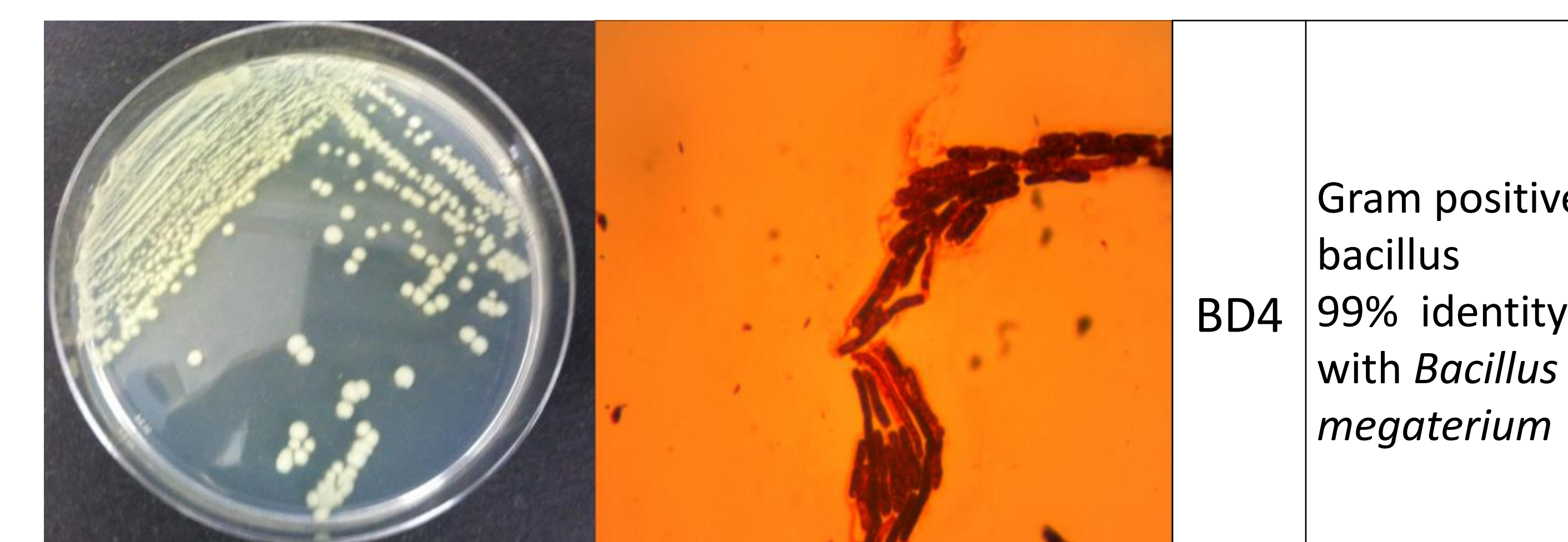
BD2

Gram positive bacillus  
99% similarity with *Bacillus songklensis*



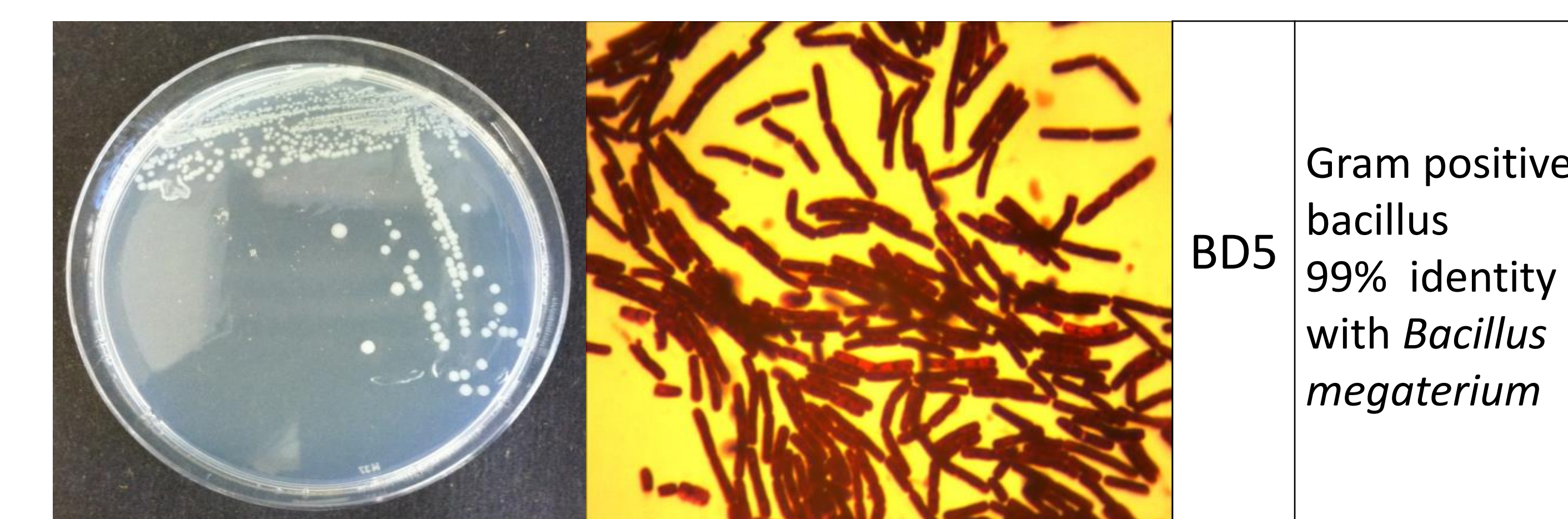
BD3

Gram positive bacillus  
99% identity with *Bacillus barbaricus*



BD4

Gram positive bacillus  
99% identity with *Bacillus megaterium*



