Online Proceedings

POSTER SESSION 1

MGH 241, Easel 136

11:00 AM to 1:00 PM

ComGen Authentic Undergraduate Research Project

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In this study, we focused on identifying specific genes that make *Pseudomonas fluorescens* L5.1-96 a potential biocontrol agent to combat wheat take-all disease. This strain is a supercolonizer meaning it suppresses fungal pathogens in the soil and colonizes the rhizosphere of plants. This strain has not yet been fully sequenced; therefore, analyzing genomic data will contribute to its classification. Our first step was increasing the amount of L5.1-96 DNA through culturing *E. coli* that contained an insert of L5.1-96 DNA. Next we extracted L5.1-96 DNA using the Qiagen miniprep kit, amplified the DNA insert using linear sequencing, and sent our cleaned DNA samples to Bellevue College for Sanger sequencing. We ran bioinformatics software (NCBI BLAST) to compare our DNA insert with previously sequenced genomic data. We found our DNA sample resulted in a 98% match with genes from *Pseudomonasbrassicacearum* strain BS3663; therefore this indicates a phylogenetic relationship between the two *Pseudomonas* species. While we did not discover any new genes or novel proteins, our sample coded for tRNA preQ1(34)S-adenosylmethionine ribosyltransferase-isomerase QueA which is a nutrient protein found in both eukaryotes and bacteria. We have contributed to the genomic analysis of strain L5.1-96 and with this information it is possible one day all of the data together will lead researchers to fully evaluate strain L5.1-96 and its ability to suppress wheat take-all disease.

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Gene Sequencing of *Pseudomonas fluorescens* L5. 1-96 to Discover Genes Associated with Wheat Take All Disease

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Mentor: Elysia Mbuja, Biology, Pierce College

Pseudomonas fluorescens is a bacterium recognized for producing an antifungal against the debilitating wheat take-all disease. In this study I am investigating the *Pseudomonas fluorescens* L5.1-96 strain to identify the genes making it a supercolonizer of take-all disease resistant soil and how it fits phylogenetically with other bacteria. This project will contribute to the understanding of how biological agents may be used to combat wheat take-all disease. To investigate this, a plasmid of DNA from strain L5.1-96 was inserted into *Escherichia coli* to make a bacterial library. I separated the plasmid from the *E. coli* and amplified it by linear sequencing then sent the DNA to Bellevue College for Sanger sequencing. I used bioinformatics to analyze my unique insert which I found did not match unknown genes, rather it coded for proteins regarding nitrogen fixation. The sequences matched those of *Pseudomonas brassicacearum*. This provides evidence to renaming the bacterial strain. A match to an anaerobic enzyme in nitrogen fixation might provide insight to its supercolonizer abilities. As we are sequencing a minor portion of the strain L5.1-96 genome, there is need for further gene sequencing in order to complete its genome.

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Genetic Sequencing of *Pseudomonas fluorescens* L5.1-96

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Wheat Take-All Disease (TAD) is caused by the effect of the soil-based fungus *Gaeumannomyces graminis var. tritici* on wheat crops. We as Pierce College Biology students in conjunction with other local institutions sequenced part of a strain of *Pseudomonas fluorescens*, L5.1-96 (P.f. L5.1-96), because of its known trait to supercolonize soil. Sequencing was initially achieved at Pierce College by linear sequencing reaction before being completed by Sanger Sequencing

at Washington State University. Resulting data was then interpreted using FinchTV, and the NIH BLAST and tBLAST programs. P.f. L5.1-96 produces 2,4-diacetylphloroglucinol (DAPG) which is a key component in Take-All Decline, the natural suppression of the fungus after several generations, in soils. Using *Escherichia coli* bacteria to clone 1.7kb long plasmids of the P.f. L5.1-96 strain, the plasmids were amplified and purified in order to discover genes relevant to the empirical objective to better understand this bacteria. Our DNA insert, the portion of data analyzed from FinchTV by cross reference with BLAST, aligned with several important enzymes related to antibiotic resistance and toxin metabolism, both of which could be key to supercolonization of soil. In addition, a phylogenetic relationship has been established using BLAST between *P. brassicacearum*, *P. chlororaphis*, and our strain of *Pseudomonas fluorescens* L5.1-96. The findings of the research could provide greater insight into what constitutes an effective biological control agent against TAD in Washington, and possibly other affected areas of the world.

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Identifying the Supercolonizer Ability and Genetic Links of *Pseudomonas fluorescens* Strain L5.1-96 to Other *Pseudomonas* Species

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Vince Le Bleu, Sophomore, Chemistry, Biology, Pierce College
Mentor: Elysia Mbuja, Biology, Pierce College

Pseudomonas fluorescens strain L5.1-96 has been known to help combat the wheat take-all fungal pathogen, *Gaeumannomyces graminis var. tritici* (Ggt). Strain L5.1-96 is maintained in the soil longer than others, so it is known as a supercolonizer, which contributes to the phenomenon referred to as Take-All Decline (TAD). The strain L5.1-96 is a non-pathogenic bacterium which grows in the rhizosphere of plants and produces an antifungal agent known as 2.4-diacetylphloroglucinol (DAPG) causing TAD. The DNA fragment we amplified, sequenced, and analyzed was a perfect match to *P. brassicacearum* strain BS3663. Through bioinformatics, we also found our fragment matched the genetic code for pyridoxal 5'-phosphate (PLP)-dependent aminotransferase, a co-enzyme which catalyzes the transamination of amino acids to alpha-keto acids. We did not identify new proteins that are responsible for the advantages that this bacterium holds against wheat take-all; although, we did find the best phylogenetic fit for this strain. These findings contribute information to the broader study at Washington State University to investigate whether strain L5.1-96 can be used as a biocontrol agent against wheat take-all.???