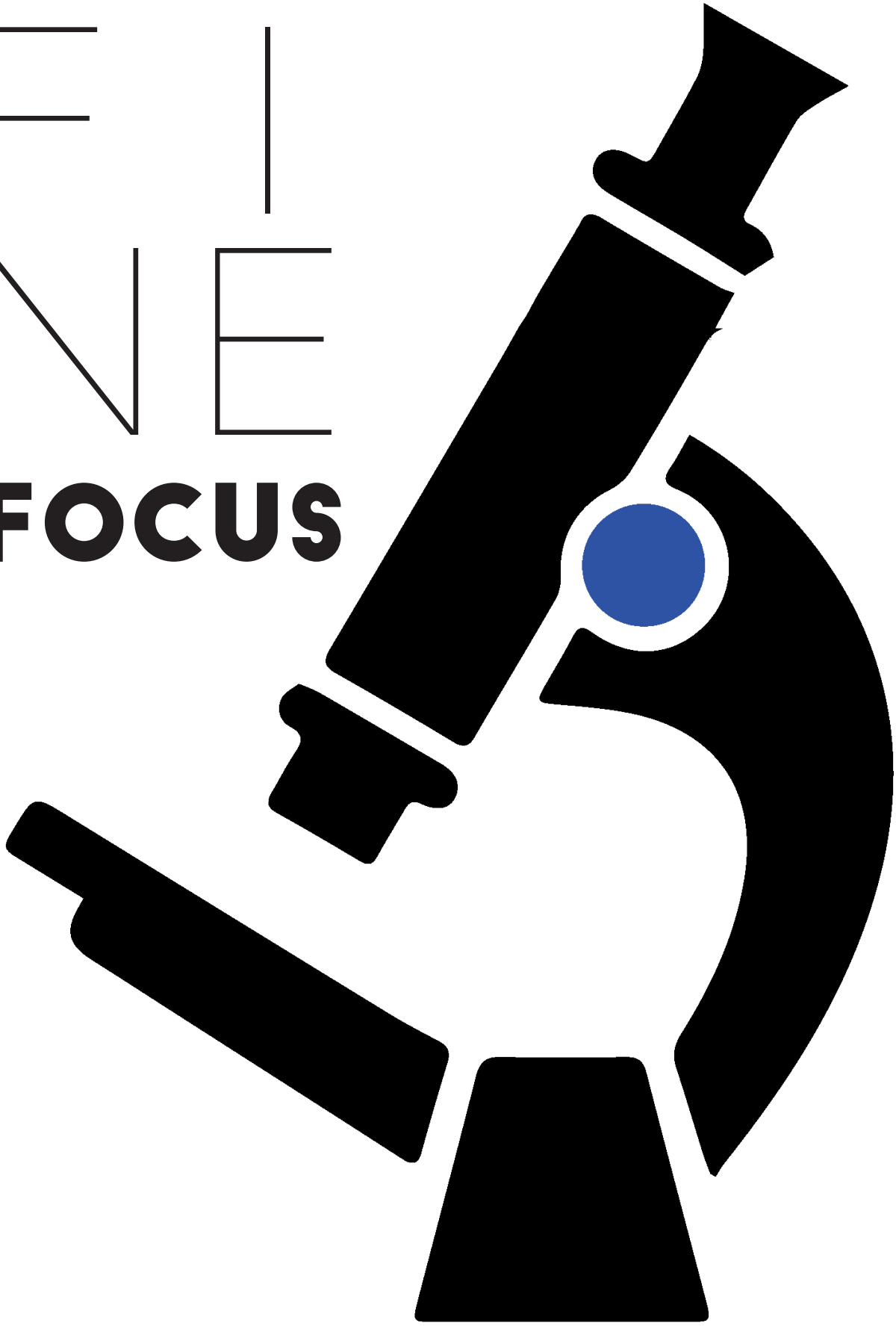
FINE FOCUS



An International Microbiology Journal for Undergraduate Research

MISSION

We publish original research by undergraduate students in microbiology. This includes works in all microbiological specialties and microbiology education.

SCOPE

We are an international journal dedicated to showcasing undergraduate research in all fields of microbiology. Fine Focus is managed entirely by undergraduate students from production to print, but utilizes and External Editorial Board of experts for double-blind peer review of manuscripts.

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OBJECTIVE LENS

Hannah DeWitt
Thesis Advisor: John L. McKillip

Ball State University, Muncie, Indiana, USA

INTRODUCTION

"This issue I have selected to use a metaanalysis by Hanna G. DeWitt (B.S., Biology, Ball State University, 2019) to provide a bit of a status report on our journal's impact and logistics to interested readers." -JLM

BENEFITS OF UNDERGRADUATE RESEARCH PARTICIPATION

The Council for Undergraduate Research (CUR) has been supporting undergraduate research initiatives since 1978 and supports over 900 colleges and universities in their endeavors and found that the benefits of undergraduate research are numerous (1). CUR published a book in 2009 entitled "Broadening Participation in Undergraduate Research: Fostering Excellence and Enhancing the Impact" in which they list twenty seven benefits in four categories: gains in knowledge and skills, academic achievement and educational attainment, fostering professional growth and achievement, and promoting personal growth (4). These benefits included a range of areas such as the increase in critical thinking and problem solving skills, enhanced oral and written communication skills, improved ability to apply knowledge from the classroom to practice, enhanced professional credentials, elevated confidence, higher graduation rates, and possibly one of the most important, higher rates of acceptance into and enrollment in graduate and professional schools. The Survey of Undergraduate Research Experiences (SURE) collected data on undergraduate researches and their progress over a 9-month period. They found that of the 1135 students that they surveyed (with a 75% response rate), over 87% responded that they had plans or had already begun further education in the sciences. An overwhelming response from students showed that their undergraduate research experiences were a positive influence in this decision with 29% stating that their decision to pursue a PhD was directly connected to the experiences of their research project (5). As students become more confident in their field of study they gain a better understanding of the potential work that might interest them in the future, leading them to continue their education to pursue a passion they might not have experienced without an undergraduate research opportunity. Having the opportunity to explore research in a safe, structured environment allows for exploration and personal discovery. Only 4.2% of students responded that their plans to pursue a postgraduate education had changed after their undergraduate research experience but even that information is valuable. This demonstrates that research experiences are key for students to be able to explore their field of study outside of the standard classroom structure to discover if research is the path they want to take. Learning that a passion for research exists is equally as important for a student as learning that research might not be the career path they want to take.

Publishing as an undergraduate can have lasting effects on future endeavors. Publishing research findings is a crucial part of expanding our understanding of the world, especially for those in the STEM (Science, Math, Engineering, and Technology) field. If a student does have the opportunity to publish work they have been conducting they gain a permanent example of their commitment to their research field. Publishing early can show graduate school and employers that a student has dedicated their spare time and effort into advancing their understanding of the scientific world to benefit the collective base of knowledge. This collaboration and show of commitment can enhance their professional credentials and establish a network of scientists that they can rely on for mentorship and further education (4). Students also become familiar with the process of scientific peer review and publication early. This can improve their understanding of what is needed to construct a solid, publishable article before it is a requirement of their education or career field. SRI International, a nonprofit scientific research institute, conducted a nationwide study of STEM undergraduate research opportunities with over 15,000 respondents. Their results showed that "(88%) of the respondents to the NSF follow up survey reported that their understanding of how to conduct a research project increased a fair amount or a great deal, 83% said their confidence in their research skills increased, and 73% said their awareness of what graduate school is like increased" as well as 29% reporting that they

had new expectations for pursuing a PhD (9). This increased confidence and awareness of scientific process elevates their potential success in future careers.

COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCES

What is CURE? CURE is defined as course-based undergraduate research experiences. They are designed to incorporate an entire classroom of students on a research topic within the course-work itself, allowing an increasing amount of students to participate in gaining experience working on research related to their field of study (2). Course-based opportunities allow institutions to provide research experience to underrepresented minorities, first generation, and at-risk students which has been found to improve retention rates in these groups significantly (7). According to the Course-Based Undergraduate Research Experience Network (CUREnet), an organization founded in 2012 to better understand the benefits and potential roadblocks to CUREs, there are five elements that must be present to enhance student learning: the use of scientific practices, discovery of knowledge previously unknown to the student and often the faculty member, work that was relevant or important to the field of study, collaboration amongst students and faculty, and iteration of the research conducted (3). CURE has been implemented across the country with positive results. The Genomics Education Partnership (GEP) has created a structure of nearly 100 universities in the United States to provide students a unique way to explore the field of genetic research. Students worked on sequencing the genome of the Drosophila, commonly known as the fruit fly. As a result of their work, more than 100 students and faculty have become coauthors on a paper that compares the F element of four species of fruit fly (8). CUREs can also come in the form of field research, such as is the case in a program started by the University of Minnesota which sought to provide students interested in ecology, animal behavior, and aquatic biology with an opportunity for hands-on work within a course-based curriculum (9). The five-week summer course resulted in positive feedback from the students and high-quality written reports that they will be able to use for future research and publication.

WHAT IS *FINE FOCUS*?

Scope: *Fine Focus* is a digital and print journal dedicated to showcasing the research of undergraduate students, internationally, in all fields of microbiology. *Fine Focus* is managed entirely by undergraduate students from production to print and coordinates double-blind peer reviews by our Editorial Board of experts from all subdisciplines of microbiology.

Mission: *Fine Focus* publishes original research by undergraduate students in microbiology. This includes works in all microbiological specialties and microbiology education. Research in other biology disciplines will not be accepted unless the main emphasis of the work centers on microorganism(s).

At a Glance: *Fine Focus* is comprised of 10-20 undergraduate Ball State University students under the direction of the faculty advisor, Dr. John McKillip. Each semester, the students serve as managing editors for the manuscripts that are submitted via the Open Journal System (OJS). The journal is managed entirely by undergraduate students from production to print, but utilizes an external editorial board of experts for double-blind peer review of manuscripts. Submission of manuscripts to *Fine Focus* is free and acceptance into the journal carries no page charges, indicating that it qualifies as an Open Access journal. Those who wish to read the journal or select manuscripts are able to receive free paper copies in the mail or access the material for free online at finefocus.org. Manuscripts that are published in *Fine Focus* are indexed and searchable through Cardinal Scholar, Ball State's online institutional repository for scholarly works contributed by students and faculty.

META-ANALYSIS

For this project, we focused on the data that has been collected on each manuscript submitted to *Fine Focus,* which included the author's names, what institution they were submitting from, the time between decisions, and a list of decisions that had been made for each manuscript. We also took time to look at the composition of each journal issue. This allowed us to dissect the information into quantifiable points that showed the progress of the journal. These points included: acceptance vs. rejection rate, international submission numbers, location of international submissions, and the composition of each journal (page numbers and manuscript type). The graphs below show the results of this analysis:

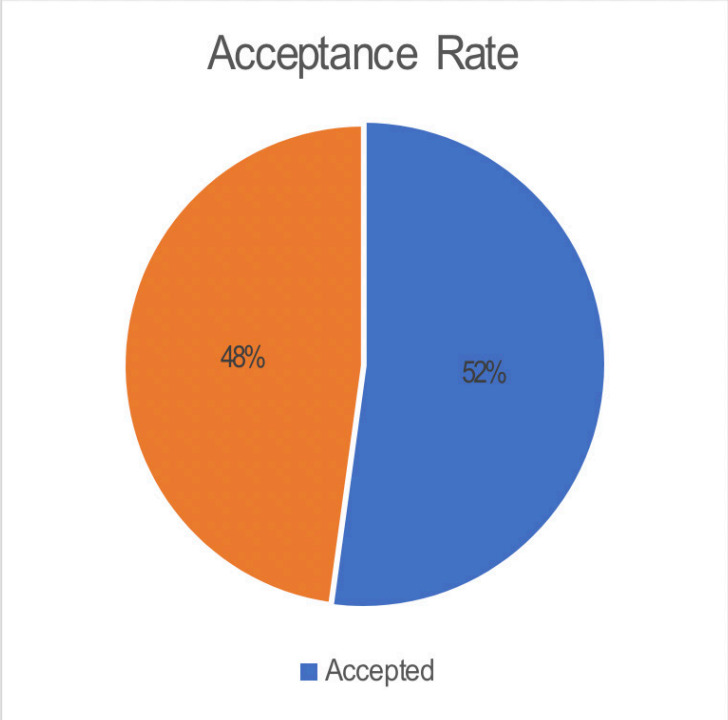

Figure 1: Acceptance rate of the *Fine Focus* journal from Issue 1 to current issue.

The acceptance rate was found to be 52%. There were no concerns with the current percentage. This number will be used to track future progress of the journal and can be used to market to potential authors who wish to publish.

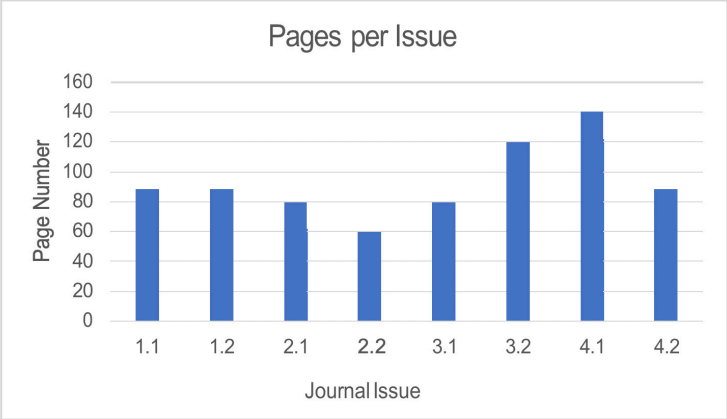

Figure 2: Number of pages for each issue of the journal.

The number of pages per issue have varied significantly from issue to issue. The average number of pages was roughly 94, with the lowest number of pages being issue 2.2 with 62 pages and the highest being issue 4.1 with 143 pages. Based on these numbers, we can suggest that if the journal were to move from publishing twice a year to just once a year, the number of pages would need to stay relatively high to ensure the quality and number of manuscripts is not sacrificed.

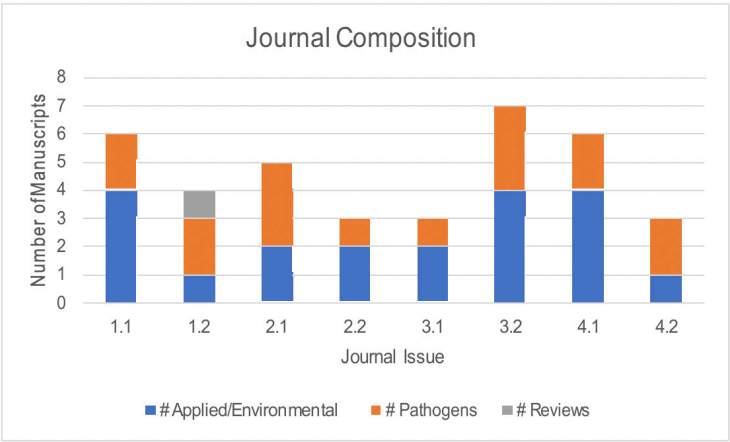

Figure 3: Composition of each journal issue based on type of manuscript published.

The graph above shows the composition of each journal based on the type of manuscript that was accepted and published in that issue. Manuscripts for *Fine Focus* tend to fall into two categories: applied/environmental and work conducted on pathogens. The journal also accepts reviews on topics related to microbiology as well as manuscripts on microbiology education. In the past, the journal has only accepted one review that

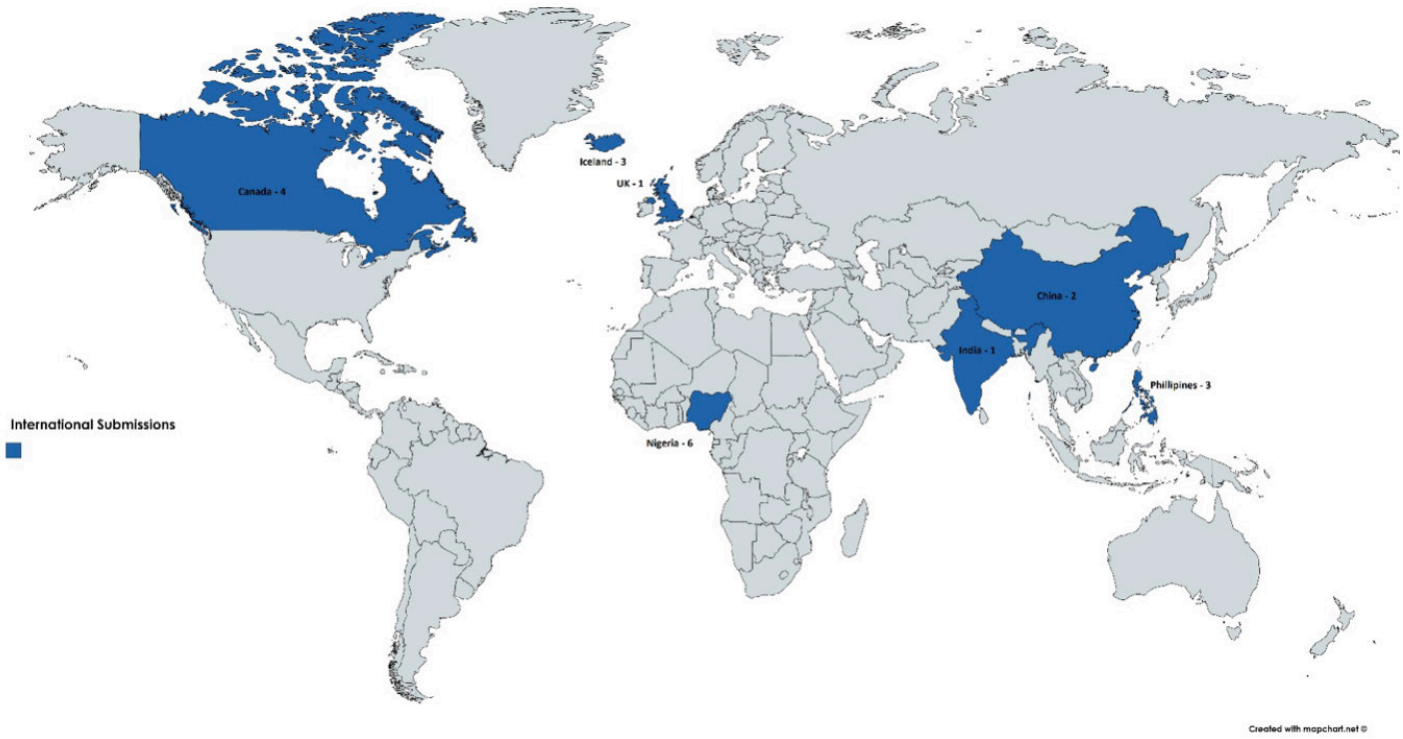

Figure 5: Map showing the location of each international submission received by the journal.

matched the standards of the journal. The recent issue showed a decrease in manuscripts published in the applied/environmental category. In the future, the journal hopes to increase this number by focusing efforts to encourage students and institutions that are working on research in this category to publish their work in the journal.

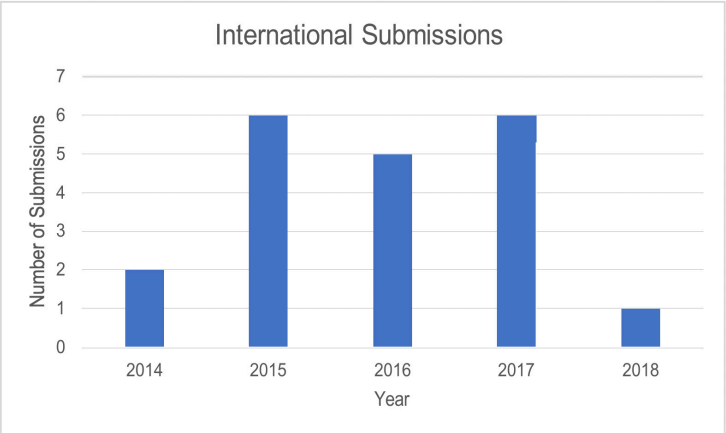

Figure 4: Number of international submissions by year.

