OMB Number: 4040-0001
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APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED | **Applicant Identifier**

4. a. Federal Identifier: AI104172

b. Agency Routing Identifier:

5. APPLICANT INFORMATION

*** Organizational DUNS:** 063690705

* Legal Name: University of Alabama at Birmingham
Department: Office of Sponsored Programs
Division:
* Street1: 1720 2nd Avenue South
Street2: AB 1170
* City: Birmingham
County / Parish: Jefferson
* State: AL: Alabama
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 352940111

Person to be contacted on matters involving this application
Prefix: Ms.
* First Name: Ellen
Middle Name: C.
* Last Name: Johnson
Suffix:
* Phone Number: (205) 975-8580
Fax Number: (205) 975-5977
Email: ecjohn@uab.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 1636005396A6

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education
Other (Specify):
Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☐ New ☒ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Hydrogen Peroxide and Capsule Production in Streptococcus pneumoniae

12. PROPOSED PROJECT:
* Start Date: 09/01/2013
* Ending Date: 08/31/2016

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT:** AL-007

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Miss
* First Name: Jocelyn
Middle Name: Renee
* Last Name: Hauser
Suffix:
Position/Title: Graduate Assistant
* Organization Name: University of Alabama at Birmingham
Department: Microbiology
Division:
* Street1: 845 19th Street South
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PROJECT SUMMARY/ABSTRACT

Streptococcus pneumoniae is a gram-positive bacterial pathogen that causes diseases such as pneumonia, meningitis, bacteremia and middle ear infections. The major virulence factor of *S. pneumoniae* is its polysaccharide capsule. The capsule enables the organism to evade host defenses by providing protection against complement-mediated opsonophagocytosis in systemic sites and by allowing the organism to successfully colonize the nasopharynx. The nasopharynx is the natural reservoir of *S. pneumoniae*. In the nasopharynx, *S. pneumoniae* is in a highly aerated (oxidizing) environment, however when it has the opportunity to bypass host defenses and invade systemic sites, it reaches environments with low aeration (reduced environments). Capsule production seems to correlate with environmental conditions. In aeration, capsule production is decreased, but it increases with decreasing aeration. Being able to rapidly modulate capsule production is important to the survival of the organism, as it experiences environmental changes throughout the stages of colonization and infection. Modulation of capsule production is controlled in part by a phosphotyrosine regulatory system that is comprised of a membrane sensing protein (Cps2C), a kinase (Cps2D) and a phosphatase/kinase inhibitor (Cps2B). In this project, we propose that capsule production may also be modulated through the production of hydrogen peroxide (H_2O_2). Under aerobic growth conditions, *S. pneumoniae* produces H_2O_2, which diffuses from the cell and accumulates in the culture medium in large quantities (>1 mM). The pyruvate oxidase, SpxB, is a major source of H_2O_2 in *S. pneumoniae*. SpxB is an important virulence factor that enhances the bacterium's ability to cause invasive disease. The goal of this project is to evaluate the dual effects of SpxB and environmental conditions on capsule production in *S. pneumoniae*. We hypothesize that capsule production is regulated in part by the effects of H_2O_2 on capsule biosynthetic enzymes. Under reduced aeration and low H_2O_2, environmental conditions similar in invasive disease, capsule production is increased thereby allowing survival of the organism. Ultimately, through this project, we may identify a posttranslational mechanism by which *S. pneumoniae* modulates the production of capsule biosynthesis as it travels from the nasopharynx to systemic sites to cause disease. The specific aims for this project are to: 1) Determine effects of H_2O_2 and *spxB* mutations on capsule production; 2) Determine effects of *spxB* mutations and H_2O_2 on enzyme activities and oxidation states; and 3) Determine effects of *spxB* mutations on virulence *in vivo*.

PROJECT NARRATIVE

Streptococcus pneumoniae is the most common cause of community-acquired pneumonia and is also a major cause of sepsis, meningitis, and otitis media (middle ear infections). The mechanisms by which this bacterium causes systemic disease are not fully understood. Identifying the regulatory element(s) *S. pneumoniae* uses to modulate a major virulence factor, the capsular polysaccharide, will provide insights into how a commensal bacterium becomes pathogenic and may lead to new therapeutics that not only target all pneumococci but organisms that use similar mechanisms.

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FACILITIES AND OTHER RESOURCES

Laboratory:

Dr. Yother's Laboratory occupies 1750 square feet of space in Bevill Biomedical Research Building. This building houses the majority of the faculty in the Department of Microbiology, including multiple investigators studying the genetics and pathogenesis of *Streptococcus pneumoniae*, *Mycobacterium tuberculosis, and Bacillus anthracis.* Faculty located in adjacent buildings are involved in studies of other bacteria, including oral streptococci, salmonella, and mycoplasma.

Core facilities

Core facilities for High Resolution Imaging; , Mass Spectrometry, Metabolomics and Proteomics; DNA sequencing/genomics/microarrays, and NMR are available on campus.

Animal:

The UAB Department of Animal Services provides an animal care core facility in the Bevill Biomedical Research Building with complete facilities for animal care and anesthesia. Professional, full-time supervisory staff and veterinarians are available for consultation as needed. We have access to a clean room where mice are housed prior to infection studies. A separate room is used for bacterial inoculations and housing until sacrifice. The facilities are fully accredited, and animals are cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Computer –

There are four MacIntosh and one PC computer located in Dr. Yother's lab that are available for use in these studies.

Office –

300 square feet of office space is located adjacent to the laboratory.

Other –

The following Core and shared facilities are available within the Department of Microbiology or UAB: DNA sequencing, Mass Spectrometry, Metabolomics and Proteomics, Protein sequencing, Hybridoma, Protein expression, Microarray, Gas chromatography/Mass Spectrometry (GC/MS). Dr. Yother is the Director of the GC/MS Shared Facility for Carbohydrate Research, and the instrument is located in her laboratory.

EQUIPMENT

Located in Dr. Yother's Laboratory
Scintillation counter; FPLC; GC/MS; ultracentrifuge; chromatography columns; fraction collectors; PCR units; microplate spectrophotometer; fluorescence/luminesence plate reader; spectrophotometer; DNA and protein electrophoresis units; pressure blotter, hybridization ovens, UV crosslinker for Southern blot analyses; low speed, high speed, and eppendorf centrifuges; 30^{o}C, 37^{o}C, 65^{o}C, and CO_2 incubators; -20^{o}C and -80^{o}C freezers; chromatography refrigerator; incubated floor shaker; water baths; balances; photography equipment; phase contrast microscope; sonicator.

Located in adjacent laboratories –
Fluorescence microscope; light cyclers; HPLC; densitometer; cold and warm rooms.

Shared facilities located on the floor –
Autoclaves, glassware washers and dryers, media prep room, dark room, bioimager.

GRADUATE PROGRAM DESCRIPTION

The Graduate Biomedical Sciences (GBS) Program at University of Alabama at Birmingham (UAB) is an interdisciplinary program that includes 8 “themes” or programs. These themes are: Biochemistry and Structural Biology, Cancer Biology, Genetics and Genomic Science, Immunology, Pathology and Molecular Medicine, Neuroscience, Cell Molecular and Developmental Biology, and Microbiology. Training in the GBS program, which typically requires five to six years, includes, research, academics, and professional development. During the first year in the graduate program, students complete a series of courses presented as part of the GBS core curriculum. The core curriculum involves courses in Biochemistry, Genetics, Cell Biology, Statistics, and Bioethics. The Bioethics course satisfies the requirements set forth for all NIH-funded trainees. The Microbiology Theme courses taken in the first year are Prokaryotic Genetics and Molecular Biology, Eukaryotic Genetics and Molecular Biology, Immunology, Virology, Microbial Pathogenesis, and Structural Biology. After the first year, students are required to take three advanced courses (total). All students are also required to complete 3 laboratory rotations in the first year. Students spend 10 weeks in a laboratory of their choosing to identify specific research areas and mentor. At the end of the rotation, students present their research in the form of a poster session.

During the second year, students complete the qualifying exam, which is a proposal and oral defense based on their research. The Admission to Candidacy occurs in the third year. The dissertation committee consists of five faculty members (including the mentor) whose research and training expertise are relevant to the project. The committee will advise and guide the student throughout the remainder of the training. Subsequent committee meetings will occur approximately every six months.

Following completion of all research and academic requirements students write their Dissertation, defend their work in a private defense attended by only the committee, and then present a final defense open to all. Students are generally expected to have at least two manuscripts published or in final preparation for publication prior to the defense.

FELLOWSHIP APPLICANT BIOGRAPHICAL SKETCH

USE ONLY FOR INDIVIDUAL PREDOCTORAL and POSTDOCTORAL FELLOWSHIPS. DO NOT EXCEED FOUR PAGES.

NAME OF FELLOWSHIP APPLICANT Jocelyn Renee Hauser	POSITION TITLE Graduate Assistant
eRA COMMONS USER NAME (credential, e.g., agency login) JOCELYN	

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
North Carolina Central University, Durham, NC	BS	2000	Biology
University of Maryland-Baltimore, Baltimore, MD	MS	2010	Biomedical Research
University of Alabama at Birmingham, Birmingham, AL	Ph.D.	2010- Present	Microbiology

A. Personal Statement

I entered doctoral training with unique perspectives, having taught science in the secondary public school system, worked as a clinical microbiologist, and then returned to school to earn a masters degree in biomedical research. My background has enabled me to shape and clearly define my goals as a graduate student as well as my ultimate career path. Teaching middle school science and math helped me realize my desire for a career in science. I decided to enter a certification program at the Washington Hospital Center to become a certified medical technologist (MT). This allowed me to further explore my interests as well as gain laboratory experience. Working in the microbiology lab allowed me to expand on my knowledge of the isolation, identification and treatment of pathogenic microorganisms. I knew then that I wanted to take my career in science a step further into the field of microbiology.

Continuing to work full-time as an MT, I entered a masters degree program at the University of Maryland at Baltimore (UMB). I worked under Dr. Amy J. Horneman studying *Aeromonas*, a bacterium that causes gastroenteritis, soft tissue infections, septicemia and meningitis in humans. The overall goal of my master's thesis project was to evaluate the ability of a molecular typing scheme, Multilocus Sequence Typing (MLST), to group 135 isolates of Aeromonads by species and distinguish between environmental and clinical strains. My work showed that MLST is an effective method for distinguishing species among Aeromonads and shows strong evidence of being able to distinguish environmental strains from clinical strains. I presented my findings at the 10th International *Aeromonas/Plesiomonas* Symposium in 2011.

Completing my masters degree helped prepare me for the Ph.D. program at the University of Alabama at Birmingham (UAB). In addition to my coursework and research project, attending seminars and workshops at UMB and other surrounding institutions such as the J. Craig Venter Institute exposed me to the different avenues within microbiology research. This helped develop my interests in the field of bacterial genetics and pathogenesis, ultimately leading me to the lab of Janet Yother where I have been studying the role of hydrogen peroxide production in the modulation of capsule polysaccharide in *Streptococcus pneumoniae*.

Since beginning my graduate studies at UAB, I have continued to define my goals and improve skills necessary to become a successful scientist. One of my goals as a graduate student at UAB is to regularly present my research at conferences and seminars. Since joining Dr. Yother's lab, I have presented several posters at conferences and retreats. Recently, I received the Richard and Mary Finkelstein Student Travel Grant and the Outstanding Poster Awards for the upcoming American Society for Microbiology 113th General meeting in Denver, CO where I will present both a poster and give a short talk.

Another one of my goals as a graduate student is to develop the skills necessary to become an independent researcher. Attending research seminars, participating in a journal club and developing my research project has allowed me to make significant progress in the doctoral program. I have learned to critically evaluate scientific literature, formulate hypotheses and solve problems. I am continually enhancing my understanding of bacterial physiology, genetics and pathogenesis and being exposed to numerous techniques. All of these things have allowed me to advance my project, which is in preparation for publication. In addition, I have successfully completed my core courses, passed my qualifying exam, and been admitted to candidacy. These milestones have been reached despite taking an unexpected academic hiatus due to a critical family emergency during my first year. Subsequently, I received a "C" and a "withdrawal" for two courses. Since that

time, I was granted permission to retake these courses, both of which I completed, receiving an A.

