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  

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>BD1</td>
<td>Gram negative bacillus 96% identity with Pantoea agglomerans</td>
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<tr>
<td>BD2</td>
<td>Gram positive bacillus 99% similarity with Bacillus songenensis</td>
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<tr>
<td>BD3</td>
<td>Gram positive bacillus 99% identity with Bacillus barbaricus</td>
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<tr>
<td>BD4</td>
<td>Gram positive bacillus 99% identity with Bacillus megaterium</td>
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<tr>
<td>BD5</td>
<td>Gram positive bacillus 99% identity with Bacillus megaterium</td>
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</tbody>
</table>

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

References  
(1) Bashan (1998) Biotechnology Advances. 16:729-770  
(5) Fang et al. (2005) Journal of Applied Microbiology 100:938-945