Fine Focus is an international journal that not only has external reviewers from nine countries but features manuscripts from all over the world. Recently, we have seen a sharp decrease in international submissions with the reason being unknown. The class of students that manage the journal have begun a program called CAP, the Campus Ambassador Program, that is seeking to establish satellite groups of students at other universities that would encourage their fellow students and faculty to submit their research to *Fine Focus*.

The map above shows the distribution of international submissions that have been received by the journal from 2014-2018. The hope is to further the reach of the journal and encourage more countries to submit their research. To reach seven countries in four years is still significant and shows that the journal is a viable choice when undergraduates from across the world want an established journal to showcase their work.

Name	Discipline	Free to publish?	Free to access?	Indexed and Searchable?	Undergrads involved in review?
Advanced Journal of Graduate Research	STEM	✓	✓	✓	-
AnthroJournal	Anthropology	✓	✓	✓	
Berkeley Scientific Journal	STEM	-	✓	✓	✓
Bios+2:25	Biology	✓		✓	
Caltech Undergraduate Research Journal	STEM	-	✓		✓
Catalyst: Rice Undergraduate Science & Engineering Review	STEM	✓	✓		✓
Columbia Undergraduate Science Journal (CUSJ)	STEM	✓	✓	✓	✓
Darthmouth Undergraduate Journal of Science	STEM	✓	✓	✓	✓
DePaul Discoveries	Science and Health	✓	✓	✓	✓
Eastern Oregon University Science Journal	STEM	-	✓		
EvoS	Evolution	✓	✓	✓	✓
Illumin	STEM	-	✓		✓
Impulse	Neuroscience	✓	✓	✓	✓
International Journal for Undergraduate Research - Science, Engineering & Technology (IJUR-SET)	STEM		✓	✓	

International Journal of Exercise Science	Exercise Science	-	✓	✓	✓
Involve - A Journal of Mathematics	Mathematics	-	✓	✓	✓
Journal of Experimental Microbiology and Immunology (JEMI)	Microbiology, Immunology	-	✓		✓
Journal of Science and Health at The University of Alabama (JOSHUA)	STEM	✓	✓		✓
Journal of Undergraduate Chemistry Research	Chemistry		✓	✓	-
Journal of Undergraduate Research in Physics	Physics	-	✓		
Journal of Undergraduate Sciences	STEM		✓		✓
Journal of Young Investigators	STEM	-	✓	✓	✓
MarSci	Marine and Aquatic Science	-	✓		✓
Missouri Journal of Mathematical Science	Mathematics		✓	✓	
MIT Undergraduate Research Journal	STEM		✓		
National Undergraduate Research Clearinghouse	STEM		✓		
Penn Bioethics Journal	Bioethics	-	✓		✓

PUMP Journal of Undergraduate Research	Mathematics		✓	✓	
Rose-Hulman Undergraduate Mathematics Journal	Mathematics		✓		✓
RURALS: Review of Undergraduate Research in Agricultural and Life Sciences	Agriculture, Life Sciences	-	✓	✓	
Saltman Quarterly	STEM		✓		✓
Scientia	STEM		✓		
SPORA - A Journal of Biomathematics	Biology, Mathematics		✓	✓	
The Catalyst	Bioengineering, Biotechnology				✓
The Graduate Journal of Mathematics	Mathematics			-	

The chart above shows information on other undergraduate research journals in the STEM fields. All the criteria is based on elements that *Fine Focus* is able to offer to its authors and readers. Checkmarks represent characteristics which the journal shares with *Fine Focus* while empty spaces represent unshared characteristics. A dash (-) represents information that was unable to be obtained from the websites of each of the journals. Most information was collected directly from the respective websites that each journal maintained as emails requesting clarifications were mostly left without answer. The chart demonstrates how *Fine Focus* fits within the established realm of undergraduate research journals and can help potential authors understand what the journal has to offer.

THE FUTURE

The future of *Fine Focus* is vibrant and powerful. *Fine Focus* was the first international journal specifically for undergraduates that wish to publish work related to microbiology. According to the Bureau of Labor Statistics, demand for microbiologists will increase by 8% between now and 2026 (11). With the growing demand for hardworking and dedicated researchers, publishing early and often has never been more important. As the journal continues to grow and develop, we hope to see an increase in submission overall as well as those from diverse countries. The class structure of the journal allows for new students to experience the publishing process, gaining a perspective on what happens to a manuscript that has been submitted for review. Not only are students able to work together to achieve a common goal of managing the journal from start to finish, they are able to gain a better understanding of what it will take to have their own research published in the future. The hope for this research is to inform the students that manage the journal semester to semester, as well as the Executive Committee, on how the journal should proceed in the future.

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PEER MENTORING: THE MISSING PIECE IN GRADUATE PROFESSIONAL DEVELOPMENT

Kateri R. Salk[1]
LeighAnn Tomaswick[2]
Allison R. Rober[3*]



[1] Nicholas School of the Environment, Duke University, Durham, North Carolina
[2] Center for Teaching and Learning, Kent State University, Kent, Ohio
[3] Department of Biology, Ball State University, Muncie, Indiana

CORRESPONDING AUTHORS

Allison R. Rober
Department of Biology,
Ball State University, Muncie, IN, 47306.
arrober@bsu.edu

KEYWORDS

- *peer mentoring*
- mentor
- mentee
- graduate
- professional development
- STEM
- future faculty

ABSTRACT

Many academic institutions offer professional development programs to prepare graduate students to meet the changing expectations of science, technology, engineering, and mathematics (STEM) faculty. Peer mentoring is not widely adopted in graduate professional development, yet incorporating this approach can better facilitate the transition from graduate student to faculty member. Using evidence from experience as peer mentors (2011–2017), we examine established characteristics of peer mentoring and evaluate their strengths in the context of a future faculty professional development program. Peer mentors coached mentees by sharing common experiences related to teaching and learning, provided a safe space for mentees to discuss their experiences, and acted as a liaison between mentees and faculty advisors. These benefits translate into increased competency for future faculty to engage in research, teaching, and mentoring.

A call for improved graduate professional development

Recognition that future faculty preparation is insufficient to meet the changing expectations of education has led to a re-envisioning of professional development programs for graduate students aiming at careers in academia (Austin 2002; Cooper et al. 2015). The focus of many existing professional development programs is preparing future faculty to implement and advance effective teaching practices (Pfund et al. 2009). However, few of these programs emphasize mentoring in their goals (Wulff and Austin 2004; Ebert-May et al. 2011). Given that new faculty are expected to fulfill diverse roles that include mentoring as well as teaching and research (Austin 2002), it is essential that professional development programs provide opportunities for future faculty to develop mentoring skills (Schussler et al. 2015).

Many professional development programs employ leadership structures led by faculty advisors, which are an integral part of graduate education (Grant-Vallone and Ensher 2000). Traditional mentoring is generally characterized by a one-way flow of expertise and skills from mentor to mentee (McManus and Russell 2008). Alternatively, peer mentoring is by nature a reciprocal relationship and thus offers the potential for additional mentorship owing to a similar hierarchical level, complementary knowledge and skills, and reciprocity between peer mentors and mentees (McDougall and Beattie 1997; Holland et al. 2012). For peer mentors, immersion in the academic process allows further development as independent scholars (Campa et al. 2000).

Including peer mentoring relationships in professional development infrastructure can provide unique benefits to mentees and peer mentors. To demonstrate these benefits, we examine the established characteristics of peer mentoring and evaluate their strengths in the context of future faculty professional development. Evidence for the efficacy of peer mentoring in future faculty preparation is provided via reflections collected from mentees and peer mentors (i.e., the authors), who are now early-career faculty and instructional consultants contributing to teaching and learning at academic institutions.

FAST: A model professional development program

The Future Academic Scholars in Teaching (FAST) program is a year-long high engagement professional development program that complements the efforts of the Center for the Integration of Research, Teaching and Learning (CIRTL) network (www.cirtl.net). The FAST program has been in existence for 13 years at Michigan State University. Throughout the FAST program, a selected cohort of 10-14 STEM graduate students participated in workshops on pedagogy and instructional design, sessions on professional development for academic careers, and completed a teaching-as-research project in a course within their discipline (Vergara et al. 2014). Participants are mentored by faculty advisors and a graduate student that has previously completed the program (the peer mentor). The peer mentor acts as a liaison between the faculty advisors and mentees and leads a reading group attended only by peer mentors and mentees, that serves as a time to discuss literature on teaching and learning, and to address successes and challenges in teaching-as-research project development.

Throughout the program, peer mentors gain practice in building a supportive learning community, providing constructive criticism, and tailoring mentoring to the specific instrumental (i.e., logistical and academic) and psychosocial (i.e., emotional and interpersonal) support needed by individual mentees. As a result, peer mentoring benefits the mentees as well as the peer mentors themselves (Figure 1). These gains contribute to peer mentors' development as future STEM professionals, particularly as the skills gained through peer mentoring are not often included in formal graduate education (Schussler et al. 2015).

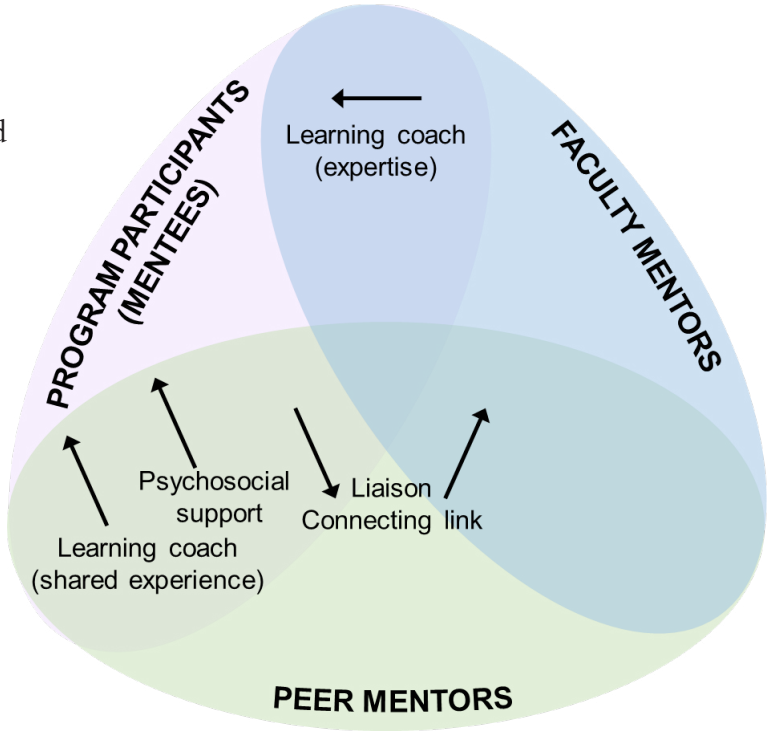

Figure 1. Diagram illustrating the roles and relationships between peer mentors, mentees, and faculty mentors in future faculty professional development.

Benefits of peer mentoring for the mentee

Peer mentoring relationships provide psychosocial support that traditional mentoring relationships are unlikely to provide (Table 1; Grant-Vallone and Ensher 2000). Research on peer mentoring has shown that mentees are more likely to display vulnerability when interacting with peer mentors than with traditional mentors (McManus and Russell 2008). The FAST reading group, attended only by mentees and the peer mentor, provided a space in which mentees were comfortable displaying vulnerability and taking risks (Table 1). Interviews of past program participants have indicated a larger degree of comfort sharing insecurities compared to meetings when faculty advisors were present. For instance, one participant reflected:

> *It's kind of less pressure to just say how you're feeling about things to group of your peers… one of the more impactful parts of the [reading group] was just having a forum, a venue to talk about your experiences with people who are experiencing similar things, or have experienced similar things.*

As both mentees and peer mentor are new to the scholarship of teaching and learning, there is reduced fear of judgment when communicating challenges of their experience (Colvin and Ashman 2010). The peer mentor has often encountered and resolved similar challenges either personally or in their interactions with other participants. The empathy inherent in this relationship encourages mentees to display vulnerability and in turn allows the peer mentor to provide encouragement and shared experience (Table 1). Evidence that peer mentoring can increase the confidence of mentees is demonstrated by a participant that noted:

> *[The peer mentor] definitely provided that… I personally need that kind of encouragement to know, 'Okay, you're seeing what I'm doing. What I'm doing is okay.' Otherwise I start to second-guess myself.*

The vulnerability and empathy displayed between peer mentors and mentees gives rise to several instrumental support roles (Table 1; Colvin and Ashman 2010). By working closely with program participants in a comfortable setting, the peer mentor can monitor mentees' progress closely and relay challenges to faculty mentors. In this way, the peer mentor acts as a liaison between the two groups (Figure 1). The faculty mentoring team can then resolve issues on an individual basis or shift the focus of future group meetings to address common challenges. Small hierarchical differences between the peer mentor and mentee allows the peer mentor to fill a unique role as a learning coach (Colvin and Ashman 2010). While traditional mentoring by faculty advisors provides a learning coach role through the sharing of expertise, the peer mentor's role as a learning coach is more commonly embodied by sharing recent experience (Figure 1). For instance, the peer mentor in the FAST program has often observed a specific strategy for success through interactions with their own cohort of participants and can help mentees overcome a problem by using this strategy in a similar context. However, it is common for mentees to encounter issues that are outside the expertise of the peer mentor. In these cases, the peer mentor can act as a connecting link with faculty advisors (Sanft et al. 2008). This role often involves consulting with a faculty advisor with relevant expertise who then works with the mentee to address topics such as assessment of student learning, innovative instructional techniques, experimental design, or data analysis.

Benefits of peer mentoring for the mentor

Given the increasingly interdisciplinary nature of education and research in academia (Adams 2007), developing skills to engage diverse audiences in a common dialogue is essential. Serving as peer mentors in the FAST program contributed to our ability and confidence to work with a diverse academic community by providing a psychologically safe environment where the fear of misguidance would not have career-ending consequences (McManus and Russell 2008). Opportunities for us to explore our own developmental needs and having nonjudgmental and supportive feedback was instrumental in facilitating our growth as mentors. The expectations for us as peer mentors were clearly outlined at the onset of the mentoring relationship, which was a crucial component in our development of self-efficacy (Hall et al. 2008). Further, positive reinforcement provided by both mentees and faculty advisors for our willingness to pass along information that we had learned to others was personally rewarding (sensu McManus and Russell 2008, Colvin and Ashman 2010). Having a safe space for peer mentoring to take place helped to foster both professional and personal empathy for our mentees. The ability to engage in dialogue with a diverse group of peers and express empathy was significant given that survey responses from mentees suggest that they found our peer-mentoring relationship to be more valuable for psychosocial support than technical or logistical support related to teaching and learning. These findings are consistent with empirical research on peer mentoring which have shown that psychosocial support in peer-mentoring relationships is a unique characteristic that distinguishes them from traditional mentoring (Kram and Isabella 1985, Grant-Valione and Ensher 2000). Collectively, these experiences have contributed to our ability to bridge differing perspectives (e.g., vocabulary, theoretical framework) and work toward a common goal, a skill that has been critical in the transition from graduate student to professionals.

As peer mentors, we gained a depth of knowledge related to teaching and learning, leadership skills to facilitate instructional improvements, and experience working with university administrators on programmatic priorities. Through this process, we not only became better equipped to contribute to the curricular goals of our future institutions but also to work across multiple administrative levels to act as agents of change (sensu Healey 2012). As a

result, we have been able to transfer many of these skills to a variety of research and education settings, such as advising teaching assistants and mentoring undergraduate and graduate researchers.

Table 1. Peer mentoring offers unique benefits to professional development programs that supplement traditional mentoring by faculty. Experiences from the Future Academic Scholars in Teaching (FAST) program provide evidence to support for the inclusion of peer mentoring as part of future faculty professional development.

Unique attributes of peer mentoring	Evidence from peer mentoring experience
Psychosocial support	
Vulnerability: *Mentees trust their concerns, doubts, and weaknesses will be met in a supportive and non-judgmental way by the peer mentor*[a,b]	Mentees during and after the FAST program commonly ask for advice about conducting teaching-as-research while also maintaining a productive disciplinary research program as well as work-life balance. Given that peer mentors are fellow graduate students who have recently completed the program, the similar hierarchical level and shared experiences helped to facilitate this exchange of information.
Empathy: *Hierarchical similarities give rise to mutual understanding*[a]	Having gone through the program previously and encountering similar challenges, peer mentors display empathy and understanding toward program participants.
Instrumental support	
Liaison: *Mentees share experiences with the peer mentor, who then relays information to traditional mentors from the perspective of the mentees*[a]	As the leader of the reading group, the peer mentor was able to monitor program participants' progress closely. In meetings with the steering committee, the peer mentor acted as a liaison by communicating challenges with traditional mentors and devising a plan together for continued progress.
Learning coach: *Peer mentors teach academic and life skills to mentees, often in the setting of one-step-ahead expertise sharing*[a]	In encountering the need to balance expectations of teaching, research, and mentoring in the FAST program, peer mentors have been able to share these experiences and act as a learning coach in other situations such as conferences and programs for early-career faculty.
Connecting link: *Peer mentors direct mentees toward relevant resources needed to complete a task*[c]	In several situations, mentees experienced challenges outside the expertise of the peer mentor. To fill this gap, the peer mentor connected program participants with faculty members who have the relevant expertise. The role of connecting link has proven useful for peer mentors as they use this expertise-gathering strategy in research and job search settings.

[a]McManus and Russell 2008, [b]Colvin and Ashman 2010, [c]Sanft et al. 2008

CONCLUSION

Mentoring is a critical skill in academia, and peer mentoring in the FAST program provided a formal opportunity to train as a mentor that would not be available in most graduate programs (Schussler et al. 2015). The unique role and support structure demonstrated by peer mentors in the FAST program represents a model that is transferable to a wide range of graduate professional development programs across disciplinary and institutional boundaries. Given increasing recognition for the need to prepare future faculty as independent teacher-scholars (Cooper et al. 2015) and the potential for peer mentoring opportunities to facilitate the transition from mentee to mentor, we advocate that formal peer mentoring opportunities should be built into existing professional development frameworks.

ACKNOWLEDGMENTS

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HOW CRISPR-MEDIATED GENOME EDITING IS AFFECTING UNDERGRADUATE BIOLOGY EDUCATION

Ethan S. Pickerill[1*]
Caleb M. Ebree[2*]
Ben A. Evans[1]
Elena R. North[1]
Gennifer M. Mager[1]
Douglas A. Bernstein[1]

[1] Department of Biology, Ball State University, Muncie, IN 47306 USA
[2] Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210 USA

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CORRESPONDING AUTHORS

Douglas A. Bernstein
dabernstein@bsu.edu
Tel: (765)-285-8851

* Authors contributed equally to this work

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ABSTRACT

In 2010, the CRISPR/Cas system of *Streptococcus thermophilus* was found necessary and sufficient to cleave bacteriophage DNA. Since this time, CRISPR went from a niche scientific field to the laboratories of major research institutions, undergraduate classrooms, and popular culture. In the future, CRISPR may stand along with PCR, DNA sequencing, and transformation as paradigm shifting discoveries in molecular biology. CRISPR genome editing is technically uncomplicated and relatively inexpensive. Thus, CRISPR-mediated genome editing has been adopted by and applied to undergraduate curricula in a wide variety of ways. In this review, we provide an overview of CRISPR-mediated genome editing and examine some of the ways this technology is being leveraged to train students in the classroom and laboratory.