Upon completing my graduate training at UAB, I would like to complete postdoctoral training that will allow me to further develop as an independent researcher. Ultimately, I would like to conduct research that improves and advances the diagnosis and treatment of disease as well as knowledge in the scientific community.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/ EMPLOYER
Medical Technologist III	8/05	7/10	Clinical Laboratory Science	Washington Hospital Center	Donna Hershberger
Microbiology Teaching Assistant	8/08	5/09	Clinical Laboratory Science Education	Department of Medical and Research Technology, University of Maryland-Baltimore	Amy J. Horneman, Ph.D.
Teacher	8/00	7/05	Education	Prince George's County Public Schools	Benjamin Covington
Instructor	06/04	8/07	Education	Center for Talented Youth, John Hopkins University	Tricia Metz
Writer/Editor	6/98	8/98	Public Affairs	U.S. Navy Bureau of Medicine and Surgery	Capt. Sheila Gram

Academic and Professional Honors

Richard and Mary Finkelstein Student Travel Grant Award (2013), American Society for Microbiology
Outstanding Student Poster (2013), American Society for Microbiology
Graduate Student Association Travel Grant (2013), University of Alabama at Birmingham
3rd Place Graduate Research Day Oral Presentation (2013), University of Alabama at Birmingham
University Scholar (1996-2000), North Carolina Central University

Memberships in Professional Societies:

American Society of Microbiology
American Society of Clinical Pathologists
American Association for the Advancement of Science
National Council of Negro Women
Beta Kappa Chi Scientific Honor Society

C. Publications

Manuscript in preparation

Hauser JR and Yother J. Hydrogen peroxide production as a redox regulatory mechanism involved in modulation of capsule production in *Streptococcus pneumoniae.*

Abstracts

Hauser JR and Yother. 2013. *Modulation of Capsule Production in Streptococcus pneumoniae by SpxB and Hydrogen peroxide.* American Society for Microbiology 113th General Meeting, Denver, CO. (Poster and talk presentations)

Hauser JR and Yother J. 2013. *Modulation of Capsule Production in Streptococcus pneumoniae by SpxB and Hydrogen peroxide.* Pulmonary, Allergy, and Critical Care Update Symposium, Birmingham, AL. (Poster)

Hauser JR and Yother J. 2013. *Modulation of Capsule Production in Streptococcus pneumoniae by SpxB and Hydrogen peroxide.* UAB Graduate Research Day, Birmingham, AL (Talk)

Hauser JR and Yother J. 2012. *Hydrogen peroxide production as a redox regulatory mechanism involved in modulation of capsule production in Streptococcus pneumoniae.* 1st Annual Graduate Biomedical Sciences Retreat, Birmingham, AL(Poster)

Hauser JR and Yother J. 2012. *Hydrogen peroxide production as a redox regulatory mechanism involved in modulation of capsule production in Streptococcus pneumoniae.* 21st Annual Department of Microbiology Research Retreat, Destin, FL. (Poster)

Hauser JR, Brady AM, and Yother J. 2011. *Modulation of Streptococcus pneumoniae Capsule Production by Hydrogen Peroxide.* 1st Annual UAB Microbiology and Host Interaction Student Conference, Birmingham, AL. (Poster)

Hauser JR, Huys G, Joseph SW, Chopra AK, Stine OC, and Horneman AJ. 2011. *Identification of Aeromonas Isolates from Clinical and Environmental Sources Using Multilocus Sequence Analysis Based on gyrB, rpoD, dnaJ and chitII.* 10th International Symposium on *Aeromonas* and *Plesiomonas*. Galveston, TX. (Poster)

Hauser JR, Chopra AK , Joseph SW, Horneman AJ, and *Stine OC. 2010. *Most Clinical Isolates of Aeromonas belong to Two Phylogenetic Groups Defined by Multilocus Sequence Typing.* United States-Japan Cooperative Medical Sciences Program 45th Joint Panel Meeting on Cholera and Other Bacterial Enteric Infections Panel, Kyoto, Japan. (Poster; presented by *)

Non-scientific publications
Hauser, JR "What a Headache: Dealing With a Migraine" *Navy and Marine Corp Medical News*, July 17, 1998. reprinted in *Sea Service Weekly*, July 24, 1998.

Hauser, JR "Tiny Tick Takes Bite Out of Summer Fun" Navy and Marine Corp Medical News, July 31, 1998. reprinted in *The Flagship*, August 1998.

D. Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE
	North Carolina Central University	
1996	Science Odyssey	
1997	Molecules and Cell Function	
1997	General Zoology	
1997	General Chemistry	
1998	General Microbiology	
1998	General Chemistry	
1998	General Botany	
1998	Genetics	
1998	Organic Chemistry I for Chemistry Majors	
1998	Organic Chemistry Lab I	
1999	Vertebrate Physiology	
1999	Organic Chemistry II for Chemistry Majors	
1999	General Physics	
1999	Human Anatomy and Physiology I	
2000	Cell Physiology	
2000	Organic Chemistry Lab II	
2000	General Physics II	
	Overall GPA: 3.136	
	University of Maryland-Baltimore	
2007	Research Design	
2007	Lab Management	
2007	Biological Chemistry	
2007	Advanced Topics in Lab Science	

YEAR	OTHER COURSE TITLE	GRADE
	North Carolina Central University	
1996	English Composition I	
1996	Society, Behavior and Spatial Organization	
1996	College Algebra/ Trig II	
1996	Intermediate Spanish I	
1997	English Composition II	
1997	World Societies	
1997	Calculus/ Analytic Geometry	
1997	Elements of Speech	
1997	Personal and Social Development	
1998	Intro to Biostatistics	
1998	Health	
1998	Rhythm Aerobics/Aerobic Train	
1998	General Psychology	
1999	Black Experience to 1865	
1999	Elementary Swimming	
1999	Arts and Humanities I	
1999	Foundations of Public Health	
1999	Community Health Education	
2000	Arts and Humanities II	
2000	Foundations of Public Health II	
	Prince George's Community College	
2001	Tests and Measurements for Teachers	
2001	Educational Psychology	

YEAR	SCIENCE COURSE TITLE	GRADE
2007	Seminar	
2008	Responsible Conduct of Research	
2008	Teaching Practicum	
2008	Academic Writing for Graduate Students	
2008	Quality Control and Regulation	
	Overall GPA: 3.48	
University of Alabama at Birmingham		
2010	Basic Biochemistry/Metabolism	
2010	Basic Genetics/Molecular Biology	
2010	Basic Biological Organization	
2010	Intro to Immunology	
2010	Prokaryotic Genetics	
2010	Lab Rotation 1	
2011	Eukaryotic Molecular Biology	
2011	Virology	
2011	Microbial Pathogenesis	
2011	Structural Biology for Microbiology	
2011	Lab Rotation 2	
2011	Intro to Biostatistics for GBS	
2011	Principles of Scientific Integrity	
2011	Lab Rotation 3	
2011	Developing Communication skills for Biological Research	
2011	Bacterial Pathogenesis Journal Club	
2012	Virology	
2012	Microbial Pathogenesis	
2012	Developing Communication skills for Biological Research	
2012	Bacterial Pathogenesis Journal club	
2012	Biology of Disease	
2012	Bacterial Pathogenesis Journal club	
2012	Developing Communication skills for Biological Research	

YEAR	OTHER COURSE TITLE	GRADE
	Overall GPA: 3.0	
Catholic University of America		
2002	Characteristics of Special Education	
2003	Methods for Secondary Reading I	
	Overall GPA: 3.75	
University of Maryland-Baltimore		
2006	Special Topics	
2009	Statistical Analysis	
	GRE Scores:	
	Verbal:	
	Quantitative:	

INTRODUCTION TO REVISED APPLICATION

We appreciate the reviewers' comments concerning the original application. Since that submission, I have made substantial progress in my training program and in my research project. In particular, I have passed my qualifying examination, been admitted to candidacy, and completed experiments critical to the underlying hypothesis of my proposal. This progress has allowed me to better focus my proposal and to address many of the critiques from the original review. In the sections below, I address major comments from the reviewers. Following that, my mentor addresses sponsor-related issues.

A particular issue I would like to address involves my grades, as more than one reviewer commented on this aspect of my record. It was correctly noted that I received one A, one C (Virology), and the remainder Bs in my first year graduate courses. One course (Microbial Pathogenesis) was also listed as incomplete. During spring 2011, I had to take a leave of absence due to the death of my mother. This leave occurred at the end of the Virology course and during Microbial Pathogenesis, resulting in the C and Incomplete grades. In my second year, I completed both courses, receiving an "A" in each (denoted in my biosketch with astericks).

A major criticism of the proposal was the development and organization of the specific aims and the lack of depth. In the last year, I have used the *Streptococcus pneumoniae* D39 strain to: demonstrate that *spxB* deletion and H_2O_2 affect capsule production; gain evidence that control is posttranslational; and show a role for SpxB in nasopharyngeal colonization of mice. I have also shown H_2O_2 alters capsule production in a second *S. pneumoniae* strain. These results have provided support for my hypothesis and allowed me to significantly reorganize the aims and propose additional studies to address the mechanism(s) of control. I have also provided further consideration of alternative strategies and potential problems.

The revision has been extensively reorganized and rewritten. Only changes representing new information, new data, or specific responses to critiques are indicated (by a line in the margin).

Critique 1 - *Alternative model, role of SpxB-Pta-AckA pathway in expression of TCS regulons* – alterations in this pathway do affect TCS regulation, but mutations eliminating only *spxB* do not. I have provided information from the literature on this point, and presented my experiments to address possible effects in the Approach.

Transcriptional analyses – these analyses showed that transcription, which appeared to be modestly reduced, was not responsible for the increase in capsule production. Additional experiments confirmed that transcription changes were not responsible for capsule increases. I discuss this data in the revision, and also present immunoblot analysis of capsule proteins.

Vertebrate animals - statistical methods are now described.

Critique 2 (Overlap with Critique 1 is not repeated)

Use of mutants vs H_2O_2 – H_2O_2 provides a means for rapidly testing effects on capsule and for "exogenously" complementing *spxB* mutations to determine if defects are H_2O_2-specific. *spxB* mutations allow for specific alterations and determining whether non-H_2O_2 factors are involved. Mutants are also critical for *in vivo* experiments. While we may find one or the other is sufficient, we will initially continue to test both.

Cps2B references – these are now provided. This work has been carried out in our lab.

Virulence of mutants – Data demonstrating colonization attenuation for the SpxB mutant is now provided.

Professional and academic training – both my mentor and I present additional information regarding new training opportunities.

Critique 3 – *Virulence studies* – I have clarified information regarding the previous studies and how my studies differ, and presented more information regarding in vivo capsule expression. I have also included more strains in the virulence studies to help address these issues.

Sponsor replies – The main issue concerned my productivity and funding. Since the original submission of the F31, I have received an R21 and a pilot award (both beginning April 2013). My R01 will enter a no-cost extension June 2013 but there are sufficient funds remaining to continue to support the project and Ms. Hauser while the renewal is in process. I also have additional departmental funding to support my research. The modest number of publications is related primarily to several changes in senior personal over the last few years, along with beginning new directions in several of our projects. I have also had a number of beginning graduate students whose projects are now maturing to the point of publication. Since the original submission of the F31, we have two papers published, two (and possibly three) in submission as of this month, and ≥ three to be submitted in the next two to three months.

The reviewer also noted that dissertation committee meetings should occur twice, rather than once per year. I agree on this point, and the program is in the process of revising its requirement. Ms. Hauser's last committee meeting was in October, and her next one will be in May.

Specific Aims

The goal of this study is to identify the roles SpxB and H_2O_2 play in the aeration-dependent reduction of capsule production in *Streptococcus pneumoniae*. The specific aims are to:

1. **Determine effects of H_2O_2 and *spxB* mutations on capsule production.** My data demonstrate that aeration-dependent reduction of capsule in *S. pneumoniae* serotype 2 is due in part to H_2O_2 produced as a byproduct of the SpxB-mediated conversion of pyruvate to acetyl-phosphate. In this aim, I will determine whether capsule production in other serotypes is similarly affected by exogenous H_2O_2. For serotypes that respond to H_2O_2, *spxB* mutations will be generated to investigate the dependence on the pyruvate oxidase. For those serotypes that do not respond to H_2O_2, I will use capsule switching experiments to determine whether the failure to respond is due to capsule-specific enzymes or elements outside the capsule genetic locus. Lastly, I will construct strains in which H_2O_2 levels are altered as a result of specific point mutations in *spxB*.
2. **Determine effects of *spxB* mutations and H_2O_2 on enzyme activities and oxidation states.** Specific enzymes involved in serotype 2 capsule biosynthesis, such as the initial glycosyltransferase Cps2E, have been shown to respond to aerated/oxidized and non-aerated/reduced environments. In this aim, I will determine the effects of mutations in *spxB* on the enzymatic activities of proteins involved in capsule production, and will determine *in vivo* oxidation states using thiol-trapping methods. I will also initiate studies to examine the global effects of H_2O_2 on cellular proteins by identifying redox-sensitive proteins using thiol-trapping and Isotope Coded Affinity Tag technology coupled with mass spectrometry
3. **Determine effects of the *spxB* mutations on virulence *in vivo*.** In this Aim, I will use mutants constructed in Aim 1 to examine the effects of alterations in SpxB and H_2O_2 on colonization and pneumonia in mice. Two parent strains, their *spxB* deletion derivatives, and derivatives containing *spxB* point mutations that alter the levels of H_2O_2 will be examined. For mutants that retain the ability to colonize or cause pneumonia, I will examine capsule production and gene expression using recovered bacteria.