BD5

Gram positive bacillus  
99% identity with *Bacillus megaterium*

## Results

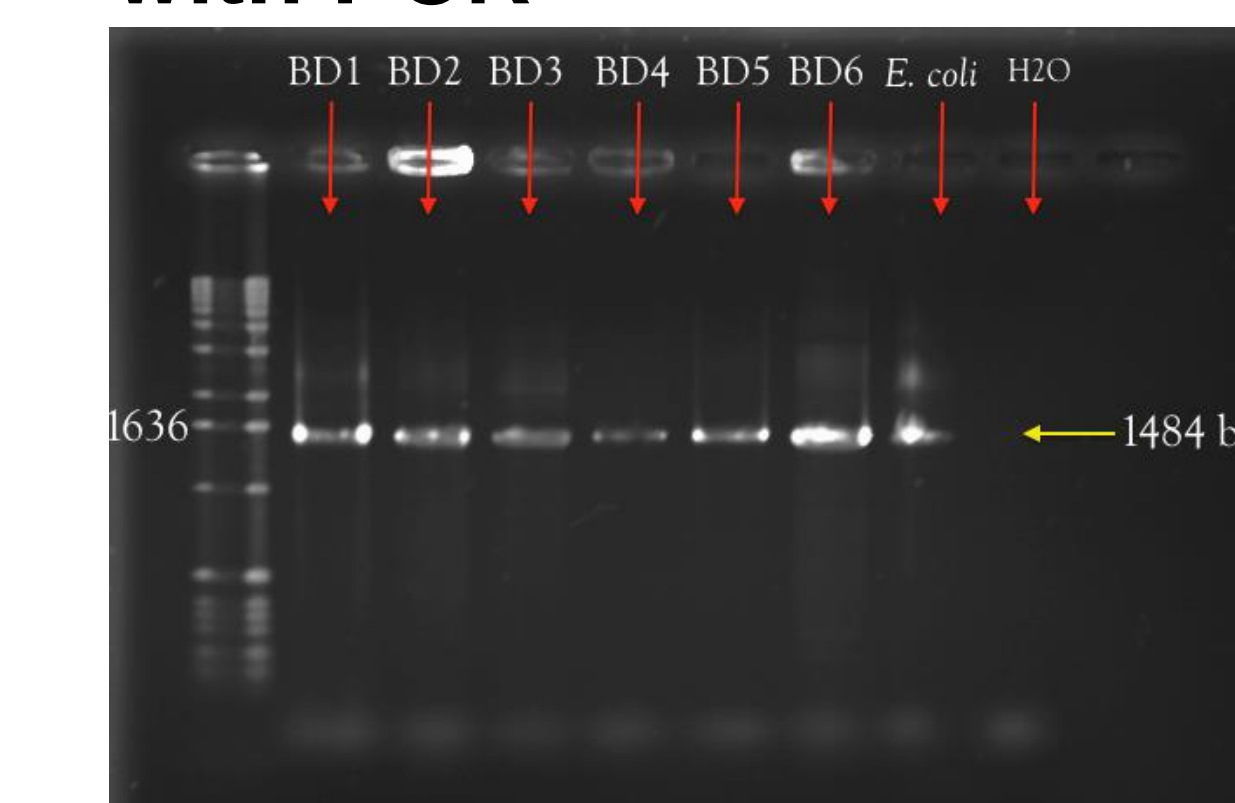
- Coated alfalfa seeds were germinated and screened for the presence of nodules
- The presence of *nif* genes was evaluated with PCR and *nifH* specific primers