CRISPR PROTECTS BACTERIA FROM VIRUSES

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are short DNA repeats found in prokaryotic genomes (1). These repeats are interspersed with non-repetitive short sequences homologous to bacteriophage DNA (Figure 1) (2). The discovery that the interspersed elements possess homology to bacteriophage genomes lead investigators to propose that these short viral elements provide bacteria with immunity against invading viruses. Indeed, CRISPR sequences were found to mediate bacteriophage resistance and investigators identified CRISPR-associated genes (Cas genes) that were also important for this viral immunity (3, 4). Bacteria can encode a wide variety of Cas proteins which play diverse functions in bacteriophage DNA cleavage reviewed in (5). Briefly, 20 nucleotide CRISPR-RNA (crRNA) guides transcribed from the spacer regions inhibit bacteriophage infection by targeting a Cas nuclease to the viral DNA using complementary base pairing. Some CRISPR-mediated viral cleavage systems also require a trans-activating CRISPR-RNA (tracrRNA) for maturation of crRNA (6). In addition, cleavage of viral genome also requires a short, 2-6 nucleotide, Protospacer Adjacent Motif (PAM) in the viral sequence (7-9). The dual requirement of the 20 base guide and adjacent PAM sequence provides a high degree of specificity limiting off-target or bacterial genome cleavage.

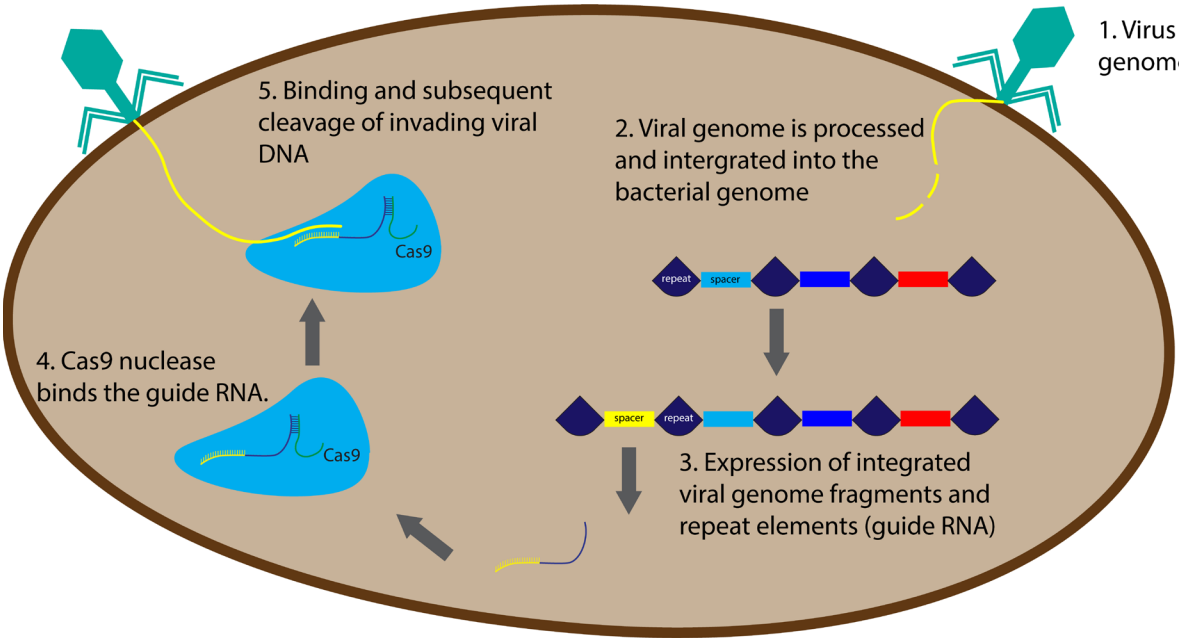


Figure 1. CRISPR is used by bacteria to cleave viral DNA. crRNA is yellow and navy blue. tracrRNA is in green.

CRISPR-MEDIATED GENOME EDITING

The discovery that crRNA guides, in coordination with Cas nuclease mediate viral genome cleavage in bacteria provided an elegant method by which bacteria defend themselves from viral invasion. This discovery however begged the question, could this system cleave DNA *in vitro* or in other organisms? Investigators determined Cas9, crRNA, and tracrRNA were sufficient to cleave plasmid DNA *in vitro* in a sequence specific manner (8). Building upon these results, multiple investigators tested and found Cas9, crRNA, and tracrRNA could cleave DNA *in vivo* in organisms other than bacteria including mammalian cells (10-12). This technique is now commonly referred to as CRISPR-mediated genome cleavage or merely CRISPR.

For CRISPR-mediated genome cleavage to occur, a guide RNA must target a Cas9 nuclease to a DNA sequence of interest. However, once cleavage occurs it is critical that the host cell's DNA repair machinery efficiently repairs the DNA break or the cell will die. DNA breaks are repaired by two distinct mechanisms. Nonhomologous End Joining (NHEJ), reviewed in (13), requires that the cleaved ends of the DNA are trimmed (Figure 2). The ends are then joined, resulting in deletion of the trimmed sequence. Such deletions can shift the reading frame causing premature translation termination. Homologous Recombination (HR), reviewed in (14), is another mechanism used to repair Cas9-mediated cleavage. During HR cells use a homologous DNA sequence to repair the cut site. DNA encoding a desired genome edit, the repair template, is cotransformed with the Cas9 nuclease and guide RNA. When the cell uses the repair template to fix the cleaved DNA, changes encoded by repair template are introduced at the site of cleavage (Figure 2). Thus by designing the sequence of the repair template, the investigator can precisely edit the genome, introducing desired point mutations, insertions, or deletions. Such precise changes are commonly referred to as CRISPR- mediated genome editing.

A number of nucleases can be used to perform genome cleavage, and these nucleases have distinct requirements. For instance, Cas9 nuclease requires a tracrRNA as well as guide RNA to mediate cleavage (6, 9), while Cpf1, another Cas nuclease, does not require tracrRNA (15). Cas9 and Cpf1 also use distinct PAM sequences and cut at different lengths away from their PAM sequence. In addition to genome editing, Cas9 can be used to alter gene expression by creating a gene fusion a transcriptional activator or repressor to catalytically active Cas9 and targeting these complexes to the gene of interest (16). Furthermore, inactive Cas9 nuclease has been used to fluorescently label the genomic loci enabling investigation of the 3D architecture of the nucleus (17). These discoveries have fundamentally changed the field of biology allowing researchers unprecedented flexibility to manipulate an organism's genome and transcriptome.

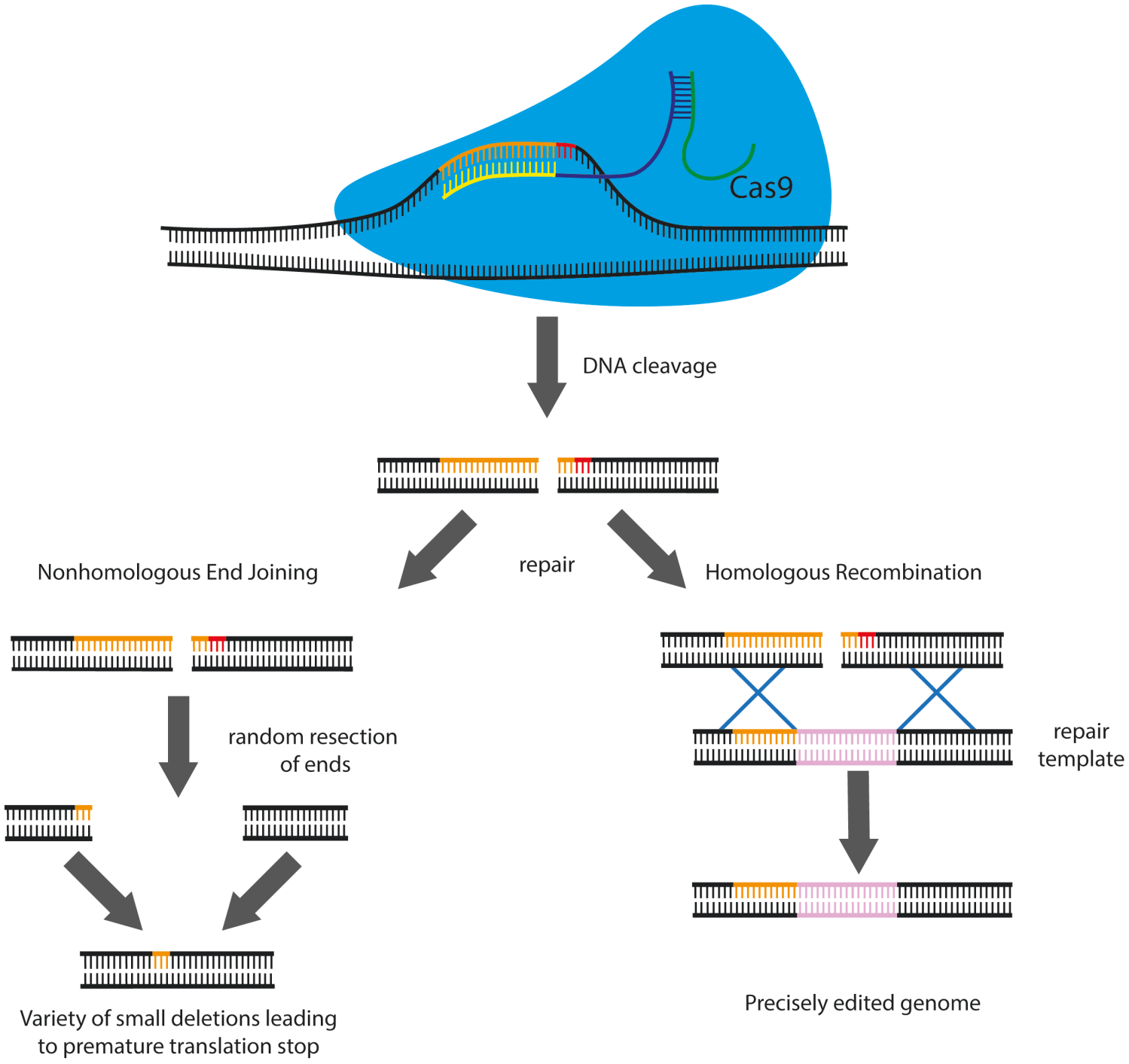


Figure 2. DNA cleaved by Cas9 is repaired by either nonhomologous end joining or homologous recombination. PAM site is red.

CRISPR MEDIATED GENOME EDITING BECOMES WIDESPREAD

The groundbreaking realization that CRISPR genome editing was broadly applicable has since lead to the development of CRISPR systems in numerous organisms. For instance, in the fungal kingdom, CRISPR-mediated genome editing systems have been established for traditional yeast model systems such as *Schizosaccharomyces pombe* (18) and *Saccharomyces cerevisiae* (19), as well as non-conventional yeast *Candida glabrata* (20), *Candida albicans* (21), Yarrowia lipolytica (22), *Kluyveromyces lactis* (23), *Pichia pastoris* (24), *Naumovozyma castellii* (25), *Ogataea thermomethanolica* (26), *Candida lusitaniae* (27), Candida parapsilosis (28), *Cordyceps militaris* (29), *Cryptococcus neoformans* (30), *Shefferomyces stipites* (31), and *Aspergillus fumigatus* (32) as well as others. In addition, CRISPR-mediated editing systems have been developed for many mammalian (33) and plant model systems (34), as well as other organisms whose genomes have historically been challenging to manipulate (21, 35). This expansion of the use of CRISPR is similarly demonstrated by online mentions. In 2004 a keyword search for CRISPR of the PubMed database would have retrieved under ten papers. In 2017 alone, over 3000 papers with the key word CRISPR were deposited and indexed on PubMed (Figure 3). Google searches for CRISPR have had a similar meteoric rise in popularity over the same time period (Figure 3) and this rise in popularity has led to articles and features in popular media (36, 37).

CRISPR's appeal is in part due to the relative ease by which it can be applied to a wide variety of biological systems. Such promiscuity gives CRISPR-mediated genome editing the potential to effect human well-being in a multitude of ways (38). Genome engineering in agriculture has the potential to increase yields and develop more nutritional food products (39). Gene drive technologies that use CRISPR Cas9 to spread genes in wild arthropod populations could fundamentally alter crop management strategies and reduce pesticide use (40). Similar gene drive strategies could be employed to limit the spread of diseases such as malaria (41). However the release of genetically modified organisms to the wild is controversial and has the potential to cause unforeseen consequences. Beyond regulating the spread of disease, increased genetic engineering efficiency via CRISPR enables investigators to better model diseases *in vivo* (42-44). Unlike transplant or *in vitro* experiments, CRISPR-Cas9 can be used to introduce a specific genotype. This is ideal for studying disease progression and variability, as such studies more closely maintain tissue microenvironments and can be performed in fully immunocompetent animals (45). Genome editing systems in human pathogens such as Trypanosoma brucei (46, 47) or C. albicans (21) may enable researchers to identify new therapeutic targets. Collectively, these models have the power to open doors to new therapies and a better understanding of human health. Finally, CRISPR-mediated genome editing could be used to alter the human genome to improve health and change phenotype (48). While CRISPR-mediated genome editing has the potential to improve human health in a variety of ways many such applications are provocative and controversial. The scientific community and public must wrestle with not only the technical but ethical challenges presented by such avenues of investigation (49).

HOW CRISPR IS BEING APPLIED TO UNDERGRADUATE EDUCATION

These potential applications can bring about significant technical challenges and require students balance complex ethical considerations as well. As such, it is imperative students are exposed to genome editing technology during their undergraduate education. This exposure will provide students the best opportunity to efficiently and responsibly apply CRISPR technology to important research problems. These experiences can come in a variety of forms (50).

Figure 3. The increase in CRISPR related academic and social materials over time. **A.** CRISPR publications indexed on PubMed. Number of publications submitted to PubMed indexed with the key word CRISPR by year. **B.** CRISPR Google search trends. Google search frequency from 2004 to 2017. Each data point is divided by the total searches of the geography and time range it represents to compare relative popularity. Time period with the highest search frequency will score 100, while the time period with the lowest frequency of searches will score 1.

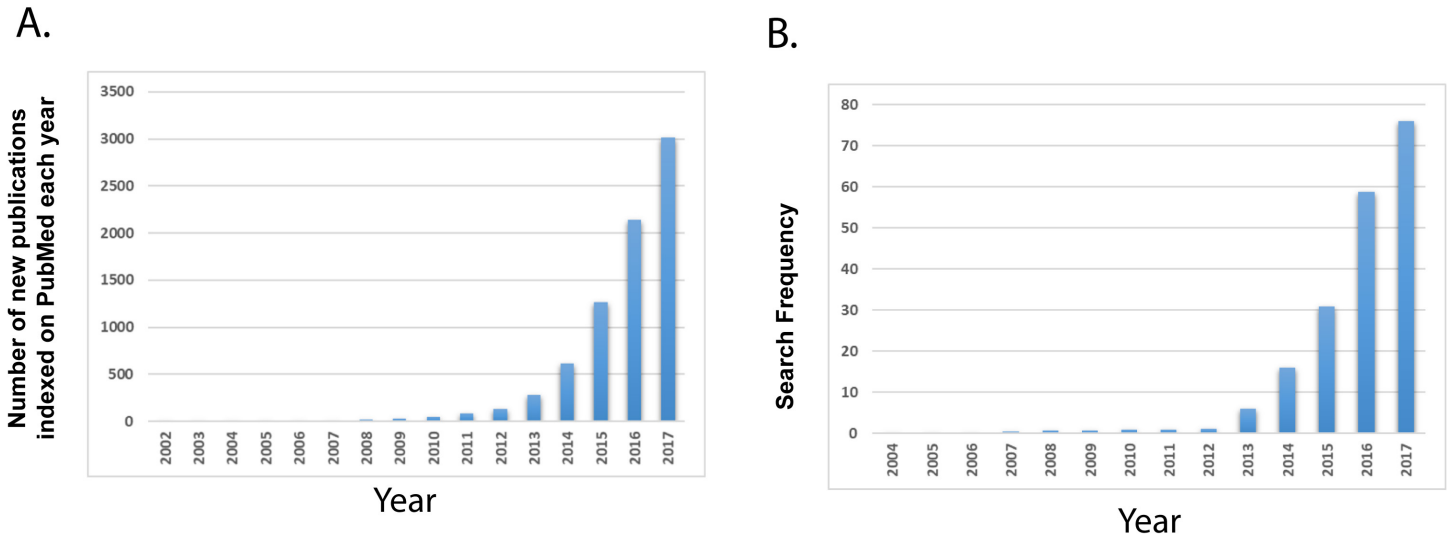
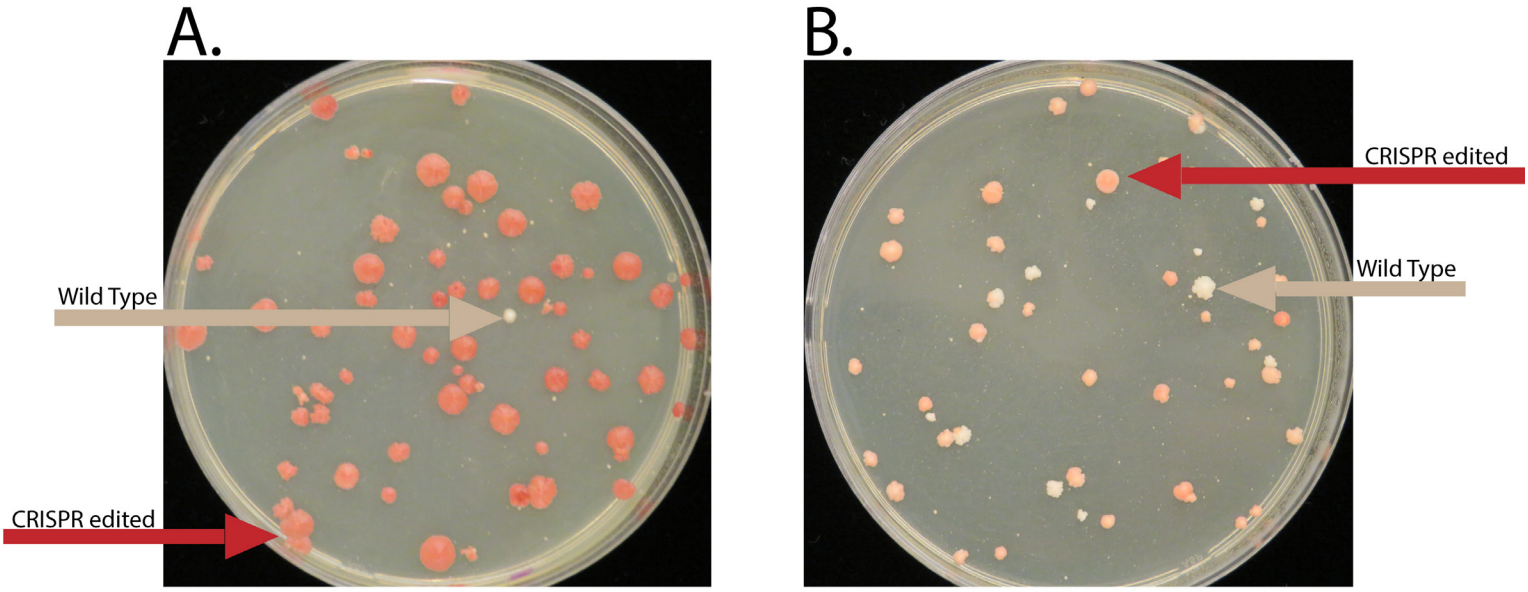


Figure 4. CRISPR mediated genome editing of *S. cerevisiae* ADE2. Repair of the cleavage sites in the two strains of yeast were shown was done by homologous recombination. Stop codons were inserted into the 5' end of the ADE2 gene generating a functional deletion. Pink and red colonies indicate successful genome editing while white colonies are unedited *S. cerevisiae*. **A.** Commercially available baking strain, Red Star Quick Rise Instant Yeast. **B.** Commercially available brewing strain, Lallemand Nottingham Ale Brewing Yeast.