RESEARCH STRATEGY

A. Significance

Streptococcus pneumoniae is a gram-positive bacterial pathogen that is a common cause of pneumonia, meningitis, bacteremia and middle ear infections. The major reservoir of *S. pneumoniae* is the nasopharynx, and passage is by person-to-person contact. According to the Centers for Disease Control, 5 to 70 percent of healthy adults are asymptomatic carriers of *S. pneumoniae* (1). The major virulence factor of *S. pneumoniae* is its polysaccharide capsule, which enables the organism to evade host defenses by providing protection against complement-mediated opsonophagocytosis and by shielding surface molecules. The polysaccharide capsules differ by sugar compositions and linkages, and these determine the capsular serotype. Currently, >90 serotypes of capsular polysaccharides of *S. pneumoniae* have been identified (8). All but two of these serotypes are synthesized by the Wzy-polymerase dependent pathway, a mechanism that is used by both Gram-positive and Gram-negative bacteria for the synthesis of many polysaccharides. In the prototypical serotype 2, synthesis begins with the initiating glycosyltransferase, Cps2E, adding a glucose-1-phosphate, to undecaprenyl-P (Und-P) (step 1 in Fig. 1, (9)). This step is followed by addition of other sugars by glycosyltransferases to form an oligosaccharide repeat unit (step 2, Fig.1, (16)) that is transported to the outside of the cell membrane where polymerization occurs (steps 3 and 4, Fig. 1). Finally, the polysaccharide chain can stay associated with the cell membrane, be attached to the peptidoglycan, or released into the environment (step 5 in Fig. 1) (38).

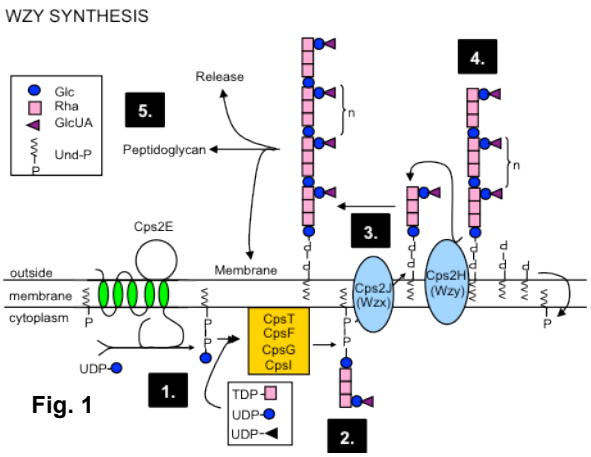

Fig. 1

Regulation of the capsule pathway appears to involve multiple mechanisms but to date, details concerning any of these mechanisms are sparse. In the host, *S. pneumoniae* encounters varying environmental conditions in the course of nasopharyngeal colonization and subsequent infections of the lung, bloodstream, and other sites. One of the environmental changes encountered is the level of aeration. In the highly aerated (oxidizing) environment of the nasopharynx, reduced levels of capsule allow exposure of adhesins for colonization (19, 35). In sites such as the blood and alveolar spaces during pneumonia, however, the bacteria encounter decreased aeration and a reducing environment. Under these conditions, increased levels of capsule, important for preventing opsonophagocytosis, are produced (35).

Alterations in capsule in response to changing oxygen levels correlate with alterations in the level of tyrosine phosphorylation of Cps2D, a protein encoded within the *S. pneumoniae* capsule genetic locus; increased oxygen levels correlate with decreased Cps2D phosphorylation and decreased capsule, and vice-versa (35). Cps2D is a tyrosine kinase that interacts with a membrane sensing protein (Cps2C) and a phosphatase/kinase inhibitor (Cps2B) (Fig. 2) (4, 5, 23). The specific roles for these proteins in capsule synthesis are not known, but deletion of *cps2C* or *cps2D* results in only short-chain capsule polymers (4). Mutations in *cps2B* cause increases in phosphorylation of Cps2D and alterations in capsule amount that are dependent on the specific mutation (4, 23). Loss of any of these genes reduces virulence and colonization. Recent studies in our lab have shown that Cps2B activity increases during aerated growth conditions, but this appears not to be responsible for the decrease in capsule production. *In vitro*, the activity of the initiating glycosyltransferase Cps2E is reduced in the presence of oxidizing agents such as hydrogen peroxide (H_2O_2) but enhanced in the presence of reducing agents such as dithiothreitol (DTT). This enzyme contains four Cys residues capable of forming disulfide bonds in an oxidizing environment (Fig. 2, asterisks). Mutation of C175 in Cps2E eliminates the ability to form disulfide bonds and results in increased activity of the recombinant enzyme in *E. coli*. Multiple enzymes in the capsule pathway, including Cps2D (Fig. 2), the glycosyltransferases Cps2TFGIK (Fig. 1), and the TDP-Rha biosynthetic enzymes Cps2LMNO, also contain Cys residues potentially involved in reduction-oxidation (redox) reactions.

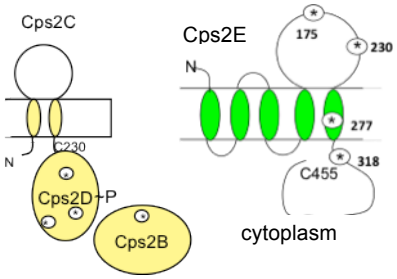

Fig. 2. Phosphotyrosine enzymes Cps2BCD and initiating glycosyltransferase Cps2E

In considering factors that might be important in redox control, we recognized that *S. pneumoniae* produces high amounts of the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) when grown under

aerated conditions (22), where levels can reach as high as 1 mM in the culture medium (3). The majority of the H_2O_2 results from the activity of the pyruvate oxidase SpxB, which is a 64-kDa cytoplasmic protein associated with the cell membrane (33). During glycolysis, in the presence of oxygen and inorganic phosphate, SpxB converts pyruvate to acetyl phosphate (AcP) (11), producing H_2O_2 as a by-product (Fig. 3). The H_2O_2 freely diffuses out of the cell and can help the organism to persist and cause disease by destroying host tissues (12, 14) and killing other competing bacteria such as *Staphylococus aureus* and *Haemophilus influenzae* (24, 30); *S. pneumoniae* is more resistant to H_2O_2 than many other bacteria (24, 25). Loss of *spxB* largely eliminates H_2O_2 and results in colonies that exhibit increased mucoidy and opacity (25, 29). **The basis for the changes in colony morphology has not been described, but the effects of oxidizing conditions on capsule production led us to hypothesize that they may be the result of alterations in capsule.**

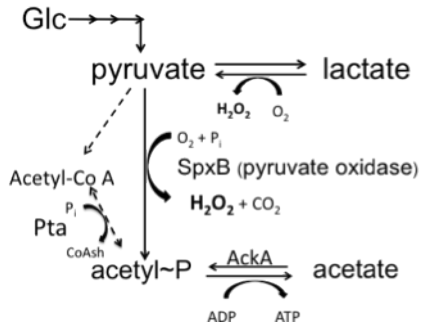

Fig. 3. Generation of H_2O_2

ROS are known to play roles in both eukaryotic and prokaryotic cellular processes such as signal transduction pathways, gene expression, and cellular growth (31). Because H_2O_2 is a mild oxidant with specificity for the oxidation of thiols, it is considered a second messenger (15). ROS can also have important antimicrobial activities as a result of inducing DNA and protein damage. An additional role for ROS, suggested by the above observations, could lie in the ability to down-regulate expression of bacterial capsules, thereby reducing the virulence potential of the organism. A study with *Campylobacter jejuni* published after the first submission of this F31 suggested a similar possibility. There, H_2O_2 reduced capsule levels and decreased tyrosine phosphorylation of an outer membrane tyrosine kinase. The kinase was necessary for capsule production, where it was shown to phosphorylate an epimerase involved in capsule synthesis (11).

In this project, we propose that capsule production in *S. pneumoniae* is regulated in part by oxidizing and reducing conditions that posttranslationally affect the activities of enzymes involved in capsule synthesis, and that H_2O_2 generated by *S. pneumoniae* plays an important role in this regulation. The results of this study may also have importance for other H_2O_2-producing bacteria, including many streptococci, as well as both H_2O_2-producing and non-producing bacteria as a result of the effects of host-generated H_2O_2.

B. Approach

Because the majority of H_2O_2 in *S. pneumoniae* results from the activity of the pyruvate oxidase SpxB (Fig. 3), I first generated *spxB* deletion mutations and characterized the effects on capsule production. Those results are described first, followed by my subsequent plans.

spxB mutants were constructed in *S. pneumoniae* D39, which expresses the serotype 2 capsule. This strain has served as a model for many studies in our lab and others (5, 9, 10, 16, 28, 29). Two independent mutants derived from separate mutagenesis experiments were generated using allelic exchange to replace *spxB* with a non-polar insertion of *aphA-3*, which encodes for kanamycin resistance and allows for direct selection of the mutants. This is a standard method used in our lab for generating deletion mutations (16, 37). Both mutants were shown to have similar phenotypes in subsequent studies.

The *spxB* mutation largely eliminated H_2O_2 production (Fig. 4A). Growth in liquid medium was not affected (Fig. 4B) but a larger and more mucoid colony morphology and less α-hemolysis were evident on blood agar plates. The latter properties are consistent with increased capsule and reduced H_2O_2 production, respectively. Capsule levels under aerated and non-aerated conditions were increased by the *spxB* mutation, as determined by ELISA and immunoblotting (Fig. 4C-D). The latter method also revealed no apparent alterations in capsule chain lengths. Release of capsule into the culturing medium is minimal for D39, and no alterations were observed for the *spxB* mutant (data not shown). Supplementation of the growth medium with exogenous H_2O_2 (0.5 mM) resulted in decreased capsule for the *spxB* mutant under both aerated and non-aerated conditions (Fig. 4E). The more modest decrease under aerated growth may reflect the more rapid loss of H_2O_2 under these conditions (data not shown). For these experiments, H_2O_2 was added only once, in the early stages of growth. In future experiments, it will be replenished over time. For the parent, H_2O_2 addition reduced capsule only under non-aerated growth, possibly due to the already high concentration of endogenous H_2O_2 under aerated growth and the more rapid loss of the exogenous H_2O_2 (Fig. 4E). The 0.5 mM concentration of H_2O_2 did not affect growth of the parent or mutant (data not shown).

The loss of *spxB* decreases cellular acetyl-phosphate ~80% (AcP, Fig. 3) (25, 28) and eliminates the ability to increase ATP production during aerated growth (25). However, only negligible changes in global transcription and regulons associated with the 13 *S. pneumoniae* two-component systems (TCS) are observed in D39 *spxB* mutants; only three of the TCS gene pairs are even marginally affected (1.1- to 2.2-fold) (28, 29). The reduction in AcP thus appears not to affect TCS regulation that might potentially affect capsule production (and no associations of TCS with capsule have been described). To more directly test this assumption, I examined capsule production for bacteria grown in 15 mM potassium acetate, which was reported to increase AcP levels in D39 and *pta* mutants (not reported for *spxB* mutants) (28). This supplement did not decrease the elevated capsule observed with the *spxB* mutant but instead, modest increases in capsule were observed for both D39 and the *spxB* mutant under aerated and non-aerated conditions (data not shown). Taken with the results obtained from addition of exogenous H_2O_2, these results indicate the phenotypic effects of the *spxB* mutation are due to the loss of H_2O_2 and not an effect on another part of the pathway shown in Fig. 3.

