**Comparative genomics of clinical isolates of *Pseudomonas fluorescens,* including the discovery of a novel disease-associated subclade.**

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Microbiology and Immunology)
in The University of Michigan
2015

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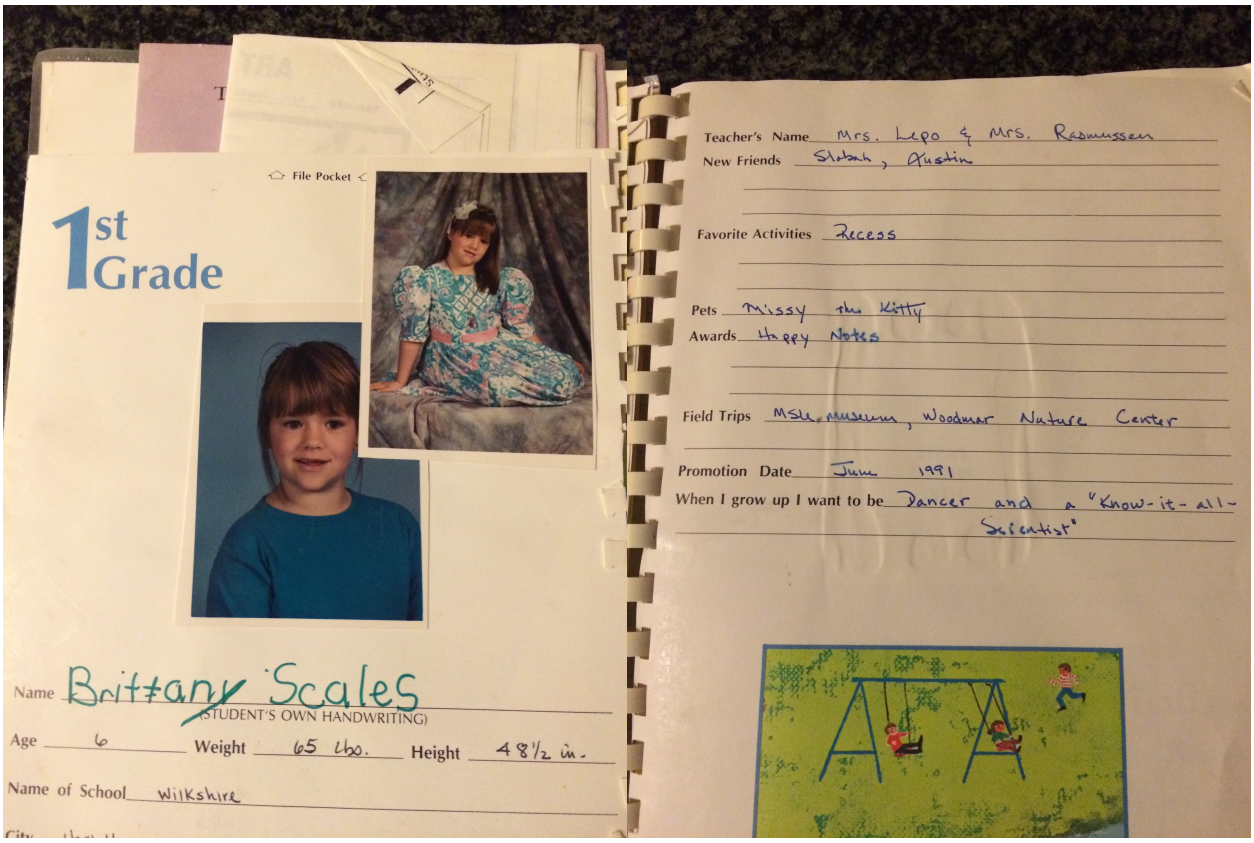
## DEDICATION

Twenty- five years ago a little girl told her mother that when she grew up she wanted to be a ‘Know-it-all-Scientist’. This dissertation is dedicated to the single mother who didn’t tell her little girl that such a goal was impossible, but instead, did everything in her power to make it happen.



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## ABSTRACT

Comparative genomics of clinical isolates of *Pseudomonas fluorescens,* including the discovery of a novel disease-associated subclade.

by

Brittan Starr Scales

Chair: Gary Huffnagle

Abstract title: Comparative genomics of clinical isolates of *Pseudomonas fluorescens,* including the discovery of a novel disease-associated subclade.

Taxonomically, there are over 52 *Pseudomonas* species that group within the *Pseudomonas fluorescens* species-complex. They are gram-negative bacteria (gammaproteobacteria) that have the ability to flourish in many different environmental niches due their metabolic adaptability. Prior to beginning the project described in this dissertation, we had identified (using culture-independent analysis) that the lung microbiome of patients with severe chronic obstructive pulmonary disease (COPD) contained abundant levels of *P. fluorescens*. This finding was highly unexpected because *P. fluorescens* colonization had not been previously reported in chronic respiratory disease (human or veterinary). Furthermore, there was no taxonomic or genome information on clinical *P. fluorescens* strains in the published literature or NCBI database. This raised the question of whether there were differences between *P. fluorescens* strains isolated from clinical vs. environmental sources. To begin to address this question, I sequenced a collection of *Pseudomonas spp.* isolates from individuals with chronic lung disease, which included 22 clinical *P. fluorescens* strains. Twelve of the 22 could be grouped within previously defined subclades I, II and III of the *P. fluorescens* species-complex. However, the other 10 strains were distinct and could be clustered as a phylogenetically unique fourth subclade, which lacked any representative genomes (clinical or environmental) in the

NCBI database. I performed additional comparative genomic analyses on all four subclades in order to identify genomic attributes that were associated with growth in humans (vs. the environment). Subclade III clinical isolates were almost indistinguishable from environmental isolates; however, the clinical isolates contained additional homologues of genes involved in metal toxicity resistance, an attribute that has been reported for chronic antibiotic exposure, such as would occur in the cystic fibrosis lung. In sharp contrast, subclade IV strains displayed marked reductions in genome size, gene diversity and GC content, as well as containing all of the elements for a Ysc family type III secretion system that was extremely similar to that found in *P. aeruginosa.* Furthermore, our subclade IV strains, collected from geographic locations across the U.S., had a very high level of shared nucleotide identity and a small accessory genome, suggesting that they may have evolved from living in multi-trophic environments to a life in a much narrower niche, perhaps human airways. We also identified that some colonies of inbred mice contained indigenous *P. fluorescens* in their lung microbiome (subclade III) and chronic inflammation was associated with an outgrowth of *P. fluorescens*. Expanding my analysis to human respiratory disease, I found an increase in *P. fluorescens* (largely subclade III) in lung samples from multiple diseases (COPD, IPF and lung transplant) but not healthy subjects. Altogether, these studies reveal that *P. fluorescens* is an under-appreciated colonizer of humans, particularly in the context of pulmonary inflammation, and lay the groundwork for future studies delineating the contribution and molecular mechanisms of this host-microbe interaction.