UNDERGRADUATE RESEARCH PROJECTS

One way students can be exposed to CRISPR-mediated genome editing is by applying it to an undergraduate research project. Well-designed undergraduate research projects both train students and move a larger research program forward. Undergraduates typically have limited time they can devote to a research project and their tenure in a lab is likely to be shorter than that of a graduate student or more senior scientist. Such limitations make it crucial that students generate strains and reagents required to test a hypothesis in a timely manner. In some fields, CRISPR-mediated genome editing has increased the speed by which investigators can generate such materials. For instance, the human fungal pathogen C. albicans is diploid (51), does not undergo meiosis, and cannot maintain plasmids. Historically, genetic manipulation of C. albicans required multiple rounds of homologous recombination as each allele must be mutated independently and screened in succession (52). As ploidy increases genetic manipulation generally becomes more laborious. These characteristics made it considerably more challenging to work with than other fungal model systems such *S. cerevisiae*. The development of CRISPR-mediated genome editing in C. albicans has greatly increased the speed at which genetic engineering can be performed and allows undergraduate researchers to generate strains quickly (25, 53). Such efficiency is important so students make progress on a project and are afforded the opportunity to witness the payoff of performing hypothesis-driven experiments.

CRISPR IN THE CLASSROOM

Applying CRISPR to a mentored research project provides a tremendous opportunity for undergraduates to develop a deep understanding of how genome editing can be applied to a research problem. However, it is unrealistic to expect that all undergraduates could gain experience in this way. Laboratory courses that employ CRISPR as a technique are an alternative method of teaching genome editing to larger groups of students. How CRISPR can be applied in an undergraduate curriculum depends upon the experience level of the students. Introductory courses pose unique challenges due to students' relative unfamiliarity with bench science and the financial and equipment constraints inherent to many such courses. Thus, it is important that exercises yield easily interpretable results while staying within the course's technical and financial boundaries. One example of a model system well suited for introductory biology courses is CRISPR-mediated genome editing of yeast ADE2. Mutation of *S. cerevisiae* ADE2 leads to a buildup of an adenine precursor pigmenting the yeast red (Figure 4) (25). The intensity of the color and efficiency of gene editing will depend upon the length of the incubation as well as S. cerevisiae strain. This easily observable phenotype, and the practicality of yeast, make them an exceptional system to expose undergraduate students to CRISPR-mediated genome editing in introductory courses (54).

As students gain more experience, the experiments they can perform and data they can interpret during laboratory classes broadens. This provides the instructor with significantly more latitude when designing exercises. There has been a shift recently in higher education away from laboratories that merely teach a technique towards Course-Based Undergraduate Research Experiences (CUREs) (55). CUREs allow students to develop and address a scientific question using a variety of methodologies and techniques. The speed and relatively simple nature of CRISPR-mediated genome editing makes it particularly well suited for upper level biology CUREs where faculty address a research question of interest. CRISPR based CUREs have been developed using a variety of different model systems including zebrafish (56), *Drosophila melanogaster* (57), and mammalian tissue culture (58). The framework for many of these courses is similar; students design and clone guide RNAs into appropriate expression vectors and then use these vectors to edit the genome of their model system. These courses allow faculty to address an important research question and introduce cutting edge genome editing technology when appropriate to students concurrently. Students then assess how edits they introduce affect phenotype. In the case that guide generation or editing takes longer than predicted, phenotypic assessment can be performed in other classes or in the research lab of the principal investigator leading the exercise.

Another way CRISPR is being applied in the classroom is by examining how CRISPR helps bacteria defend against viruses. For example, labs have been developed where undergraduates infect *Streptococcus thermophilus* with bacteriophage and sequence the bacterial genomic DNA to see if viral DNA has been incorporated to the CRISPR array (59). Modified bacteria gain resistance to later phage infections and subsequent experiments can be performed that further examine the CRISPR-mediated resistance. Furthermore, CUREs have been developed to identify CRISPR repeats in uncharacterized strains of *Escherichia coli* (60). The CRISPR loci found among different strains and species of bacteria should be distinct and would depend upon the viruses they have encountered. By examining the sequences of CRISPR arrays, students can identify phages the bacteria have encountered. While students that perform these exercises are not editing genomes, they learn about CRISPR biochemical mechanisms and gain an appreciation for how fundamental discoveries can have broad applications.

The exercises described above provide examples of how CRISPR is being applied to undergraduate curriculum. However, CRISPR-based CUREs go beyond just genome editing, exposing students to a variety of other molecular biology techniques. For instance, to clone a guide RNA into an expression vector students need to perform a variety of additional techniques including restriction digestion, plasmid purification, and DNA ligation. CRISPR CUREs therefore represent comprehensive approaches that integrate and expose students to genome editing and foundational molecular biology techniques. CRISPR's broad utility and practicality make CRISPR CUREs attractive pieces from which to shape undergraduate molecular biology curricula. As CRISPR is now part of the molecular biology tool box, instructors should consider adoption of it alongside classic molecular biology tools when developing classroom activities.

CRISPR AND SCIENCE POLICY

In undergraduate curricula, the most immediate impact of cutting edge genome editing technologies like CRISPR is being felt in biology classrooms as the development of genome editing technologies directly affects course material being taught. However, the remarkable rise and accessible nature of CRISPR, combined with the powerful prospect that it will enable us to manipulate genomes at will, brings with it a variety of moral and ethical questions that society must examine. For all its promise, CRISPR raises apprehension regarding human germline manipulation, unforeseen risks, and the potential for these technologies to further social inequities (61). These are by no means novel concerns; since generation of the first transgenic organism, the scientific community has debated the lines that separate the unethical from ethical and the reckless from the enthusiastic. While these debates continue in laboratories and scientific conferences across the world, the discussion should extend beyond the broader science community to the general public and society at large. Evidence for this shift can already be seen by the inclusion of CRISPR debates in academic courses on public policy and science law (62, 63). Diverse enrollments in these types of classes will provide opportunities to engage in discussions and hear various viewpoints relating to genome editing technologies. It is imperative that the scientific community continue to inform the public of the remarkable promise of genome editing, as well as the limitations and hazards it presents. The inclusion of these discussions in classrooms outside of biology departments will provide the best chance for us to make informed decisions on how we choose to apply genome editing technology as a society.

SUMMARY

When the students involved in writing and researching this article started high school, CRISPR-mediated genome engineering had not been invented and now it is being taught in undergraduate curricula across the world. In just one decade, a little known bacterial immune response has profoundly changed

research and molecular biology undergraduate education in ways few envisioned. The potential for CRISPR- mediated genome editing to profoundly affect research, medicine, and society over the next decades is significant. The initial version of any technology invariably requires refinement. Advancements to CRISPR- mediated genome editing will continue as more bacterial CRISPR systems are characterized and the molecular biology of systems in use is further refined. Improvements to genome editing technologies along with the likely continued drop in the price of gene synthesis will open the door to further scientific advances. In addition, as CRISPR-mediated genome editing systems are developed for more organisms, the limitations traditional model systems impose on research may begin to dissipate. Such progress is especially exciting in the context of undergraduate education. A recent paradigm shift has placed more focus on laboratory exercises that not only teach techniques, but also explore important biological problems through guided semi-independent research in the classroom. The confluence of this shift with advances in genome editing technology have the potential to allow students to make significant progress on research questions previously impractical for undergraduate curricula.

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TATC2 IS IMPORTANT FOR GROWTH OF *ACINETOBACTER BAYLYI* UNDER STRESS CONDITIONS

Justin M. Langro[1]
Megan M. Chamberland[2]
Celena M. Gwin[3]
Natalia Prakash[4]
Danielle T. Velez[5]
Nathan W. Rigel[6]

1.Department of Biology, Hofstra University, Long Island, New York City, USA

3. Present address: Department of Microbial Pathogenesis, Yale University, New Haven, CT, USA

5. Present Address: Regeneraon Pharmaceuticals, Tarrytown, NY, USA

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CORRESPONDING AUTHORS

Department of Biology
Hofstra University
318A Gittleson Hall
Hempstead, NY 11549
nathan.w.rigel@hofstra.edu
Phone: 516-463-6542
Fax: 516-463-5112

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- cell envelope
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- *tatC2*

ABSTRACT

Protein export pathways are important for bacterial physiology among pathogens and non-pathogens alike. This includes the Twin-Arginine Translocation (Tat) pathway, which transports fully folded proteins across the bacterial cytoplasmic membrane. Some Tat substrates are virulence factors, while others are important for cellular processes like peptidoglycan remodeling. Some bacteria encode more than one copy of each Tat component, including the Gram-negative soil isolate *Acinetobacter baylyi*. One of these Tat pathways is essential for growth, while the other is not. We constructed a loss-of-function mutation to disrupt the non-essential *tatC2* gene and assessed its contribution to cell growth under different environmental conditions. While the *tatC2* mutant grew well under standard laboratory conditions, it displayed a growth defect and an aberrant cellular morphology when subjected to high temperature stress including an aberrant cellular morphology. Furthermore, increased sensitivities to detergent suggested a compromised cell envelope. Lastly, using an *in vitro* co-culture system, we demonstrate that the non-essential Tat pathway provides a growth advantage. The findings of this study establish the importance of the non-essential Tat pathway for optimal growth of *A. baylyi* in stressful environmental conditions.

INTRODUCTION

Transport of proteins between membrane-bound compartments is a fundamental biological problem faced by all cellular organisms, including bacteria. Protein transport is carried out by multi-subunit, membrane-spanning molecular machines (12, 23). In Gram-negative bacteria, most exported proteins cross the inner membrane *via* the General Secretion (Sec) pathway. Proteins that take this path must remain unfolded in order to pass through the SecYEG inner membrane translocase. However, some proteins are exported in a fully folded state by using a separate machine called the Twin-Arginine Translocase (Tat) (15). Proteins destined for export *via* Sec or Tat contain a recognizable tripartite signal peptide composed of a positively charged region at the N-terminus, followed by a hydrophobic region in the middle and a polar region at the end (30). However, there are several key differences between Sec and Tat signal peptides; among them the charged region of canonical Tat signal peptides contains an arginine dipeptide within a SRRXFLK sequence motif (6, 8). Signal peptides are recognized by the Sec and Tat machinery during the initial stages of protein translocation, and are cleaved by periplasmic signal peptidases upon export (29).

The Tat machinery is composed of *TatA*, *TatB*, and *TatC* proteins; some bacteria lack *TatB*, but in these cases the role of this protein is performed by *TatA* (17, 22, 45). Other organisms may also encode TatE, which acts as a functional homolog of *TatA* (39). To achieve substrate transport, the *TatB* and *TatC* proteins form a subcomplex in the inner membrane that recognizes the Tat-specific signal peptide (1). Recent evidence suggests that *TatA* protomers are part of the *TatB*C receptor complex and that after binding signal peptide, additional *TatA* subunits are recruited to form the translocase (2, 18). The active translocase requires the proton motive force for translocation of the substrate across the inner membrane. Upon export of the substrate, the *TatA* protomers dissociate from the recognition complex and the cycle can begin again.

To date, most studies of this protein transporter have been performed using *Escherichia coli* as the model. Recent analyses of bacterial and archaeal genomes reveals that in some organisms, multiple tat homologs are present (29). In fact, there are reports of Gram-negative bacteria with two distinct Tat translocases (24, 33). Similarly, there are two distinct Tat translocases in the Gram-positive bacterium *Bacillus subtilis*, though in this case only *TatA* and *TatC* components are found. In some cases, these extra Tat components are functionally redundant (4), while in other cases they are used to export specific substrates (13). There are few studies of protein transport in organisms with multiple Tat components, and thus the significance of possessing two distinct Tat pathways is not fully appreciated. In order to better characterize the importance of dual Tat pathways, we used the Gram-negative soil bacterium *Acinetobacter baylyi* as a model. Scanning the genome of *A. baylyi* reveals the presence of two distinct Tat pathways.

Remarkably, comprehensive mutagenesis of *A. baylyi* suggests that the components of one Tat machine are essential for growth, while the components of the other machine are dispensable (11). For clarity, henceforth we refer to the essential Tat genes as *tatA1*, *tatB1*, and *tatC1* and the non-essential Tat genes as *tatA2*, *tatB2*, and *tatC2*. In this report, we describe experiments characterizing the non-essential Tat pathway of *A. baylyi*. Specifically, we sought to understand how Tat-dependent protein export enables the growth of *A. baylyi* under different environmental conditions. We uncovered a role for the non-essential Tat pathway in maintaining normal cell envelope integrity and cellular morphology under stress conditions. Using co-culture experiments, we found that the non-essential Tat pathway provides a growth advantage to *A. baylyi* in competition with Pseudomonads that also possess two Tat machines. Taken together, our findings provide insight into the function of the non-essential Tat pathway of a model soil microbe.

MATERIALS & METHODS

Strains and growth conditions. Unless otherwise noted, growth of *A. baylyi* wild-type strain ADP1 (strain 33305 from the American Type Culture Collection; Manassas, VA) was performed at 30 degrees Celsius. For routine culturing, all bacterial strains used in this study were grown in LB Lennox (10 g Bacto-tryptone, 5 g yeast extract, and 5 g NaCl per liter; Fisher Scientific). For growth curve experiments, each strain was grown overnight in LB and then diluted 1:100 in fresh media. These cultures were incubated with intermittent shaking at 200 rpm and optical density (OD) measurements (at 600 nm) were taken every 30 minutes for 8-10 hours. All strains were grown in triplicate.

Construction of an *A. baylyi tatC2*::kan insertion-deletion mutant. The *A. baylyi tatC2* mutant was constructed using an overlap-extension PCR strategy described previously (3). Briefly, upstream and downstream sequences flanking *tatC2* (ACIAD0521) were amplified by colony PCR from the *A. baylyi* wild-type strain ADP1 (Fig. 1A). The upstream flanking sequence was amplified using primers A (5'-TGGAG*TATA*TAAAAATGGC-3') and B (5'-ATTGTTTTAGTACCGAGCTCCTTG-GGCAGGCATGATGTC-3'). The downstream flanking sequence was amplified using primers C (5'-GCCATTTATTATTTCCTTCGATCCTC-GAAAAACGTAG-3') and D (5'-GATTACCTTTG-GCATCAAC-3'). A 795 bp kanamycin-resistance cassette was amplified from plasmid pIM1445 (gift from Ichiro Matsumura) (28) using primers E (5'-GAGCTCGGTACTAAAACAAT-3') and F (5'-GAAGGAAATAATAAATGGC-3'). These three products were mixed together in equal molar ratios in a new tube and joined together in a final PCR using the outer-most upstream and downstream flanking primers (A and D), resulting in the ::kan insertion-deletion allele. This PCR product was purified and then used to transform naturally competent wild-type *A. baylyi* (14). Incorporation of the insertion-deletion allele was confirmed by PCR analysis and DNA sequencing.

Construction of *pTatC2* expression plasmids. To perform complementation experiments on the *tatC2*::kan mutant, the wild-type *tatC* gene was cloned into the BamHI site of pWH1266 by custom gene synthesis (GenScript, Piscataway, NJ) using sequences from the *A. baylyi* genome (www.biocyc.org). Plasmids were transformed into *A. baylyi* as follows. From an overnight culture of wild-type strain ADP1, 0.1 ml of cells was gently mixed with 100 ng of plasmid DNA. The mixture was allowed to incubate at room temperature for 1 hour. To allow outgrowth of any transformed cells, 0.9 ml of LB was added to the mixture followed by overnight incubation at 30°C while shaking. The next day, 0.1 ml of the transformation mixture was plated onto LB-ampicillin agar and grown overnight at 30°C to select transformants.

Detergent sensitivity assay by Efficiency of Plating method. Cultures of wild-type, *tatC2* mutant, and complemented *tatC2* mutant bacteria were grown overnight in LB broth, then diluted in series in fresh LB in a 96 well plate. Using a multi-prong metal replicator, approximately 2 °l of each dilution was transferred to the surface LB agar or LB agar supplemented with 2% sodium dodecyl sulfate (SDS) and 1 mM ethylenediaminetetraacetic acid (EDTA). Plates were incubated overnight at 30°C and growth on the plate was assessed the next day.

Microscopic examination of cellular morphology. Wild-type and *tatC2* mutant starter cultures were grown at 30°C overnight. The next day, each strain was diluted 1:100 in fresh LB broth in duplicate. One set of strains was grown at 30°C while the duplicates were grown at 42°C until late exponential phase. Ten microliters of each culture was spotted onto a glass slide and the smears were allowed to air dry. Following heat fixation, the smears were stained with crystal violet and visualized using an Olympus BX41 microscope under the 100X objective with immersion oil. Images shown are a representative field from at least three independent experiments (Figure 2).

Competitive co-culture experiments. Overnight cultures were used to inoculate flasks containing 50 ml of fresh LB broth for competition assays. The optical density (OD600 nm) of each culture was normalized to obtain a final OD of 0.1 in the flask. Flasks were incubated at 200 rpm on a platform shaker at 30°C for 24 hours. One hundred microliter aliquots were taken from the initial inoculum and at

the 24-hour time point, diluted in 10-fold series to a final dilution of 10-7. To enumerate colony forming units (CFUs), 0.1 ml aliquots were plated from the dilutions to obtain well-isolated colonies. Individual competition assays were performed in triplicate. Input and output CFU counts were used to determine the competitive index (CI) for each *A. baylyi* strain using the following formula: CI = (Af/Ai)/(Bf/Bi), where A represents the *A. baylyi* strain and B represents the competitor strain, with f and i denoting the final and initial CFU counts, respectively. CI values were subsequently analyzed using one-way ANOVA.