To determine whether transcriptional effects were involved, I performed real-time PCR with primers specific for the first five genes of the capsule operon (*cps2ABCDE*). The results were similar for each gene and demonstrated no significant changes for aerated vs non-aerated and no significant changes for D39 vs the *spxB* mutant (data not shown). I also used western immunoblotting to examine the levels of Cps2A, Cps2B, Cps2D, and Cps2E, and observed comparable levels of proteins under the different aeration conditions and for the parent and mutant (Fig. 4F). Although there may be a modest difference in Cps2E levels, this is not attributable to transcription, based on the real-time PCR results. These results, together with the reported minimal effect of *spxB* deletion on global transcription (28, 29), indicate that the increase in capsule resulting from the *spxB* deletion may occur through a posttranslational mechanism.

Using adult mice, I observed reduced nasopharyngeal colonization with the *spxB* mutant compared to D39 (1/10 vs 9/10 colonized; $P < 0.01$). This result is consistent with our hypothesis that colonization requires reduced capsule production, which is impaired in the mutant *in vitro* and possibly *in vivo*, as well.

To address the general nature of my findings, I examined the effect of exogenous H_2O_2 on a strain representing a different serotype. As with the serotype 2 D39 strain, reduced capsule was observed with the serotype 4 strain TIGR4, when exogenous H_2O_2 was added to non-aerated cultures (Fig. 4G).

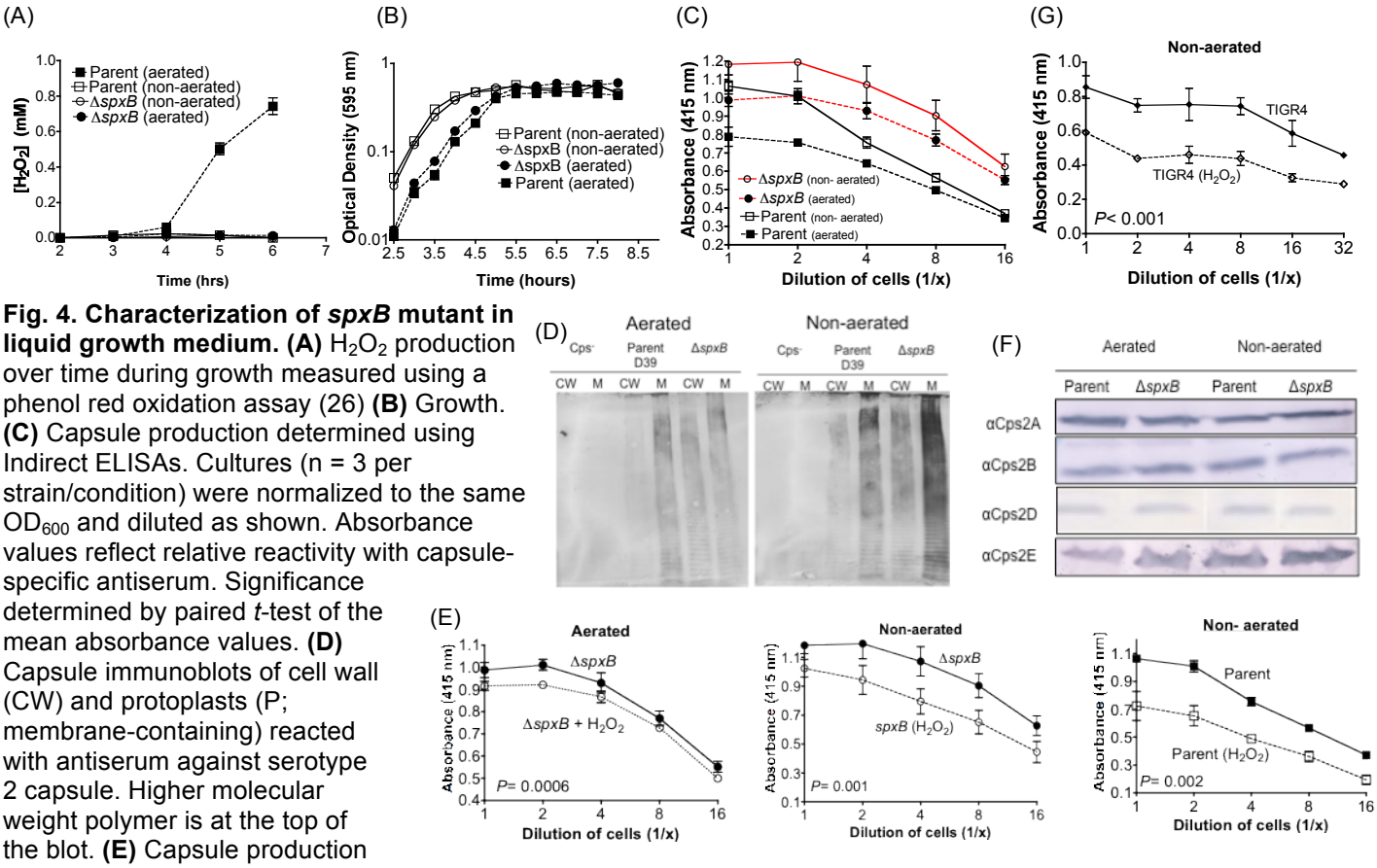

Fig. 4. Characterization of *spxB* mutant in liquid growth medium. (A) H_2O_2 production over time during growth measured using a phenol red oxidation assay (26) **(B)** Growth. **(C)** Capsule production determined using Indirect ELISAs. Cultures (n = 3 per strain/condition) were normalized to the same OD_{600} and diluted as shown. Absorbance values reflect relative reactivity with capsule-specific antiserum. Significance determined by paired *t*-test of the mean absorbance values. **(D)** Capsule immunoblots of cell wall (CW) and protoplasts (P; membrane-containing) reacted with antiserum against serotype 2 capsule. Higher molecular weight polymer is at the top of the blot. **(E)** Capsule production (determined by indirect ELISA) during growth with exogenous H_2O_2 (0.5 mM). Growth was not affected by H_2O_2 addition,

as determined in preliminary experiements to identify the appropriate H_2O_2 concentration. **(F)** Protein immunoblots. Bacteria grown under each condition were separated by SDS-page and transferred to nitrocellulose membranes. Protein levels were detected using rabbit polyclonal antiserum against Cps2A, B, D, E. **(G)** TIGR4 capsule production (determined by indirect ELISA) during growth with exogenouse H_2O_2 (0.5 mM) under non-aerated conditons.

My results to date indicate that SpxB plays an important role in the aeration-dependent reduction in capsule, and this reduction is related at least in part to H_2O_2. I will further address these outcomes and the underlying mechanisms in my further studies.

B1. Specific Aim 1. Determine effects of H_2O_2 and *spxB* mutations on capsule production. In this aim, I will further address the effects of H_2O_2 and SpxB, as well as their roles in other serotypes. I first describe the other serotypes to be examined and then the approaches, as these will be similar for all. If general principles are established, I expect to focus later studies mainly on the serotype 2 D39 strain.

B1.1. Additional *S. pneumoniae* strains. I will initially use strains that, like serotype 2, synthesize capsule by the Wzy pathway but contain initiating glycosyltransferases that are in different families (21). Cps2E of serotype 2 is in the most common family (WchA, 69 of the serotypes), and I will also determine whether other serotypes in this family respond similarly. The other families (WciI, WcjG, WcjH) are distinguished by the addition of an initial sugar other than glucose and the lack of the outer loop shown in Fig. 2. The observation that serotype 4 (WciI) produces reduced capsule in the presence of H_2O_2 argues that the outer loop is not essential for this response, though data for Cps2E described in the significance section suggest that it has some role in redox reactions. Although we may ultimately find these enzymes are not critical to the H_2O_2 response, this will provide an initial basis for selection of strains for my further studies. I will also examine a serotype 3 strain because its synthesis occurs by the synthase-dependent pathway where, in distinct contrast to the Wzy pathway, a single glycosyltransferase (the membrane-localized synthase) is responsible for all the steps necessary to synthesize the glucose-glucuronic acid polymer; additionally, the phosphotyrosine regulatory pathway is not involved (38).

I will initially test these strains for their response to H_2O_2, as it provides a rapid means to determine whether capsule will be affected. Because I know the TIGR4 serotype 4 strain is affected, I will construct *spxB* deletion mutants using allelic replacement with the Km-resistance cassette, as described above for D39. Mutants in other serotypes will be constructed depending on the H_2O_2 outcomes. Allelic replacement with the parental gene and screening for loss of Km-resistance will be used to repair mutations to ensure no other mutations are responsible for the observed phenotypes. *spxB* is not in an operon, and no polar effects have been observed with similar mutations (29).

Failure of other serotypes to respond to H_2O_2 could reflect a difference related specifically to the respective capsule enzymes, or it may suggest that the response lies outside the enzymes encoded within the *cps* locus (i.e., other factors in the genetic background of the responder strains are necessary). I will test these possibilities by examining additional strains within serotypes that do not respond, and by examining capsule-switched strains in which the capsule locus of a non-responding serotype is introduced into the D39 (responder) background. Our lab previously generated derivatives of D39 (and other strains) that express the type 3 capsule (2, 17), and other switched strains can be generated as needed.

B1.2. Effects of exogenous catalase. The observation that the *spxB* mutation resulted in increased capsule production under non-aerated growth suggests that the parent produces low levels of SpxB-generated H_2O_2 under this condition. To test this hypothesis, I will add catalase to the growth medium and test the effect on capsule produced by the parent and mutant under aerated and non-aerated growth. Catalse catalyzes the conversion of H_2O_2 to H_2O and O_2, and is produced by many cells but not by *S. pneumoniae*. I anticipate that catalase will increase capsule under aerated, and possibly non-aerated growth for the parent. If an increase is observed with the *spxB* mutant under either condition, it likely indicates that this mutant produces H_2O_2 in amounts that are too low to be detected by our assay. This possibility would not be unexpected since low amounts of H_2O_2 do occur from the activities of other pathways (34). Initial experiments will determine the appropriate concentration of catalase by assaying for reductions in H_2O_2 and ensuring that growth is not affected.

B1.3. SpxB mutants with altered activities. It has previously been shown that polymorphisms exist within *spxB*, and these affect the amount of H_2O_2 produced. An Ala282Thr alteration decreases H_2O_2 production by ~3-fold, likely by decreasing the affinity for FAD cofactor binding, whereas an Ala282Pro change has little effect (29). Our serotype 2 D39 strain contains Thr at this site, whereas our serotype 3 and the serotype 4 TIGR4 strains have Pro (29). Using site-directed mutagenesis, I will change the D39 Thr to Ala, and the Pro to Thr in the respective genes. The mutations will be introduced into the deletion strains

using allelic replacement of the Km-marker with screening for loss of Km-resistance. The mutations will be confirmed by sequencing and the phenotypes determined as above. These mutants will allow us to determine the effects of varying amounts of H_2O_2 and whether loss of the SpxB protein itself is important in the phenotypes we observe. Our lab routinely performs these types of allelic exchanges to introduce point mutations. If difficulties are encountered, we use the alternative method of introducing the mutant gene into the deletion strain at an ectopic site via insertion with an integration vector.

B2. Specific Aim 2. Determine effects of *spxB* mutations and H_2O_2 on enzyme activities and oxidation states. As noted above, the initiating glycosyltransferase and enzymes of the phosphotyrosine regulatory pathway respond to oxidizing and reducing conditions. These enzymes contain cysteine residue(s) that could undergo rapid oxidation by H_2O_2 to form reversible sulfenic acids or disulfide bonds, or irreversible sulfinic and sulfonic acids. These oxidations may affect enzyme activities directly through disulfide bond formation, by preventing phosphorylation, or by affecting catalytic Cys residues (27). In this aim, I will determine the effects of SpxB and H_2O_2 on the activities of enzymes involved in capsule synthesis. I will also initiate studies to examine more global effects of SpxB and H_2O_2.

B2.1 Effects on glycosyltransferase activity. The initiating glycosyltransferase, Cps2E, has been shown to be an important point of capsule regulation (9, 16, 37). In this aim, I will examine Cps2E activity in membranes isolated from the *spxB* mutants and the parent strain grown under aerated and non-aerated conditions and +/- H_2O_2. In these assays, activity is measured as the ability to incorporate [^{3}H] Glc from UDP-[^{3}H]Glc into an organically soluble product, indicative of the ability of Cps2E to transfer Glc-1-P from UDP-Glc to the membrane lipid acceptor undecaprenyl-phosphate (9). Radioactivity incorporated into the organic phase will be measured by liquid scintillation counting. I anticipate that Cps2E activity in mutants producing reduced H_2O_2 will be higher than the parent, regardless of the growth conditions. Likewise, mutants producing elevated H_2O_2 are expected to exhibit reduced Cps2E activity. We also have the assays in place to similarly analyze the other glycosyltransferases in the type 2 capsule pathway (16).