Alfalfa plant

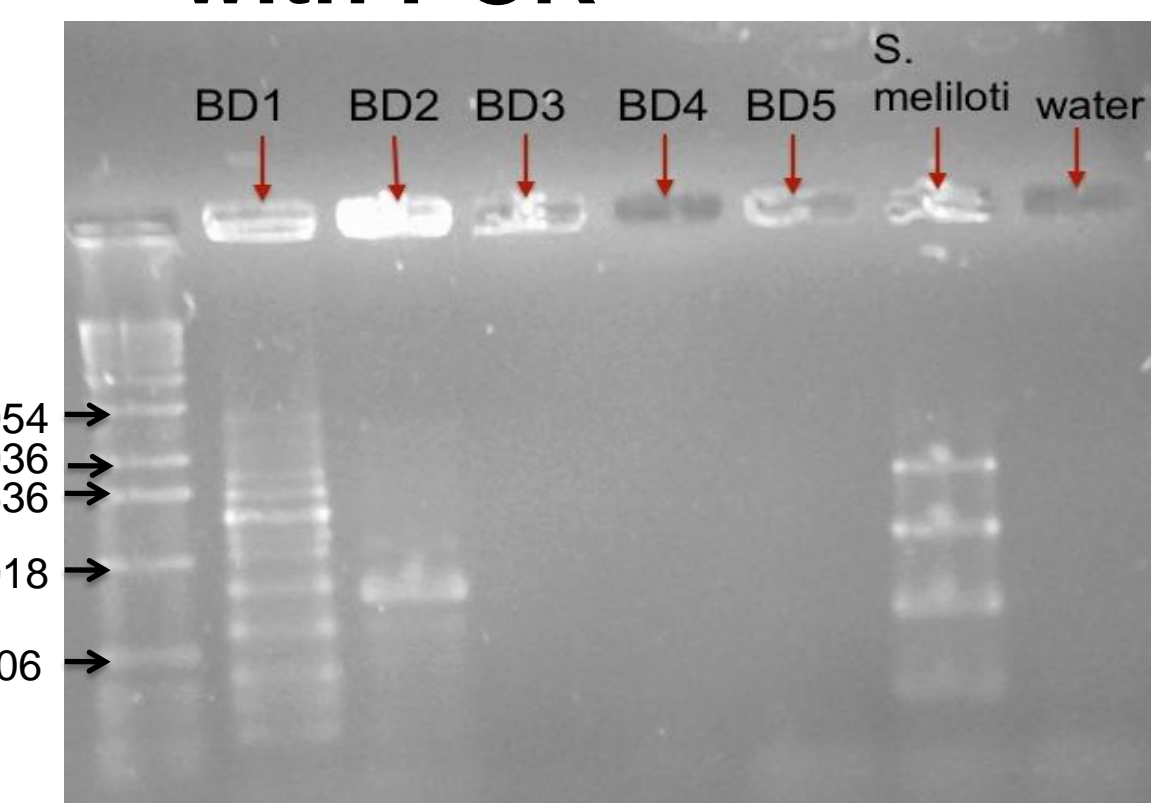
Nodule



### 16S rRNA genes amplified with PCR



### *nifH* gene amplified with PCR



## Conclusion

- Interestingly, *Sinorhizobium meliloti* was not among the isolated strains. However, the plant inoculation assay with coated alfalfa seeds indicated 6 nodulated plants out of 20 plants. This suggests a low abundance of *S. meliloti* in the coated seeds.

- Among the strains isolated, only BD1 and BD2 contain the *nifH* gene

## Acknowledgments

We would like to thank the Department of Biological Sciences at Western Michigan University for funding, and the Michigan ASM Branch and the Annual Research and Creative Activities Poster Day for providing the opportunity to present this poster.

## References

- (1) Bashan (1998) *Biotechnology Advances*. 16:729-770
- (2) Chowdhury et al. (2009) *European Journal of Soil Biology* 45:114-122
- (3) Comacho et al. (2001) *Canadian Journal of Microbiology* 47:1058-1062
- (4) Ding et al. (2005) *Journal of Applied Microbiology* 99:1271- 9281
- (5) Fang et al. (2005) *Journal of Applied Microbiology* 100:938-945