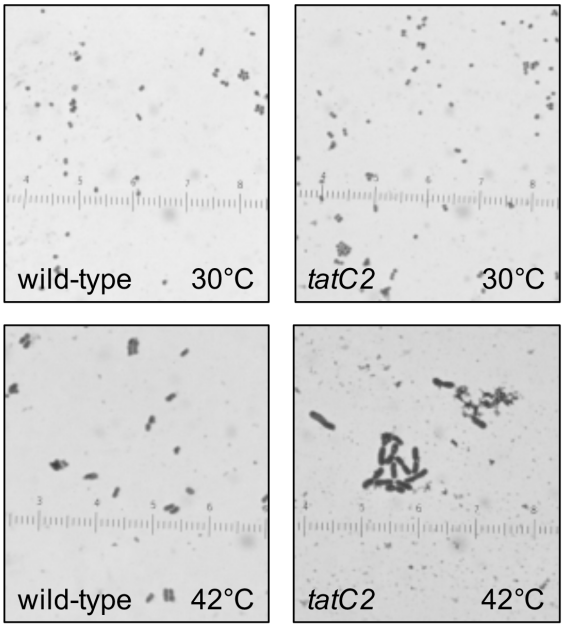
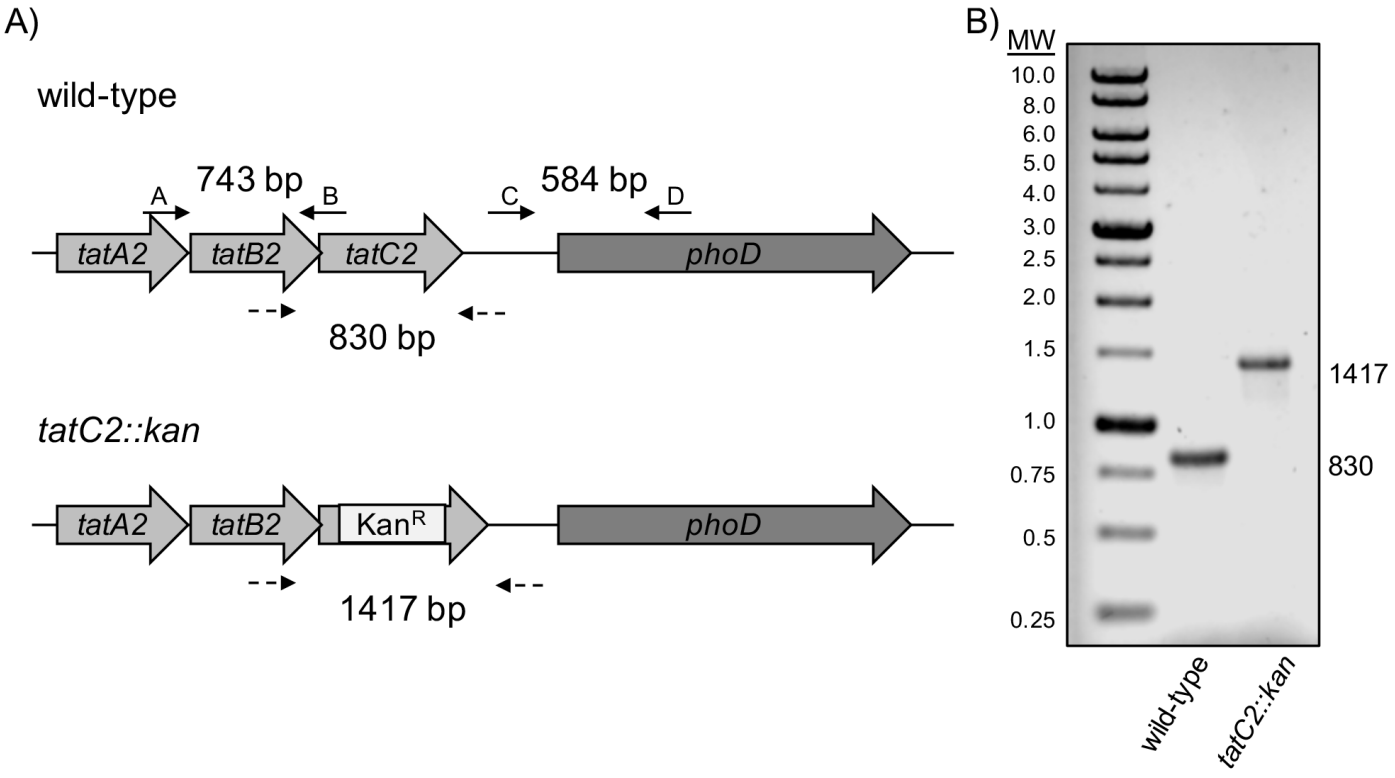


Figure 2. The *tatC2* mutant displays abnormal cellular morphology under high temperature stress conditions. Overnight cultures of wild-type (left column) and *tatC2* mutant (right column) bacteria were diluted 1::100 into fresh LB broth and grown to late exponential phase at 30°C (top row) or 42°C (bottom row). Aliquots from each culture were heat-fixed to a glass slide and stained with crystal violet. Images were captured using the 100X objective. Each tick in the scale bar equals 1 um.

Figure 1. Strategy to construct a *tatC2*::kan insertion-deletion mutant. A) Primers used to generate the upstream (A and B) and downstream (C and D) regions flanking *tatC2* are indicated by solid arrows. Primers used to for PCR analysis of KanR recombinants are indicated by dashed arrows. B) Colony PCR analysis of wild-type and *tatC2*::kan strains. PCR products were resolved on a 0.7% agarose gel and visualized by staining with ethidium bromide. Molecular weight (MW) size standards are shown (in kb) for comparison.



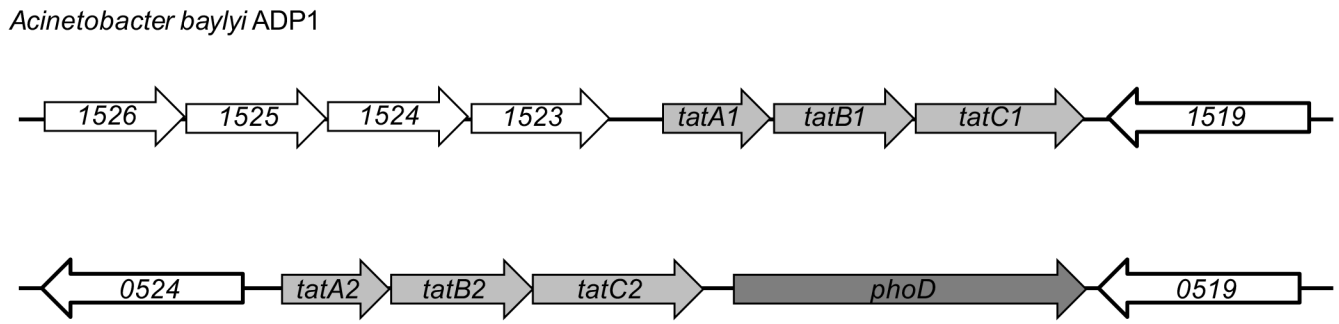


Figure 3. Genome architecture of the tat genes of *Acinetobacter* spp. Homologs of *tatA*, *tatB*, and *tatC* have light shading. Putative alkaline phosphatase *phoD* has dark shading. Flanking genes of unknown function are labeled with ACIAD genome reference numbers.

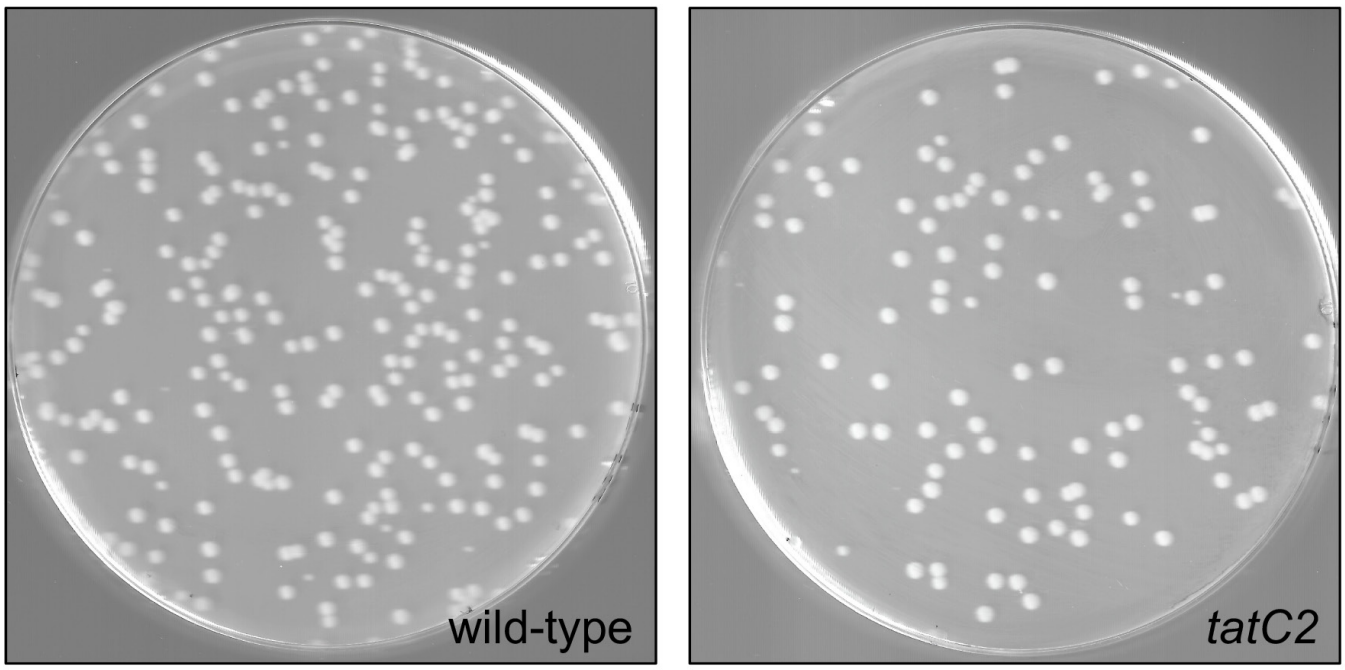


Figure 3. Colony morphology of wild-type and *A. baylyi tatC2* mutant strains. Wild-type (left) and *tatC2* mutant (right) *A. baylyi* strains were grown overnight in LB broth cultures at 30°C. Then serial dilutions of each strain were plated onto LB agar. The plates were incubated overnight at 30°C prior to imaging.

RESULTS

The genome of *Acinetobacter baylyi* encodes homologs of the Twin-arginine translocation (Tat) pathway. The genus *Acinetobacter* has recently drawn increased attention due to the rise of drug resistant isolates of *Acinetobacter baumannii* (31). In addition, *Acinetobacter baylyi* is of growing interest as a model Gram-negative organism due to its ease of genetic manipulation and diverse metabolic capabilities (14). While exploring the genome of *A. baylyi* for evidence of known protein export machines, we located homologs of the Tat pathway. Interestingly, when we examined the *A. baylyi* genome, we noted two homologs each of *tatA*, *tatB*, and *tatC* (Fig. 3). We found that the *TatA* homologs have the highest sequence identity to each other at 86%, while the sequence identity of the *TatB* and *TatC* homologs is lower scoring 62.3% and 70.8%, respectively (Table 1). In addition to the differences in amino acid sequence, there was also a reported difference in function between these two putative Tat pathways. De Berardinis and colleagues reported that the genes encoding one Tat pathway were essential for growth (*tatA1B1C1*), while genes encoding the other (*tatA2B2C2*) were dispensable for growth (11). This finding suggests the possibility that each of these Tat pathways has a different function in secreting proteins out of the cytoplasm. In particular, it could be the case that some Tat substrates are specifically routed through one Tat pathway rather than the other. As precedent, the dual Tat pathways of *Bacillus subtilis* are known to display substrate specificity (32). To clarify the role played by each Tat pathway in *A. baylyi* physiology, we focused on the non-essential Tat machine. Given the established role of *TatC* homologs in substrate recognition (19, 20, 35), we started our analysis by creating a loss-of-function *tatC2* mutant strain.

Construction and phenotypic analysis of a *tatC2* mutant. We constructed an insertion-deletion *tatC2* mutant using a previously described method (3). The *tatC2*::kan mutant allele was transformed into the wild-type strain of *A. baylyi* ADP1, and kanamycin-resistant colonies were selected on LB agar supplemented with kanamycin. Recombination of each mutant allele into the bacterial chromosome was confirmed by PCR and DNA sequence analysis (Fig. 1). We obtained numerous transformants for the *tatC2*::kan allele, suggesting that this gene is dispensable for growth under standard laboratory conditions, in agreement with previous findings (11). To begin characterizing these mutants, the mutant cultures were assessed for their growth properties. Colonies of the *tatC2* mutant are indistinguishable from wild-type *A. baylyi* when grown on LB plates at 30°C (Fig. 4). When grown in LB broth at 30°C, the *tatC2* mutant exhibited similar growth kinetics as wild-type *A. baylyi* (Fig. 5A). A similar growth trend was observed when we plated for viable counts (data not shown). In some organisms, loss of a functional Tat pathway has been associated with defects in virulence, while in other organisms tat mutants exhibit aberrant morphological and physiological phenotypes under *in vitro* stress conditions, including when subject to high salt or detergent concentrations (10, 42, 44). We next tested the ability of the *tatC2* mutant to grow when challenged with various environmental stresses.

The *tatC2* mutant is sensitive to detergents and growth at high temperature.

Defects in bacterial protein export machines, including the Tat pathway, are often accompanied by increased sensitivity to toxic small molecules (21, 25, 26, 36). In *E. coli*, the Tat pathway exports proteins that help maintain the structural integrity of the cell envelope. Failure to export these proteins weakens the cell envelope, thereby increasing its permeability. To determine if the non-essential Tat pathway is important for cell envelope integrity in *A. baylyi*, we tested the ability of the *tatC2* mutant to grow in the presence of the detergent SDS. Using an efficiency of plating assay, we found that growth of the *tatC2* mutant is impaired by treatment with detergent (Fig. 6). Importantly, we observed that complementation of the detergent sensitivity phenotype of the *tatC2* mutant could be achieved by constitutive expression of *tatC2* on a plasmid.

Given the increased cell envelope permeability associated with the *tatC2* mutant, we wondered if the non-essential Tat machine was important to resist temperature stress. Although the *tatC2* mutant grew as well as wild-type at 30°C, its growth was impaired at 42°C (Fig. 5B). At later time points, growth of the *tatC2* mutant slowed as compared to wild-type cultures grown at 42°C. This finding is consistent with the the function of Tat in other organisms and suggests that the non-essential Tat pathway of *A. baylyi* plays a role in cell envelope biogenesis (10). Given the established role of Tat in maintaining normal cellular morphology (7), we next examined the cellular morphology of the *tatC2* mutant by microscopy (Fig. 2). We found that the *tatC2* mutant cells grown at

30°C were indistinguishable from the wild-type cells. Both strains appeared as small coccobacilli, which is characteristic of the *Acinetobacter* genus (41). At 42°C, cells of wild-type *A. baylyi* were slightly larger than those grown at 30°C. Cells of the *tatC2* mutant were longer and thicker when grown at 42°C. Thus *TatC2* is important for maintaining normal cellular morphology of *A. baylyi* under high temperature stress conditions.

The non-essential Tat pathway provides a competitive advantage to *A. baylyi* grown in co-culture.

We showed that *TatC2* is important for maintaining normal cellular morphology under stressful growth conditions in the laboratory. This led us to wonder how important the non-essential Tat machine is for *A. baylyi* to grow in the environment. The soil is home to complex microbial communities; these microbes are often in competition with each other for scarce nutrients. In such an environment, some protein secretion systems are important for nutrient acquisition (5, 27, 37), while others are used to directly inhibit growth of microbial competitors (16, 38, 43). With this in mind, we tested whether the non-essential Tat pathway, and *TatC2* in particular, was important for the growth of *A. baylyi* in a competitive co-culture system. *Pseudomonas* species are closely related to *Acinetobacter* species, and often share similar environmental niches. Therefore, we selected a subset of *Pseudomonas* species for the co-culture experiments. As shown by the competitive index (CI) values in Table 2, all three *Pseudomonas* strains tested grew significantly better than both wild-type and *tatC2* mutant strains of *A. baylyi*. However, when comparing the relative CI values between wild-type and the *tatC2* mutant, we noticed that the *tatC2* mutant was significantly impaired when grown in competition with *P. putida*. In competitions with both *P. aeruginosa* and *P. fluorescens*, there was no significant difference in CI between wild-type and the *tatC2* mutant. The difference in CI does not appear to be due to a secreted toxin as *P. putida* grown in spent media from either wild-type *A. baylyi* or the *tatC2* mutant showed no difference in growth compared to control (data not shown).

Table 1. Similarity between *A. baylyi* Tat proteins and their homologs.

Tat Protein[a]	Accession no.	% similarity	
		Tat1	Tat2
Acinetobacter baylyi TatA1	WP_004925257	100.0	86.0
Acinetobacter baylyi TatA2	CAG67449.1	86.0	100.0
Acinetobacter baylyi TatB1	WP_004925259	100.0	62.3
Acinetobacter baylyi TatB2	WP_004920102	62.3	100.0
Acinetobacter baylyi TatC1	WP_004925261	100.0	70.8
Acinetobacter baylyi TatC2	WP_004920104	70.8	100.0
Escherichia coli TatA	WP_001295260	69.5	66.2
Escherichia coli TatB	WP_053879116	54.2	53.3
Escherichia coli TatC	WP_072859488	66.4	64.8
Escherichia coli TatE[b]	WP_047613167	77.3	76.9

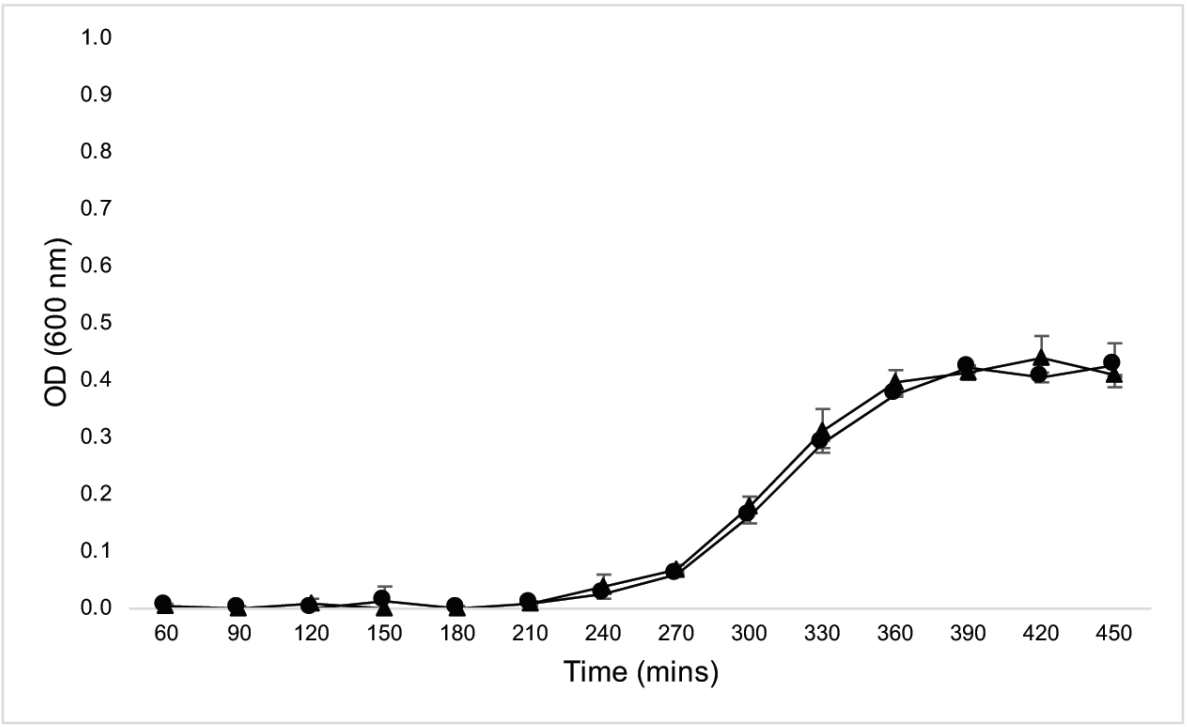
[a] Protein sequences were obtained from NCBI for *A. baylyi* ADP1 and *E. coli* MG1655 and were analyzed by pairwise BLAST. [b] Similarity as compared to *A. baylyi* TatA1 or TatA2.

Table 2. Competition between *A. baylyi* and *Pseudomonas* spp.

A. baylyi strain	*Pseudomonas* strain	Mean CI[a]	SD[b]
wild-type	*P. aeruginosa*	0.086	0.014
tatC2	*P. aeruginosa*	0.074	0.013
wild-type	*P. fluorescens*	0.133	0.023
tatC2	*P. fluorescens*	0.146	0.021
wild-type	*P. putida*	0.148[c]	0.040
tatC2	*P. putida*	0.043[c]	0.011

[a] $CI = (A_f/A_i)/(B_f/B_i)$; A represents the *A. baylyi* strain and B represents the *Pseudomonas* strain, with *f* and *i* denoting the final and initial CFU counts, respectively. [b] Standard deviation (SD) calculated from the mean of triplicate experiments. [c] $P < 0.05$ as calculated by ANOVA.