Our lab has shown that mutations in Cys175 in recombinant Cps2E cause increased enzymatic activity in *in vitro* assays (unpublished data). The data suggest that Cys175 may facilitate disulfide formation, which ultimately decreases enzymatic activity. I will introduce the Cys175Ala mutation into the parental strain and the *spxB* mutants to determine the effect of H_2O_2 on disulfide bond formation in this enzyme. I will compare enzyme activity as well as capsule production in aerated and non-aerated conditions. I anticipate the C175 mutants will produce more capsule under aerated conditions than the parental strain, and will be less affected by the *spxB* mutations.

B2.2 Effects on enzymes involved in the phosphotyrosine regulatory pathway. The effects of *spxB* mutations on the phosphotyrosine system will be determined using bacteria grown under aerated and non-aerated conditions. Phosphatase activity of Cps2B is assayed using permeabilized cells incubated with para-nitrophenylphosphate (pNpp). Cleavage of this substrate, which can be measured by increases in absorbance at 405 nm, is indicative of Cps2B activity (4). A *cps2B* deletion mutant (4) will be used a negative control. Cps2D activity will be measured by the ability of this enzyme to be phosphorylated on tyrosine residues, as detected using Immunoblotting with α-pTyr antibody (4). If H_2O_2 affects these enzymes, we anticipate an increase of Cps2D phosphorylation and a decrease of Cps2B phosphatase activity for the *spxB* mutants as compared to the parental strain due to the absence of H_2O_2.

B2.3 Oxidation states of capsule proteins. To visualize the *in vivo* oxidation state of Cps2E and other capsule-specific proteins under aerated and non-aerated conditions, I will use a double trapping method with iodoacetic acid (IAA) and iodoacetamide (IAM) (6). This treatment traps reduced Cys residues to prevent oxidation during processing and reduces Cys residues that were oxidized *in vivo*. Cell lysates from the *spxB* mutants and the parent strain will be treated, separated by non-reducing polyacrylamide gel electrophoresis (PAGE), and immunoblotted with antisera specific for the respective capsule proteins. Mobility standards of fully oxidized and reduced proteins will be used to determine the oxidation states of the enzymes. For Cps2E, which we know undergoes redox-mediated changes in disulfide bond formation, I anticipate the mutants producing decreased amounts of H_2O_2 will be in a more reduced state than the parent under both conditions. The mutants producing elevated H_2O_2 are expected to be in a more oxidized state.

B2.4 Global effects of SpxB and H_2O_2. Combined with earlier studies (28, 29), my results suggest that SpxB and H_2O_2-mediated effects on capsule occur through posttranslational rather than transcriptional effects. While we hypothesize that capsule enzymes are affected, they may not be the only, or possibly even the main players. Our lab has therefore initiated a collaboration with Dr. Stephen Barnes, Director of the UAB Targeted Metabolomics and Proteomic Laboratory, to analyze the oxidation state of cellular proteins, i.e., the

redox proteome (letter appended). Specifically, we will analyze D39 and its *spxB* mutant under aerated and non-aerated conditions and +/- H_2O_2. Our initial studies will use OxICAT technology, which identifies Oxidation states through thiol-trapping and Isotope Coded Affinity Tag technology coupled with mass spectrometry (7, 18). This technique allows for the identification of proteins affected by oxidation, as well as the redox-sensitive cysteines. Additional approaches are available to identify other oxidation modifications that may be occurring (13, 32). Dr. Barnes is an expert in mass spectrometry and proteomics, and his lab has carried out numerous studies relevant to our work. As part of this study, I will participate in a 4-day NIH-funded Metabolomics Workshop directed by Dr. Barnes and held annually at UAB. Here, I will learn about experimental design, sample preparation, analytical methods, and data analysis in preparation for my studies.

B3. Specific Aim 3. Determine the effects of *spxB* mutations *in vivo*. My results demonstrate that deletion of *spxB* reduces the ability of D39 to colonize the mouse nasopharynx. Similar results were obtained using D39Δ*spxB* in rabbits (33), but no effect of *spxB* deletion on colonization in neonatal rats was observed with TIGR4 or a serotype 6B strain (20, 30). These results may reflect bacterial strain or animal differences. I will address this point within these studies. Other studies have indicated a role for SpxB in pneumonia using Δ*spxB* mutants of D39 in rabbits and mice (29, 33).

For these studies, I will focus on six strains – D39, D39Δ*spxB,* D39(SpxB^{Thr282Ala}), TIGR4, TIGR4Δ*spxB*, and TIGR4(SpxB^{Pro282Thr}); and two mouse models – colonization and pneumonia. This approach will allow me to assess the loss of SpxB, test the effects of specific mutations that result in alterations of H_2O_2 levels (Thr produces less H_2O_2 than Ala/Pro; discussed in section B1.3), and clarify the effects of SpxB in two different animal models and strains that are frequently used in studies of *S. pneumoniae* virulence. Properties resulting in virulence differences for D39 and TIGR4 are no doubt multiple, and the purpose is not to assign these differences to SpxB, but rather to compare the effects of comparable SpxB proteins within each strain. Studies from section B1.3 will clarify the *in vitro* phenotypes of these strains, but we anticipate that D39(SpxB^{Thr282Ala}) will produce more H_2O_2 and less capsule than the parent D39, and TIGR4(SpxB^{Pro282Thr}) will produce less H_2O_2 and more capsule than the parent TIGR4. These strains may prove most useful in addressing the effect of SpxB/H_2O_2 on colonization (and possibly pneumonia) as, unlike the D39 *spxB* deletion mutant, they may retain the ability to colonize, thereby allowing us to recover bacteria and detect differences in their numbers as well as test for whether alterations in capsule are occurring *in vivo.*

Each of our animal studies uses factorial design and the resource equation to estimate sample size and to compare across multiple parameters, thus minimizing the number of mice needed for statistical significance. Initial experiments utilize groups of 3 – 5 mice and are repeated at least once. For planning purposes, we factor 10 mice per group. This design has resulted in reproducible and statistically meaningful data in past experiments (2, 4, 17, 19). Results are analyzed using ANOVA for multiple comparisons or unpaired *t*-tests and Fishers' exact test if doing direct comparisons of bacterial recovery or numbers of mice colonized, respectively.

B3.1. Colonization. For colonization, 10^7 bacteria contained in 10 µl of Ringers-lactate are introduced into the nares of non-anesthetized mice (19). By this method, bacteria remain in the nasopharynx and do not reach systemic sites (36). At 7 days post-inoculation, mice will be sacrificed and nasal washes and nasal tissues obtained (19). We will also obtain hearts and lungs to confirm no bacteria are present in these organs. Bacteria will be enumerated by plating and colony counts. The recovered samples will be stored and, if bacteria are recovered, assayed without further growth for capsule production by ELISA and capsule gene expression by real-time PCR, as described in previous sections. For six bacterial strains, these experiments will require 60 mice.

B.3.2. Pneumonia. To allow bacteria to reach the lungs, mice are anesthetized and inoculated with 10^7 bacteria in 50 µl of Ringers-lactate (36). At 7 days, or sooner if moribund, mice will be sacrificed. Nasal washes, tissues, hearts, and lungs will be obtained as above. Bacteria from all sites will be enumerated and, if recovered, capsule production and capsule gene expression will be determined as above. For six bacterial strains, these experiments will require 60 mice.

VERTEBRATE ANIMALS

1. Mice will be used in these studies to determine the relative levels of virulence and colonization of *S. pneumoniae* and to assess expression of virulence factors *in vivo*. Eight to ten week old C57BL/6J male mice will be used. Inoculations will be by the intranasal route, with and without sedation as models for pneumonia and colonization, respectively. For colonization, 10^7 bacteria are inoculated in a 10-µl volume. For pneumonia, 10^7 bacteria are inoculated in a 50-µl volume. Mice are sacrificed at 7 days (or sooner if moribund). Nasal washes, tissues, hearts, and lungs are collected to enumerate bacteria, capsule amount, and capsule gene expression. We anticipate the need for approximately 120 mice for the complete study, based on six *S. pneumoniae* strains, two models (pneumonia and colonization), and 10 mice per model.

2. C57BL/6J male mice will be used because they are immunologically normal, because they are susceptible to pneumococci infections, and because we have information with them for the specific *Streptococcus pneumoniae* parent strains to be used in this study. The numbers of mice to be used are based on factorial design experiments using the resource equation to calculate sample sizes. Sufficient animals are included to allow for repeating each experiment at least once. The numbers proposed (3 – 5 group, repeated at least once) have provided reproducible and statistically meaningful results in past experiments. Results will be analyzed using ANOVAs, unpaired *t*-tests, or Mann-Whitney two-sample rank test, as appropriate for the specific study.

3. Animals are housed in the Animal Care Facility in the Bevill Building, where our labs are located. Animals are monitored by the Animal Services Department and by our lab personnel. After inoculation, animals are moved from clean rooms to rooms specifically for mice infected with *S. pneumoniae*. Mice will be checked every six to eight hours following inoculation to ensure their healthy condition, and more frequently if ill. Mice will be sacrificed if they become moribund (as determined by surface temperature $<26^oC$) before the planned end of the experiment. All animal care and experimentation will be under the supervision of our veterinary staff of our Animal Services Department. Our animal facilities have AAALC accreditation.

4. Mice will be anesthetized when inoculated intranasally to achieve pneumonia. Isoflurane will be administered by the microdrop method (2% in chamber), according to the guidelines of our Animal Services Department.

5. Mice will be sacrificed by CO_2 asphyxiation. This method was selected because it should be no more painful than administration of injected drugs and it will allow us to recover bacteria that might be otherwise affected by the use of drugs. This method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. UAB Animal Services maintains a CO_2 euthanasia station within the animal facility.

RESPECTIVE CONTRIBUTIONS

Although the initial stages of this project began before I joined the lab, I further developed the research plan, which involved writing and editing the proposal, as well as performing all experiments presented in the proposal. My mentor provided general comments and edits to the proposal.

Throughout this project, my mentor and I have had daily informal meetings to discuss day-to-day results and direction of the project. I have participated in lab meetings, poster and oral presentations, for which I have analyzed, presented and discussed results and ideas with not only my mentor but with other lab mates and fellow graduate students. I will continue to participate in these activities for the duration of this project,

I have begun preparing my first manuscript for publication. During this process, my mentor and I will work together to complete the final versions for submission to peer-reviewed journals.

In addition to meeting and working with my mentor, I am also required to have committee meetings every 6 months. My committee is made up of four faculty members and my mentor. The committee members are faculty members that are involved in both closely related research as well as research areas outside of microbiology. Their expertise will also help shape my research project. As the project progresses, I will continue to expand the research plan and make decisions on the directions with general guidance from my mentor.

SELECTION OF SPONSOR AND INSTITUTION

I chose to pursue my graduate studies at the University of Alabama for many reasons. I was initially attracted to UAB for its outstanding record as a research institution. When I visited the campus I found the faculty and staff to be very welcoming and encouraging. I wanted to study in an environment that had a strong curriculum, small class sizes, diversity among faculty and students and an overall environment where I felt I could grow as an independent scientist. After speaking with a former student who had a similar background, I was more than convinced that UAB was where I would continue my graduate training.

When I came to UAB, I was specifically interested in studying bacterial genetics and pathogenesis. I completed my three laboratory rotations in labs that study different aspects of Streptococcal genetics and pathogenesis. One of those rotations was in the lab of Janet Yother. While rotating in Dr. Yother's lab, I studied antimicrobial peptides and their effects on capsule polysaccharide of *Streptococcus pneumoniae* serotype 3. During my rotation I was able to gain insight into the type of projects and lab techniques used to study bacterial genetics. I found Dr. Yother to be a great mentor who I felt I could go to at anytime to talk about my project. Outside of the lab, I also had interaction with Dr. Yother as the Microbiology Theme director for the Graduate Biomedical Science program and through my courses, specifically Prokaryotic Genetics in which she was the course master. Dr. Yother is an excellent teacher who is able to convey information in a way that anyone can understand. For those reasons I chose to join Dr. Yother's lab. I am confident that training under Dr. Yother, I will leave my graduate career with all the necessary tools to be a successful scientist in the field of bacterial genetics and pathogenesis.

