

Christopher Spencer, Biochemistry

University: Northwest Missouri State University
Year in School: Sophomore
Hometown: Rogersville, Missouri
Faculty Mentor: Steve Witzig and Dr. Joe Polacco, Biochemistry
Funding Source: Missouri Academy

Removing pink pigmented facultative methylotrophs (PPFMs) from *Arabidopsis thaliana* seeds

Christopher Spencer, Alejandro Pineda, Steve Witzig, and Joe Polacco

Arabidopsis thaliana is an important model plant. When grown under axenic conditions, standard practice is to surface sterilize the seeds before plating. This is effective at removing surface-dwelling organisms; however, it does not rid the seed of microorganisms residing within the seed, i.e. under the seed coat. *Methylobacterium sp.* is one such microorganism. Several protocols for sterilization of seeds were used to attempt to eliminate the bacteria located within the seeds: ethanol and bleach, bleach alone, and chlorine gas. We also performed dry heat treatments at 50, 65, 85, and 100 degrees C ranging from 1 day to 4 weeks in some treatments. Some untraditional methods were used as well, such as microwave and autoclave treatments. The ethanol/bleach and bleach treatments both were effective at removing the surface contaminants and treated seed had near 100% germination rates. The chlorine gas treatments also showed no contaminants and near 100% germination. All of the seeds from the dry heat treatments showed contamination. Prolonged heating at 65° C for 3 weeks, lowered the germination to about 75% on surface sterilized seeds. Data from the microwave treatments are still being gathered. The autoclave treatment showed no germination except for one plant at the one-minute interval. That plant is now growing in soil. A goal of the autoclave treatment was to determine if submerging the seeds in water affects germination rates. The data from that experiment are still being gathered. Results gathered to date show that the chlorine gas treatments as well as other surface sterilization techniques produce the highest germination rates without seedling contamination. Experiments to select for bacteria within the seed are in progress. Briefly, axenic plants have been ground to select for *Methylobacterium* in the macerate that have eluded the sterilization treatments.