A)



B)

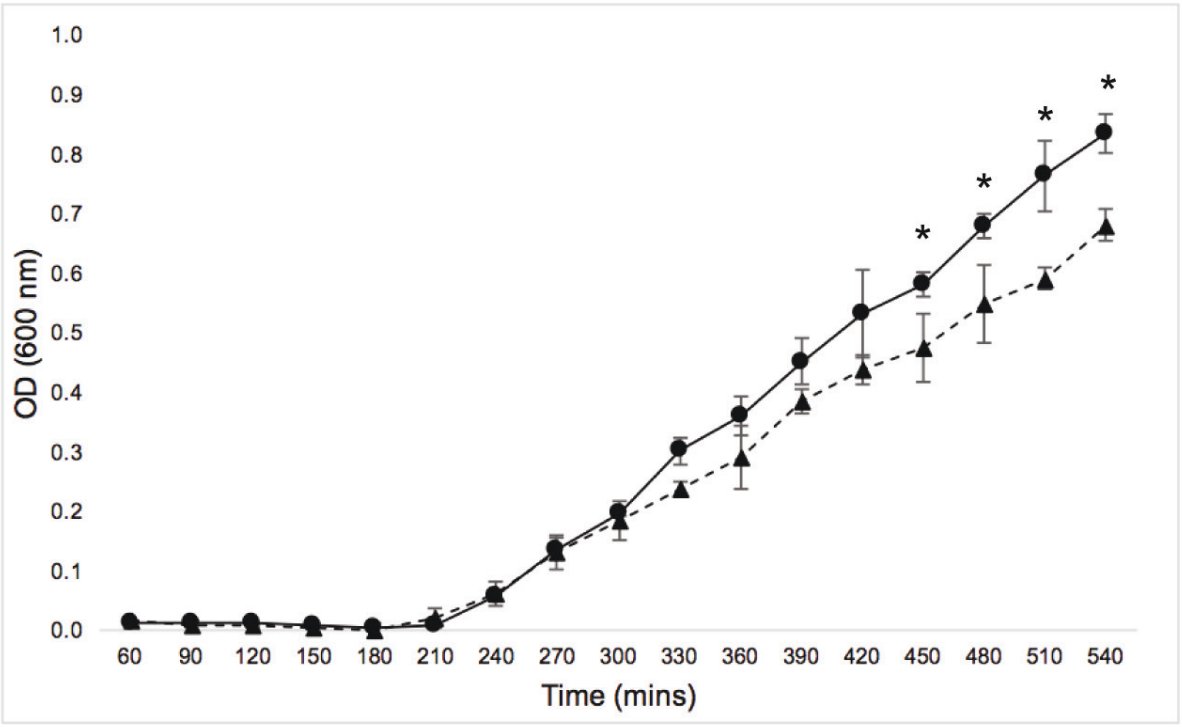


Figure 5. Growth of wild-type and *A. baylyi tatC2* mutant strains in liquid culture. Wild-type (●) and *tatC2* mutant (▲) *A. baylyi* strains were grown at 30°C (A) or 42°C (B) in LB broth cultures. Overnight cultures of each strain were diluted 1:100 in fresh LB at the start of the experiment. Optical density (OD600 nm) was measured periodically. Each data point represents the mean of triplicate experiments. Error bars indicate standard deviation from the mean. ⋆, $P < 0.05$ by one-way ANOVA.

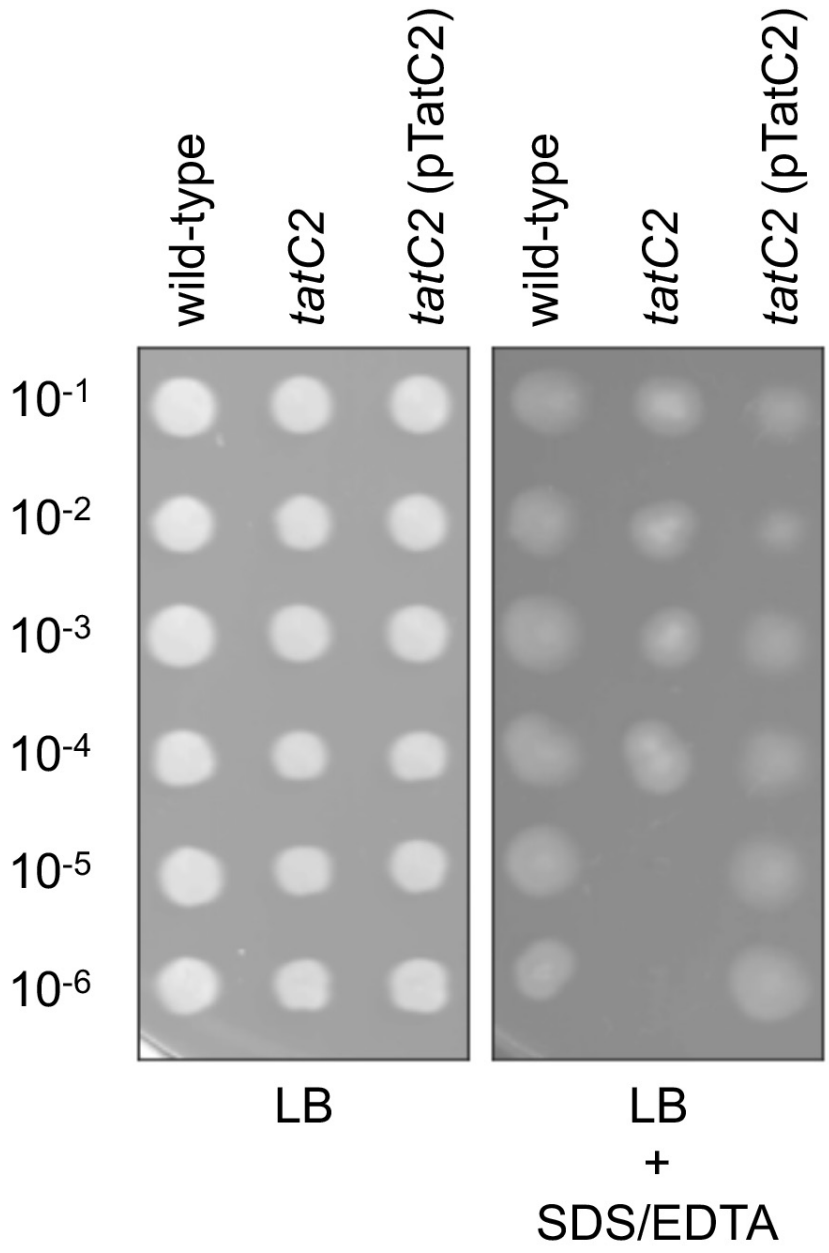


Figure 6. The *A. baylyi tatC2* mutant is sensitive to detergents. The indicated strains were tested for detergent sensitivity using an efficiency of plating assay. Overnight cultures of each strain serially diluted and plated onto LB agar or LB agar supplemented with 2% SDS + 1 mM EDTA. Each plate was incubated overnight at 30°C prior to imaging. Shown is a representative experiment from triplicate trials.

DISCUSSION

Despite the fundamental nature of protein export systems, the important contributions of these pathways to bacterial physiology and growth is often underappreciated. Since its discovery, much has been learned about the structural features and mechanistic details of how the Tat pathway transports proteins. Yet there are still key questions to be addressed. Specifically, how do organisms with two Tat pathways sort their cargo to the correct translocase? What is the benefit to having two Tat pathways? Using *A. baylyi* as a model system, we have started exploring these questions in detail.

By disrupting the non-essential Tat pathway by mutation of *tatC2*, we uncovered a role for this machine during stress conditions. When exposed to detergents, viability of the *tatC2* mutant was decreased as compared to wild-type. This finding supports a role for the non-essential Tat pathway in maintaining integrity of the bacterial cell envelope. We also found that the *A. baylyi tatC2* mutant displays aberrant cellular morphology under high temperature stress. Similar phenotypes have been observed in tat mutants of other bacterial species; notably, cells of *E. coli* tat mutants form long chains because the cell wall amidases important for proper cell division are Tat substrates (7). Our search of the *A. baylyi* genome did not reveal homologs to these particular amidases. Given that *E. coli* only possesses a single Tat machine, it is possible that there is a more diverse collection of Tat substrates in organisms with two Tat machines like *A. baylyi*. We believe that biochemical methods will be needed to identify the full suite of Tat proteins in *A. baylyi*.

While the role of protein export systems in bacterial pathogenesis has been well-studied, the importance of these machines with respect to growth in polymicrobial communities is poorly understood. By using a simple co-culture assay, we uncovered a role for the non-essential Tat pathway when grown in competition with other bacterial species. Interestingly, this phenotype was only evident when *A. baylyi* was grown in competition with *P. putida*, an organism that also encodes two distinct Tat translocases. To explain this difference in CI among the *Pseudomonas* strains, we considered the basic physiology of all three organisms. All three grow at a similar rate and under similar conditions. *P. aeruginosa* is a human pathogen but can also be isolated in environmental samples (40). Neither *P. fluorescens* nor *P. putida* are human pathogens, however, both are associated with the plant root microbiome (9, 34). Interestingly, when we examined the genomes of each species for tat gene homologs, we found that *P. aeruginosa* and *P. fluorescens* possess genes encoding a single Tat pathway, while *P. putida* encodes genes for two separate and distinct Tat pathways. Perhaps the non-essential Tat pathway of *A. baylyi* is important when facing competition from other dual-Tat pathway bacteria, particularly when growing in complex microbial communities in soil. More experiments are needed to rigorously test this possibility.

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THE PERSISTENCE OF *STAPHYLOCOCCUS AUREUS* ON HOSPITAL PRIVACY CURTAINS.

Sarah E. Cole[1]
Brittany J. Gasper*

[1] Current address - 11200 SW 8th St, Miami, FL 33199
* Center for Teaching and Learning, Kent State University, Kent, Ohio

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CORRESPONDING AUTHORS

Sarah E. Cole
sarahecole95@yahoo.com

Brittany J. Gasper
bgasper@flsouthern.edu

ABSTRACT

While healthcare professionals are working in hospitals, they will often manipulate the privacy curtains during the care of their patients. Studies have shown that the transfer of bacteria from hands to the curtains and *vice versa* is possible. Despite the possibility of hospital curtains being a mode of infection transmission, studies have shown that 53% of hospitals surveyed did not have a policy for cleaning or changing their curtains. The question that this study focused on was whether curtain material affects the persistence of *Staphylococcus aureus*. In this study, five different curtain types were inoculated with overnight, diluted, and finger imprint cultures of *S. aureus*. They were incubated at room temperature and were sampled for growth regularly onto Mannitol Salt Agar plates. The colonies were counted, and one-way ANOVA statistical analysis was completed on the data. The statistical analysis showed that the length of persistence of liquid cultures of *S. aureus* on the curtains was not dependent upon initial concentration. Finger imprint inoculations of four curtain varieties had statistically significant longer persistence times than the liquid cultures. Only the curtain type composed of 100% antimicrobial polyester with water repellant had significantly lower persistence times for the finger imprint culture than the other four curtains. The results suggest that the 100% inherently flame resistant antimicrobial polyester curtain material reduces *S. aureus* persistence times and that it may benefit hospitals to use this type of curtain.

KEYWORDS

- persistence
- health-care
- associated infection
- nosocomial infection
- hospital curtain

INTRODUCTION

The environment of the hospital has an important role in the transmission of health-care associated pathogens which cause nosocomial infections. Studies have found that hospital privacy curtains are quickly contaminated by microorganisms that can be transferred to the hands of healthcare workers, the patient, and the surrounding environment (21, 30). In 1988, there was an outbreak of multiple-antibiotic-resistant *Acinteobacter baumannii* in the Queen Elizabeth Hospital in Birmingham, UK (7). After an investigation, carbapenem-resistant *A. baumannii* was isolated from surfaces of equipment, beds, mops, and curtains. It was determined that the curtains were the main source of the outbreak because they had the highest number of the organism present on them (7).

In addition to the curtains being easily contaminated with pathogens that can be transferred via the hands to people or other surfaces, some bacteria can persist on hospital surfaces, including curtains, for weeks and even months (15, 19, 20). Persistence in this case refers to the ability of an organism to survive and be cultured from a surface (15). A study by DeAngelis and Khakoo (8) indicated that these lengthy persistence times are problematic. Their study included surveys completed by hospitals on curtain cleaning policies. Over half of the hospitals surveyed (53%) said they do not have a policy for cleaning the hospital curtains, 37% said they only clean their curtains when visibly soiled, 13% clean the curtains every year, 13% clean their curtains every three months, and only 13% clean their curtains every month. In other words, the most frequently some hospitals change their curtains is every month. Since only 13% of the total hospitals surveyed clean their curtains that often, this means most of the hospitals either do not have a cleaning policy or change the curtains every three months or greater. Mitchell et al. (2015) noted that the attention

put into cleaning porous, soft surfaces such as privacy curtains and room furnishings is far less than that given towards disinfection of porous surfaces (18). As there are nosocomial pathogens such as *Staphylococcus aureus, Klebsiella quasipneumoniae* subsp. *similipneumoniae* (formerly known as *K. pneumoniae*) (10), and *Candida albicans* that can last on hospital surfaces for longer than four months (15), this three month cleaning interval may not be frequent enough. This suggests there needs to be more frequent cleaning and a change of policy.

Despite the importance of the topic, there has not been a large amount of research completed on the topic of hospital curtains and bacteria or bacterial infections. The studies completed so far have varied substantially in their inoculation technique and their analytical methods. Some have taken finger imprints from curtains actively hanging in hospital rooms (3, 14, 16, 17, 21, 22, 24). Others have inoculated known concentrations of bacteria onto specified areas of curtain swatches (13, 27) or hospital fabric (20). These studies have also differed in duration from as short as seven days (17) to as long as six months (3, 14, 25). There are different varieties of hospital curtains available including those containing antimicrobial fabric and some coated with water repellant. However, most studies only used one type of curtain and this varied from vinyl (21) to flame resistant (FR) (22), propylene (14), disposable sporicidal (13), and polyester (16, 24), amongst others. Only two studies (20, 24) were shown to compare more than one type of curtain, and only three studies used statistical tests to analyze their results (3, 17, 24). Contradictory results of previous studies include some that have showed a high rate of Methicillin Resistant *S. aureus* (MRSA) on curtains (21) and those that have not (16). Kurashige (2016) mentions that curtain type may influence these results and should be further examined.

This study intended to expand on the existing research. The focus was on *S. aureus*, a species of pathogenic bacteria that causes nosocomial infections and has been shown to be capable of developing antibiotic resistance to become hospital-associated MRSA (9). This study combined aspects of previous findings by measuring the persistence times of *S. aureus* inoculated both directly with a known concentration or by fingerprint on five different curtain types. These curtain types included three different combinations of FR and non-FR polyester with no antimicrobial properties and two antimicrobial polyester curtains, one with and one without water repellant. We hypothesize that the composition of the different curtain types, specifically the presence of antimicrobial properties, will influence the persistence of *S. aureus*.

MATERIALS AND METHODS

Curtain Varieties and Codes

Five different curtain varieties were used in this study. Each curtain was given a code for ease of designation and to prevent bias on the part of the experimenter when counting colony-forming units (CFUs) and persistence times. The curtain varieties and codes used in this study can be found in Table 1.

Curtain Code	Curtain Material
A	46% FR polyester, 27% post-consumer recycled polyester, and 27% post-industrial recycled polyester
B	87% polyester, 13% Avora FR polyester
C	52% post-consumer recycled FR polyester, 48% FR polyester
D	56.6% Amy antimicrobial polyester, 43.4% recycled FR polyester
E	100% inherently FR antimicrobial polyester with water repellant

Table 1: Composition and codes of the five different curtains used in this study.

INOCULATION OF CURTAINS

Each curtain variety was cut into 2 x 2 cm squares that were sterilized by autoclaving. Sterile curtain squares were inoculated with *S. aureus* by three different methods. For each method, six squares of each curtain type were inoculated. All overnight (O/N) cultures of *S. aureus* (ATCC 12600) were grown in liquid tryptic soy broth (TSB) media at 37°C for 24 hours.

Method one (Concentrated liquid culture technique) – Curtain squares were inoculated with 20 µl of a liquid O/N culture of *S. aureus*. This was the most concentrated solution inoculated and was intended to simulate an extreme contamination event such as

direct contact of a contaminated fluid sample with the curtain. Measurement of the OD600 on a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific) indicated this was equivalent to a total bacterial load of 2.0 x 107 cfu or a density of 5.0 x 106 cfu/cm2. Method two (Diluted liquid culture technique) – Curtain squares were inoculated with 20 µl of a liquid O/N culture of *S. aureus* diluted to an OD600 of 0.08 in a sterile 0.9% (w/v) sodium chloride solution. This was equivalent to a total bacteria load of 1.3 x 106 cfu or a density of 2.7 x 104 cfu/cm2. This OD600 was chosen as the diluted culture standard as it closely represents the OD¬600 value of the 0.5 McFarland standard used for inoculated samples during antimicrobial susceptibility testing (6). A diluted sample was included to see if the persistence of *S. aureus* was dependent on the initial concentration inoculated and to represent direct contact between curtains and diluted fluid samples. Method three (Colony finger imprint technique) – Curtain squares were inoculated with a colony of *S. aureus* by touching the colony with a gloved finger and pressing it onto the curtain piece, making sure to equally touch each area of the curtain. The colonies of *S. aureus* used for this step were similar in size and obtained from a quadrant streaked mannitol salt agar (MSA) plate that was incubated at 37°C for 48 hours. Curtain E was the only curtain where the overnight and diluted cultures were not inoculated onto the curtain. Only the finger imprint technique was used to inoculate this curtain because it is water repellant and was not able to absorb the liquid cultures. After the curtain squares were inoculated, they were left to dry in separate sterile petri dishes for 20 minutes.

MEASUREMENT OF *S. AUREUS* PRESISTENCE

After inoculation and drying, an initial measure of bacterial growth was aseptically taken from each curtain square. A flame sterilized pair of tweezers was used to hold the curtain square steady while a sterile cotton swab dipped in a sterile 0.9% (w/v) sodium chloride solution was used to evenly sample each curtain with 10 strokes (five down and five up). This cotton swab was then used to evenly, continuously streak onto an MSA plate that was incubated at 37°C for 48 hours. After incubation, the number of CFU on each plate was counted. If more than 300 CFU were present on the plate, it was recorded as too numerous to count. Curtain squares were maintained at room temperature in sterile lidded petri dishes to represent the equivalent environment of a hospital setting. Swabbed samples of *S. aureus* were taken from the squares at a maximum of three day intervals until growth of five CFU or less was seen on an MSA plate incubated at 37°C for 48 hours for two consecutive samplings. To conserve resources, this was modified from Neely and Maley (2000), who sampled until no CFU were seen for two consecutive samplings, as five or fewer CFU was considered sufficient decline to end the measurement of persistence. Once all of the data were collected, the number of days that the *S. aureus* persisted on each sample of each curtain type was counted. The day that the curtain pieces were inoculated and initially swabbed was considered day zero. All of this data were compiled and analyzed using one-way ANOVA statistics with the Tukey honestly significant difference test.

RESULTS

The persistence time of *S. aureus* was measured (in days) and compared for the three different inoculation methods on curtain varieties A, B, C, and D. The comparisons of the mean persistence times from the three inoculation techniques for each type of curtain are shown in Fig. 1.

All curtain varieties in Fig. 1 showed significantly longer persistence times after inoculation of a colony using the finger imprint technique compared to inoculation of a concentrated or diluted O/N liquid culture. Curtains A, B, and C showed a p-value of < 0.0001 while the p-value for D was less than 0.05. There was no significant difference in the persistence times between inoculation of a concentrated or diluted O/N culture for any of these curtain types.

The mean persistence times for *S. aureus* inoculated using concentrated O/N liquid cultures and diluted liquid cultures was compared for the four curtain varieties (A, B, C, and D). These mean persistence times are shown in Figs. 2 and 3, respectively. The persistence time of *S. aureus* on curtain C was significantly longer than the other curtain types (p-value $<$ 0.01). The mean persistence time for curtain varieties A, B, and D ranged from 7.76 to 10.0 days while the

mean persistence time for curtain C was 14.5 days. Interestingly, there was no statistically significant difference between the mean persistence times of *S. aureus* on these four curtain varieties when inoculated with a diluted O/N culture. The mean persistence times ranged from 9.83 to 13.3 days.