Responsible Conduct of Research

Training in the Ethical Conduct of Research

As a required by the Graduate School, all graduate students must pass a two-credit course in the Responsible Conduct of Research. The semester long course familiarizes students and faculty with Federal and University guidelines and regulations on to how conduct research responsibly. Topics discussed in the course included: utilizing moral reasoning skills, handling ethical dilemmas, intellectual property, data sharing and ownership, IRB polices and procedures for human and animal subjects research, data acquisition, mentoring and management. These discussions were facilitated using small group discussions and exercises, videos and online modules. Representatives from the Graduate School and the Office of Scientific Integrity were also involved in instruction. This course is offered on a Pass/Fail basis with a passing grade being 75% or better. I completed and passed this course on August 3, 2011. To ensure I am aware of the current Federal and University regulations of the guidelines, I will continue to attend seminars and other venues in which ethical issues in research are discussed. The UAB Center for Ethics and Values in the Sciences frequently sponsors seminars and conferences on ethical conduct in science.

Training in the Ethical Conduct of Experiments Involving Animals.

All investigators and trainees are required to pass the courses “Using Animals for Teaching, Testing, or Research at UAB” and “Species Training” before initiating research with animals. Both courses are sponsored by the Institutional Animal Care and Use Committee (IACUC) and are completed online. Through the two courses, students learn about animal welfare, procedures involving pain and the use of anesthesia, appropriate methods for euthanasia, requirements for housing and veterinary care, and specific requirements for working with different animal species.

“Using Animals for Teaching, Testing, or Research at UAB” consists of three modules: An Introduction to the IACUC; Regulations, Policies, and Procedures; and Health and Safety. Completion of each module requires passing of a quiz with a score of 80% or better. “Species Training” is for the species that will be used and consists of online training and passing of a quiz, as above. The topics of the course are designed to teach issues relating to physiological and environmental considerations, animal handling and transport, operations, and monitoring. I have completed all necessary training to perform animal studies proposed in this study.

Goals for Fellowship Training and Career

During the course of my graduate training at the University of Alabama at Birmingham, my goals are to (1) gain a thorough understanding of bacterial physiology, genetics and pathogenesis; (2) present and publish my research at national meetings and in peer reviewed journals, respectively; and (3) successfully secure independent funding for my research. Accomplishing these goals will prepare me for a career in bacterial genetics and pathogenesis research.

Obtaining my graduate training in the lab of Dr. Janet Yother will allow me to understand the regulation of capsule biosynthesis in *Streptococcus pneumoniae*, a gram positive pathogen that commonly causes pneumonia and middle ear infections. Throughout this project, I will learn how to critically analyze results, formulate scientific questions and translate these questions into experiments. I will also be exposed to techniques used to study bacterial physiology, genetics, and pathogenesis. I will also be able to develop assays and or techniques that will allow me to gain insight about my project using the literature and information learned in my courses. My ultimate goal is to be able to develop skills that I can use during my postdoctoral studies and ultimately my own lab.

During my graduate training it is my goal to regularly present my research at national and international meetings. Presenting at weekly lab meetings, joint lab meetings, and seminars has allowed me to obtain the necessary skills to improve my writing and research presentation skills which are necessary for a successful career in a field in which writing grants and presenting original research are the basis. Although I taught science and math for a number of years, I would like to continue to work on effectively and confidently presenting my research in a way that tells a story and helps me prepare for presentations at national meetings. I will be presenting a poster and a short talk at my first national meeting this spring at the American Society for Microbiology General meeting in Denver, CO. In addition, it is my goal to have at least 2 publications in peer review journals by the time I graduate and to present at a national meeting at least once a year. At this time, I have presented several posters and I am currently preparing my first manuscript.

Preparing this fellowship application has helped me understand the grant writing and review process. I plan to attend workshops offered by the university and work with my mentor to write a proposal that will help me to secure future funding. I plan to continue to develop my writing skills and my understanding of how to write proposals.

Ultimately, I not only desire to use my training to advance my career but to also educate and mentor others who are underrepresented in this field. As a teacher, I learned first hand that many students are not aware of the different career paths that are available in the science field. Pursuing a career in microbiology research, ultimately leading my own lab, I want to create opportunities that would help train students who would not normally be exposed to a career in research, arming them with skills that would make them valuable to the field.

Upon completing my graduate training at UAB, I would like to complete postdoctoral training that will allow me to further develop as an independent researcher. I plan to conduct research that will combine my knowledge and expertise in the clinical sciences as well as basic research. For example, working in the clinical laboratory, I understand that there is a need for more rapid detection systems and novel antibiotic therapies needed to identify and treat infections and diseases caused by microorganisms. To achieve these advances in healthcare, we must understand the mechanistic basis of pathogenicity. Ultimately, I would like to conduct research that improves and advances the diagnosis and treatment of disease as well as knowledge in the scientific community.

ACTIVITIES PLANNED UNDER THIS AWARD

Year	Research	Coursework	Travel	Teaching
First	92%	6%	2%	0%
Second	95%	3%	2%	0%
Third	96%	2%	2%	0%

Under this award, my training plan will consist of: 1) completing advanced courses and taking required journal clubs and seminars, 2) developing and executing my dissertation project; and 3) presenting and publishing my research.

Currently, I am in my third year of graduate training in the Microbiology Theme of the Graduate Biomedical Sciences (GBS) program at the University of Alabama at Birmingham. I have successfully completed the core curriculum courses, passed my qualifying exam, and been admitted to candidacy. As required by the program, I must take three advanced courses. The program offers advanced courses such as Streptococcal Biology, Bacterial Physiology, Biology of Lung Diseases, Advanced Bacterial Genetics and Pathogenesis, Immune Responses to Pathogens, and Post-transcriptional Regulatory mechanisms. I have completed one advanced course entitled Biology of Disease, a pathology course that discusses the molecular basis of disease. In addition to formal courses, I participate in the Bacterial Pathogenesis Journal club, which meets weekly to critique and discuss current research articles from peer review journals. I will also participate in a monthly student seminar course in which we learn to give effective seminar presentations through preparation and presentation of our research to faculty and other students. In this course, I present my work at least once a year.

Throughout this training award, the majority of my time will be devoted to achieving the specific aims stated in the research-training proposal. There there will be many opportunities for me to continually develop my research plan and receive feedback from my peers and faculty in my field. Our lab holds weekly lab meetings, which will allow me to present and discuss issues and future directions for my project. I will also participate in a monthly lab meeting with other labs on campus that also study streptococci. Committee meetings will occur every six months. These meetings will ultimately help me to develop my project into published works.

Lastly, I will present at local, national and international meetings under this award. Local meetings I attend include the UAB Microbiology Department Retreat, which is a weekend that includes talks and poster sessions; the UAB Graduate Biomedical Sciences retreat; and the biannual Pneumococcal Symposium, which features pneumococcal research at UAB as well as surrounding universities. To date, I have presented a poster at both the UAB Microbiology Department Retreat and the Graduate Biomedical Sciences Retreat. National and Internationals meetings I will attend include the American Society of Microbiology (ASM) General Meeting and the Federation of American Societies for Experimental Biology (FASEB) Microbial Polysaccharides Conference. I will be presenting my work at the 2013 ASM General Meeting as both a poster and talk, as my abstract was selected for a Richard and Mary Finkelstein Travel Grant and as an Outstanding Student Poster.

DOCTORAL DISSERTATION AND RESEARCH EXPERIENCE

While majoring in biology at North Carolina Central University (NCCU), my focus had been primarily in medicine. The summer following my junior year, I was placed as an intern with Dr. Laura Svetkey, a hypertension specialist at the Duke University Medical Center. This was my first research experience. As an intern, I assisted with the clinical research project, Dietary Approach Stop Hypertension II study (DASH 2 study). The DASH2 study was a national clinical trial that evaluated the effects of controlling hypertension in patients with normal to high blood pressure through a specialized diet, the DASH diet, and restriction of daily sodium intake. For this study, patients ate specialized meals at the hypertension center and periodically wore ambulatory blood pressure monitors. I assisted with the study by collecting data on study participants obtained from the ambulatory blood pressure monitors, logging patient food diaries and prepared blood and urine samples to be sent for laboratory analysis. Through this experience, I learned all aspects involved in conducting clinical research trials such as study design and human subject regulations.

For my Masters thesis project, I worked under Dr. Amy J. Horneman, who studies *Aeromonas,* a gram-negative bacterium. Aeromonads are a group of emerging human pathogens that have been isolated from an increasing number of cases of gastroenteritis, soft tissue infections, septicemia and meningitis in humans. As part of a research grant with the Environmental Protection Agency, the overall goal of my thesis project was to determine whether a typing scheme, Multilocus Sequence Typing (MLST), would be able to identify and group clinical and environmental isolates by species and possibly distinguish pathogenic from non-pathogenic strains. MLST is a relatively new molecular typing method, in which the DNA sequences of particular loci on the chromosome are used to type organisms. Developing a molecular typing method would allow for rapid species identification and could possibly differentiate pathogenic from non-pathogenic *Aeromonas* isolates in drinking water. To do this I used molecular techniques such as DNA isolation, polymerase chain reaction (PCR), gel electrophoresis and DNA sequencing to analyze sequence data of four gene loci, consisting of housekeeping and virulence genes. I presented my work at the 10th International *Aeromonas/Plesiomonas* Symposium in Galveston, Texas in May 2011. Working on this thesis project prepared me to continue on to a PhD at The University of Alabama at Birmingham.

Upon entering the program at UAB, I completed in three laboratory rotations in lab that study different aspects of streptococcal pathogenesis. My first laboratory rotation was in Dr. Janet Yother's lab where she studies capsular polysaccharides of *Streptococcus pneumoniae*. One of the projects in the lab was determining the effects of Antimicrobial Peptides (AMP) on capsule. AMPs are a part of human innate immunity, providing the first barrier of defense against pathogens. AMPs have broad-spectrum activity. They are effective against bacteria, fungi, and some viruses. These molecules are diverse in size and structure and have a net positive charge. Negatively charged capsule polysaccharides of *Klebsiella pneumoniae, Pseudomonas aeruginosa, and S. pneumoniae* have been shown to block the activity of positively charged AMPs by binding to them. AMPs have also been shown to cause release of capsular polysaccharides from some bacteria. The purpose of my project was to further examine this effect on *S. pneumoniae.* Throughout my time in the lab, in addition to learning about *S. pneumoniae*, I learned basic techniques used to study capsule such as chloroform extraction, stains-all assay and an antimicrobial resistance assay. Through my project, we were able to conclude that the capsule appeared to have some protective effects against AMP activity, as the MICs for Polymyxin and Bacitracin were decreased for the type 3 and type 2 capsule mutants, respectively. At the end of my time in the lab, I presented my data in the weekly lab meeting and at a poster session in which students present their projects to faculty and students within the GBS program. Presentations are judged by faculty outside of their field as part of my grade for the rotation. Students are judged based on their oral presentation, the poster, and overall understanding of the project. For my presentation, i received excellent evaluations.

My second rotation was in the laboratory of Dr. Moon Nahm, the primary research focus was the study host-bacterial pathogen interactions and to improve pneumococcal vaccines. During my time in the lab, I examined serotype variants within *S. pneumoniae* Serotype 6. Members within

serogroup 6 are commonly found in invasive disease. Within this group there are 4 known capsule types: 6A, 6B, 6C, and 6D. Members of this group share many genes in the cps locus but have variations within glycosyltransferase genes, *wciN* and *wciP*. There have been reports of serogroup 6 strains with capsules expressing an epitope cross-reacting to monoclonal antibodies against serotypes 6A and 6B. The report also observed single amino acid polymorphisms within the wciP region of 6A and 6B. The overall goal of my project was to examine whether the single amino acid polymorphisms are responsible for the observed serotype differences. To do so, I learned such techniques as Enzyme-Linked Immunosorbent Assays (ELISA), Flow Cytometric Serotyping Assay (FCSA) assay and other molecular biology and genetics techniques. I received an "A" from both judges for my poster presentation at the end of the rotation.

My final rotation was in the lab of Dr. Hui Wu. The purpose of my project was to identify a small molecule, derived from sea sponge products, which could inhibit and or disperse biofilms of oral pathogens such as *Streptococcus mutans, Streptococcus gordonii and Staphylococcus aureus.* Using a biofilm assay, I was able to identify several molecules that were able to inhibit biofilm formation *S. aureus* and *S. mutans*. I found that some small molecules not only inhibit biofilm formation but also inhibit bacterial growth. Some of the molecules I identified could possibily disperse biofilm formation.