A comparison of the mean persistence times for *S. aureus* inoculated using the colony finger imprint technique on all curtain varieties is shown in Fig. 4. The mean persistence time of *S. aureus* inoculated onto curtain variety E was significantly less than the four other curtain varieties (p-value < 0.0001). The mean persistence time for the four other curtain varieties ranged from 22.0 days to 27.0 days while it was only 4.0 days for curtain E.

There was no significant difference in the number of *S. aureus* CFUs recovered over time from each curtain type (data not shown). Regardless of the inoculation method, all curtain types A-E showed an initial high number of CFUs that decreased at a steady pace before the bacteria were no longer able to persist. Great variation independent of curtain type was seen in the specific numbers of CFUs at any given time point, but the general decline was consistent.

DISCUSSION

When comparing the mean persistence times of the O/N and diluted cultures of each of the curtains A, B, C, and D, there was no difference between the O/N and diluted cultures. In other words, the persistence following liquid inoculation was not dependent on initial concentration. On the other hand, there was a statistically significant difference between mean persistence times of the finger imprint cultures and both the overnight and diluted cultures for all of these curtains. In addition, mean persistence time of *S. aureus* inoculated by the finger imprint lasted much longer than the other cultures curtain varieties A, B, C, and D, implying that the finger imprint inoculation method did have an effect on persistence times, lasting more than 10 days longer than the O/N or diluted cultures. There was no significant difference in the initial CFU counts for any of the inoculation methods (data not shown). These data suggested that the solid inoculation of a bacterial colony increased the persistence of *S. aureus* compared to a liquid inoculation.

To our knowledge, no research comparing the persistence times of bacteria inoculated from both liquid and solid cultures has been published. Kotsanas et al. (2012) inoculated curtain samples with liquid cultures and took finger imprints at specified time points to culture from the curtain and measure persistence (13). They found *S. aureus* to persist for 10 days but show no growth at 2 months. This is consistent with the range of mean persistence times of 8.50 to 14.5 days we measured from our liquid inoculations. Interestingly, they found < 10 CFU after one day of incubation when culturing by the finger imprint method and incubating at 37˚C on horse blood agar, an enriched media (11). By comparison, our CFU values from curtains incubated for one day following liquid inoculation and cultured by swabbing and incubating at 37˚C on MSA were consistently higher than 10 CFUs for all curtain types (data not shown). The counts ranged from a low of 13 CFUs to a countable high of 123 CFUs. Of the 33 samples for which the CFU count after one day is available, 15 were found to be too numerous to count. The curtains in Korvath et al. (2012) were inoculated with 4 x 105 CFU/cm2 of culture, comparable to the 5.0 x 106 and 2.7 x 104 CFU/cm2 in the O/N and diluted samples, respectively. These data suggested the swabbing technique may be a more thorough method for culturing from the entire curtain sample than taking a finger imprint from the center of the curtain (13).

With the comparison of the persistence time from *S. aureus* inoculated from concentrated O/N cultures on curtains A, B, C, and D, there was statistical significance between curtain C and A, B, and D ($p < 0.01$). C had the longest persistence time of all of the overnight cultures with an average of 14.5 days as opposed to the averages of 8.50, 10.0, and 7.67 days for curtains A, B, and D, respectively. This statistical significance suggested that the curtain material of C had an effect on the persistence of the *S. aureus* concentrated O/N culture. Interestingly, these results were not reproducible when comparing persistence of *S. aureus* grown from diluted O/N cultures. This suggests a possible concentration-dependency on the persistence of *S. aureus* on this particular curtain material though the lack of statistical difference between the persistence of *S. aureus* from concentrated and diluted O/N cultures would conflict this. Additional research is needed to address these conflicting results.

The main distinction in curtain material between curtain C and the others is that it had the highest percentage of post-consumer recycled FR polyester

Figure 1

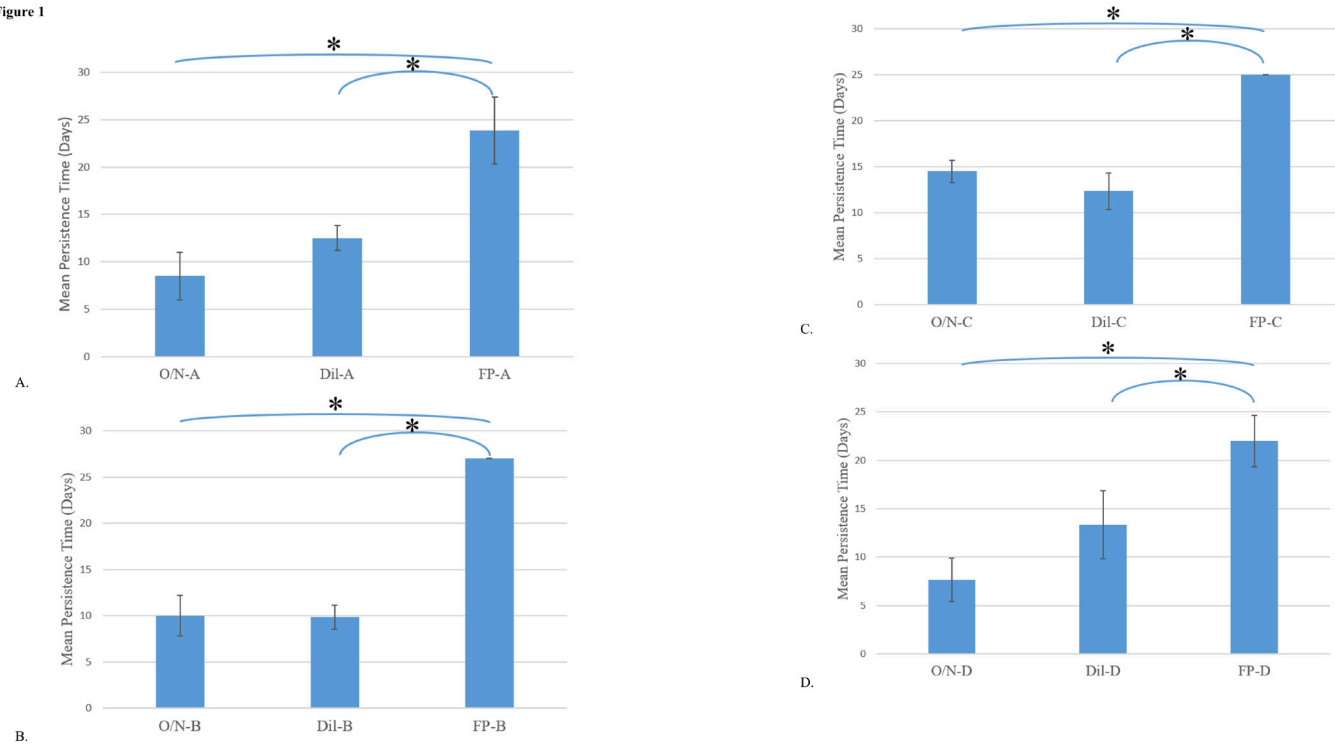

Figure 1: Mean Persistence Times of *S. aureus* from Different Inoculation of Techniques: The mean persistence times in days for curtain varieties A (Panel A), B (Panel B), C (Panel C), and D (Panel D) are shown after inoculation by concentrated O/N culture, diluted O/N culture (Dil), or colony finger imprint technique (FP). The O/N and D cultures contained 2.0 x 107 and 1.3 x 106 CFU, respectively. Each bar represents the mean persistence time ± standard deviation of n = 6 inoculated samples. Statistical analysis was completed using a one-way ANOVA (⋆ indicates statistical significance with a p-value < 0.05).

Figure 2

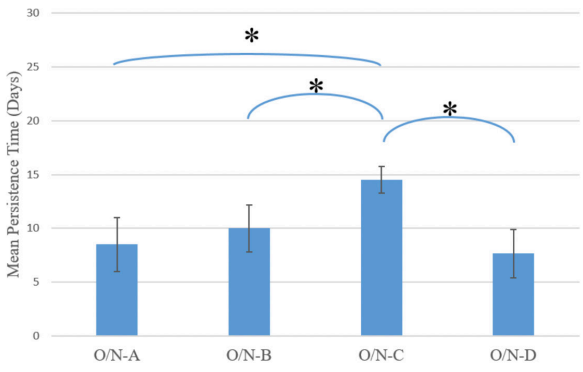

Figure 2 – Mean Persistence Times of *S. aureus* Inoculated from a Concentrated Liquid O/N Culture: The mean persistence times in days for curtain varieties A, B, C, and D inoculated with a concentrated O/N culture of *S. aureus* are shown. The O/N culture contained 2.0 x 107 CFU. Each bar represents the mean persistence time ± standard deviation of n = 6 inoculated samples. Statistical analysis was completed using a one-way ANOVA (⋆ indicates statistical significance with a p-value < 0.05).

Figure 3

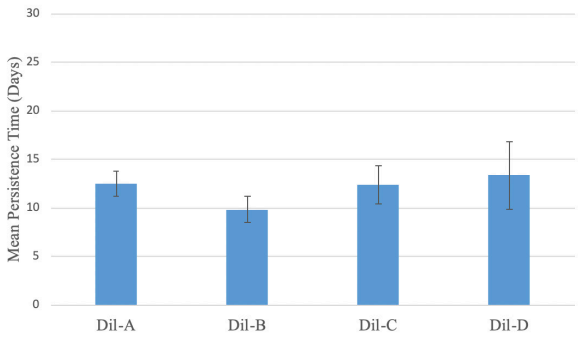

Figure 3 – Mean Persistence Times of *S. aureus* Inoculated from a Diluted Liquid O/N Culture: The mean persistence times in days for curtain varieties A, B, C, and D inoculated with a diluted (Dil) O/N culture of *S. aureus* are shown. The D culture contained 1.3 x 106 CFU. Each bar represents the mean persistence time ± standard deviation of n = 6 inoculated samples. Statistical analysis was completed using a one-way ANOVA.

Figure 4

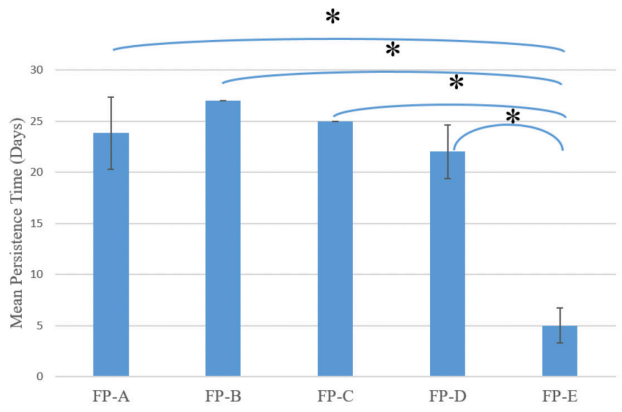

Figure 4 – Mean Persistence Times of *S. aureus* Inoculated by Finger Imprint of a Bacterial Colony: The mean persistence times in days for curtain varieties A, B, C, D, and E inoculated with a colony of *S. aureus* by the finger imprint technique (FP) are shown. Each bar represents the mean persistence time ± standard deviation of n = 6 inoculated samples. Statistical analysis was completed using a one-way ANOVA (⋆ indicates statistical significance with a p-value < 0.05).

at 52%, higher than the 27% found in curtain A, the only other curtain containing this material. Since curtain A is composed of the greatest percentage of total recycled polyester (54% including post-consumer and post-industrial), it seems unlikely that recycled polyester alone could account for the longer persistence time seen with inoculation of O/N cultures. Further research will compare the persistence times of *S. aureus* inoculated from a range of dilutions onto curtains A, B, and C, composed of mixed recycled, no recycled, and post-consumer recycled polyester, respectively. Most studies have only used one type of curtain. Neely and Maley (2000) compared the persistence of *Staphylococcus* and *Enterococcus* on multiple types of hospital fabrics (20). Their values for persistence of methicillin sensitive *S. aureus* after liquid inoculation onto 100% polyester range from 10 – 56 days, comparable on the lower end to the range of liquid culture persistence from this study. Compared to other fabrics, polyester and polyethylene provided the longest persistence times for *S. aureus* while inoculation onto 100% cotton, terry cloth, and a 60-40% cotton-polyester blend resulted in comparably shorter persistence times (20).

While curtain varieties A, B, C, and D all showed increased persistence of *S. aureus* inoculated by the finger imprint method, curtain variety E had significantly decreased persistence times by comparison ($p < 0.01$). The mean persistence of *S. aureus* on curtain E (4.0 days) is more than 15 days shorter than the averages of the other four curtains, suggesting the material of this curtain did have an effect on the persistence time. Curtain E is the only variety to contain 100% inherently antimicrobial FR polyester with a water repellant. Curtain D also has antimicrobial polyester (56.6%), but it does not contain a water repellant. Interestingly, the lower antimicrobial composition of curtain D did not seem to have an effect on persistence times as it showed no significant difference from curtains A or B that contain no antimicrobial composition. It is not clear if the significantly decreased persistence of curtain E is due to the 100% antimicrobial polyester, the water repellant nature, or both. In order to determine how the water repellant affected the persistence times from the finger imprint inoculation, additional experimentation would need to be completed comparing 100% FR antimicrobial polyester with water repellant materials to the same curtain material without the water repellant treatment. In addition, to determine how this curtain material affects the liquid cultures, experimentation would be

needed comparing the persistence times of curtains A, B, C, and D to the 100% inherently FR antimicrobial polyester without water repellant.

These results have an important implication in the hospital setting because the finger imprint cultures are a better representation of the inoculation method that would take place in the hospital. For example, in the hospital, healthcare professionals might touch the source of infection with their gloved hands and then touch the curtain. It may benefit hospitals to use this type of high percentage antimicrobial water repellant curtain to reduce persistence times within the hospital, especially in areas like the Emergency Department where contamination can occur quickly and frequently with high patient turnover rates. However, as curtain E was the only water repellant curtain examined in this study, more investigation with water repellant curtains both with and without antimicrobial composition would need to be done to confirm this.

For the purposes of resources, the persistence times of *S. aureus* alone were investigated in this study, and samples from the curtains could only be taken every 1-3 days instead of every day. While *S. aureus* is important to study because it is a type of MRSA, it is also a commensal organism that colonizes up to 30% of the human population (32) and is a leading cause of bacteremia, infectious endocarditis, and device-related infections (5, 28). Additionally, there are other important nosocomial infection-causing bacteria to study, including *K. quasipneumoniae* subsp. *similipneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (1, 2, 4, 23, 29). These other bacteria may have different persistence times on these curtains than *S. aureus* and may be affected differently by the curtain materials. The persistence times of a variety of bacteria on various curtain types should be measured to determine how different curtain material can affect persistence. Ideally these persistence times would be measured by culturing from the curtain samples every day. Future studies will specifically measure the persistence times of *Enterococcus faecalis, Escherichia coli*, and *P. aeruginosa* on these different curtain types as these represent three common causes of nosocomial infections (12, 26, 31) and exemplify three species that contain multi-drug resistant strains including vancomycin-resistant *Enterococcus* for *E. faecalis*, extended-spectrum cephalosporin-resistant *E. coli*, and carbapenem-resistant *P. aeruginosa* (26).

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MIXTURES OF MYCORRHIZAL FUNGI IMPROVE GROWTH OF *LACTUCA SATIVA* AND REDUCE LEVELS OF ZINC IN CONTAMINATED SOIL

John A. Haradean[1]
Tyler Ralph-Epps[1]
Zach Whiteacre[1]
Sarah Neumann[1]
Donna M. Becker[1]

[1] Department of Biology, Northern Michigan University, Marquette, MI 49855

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CORRESPONDING AUTHOR

John A. Haradean
jharadea@nmu.edu
Department of Biology,
Northern Michigan University
Marquette, MI, 49855.

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- *Lactuca sativa*
- Zinc

ABSTRACT

Bioremediation is currently under investigation as a viable way to remove many environmental pollutants and most commonly involves the use of microorganisms to extract organic pollutants or heavy metals from water or soil. One of the most abundant heavy metals found in industrially polluted sites is zinc (Zn); it is often found alongside metals like lead (Pb), arsenic (As), and mercury (Hg). This experiment investigated the potential bioremediation of pasteurized soil contaminated with zinc using different vesicular arbuscular mycorrhizal fungi (VAM) species and lettuce plants (*Lactuca sativa*). Soil was amended with 0.4 g of zinc chloride ($ZnCl_2$) per kg of soil. Amended and unamended soils were inoculated with two different mixes of VAM, BioAg VAM-Endo™ and MycoBloom. For each treatment, *L. sativa* plants (15 pots per treatment) were grown in a greenhouse setting. Plant diameter was measured weekly. Plants were harvested after 55-days and the wet weight of leaf tissue was measured before the tissue was sent for analysis of zinc levels. Roots were assessed for mycorrhizae using a trypan blue staining procedure. The BioAg VAM-Endo™ mix was the most successful at removing $ZnCl_2$ from the soil. *L. sativa* inoculated with VAM mixes formed mycorrhizae, grew healthier and removed more zinc from the soil than the non-inoculated group. We propose further investigation into the use of mycorrhizal fungi paired with other plant species to remove zinc from contaminated sites with harmful levels of zinc.

INTRODUCTION

Bioremediation uses an introduced or naturally present organism to break down environmental pollutants. Several subcategories of bioremediation exist, three of which are phytoextraction, rhizosphere degradation, and mycoremediation. Phytoextraction uses plants to take up a contaminant to be stored within its tissue and the tissue is then harvested and typically destroyed. Rhizosphere degradation takes advantage of the soil microbiome to help breakdown the contaminant. In mycoremediation fungi are used to degrade or capture contaminants (9, 14).

Phytoextraction, alone, has not been determined to be an effective method of removing heavy metals from contaminated sites, as of yet (29). However, when multiple bioremediation methods are used in concert, contaminant uptake can be increased. When vesicular-arbuscular mycorrhizal fungi (VAM) are associated with a plant, zinc uptake and biomass are significantly increased, in lab and field studies (17, 21).

Many fungi have powerful enzymes that can break down pollutants directly and they also contain heavy metal chelators, making them particularly suited to remediate areas contaminated with heavy metals (4, 14, 30). Fungi can bind these metals to chelators anchored within their cell wall, intracellular chelators within the cytosol, or bind them to extracellular chelators. Each of these methods render the metals inactive which can ultimately save organisms, like plants, from bioaccumulation of these toxic agents in tissues, or humans from ingestion of food crops containing toxic quantities (16, 30). Fungi have successfully been used to remediate areas of concern, but more research needs to be done in this area to make them more effective. *Glomus intraradices* is one such species of fungus that has been studied extensively. *G. intraradices* is a species of

VAM that associates with plant roots in a symbiotic relationship. A few studies have showed *G. intraradices* has a higher tolerance for heavy metals when compared to other VAM grown under the same conditions (11, 20).

Heavy metals are released into the environment through a number of processes. They often accumulate, naturally, through erosion of parent material, such as exposed bedrock and other parts of the earth's crust. Human activity has caused a sharp increase in release of heavy metal pollutants within the environment. One example of a heavy metal is zinc, which can be found in toxic concentrations in many anthropogenically contaminated sites (6). In 2005, 985 of the 1,662 hazardous waste sites that were proposed for inclusion on the EPA National Priorities List contained zinc, although it is unknown how many of those sites were evaluated for toxic quantities of zinc (1).