My current dissertation project focuses on the role of hydrogen peroxide in capsule proction in *S. pneumoniae*. The capsule polysaccharide that surrounds the bacterium contributes to its virulence by preventing it from being recognized and killed by the host. Another factor that contributes to its virulence is the bacterium's ability to produce millimolar amounts of hydrogen peroxide in aerobic growth conditons. Mutations in the pyruvate oxidase (SpxB), the protein that produces the majority of the hydrogren peroxide in *S. pneumoniae*, result in increased colony mucoidy and opacity. We hypothesize this change in colony morphology is the result of a change in capsule production. The goal of my project is to evaluate the dual effects of SpxB and environmental conditions on capsule production in *S. pneumoniae*. Since joining the Yother lab, I have been able to use my experiences from other laboratories and apply them to my project. Through my project, I have learned more techniques used to study capsule production such as immunoblotting, real-time polymerase chain reaction (pcr), and molecular techniques such as cloning and site-directed mutagenesis. Thus far, I have presented my results as poster presentations at numerous retreats and symposiums such The Graduate Biomedical Sciences Retreat and the Microbiology Departmental retreat. I have also given short talks at our Microbiology monthly meeting as well as the UAB Graduate Research Day, for which I won third place for my oral presentation.

SECTION II – SPONSOR INFORMATION

A. RESEARCH SUPPORT AVAILABLE TO MS. HAUSER

B. SPONSOR'S PREVIOUS FELLOWS/TRAINEES –

As a mentor, I have trained 20 graduate students (including Ms. Hauser) and three postdoctoral fellows. FIFTEEN of the students received their Ph.D. degrees, two received masters degrees, and THREE Ph.D. students are still in training. FIVE of the past Ph.D. students were from underrepresented minorities. I have served on the dissertation committees of 23 other Ph.D. students, been the rotation advisor for 17 other Ph.D. students, served on the qualifying examination committees of 25 other Ph.D. students, been the director of an NIH-supported T32 lung diseases training grant, and am currently the director of the UAB Graduate Biomedical Sciences Microbiology Graduate Theme. All of my past trainees, with the exception of two who are currently in transition between positions, are in science-related professions.

Representative Trainees. The following five students were selected from fifteen Ph.D. graduates from my lab. Other students are currently in positions with academic institutions, independent research organizations, and pharmaceutical companies.

Dr. Joseph Dillard was the first student to graduate from my lab (Ph.D., 1994). He received postdoctoral training with Dr. Hank Siefert at Northwestern University studying *Neisseria gonorrhoeae*. He has continued that work in his own lab at the University of Wisconsin-Madison, where he is an Associate Professor.

Dr. Tanya Kelly McKinney was the first underrepresented minority student to graduate from my lab (Ph.D., 1996). She was a postdoctoral fellow with Dr. Gordon Archer at the Medical College of Virginia and then a faculty member at Xavier University in New Orleans. Following Hurricane Katrina, she relocated to Delta State University, where she is now an Associate Professor.

Dr. Ashalla Magee received her Ph.D. in 2004. She is from an underrepresented minority. Dr. Magee performed postdoctoral work with Dr. Robert Bourret as part of a SPIRE Fellowship at the University of North Carolina at Chapel Hill. She is now the Director of Diversity Affairs in the Office of Graduate Education at that institution.

Dr. Bobbi Xayarath received her Ph.D. in 2007. She was initially a postdoctoral fellow and is now a Research Scientist with Dr. Nancy Freitag at the University of Chicago, where she studies *Listeria monocytogenes*.

Dr. David James received his Ph.D. in March 2013. He is from an underrepresented minority. He is now moving to a postdoctoral fellowship with Dr. Victor Torres at New York University, where he will be studying staphylococcal pathogenesis.

C. TRAINING PLAN, ENVIRONMENT, RESEARCH FACILITIES –

Ms. Hauser's training includes research, academics, and professional development. Her training is supported and enhanced by resources from within my lab, the Department of Microbiology, her graduate program, and UAB. Ms. Hauser is currently in her third year of training. She has passed her qualifying examination and been admitted to candidacy.

Research Project. Ms. Hauser's project is both an integral part and an extension of the ongoing work in my lab. Over the last 20 years, we have defined major aspects of the genetics, biochemistry, and virulence associated with capsules produced by *Streptococcus pneumoniae*. An important unanswered question in this work is how capsule production is regulated and to what environmental signals it responds. Ms. Hauser's work will bring together many of the observations we have made over the last several years. Specifically, are the alterations in capsule production that occur in aerated and non-aerated environments due in part to an effect of reactive oxygen species (ROS), in particular hydrogen peroxide (H_2O_2)? ROS produced by host cells have antimicrobial activities that are attributed in large part to effects on DNA and cell viability. However, *S. pneumoniae* is somewhat unusual in that during aerated growth it produces high levels of H_2O_2 that diffuse from the cell. During in vitro culture, H_2O_2 concentrations in the medium can reach 1 mM. While diffusion may diminish this concentration in host environments, local concentrations are still expected to be high. *S. pneumoniae* is significantly more resistant to H_2O_2 than other organisms, such as *Escherichia coli*, but the mechanisms for this have not been fully defined.

In our studies of the *S. pneumoniae* polysaccharide capsule, it has become apparent that less capsule is produced in aerated environments, and this coincides with alterations in the activities of enzymes responsible for capsule synthesis. Many of the enzymes contain cysteine residues that allow for oxidation and reduction, effects that may alter the enzymatic activity. During in vitro assays, we observed these alterations using H_2O_2 or other oxidizing agents, and using reducing agents. These observations led us to hypothesize that the H_2O_2 produced by *S. pneumoniae* might play a role in post-translationally regulating capsule enzymes and thus capsule production.

Ms. Hauser's project will further explore the role of H_2O_2 using specific mutants of *S. pneumoniae*. Her work will involve genetics, biochemistry, proteomics, and animal studies. Her training will be extensive and diverse, providing her with a strong foundation for her future career. The findings from Ms. Hauser's studies could describe a novel mechanism of regulation that may be relevant for many pathogens, whether the H_2O_2 is produced by the bacterium or by the host. I expect her to have several high quality publications that will be of great significance to the field.

***Required coursework and laboratory rotations*.** As a student in the Microbiology Theme, Ms. Hauser has completed the required first year coursework and ONE OF THREE required advanced courses. The first year core curriculum required of all students consists of Biochemistry, Genetics, Cell Biology, Biostatistics, and Bioethics. The Microbiology Theme Core that follows those courses consists of Prokaryotic Genetics and Molecular Biology, Eukaryotic Genetics and Molecular Biology, Immunology, Virology, Microbial Pathogenesis, Structural Biology. The Theme core involves small classes (<10 students), close interactions between faculty and students, student presentations, class discussions, and problem-solving exams. Three advanced courses, taken in the second or subsequent years are also required. Ms. Hauser has COMPLETED Biology of Disease and will take subsequent courses relevant to her training. Course offerings vary from year-to-year and include Streptococcal Biology, Bacterial Physiology, Biology of Lung Diseases, Advanced Bacterial Genetics and Pathogenesis, and Immune Responses to Pathogens. Students can take classes offered by other themes and departments, thus providing a wide variety of courses to broaden their training.

In her first year, Ms. Hauser completed three 10-week lab rotations and presented a poster after each. In addition to my lab, Ms. Hauser rotated with Dr. Hui Wu (bacterial glycoproteins and biofilms) and Dr. Moon Nahm (bacterial pathogenesis and vaccines). The evaluations from each of her mentors and for her presentations were among the best of any first year student.

***Journal Club and Seminar Series*.** Since her second year, Ms. Hauser has participated in a weekly Bacterial Pathogenesis Journal Club, for which I am the faculty sponsor. Here, one student takes the lead in presenting a current paper, but each student is required to present a part of the paper, ensuring full participation. All students participate in the Microbiology Theme student seminar series that trains students in effective means of preparing and delivering research results. Students present their work to an audience of faculty and students at least once a year in this forum. An extended question session requires students to more critically evaluate their work and provides them with practice in handling questions from a diverse audience. Students receive both written and verbal feedback from faculty and other students. Ms. Hauser has presented her work TWICE, and has received excellent reviews as well as helpful feedback each time.

***Qualifying Exam, Admission to Candidacy, and Committee Meetings*.** At the beginning of Ms. Hauser's second year (fall 2011), she held the required preliminary meeting with two members of her dissertation committee and her mentor. The goal of this meeting was to review Ms. Hauser's progress to date and to ensure that she had begun to develop her research plan.

In the spring of her second year (2012), Ms. Hauser completed her qualifying exam. As part of this exam, she prepared a proposal on her research topic in the form of an NIH fellowship. The proposal was reviewed by three members of her dissertation committee (not including her mentor) and, following minor revisions, Ms. Hauser defended the proposal in an oral exam that required her to provide a presentation of the proposed work, including the hypothesis, approaches, and expected outcomes and alternative directions or interpretations. Questioning focused on the proposal as well as general knowledge that she was expected to have acquired to that point. Ms. Hauser passed the exam without stipulation, receiving high marks for her proposal and even higher marks for her oral defense.

In the fall of her third year (2012), Ms. Hauser became the first student in her class to complete her Admission to Candidacy. This step involved the first meeting with her full dissertation committee (four faculty plus her mentor), and involved in depth discussion of her scientific and academic progress, the future directions of her project, and specific details of her results. The members of Ms. Hauser's committee were selected based on their research and training expertise and relevance to Ms. Hauser's research. Specifically, the committee members are Dr. Kevin Dybvig (mycoplasma genetics and pathogenesis, Dept of Genetics), Dr. Hubert Tse (oxidative stress and type 1 diabetes, Dept of Microbiology), Dr. Hui Wu (bacterial glycoproteins and biofilms, Dept of Pediatric Dentistry), and Dr. William Benjamin (bacterial pathogenesis and clinical microbiology, Dept of Pathology). The committee will advise and guide Ms. Hauser through the remainder of her training. Subsequent to the Admission to Candidacy, committee meetings will occur approximately every SIX MONTHS until graduation.

Requirements for Graduation and Tracking of Progress. Following completion of all research and academic requirements, Ms. Hauser will write her dissertation, defend her work in a private defense attended only by her committee, and then present a final defense open to all. Students are expected to have at least two manuscripts published or in final preparation for publication prior to the defense. These manuscripts represent the major body of the dissertation, which also includes a scholarly introduction and overall discussion of the field and the significance of the present work on that field. The typical training program requires five to six years.

Ms. Hauser's progress will be tracked by the Microbiology Theme (of which the sponsor is the Director) and by the GBS. For each committee meeting, students submit an updated Biosketch, Career Plan, and written progress report detailing accomplishments, difficulties, and plans for the next 3 – 6 months. As graduation nears, timelines and specific goals become more defined and the committee ensures that the student begins to pursue postgraduate opportunities and fellowship applications. After each meeting, the committee completes a written report to be reviewed by the Microbiology Theme and GBS Steering Committees, who are tasked with ensuring that all students are making appropriate progress and receiving the guidance necessary to complete the requirements of the program. Strengths and weaknesses are discussed with Ms. Hauser during each meeting, and plans for her next steps are defined.

***Professional and Career Development*.** During the course of her training, Ms. Hauser will annually attend national and/or international meetings where she will present her work. In May 2013, Ms. Hauser will present both a poster and a talk at the American Society for Microbiology General Meeting in Denver, Colorado. These presentations will be based on the work described in this application. Following submission of her abstract, Ms. Hauser was awarded an ASM Student Travel Grant (given to 25 – 30% of applicants) and subsequently chosen to receive a Richard and Mary Finkelstein Student Travel Grant (given to six students presenting in the area of microbial pathogenesis). She will present a poster on her work in a regular session, and will present a short talk during the Division business meeting in conjunction with the Finkelstein Award. In addition, her abstract was selected as an Outstanding Student Poster (given to about 40 students across all divisions), and her poster will be displayed in a special section throughout the meeting. Ms. Hauser will also attend the day-long Career Development Workshop sponsored by ASM in conjunction with the meeting. This workshop allows students and postdoctoral fellows to meet scientists from varied career paths and learn about the opportunities available to microbiologists and the training necessary to pursue them.

In November 2013, Ms. Hauser will attend the National Institute of Allergy and Infectious Diseases (NIAID) "Bridging the Career Gap for Underrepresented Minority Scientists" workshop in Bethesda, Maryland. This meeting allows students to engage in scientific discourse, learn about opportunities for grant applications and career paths, and develop mentors with NIAID staff. Ms. Hauser is eligible to attend this

meeting because she is currently supported by an NIAID-sponsored Diversity Supplement to my R01 award. Past students in my lab who attended this conference found it a highly valuable experience that allowed them to develop networks and expand their horizons with regard to their careers.

Students from the GBS themes represent UAB at the ASM-sponsored Annual Biomedical Research Conference for Minority Students. Attended mainly by undergraduates, this conference allows them to hear presentations from leading scientists, present their own research, explore career opportunities, and learn about graduate programs. Our students serve as ambassadors to our program by presenting their research and providing information about graduate training at UAB. This conference serves an important role in recruiting students to UAB and in the career development of our student representatives. Previous students in my lab have served in this role, and Ms. Hauser is expected to be one of UAB's representative in 2013.

In addition to ASM, students in my lab regularly attend the FASEB-sponsored Summer Research Conference on Microbial Polysaccharides as well as other smaller meetings relevant to their research interests. These meetings will provide Ms. Hauser with the opportunity to present her research findings and to begin to meet and interact with leading researchers in the field. Ultimately, these interactions will help her identify future directions for her research and career, and possible mentors for postdoctoral training.

In conjunction with her studies, Ms. Hauser will participate in the NIH-funded four-day Metabolomics Workshop, which is led by our collaborator Dr. Stephen Barnes and held annually at UAB. Here, she will learn about experimental design, sample preparation, analytical methodologies, and data interpretation. She will also participate in the monthly "Lunch and Learn" series directed by Dr. Barnes that provides continuing updates on metabolomics and proteomics.

Locally, Ms. Hauser will participate in multiple research conferences. UAB holds an annual Graduate Student Research day where students across all disciplines present short talks. In the spring 2013 conference, Ms. Hauser received a Third Place award for her presentation. She has also presented her work in poster format at the UAB Department of Microbiology Annual Research Retreat, the Microbiology Theme Annual Student Conference, the GBS-wide Research Conference, and the UAB Pulmonary & Critical Care Medicine Research Conference. The Microbiology retreat is held out-of-town over two days, and students present their work via posters or short talks. A former student or postdoctoral fellow of the department returns as an invited speaker specifically to interact with current trainees in discussions of science and careers. The GBS-wide Conference and Microbiology Student conference are organized by students and involve one-day retreats for students to present their work as posters or short talks. The Pulmonary Conference brought leaders in the field of lung biology and disease for oral presentations, with students and postdoctoral fellows presenting posters. In addition, Pneumococcal Research Day occurs every other year and includes researchers from our large streptococcal community at UAB, as well as collaborators from other parts of the southeast. We also participate in a monthly meeting with other streptococcal groups at UAB, where students and postdoctoral fellows give oral presentations. Ms. Hauser will speak at this meeting once a year. Additionally, my laboratory holds a weekly lab meeting where each member presents a detailed update of their research on a rotating basis and everyone contributes to the discussions regarding interpretation, troubleshooting, and future directions. All lab members also provide a short weekly update of their research. Ms. Hauser participates fully in all of the activities discussed above.

Other career development activities include the UAB Graduate Career Awareness and Trends (GCAT) seminar series and workshops and the UAB Professional Development Program of the UAB Graduate School. The GCAT is run by graduate students for the purpose of introducing students to varied career opportunities through meetings with professional scientists. All of my students participate in this program, and past graduates of my lab (Dr, Matthew Bender, Principal Investigator, National Biodefense Analysis and Countermeasures Center; Dr. Karita Ambrose, Medical Science Liaison, Novartis Vaccines and Diagnostics) have returned as guest speakers. The UAB Professional Development Program provides courses (semester-long) and workshops (day-long) focusing on academic writing, professional speaking, and teaching. The courses and workshops are held on a recurring basis throughout the year. All of the offerings are available in traditional classroom settings and many are also offered online.

Mentorship. As noted above, the sponsor has mentored FIFTEEN students who have completed their Ph.D. degrees and continued on to postdoctoral and ultimately permanent positions. The sponsor's office is located in the laboratory and thus interactions occur on a daily basis, along with our weekly laboratory meeting. Ms. Hauser also has the benefit of daily mentorship from many faculty experienced in graduate student training. These faculty include those on her committee, others involved in related research who participate in the various lab meetings described above, and other faculty with broad interests who participate in the Microbiology Theme student seminar series, Microbiology Departmental retreat, other

seminar series and events. Throughout Ms. Hauser's training, we will focus on preparing her for the next stages of her career. As Ms. Hauser nears the end of her graduate training, the sponsor and other faulty members will help her in the identification and evaluation of possible postdoctoral opportunities.

Ms. Hauser will be mentored in the skills of scientific speaking and writing through the preparation of her research talks, dissertation proposal, committee reports, presentation abstracts, research manuscripts, and proposals for outside fellowships. In addition to potentially securing funding for her training, preparing fellowship applications will provide her with experience in the grant writing process that will be important in the next stages of her career. In addition to the F31 application, Ms. Hauser has also prepared a UNCF Merck Graduate Fellowship that is currently in review. In writing manuscripts and other documents, all trainees in my laboratory write the complete first drafts, and we then work together to polish the writing and the thinking relevant to the research for the final version. We are now in the process of developing Ms. Hauser's first manuscript. Similarly, we work to develop presentation skills through jointly polishing Ms. Hauser's initial slide preparation and then practicing the talk.

Environment. The UAB Microbiology Theme and Microbiology Department provide an interdisciplinary environment for students interested in the microbial sciences. Faculty in our program are from many different basic science and clinical departments. Most relevant for Ms. Hauser are faculty from the Microbiology, Pathology, Genetics, Biochemistry, Medicine, and Oral Biology Departments, as we have many collaborators in these groups. Research in my lab focuses on the genetics, biochemistry, and regulation of capsules and other virulence factors of *Streptococcus pneumoniae*. We regularly interact with other groups at UAB studying this organism (Drs. David Briles and Moon Nahm), as well the oral streptococci (Dr. Hui Wu), mycoplasmas (Dr. Kevin Dybvig), *Mycobacterium tuberculosis* (Dr. Michael Niederweis), *Bacillus anthracis* (Dr. Chuck Turnbough), and *Escherichia coli* (Drs. Chuck Turnbough, Pat Higgins). Research in these labs is focused on bacterial genetics, molecular biology, physiology, pathogenesis, vaccine development, regulation, and carbohydrate biochemistry. Our research is also enhanced by interactions with collaborators working on reactive oxygen species (Dr. Hubert Tse), mucosal immune responses (Dr. Sue Michalek), and metabolomics / proteomics (Dr. Stephen Barnes). We are located in close proximity to these labs (same floor, same building, or adjacent buildings), and there is a constant sense of interaction and co-mentoring among faculty and students. Day-to-day, there is regular sharing of reagents, equipment, and ideas that results in many collaborative projects.

In her daily interactions, Ms. Hauser is exposed to scientists at all levels of training – from undergraduates and technicians to graduate students, medical students, postdoctoral fellows, senior scientists, and faculty members. We have expertise in all areas relevant to Ms. Hauser's project, and we have access to a wide variety of resources both in my laboratory and in our collaborators' laboratories and core facilities at UAB. These resources include Gas Chromatography/Mass Spectroscopy, Dionex HPLC for carbohydrate analyses, all standard molecular biology and genetics tools, High Resolution Imaging, Mass Spectrometry and Proteomics, Flow Cytometry, DNA sequencing / genomics / microarrays, NMR, and animal facilities. For any of the core facilities that Ms. Hauser utilizes for her research, she will have the opportunity to gain hands-on experience with the instrumentation.

Departmental and program seminars occur weekly, along with several endowed seminars throughout the year. Each of these series brings in leaders of their fields. Students meet with the speakers, and are responsible for inviting and hosting several speakers each year.

In addition to the mentor, support for student training and activities is derived from the Department, the Graduate Programs, and the University. Among many efforts, the Graduate School provides first year student stipends and sponsors Graduate Student Research Days; the GBS program, the Microbiology Theme, and the Microbiology Department each support retreats; the Microbiology Department, the Graduate Student Association and multiple endowed programs support travel awards for students to attend meetings, courses, and workshops, and to travel to national and international laboratories for specialized training. Ms. Hauser has already benefited from these awards, and she will continue to compete for them.

***Relationship of training to applicant's goals*.** Ms. Hauser's research project will provide her with a broad training experience in the genetics and physiology of a bacterial pathogen. She will learn microbiological techniques for working with a pathogen, genetic and molecular techniques for manipulating *S. pneumoniae* and *E. coli*, immunologic analyses for detecting bacterial antigens, biochemical techniques for analyzing carbohydrates and enzymes, mass spectrometry and proteomic techniques for analyzing global responses, and methods for performing virulence studies in mice. The skills she learns will be applicable to essentially any area of science she wishes to pursue. Ms. Hauser comes from a teaching background, and one of her goals is to train young students in microbiology research. As she progresses in her own training,

she will help mentor new students as they enter the lab and thus gain experience in this area. In this regard, she has already been instrumental in mentoring an undergraduate student in the lab. Ms. Hauser's experience as a teacher and knowledge of her research area are evident in her interactions with this student, and it is clear that she will be an outstanding mentor and teacher of her own trainees in the future. In addition to this type of experience, my students frequently participate in UAB-sponsored programs to introduce area students and teachers to laboratory science through hands-on experiments and classroom experiences.

D. NUMBER OF FELLOWS/TRAINEES TO BE SUPERVISED DURING THE FELLOWSHIP

Throughout my career, I have averaged five predoctoral students in training. At this time, I am supervising THREE predoctoral students and ONE undergraduate honors student.

E. APPLICANT'S QUALIFICATIONS AND POTENTIAL FOR A RESEARCH CAREER

Ms. Hauser completed her undergraduate training at North Carolina Central University in Durham, North Carolina (B.S., Biology, 2000) and then became a science teacher at the middle school level. During this experience, she fully recognized her passion for laboratory science and the desire to further her education. She then returned to school, first to become a medical technologist specializing in clinical microbiology and then to pursue her masters degree in biomedical research at the University of Maryland at Baltimore (M.S., 2009). Her thesis project involved molecular DNA typing in phylogenetic analyses of *Aeromonas*. While in graduate school, she was exposed to many different scientists and research interests due to her location in the Baltimore area. These interactions led to her desire to continue her education and focus on the regulation of virulence factor expression in bacteria.

Ms. Hauser was recruited to UAB because of her strong and varied background in research, clinical microbiology, and teaching. It was evident from her record and from her interview that Ms. Hauser was determined to continue her education and to ultimately become an independent researcher. Her time at UAB has proven that she has the ability and commitment to do just that.

During her first year, Ms. Hauser excelled in her lab rotations and was doing well in her classes until the unexpected illness and sudden passing of her mother in spring 2011. She returned to Baltimore for a short time and ultimately received a C in the Virology course that was in progress. She retook this class the following year (spring 2012), earning an A. The short nature of the individual first year courses required Ms. Hauser to miss much of the subsequent course (Microbial Pathogenesis, spring 2011, Incomplete / Withdrawn), and she was allowed to postpone that class until the second year, when she received an A.

I mention these events in part to explain Ms. Hauser's grades, but more importantly to reflect on what they say about her. The progress Ms. Hauser made in her project and training in the months subsequent to her first year has been impressive for any student, but that it came while dealing with a personal loss is even more significant. Ms. Hauser could easily have returned home to be closer to her family, as she already had all the credentials needed to obtain any number of jobs. Instead, she returned to UAB where she remained determined to develop into a first rate scientist. During Ms. Hauser's first three months in my lab, we submitted and were subsequently awarded a Diversity Supplement to my NIH R01 project; hers was ranked one of the top applications. Since that time, Ms. Hauser has accomplished all of the things described above – she completed the two carryover first year classes and an advanced course, submitted the first version of this F31, passed her qualifying exam with no stipulations, was the first in her class to be admitted to Ph.D. candidacy, and submitted a UNCF Merck Fellowship and this application. Throughout, she proceeded with her research project, making tremendous progress since the original submission of this application, and presenting her work in multiple conferences. That work is now nearing publication stage and has been recognized with awards from ASM and the UAB Graduate School.

Ms. Hauser chose my lab for her dissertation work because of our strong research in bacterial genetics, physiology, regulation, pathogenesis, and biochemistry. She has taken advantage of the opportunities and has now taken the lead in her research project. She functions in a largely independent manner, both with respect to her research and activities such as this fellowship proposal and other written and oral communications. With her preparation for graduate training, her natural talent and inquisitiveness, and her dedication to continue to move forward with her career preparation even in the face of great personal adversity, Ms. Hauser easily ranks among the best of my students at this stage in her career. She is an excellent scientist and teacher, and one of the best communicators I have known. I have no doubt that she will develop a successful independent career and that she will use her talents to bring other minorities into science careers. Ms. Hauser is one of those students who makes you want to be involved in education and watch as she develops into the scientist and educator she is destined to become.