Zinc has many important industrial uses (e.g., manufacturing of batteries, use in paint and rubber products, and zinc oxide). Another major source of zinc contamination is through mining, which includes leftover tailing piles, by-products of smelting, and hydraulic fracturing (7). Hydraulic fracturing, otherwise known as fracking, is a mining technique used to extract oil and natural gas. Since natural gas has gained popularity as a transitional fuel in the race to find a sustainable source of energy, hydraulic fracturing has increased in popularity. In 2015, there were 623 reported fracking fluid spills in the United States, according to the Colorado Oil and Gas Conservation Commission (27). This is down from the highest recorded number of spills in 2014, which is 786, also reported by the Colorado Oil and Gas Conservation Commission (27). The use of hydraulic fracturing, like any other form of mining, generates waste. In this case, it is the contaminated water expelled from the hydraulic fracturing wells along with the contents of whatever was in the ground (12). This waste often contains high concentrations of zinc brought up from deep within the earth. Inevitably when this water is expelled into the area around the well, zinc as well as other pollutants can accumulate in the soil often in the form of zinc chloride and other organic materials (12, 15).

In plants, zinc is used as a constituent in many enzymes and proteins and it is vital to the production of chlorophyll, to the development of cold resistance, and to the conversion of starches to sugars (5, 25). The normal concentration of zinc in healthy leaf tissue is measured to be in the range of 10-15 ppm (5, 19). In toxic concentrations of zinc, plants experience stunted growth, chlorosis of the leaves, and changes in root growth (22). High zinc concentrations also interfere with the uptake of other nutrients such as phosphorus, iron, manganese or copper, ultimately leading to their deficiency. If the toxicity is severe enough, partial or whole plant death will occur (5). Noticeable symptoms of toxicity generally occur when zinc levels accumulate to greater than 200 ppm (5, 19). The presence of elevated zinc levels in the environment by things like hydraulic fracturing wastewater has an indirect effect on us. Contamination can have detrimental effects on many staple crops, as well as native species. Zinc accumulation can be detrimental to a multitude of ecosystems and human well-being.

Knowing that VAM often form large multi-species complexes in nature, mixtures were chosen for this current study rather than using only a single species. These mixes were paired with a popular variety of *L. sativa*. *L. sativa* is a common crop grown around the world with a fast maturation rate. *L. sativa* is also susceptible to different types of environmental pollution, making it a good candidate for study on the effects of soil contamination on plants. This experiment used the same variety of *L. sativa* but with different mixes of VAM, one containing species native to the great lakes region (MycoBloom) and another sold for commercial agricultural species (BioAg VAM-Endo™). It was hypothesized that the presence of the VAM mixes would reduce zinc concentrations within the soil and that different types of VAM will have a different impact on the uptake and bioaccumulation of zinc.

MATERIALS AND METHODS

SOIL PREPARATION

Approximately 155 kg of agricultural soil was gathered from Marquette, Michigan. This soil was pasteurized, in a single batch at Northern Michigan University, twice at 75 °C for 1-hour, with a 24-hour resting period in between pasteurizations. A soil sample was then sent to A&L Great Lakes Laboratory, Inc. to determine the nutrient profile of the soil before amending.

SOIL TREATMENTS

Six treatment groups were created, each consisting of 15 pots with 1.78 kg of soil in each pot (5.5 inch Square Jumbo Dillon Greenhouse Pots, Growers Solution, Cookeville, TN). This pot size and volume of soil allowed for proper *L. sativa* plant growth (Whitacre and Neumann, unpublished). The first treatment group, T1, consisted of unamended pasteurized soil. The second and third treatment groups, T2 and T3, consisted of pasteurized soil amended with the mycorrhizal inoculants. T2 was amended with BioAg VAM Endo-Mix™. T3 was amended with MycoBloom. BioAg VAM Endo-Mix™ consists of several different species of VAM, the predominant one being *Glomus intraradices* (65.8% of the species mix). Smaller amounts, each comprising 5.7% of the species mix, of *Glomus claroidium, Glomus deserticola, Glomus mosseae, Glomus etunicatum, Gigaspora albida* and *Glomus clarum* were also included. MycoBloom consists of seven different VAM species native to the great lakes region. The species of VAM include: *Claroideoglomus claroideum, Funneliformus mosseae, Cetraspora pellucida, Claroideoglomus lamellosum, Acaulospora spinosa, Racocetra fulgida* and *Entrophospora infrequens*. The other three treatment groups, T4, T5, and T6, used the remaining half, 38.75 kg, of the pasteurized soil. This soil was moved to a separate container where a 1 L solution of water containing 31.14 g of $ZnCl_2$ was mixed in using a shovel. This was done for approximately 10-minutes to ensure uniformity mixing of the $ZnCl_2$. This process added approximately 0.4 g of $ZnCl_2$ per kg of soil. The pasteurized soil with $ZnCl_2$ added was then split up into the other 45 pots. This amount of $ZnCl_2$ was chosen due to the results of previous trials consisting of 0.5 g of $ZnCl_2$ per kg of soil (Whitacre and Neumann, unpublished), where plants across all zinc amended groups died in significant amounts.T4 consisted of the pasteurized soil amended with 0.4 g $ZnCl_2$ per kg of soil. T5, in addition to $ZnCl_2$, was mixed with BioAg VAM Endo-Mix™. T6 was mixed with MycoBloom™. The mycorrhizal inoculants for all respective treatment groups were mixed in by hand at 5.6 g per pot. Each pot contained approximately 1.78 kg of soil or soil with inoculate. All measurements made were made separately on a Taylor digital kitchen scale to the nearest 100th decimal place.

SEED GERMINATION AND PLANTING

Once each of the treatment groups were properly amended, the pots were immediately moved to Northern Michigan University's greenhouse. *L. sativa* seeds (Black Seeded Simpson, Johnny's Selected Seeds Co.) were germinated using a wet paper towel placed inside a plastic bag, for a period of 48-hours. Once germinated, two seeds were planted in each of the 90 pots to ensure survival of at least one plant. The pots were covered with plastic wrap and left for 10-days until the seedlings were culled to one seedling per pot. After 2-weeks since planting, the plastic wrap was removed. Another 2-weeks from this point, the plant diameter was measured every week for 4-weeks. A growth period of 55-days was chosen to ensure full maturity was reached, according to Johnny's Selected Seeds description. Plants were watered as needed throughout the experiment. The computer control system of the greenhouse conditions were set to not exceed 29 °C or fall below 16 °C. However, 3-days of the experiment temperatures had reached 38 °C due to a malfunction of the greenhouse cooling system. The light conditions consisted of 14-hours of sunlight, and 10-hours of dark.

SOIL AND TISSUE SAMPLING

After a total growth period of 55-days the *L. sativa* leaf and stem tissues were harvested. The harvesting was done by cutting the stem just below the basal rosette, at roughly the same place on each plant. Wet weight was taken for each plant to create an average, and then all the plants in each group were weighed together for a total group biomass. The soil from each treatment group was then bulked, taking care to keep each treatment group separate. Soil samples were then taken for each separate treatment group.

The soil and tissue samples were stored overnight in a cold room at 4 °C. The next day the samples were shipped overnight, unrefrigerated to A&L Great Lakes Laboratory (Fort Wayne, Indiana) and tested for their nutrient profile. The plant tissue was tested for total nitrogen using the Dumas method using an Elementar rapid-N cube, while mineral analysis was conducted using inductively coupled argon plasma using a Thermo iCAP 6500. The soil analysis procedures were done in accordance with "Recommended Chemical Soil Test Procedures for the North Central Region."

STAINING OF ROOTS FOR VAM

The roots were stained and analyzed for VAM colonization. They were first washed of any remaining soil with deionized water (DI water) and placed in separate beakers. Each group was then placed in 5 % potassium hydroxide (KOH) (w/v) and allowed to soak for a period of 2-days, until they appeared translucent and white (or as some would say, looked like "rice noodles"). The roots were rinsed with DI water in between soaking periods. The roots were then rinsed with DI water again and placed in a 5% hydrochloric acid (HCL) (v/v) solution to soak for 1-minute. A trypan blue stain was then created using 500 mL of glycerol ($C_3H_8O_3$), 475 mL of deionized water, 25 mL of acetic acid (CH_3COOH) and 0.1 g of trypan blue. The roots were allowed to sit in the trypan blue stain ($C_{34}H_{28}N_6O_{14}S_4$) for 7-days and were then observed under a microscope.

RESULTS

PLANT GROWTH AND BIOMASS DATA

The relationship between treatment groups and mean weekly plant diameter can be seen in Figure 1. T4, which contained zinc only (no VAM present) had stunted plant growth compared to all other treatments for all 4-weeks of measurements, however these plants had a clear linear growth rate throughout the 4-weeks. With all other treatments (either the control containing no zinc, or treatments with zinc plus VAM), plant diameter measurements were similar as shown by the clustering of the data (Fig. 1.). T5 had the greatest plant diameter at the end of the experiment, while T4 had the smallest diameter. The final plant diameters of T4 significantly differed from T1 ($p<0.005$), T2 ($p<0.05$), T5 ($p<0.00005$) and T6 ($p<0.0005$). Other treatments that differed significantly from each other, are T5 when compared to T3 ($p<0.05$), as shown in Figure 2.

The mean final biomass of *L. sativa* tissue is shown in Figure 3; the final biomass correlates similarly with the results of the mean final diameter. T3 and T4 had statistically significant lower biomass values than all other treatments ($p<0.005$), but they were not significantly different from each other. All the other treatments (T1, T2, T5, and T6) were not significantly different from each other.

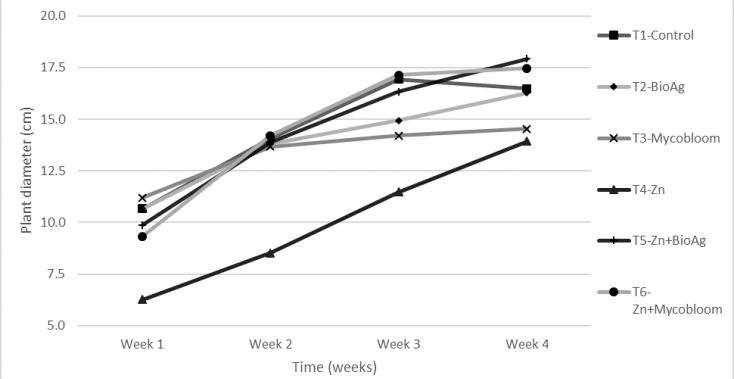

Figure 1. Mean *L. sativa* diameter (cm) at the end of each week of growth.

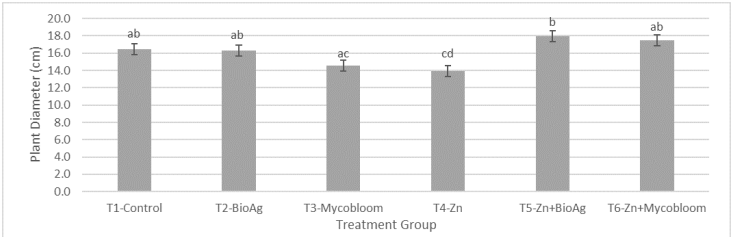

Figure 2. Mean final diameter of *L. sativa*. Bars represent standard error (n=15). Letters indicate significant difference, as indicated by Tukey's honest significant difference test.

ZINC CONCENTRATION COMPARISONS

The zinc concentrations in the above ground tissue of *L. sativa* are shown in Figure 4. Plant leaves from all 15 pots were bulked for zinc analysis (due to cost constraints) thus no statistical tests could be run. However, the tissues of the T4 samples contained a considerably larger amount of zinc, 952 ppm, than any of the other zinc amended groups. The tissues of the treatments treated with mycorrhizae and zinc, T5 and T6, contained 573 ppm (60.2% of the amount of zinc as T4), and 476 ppm (50% of the amount present in T4) of zinc respectively. The tissues of the treatments that were not amended with zinc, T1-T3, all contained similar small amounts of zinc at the end of the experiment.

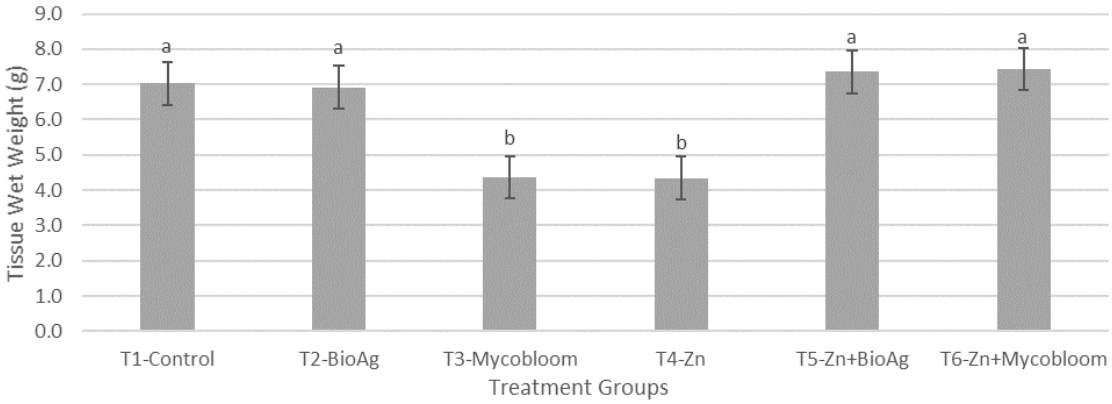

Figure 3. Mean tissue wet biomass of *L. sativa*. Bars represent standard error (n=15). Letters indicate significant difference, as indicated by Tukey's honest significant difference test.

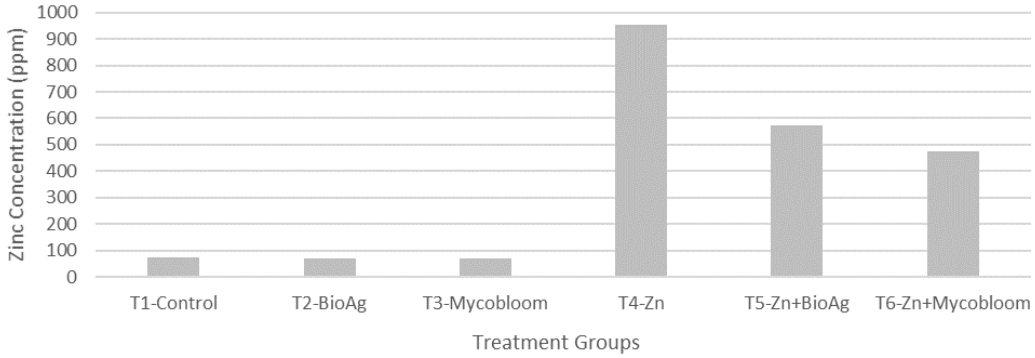

Figure 4. Mean zinc concentrations of *L. sativa* above ground tissue. Tissue from each treatment group was bulked together and tested for its nutrient profile.

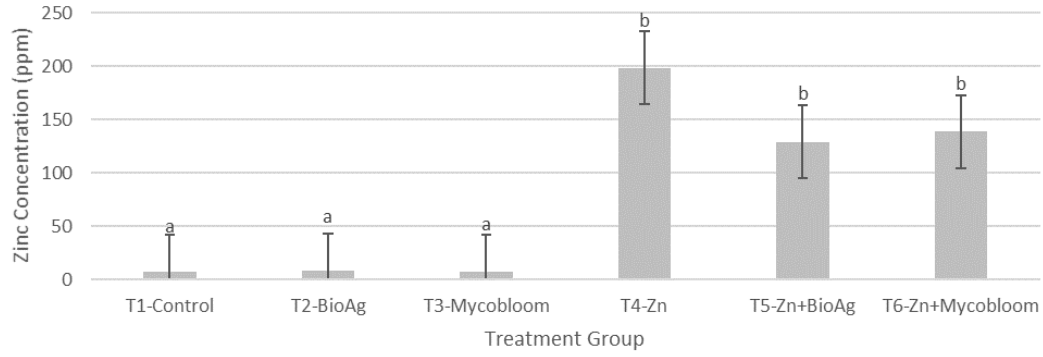

Figure 5. Mean amount of zinc remaining in soil after *L. sativa* was harvested. Bars represent standard error (n=15). Letters indicate significant difference.

The concentrations of zinc remaining in the soil are displayed in Figure 5. Soil from all 15 pots were bulked for zinc analysis (due to cost constraints) thus no statistical tests could be run. The treatment groups that were not amended with zinc all displayed similarly low levels of zinc. T4 clearly had the highest amounts of zinc in the soil. While the zinc concentrations of T5 and T6 had higher levels of zinc than unamended treatments (T1-T3), the levels of zinc are clearly lower than T4 that did not have any VAM treatment.

To ensure mycorrhizae had formed between the fungi and the roots in treatments T2, T3, T5 and T6 and that T1 and T4 were not colonized by VAM, root tissue was stained and observed under microscope. Figure 6 serves as a representative image of roots colonized by VAM, which were evident in all treatments inoculated with the mycorrhizal fungi mixes. T1 and T4 had an absence of mycorrhizae (data not shown).

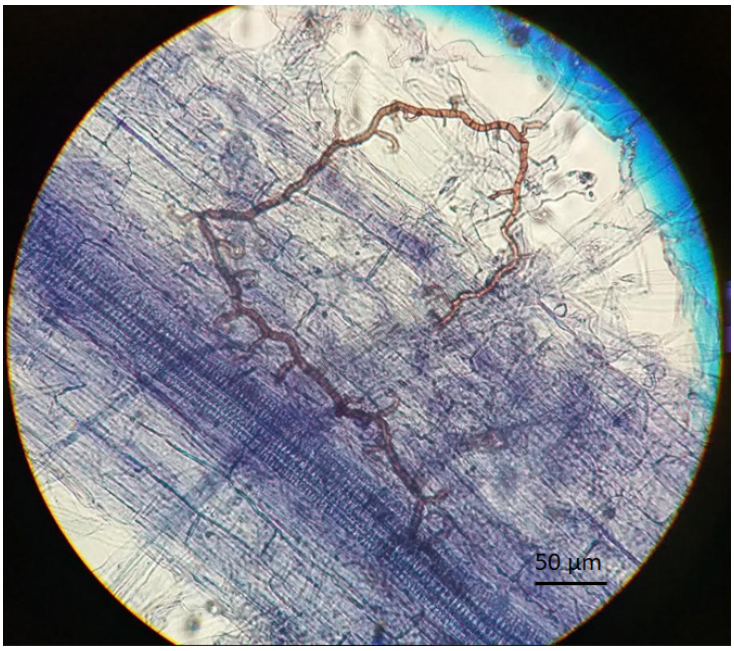

Figure 6. A representative image of *L. sativa* root tissue colonized by VAM.

STATISTICAL ANALYSIS

The results of this experiment were analyzed using the Tukey's honest significant difference test, conducted in the program R, to assess the diameters and biomasses of the various test groups.

DISCUSSION

The *L. sativa* plants, which were treated with only zinc, experienced stunted growth initially and throughout the experiment (Fig. 1). But, plants treated with VAM (T5, T6) in zinc amended soils had similar weekly plant diameters and biomass as the treatment groups that had no zinc present (Fig. 1). Taken together, this suggests the presence of the VAM aided in plant growth, likely by protecting the plant from growth-inhibitory or toxic levels of zinc. Previous studies mentioned plant biomass had significantly increased due to additions of zinc and VAM, but those studies applied lower concentrations of amended zinc (2, 16, 20). At the end of this experiment, T4 (treated only with zinc) exhibited significantly lower growth, biomass, and mean diameter compared to every other treatment group, besides T3 (Fig. 2 and Fig. 3). There were also two plant deaths that occurred in T4. During statistical analysis, the size measurements for the dead plants were counted as values of zero. The values were used in the analysis, because the deaths were believed to be caused by zinc, due to leaf chlorosis. Since this occurred, the treatments amended with zinc were on the threshold of zinc toxicity. Unexpectedly, without the addition of zinc, the plants treated with Mycobloom (T3) at the completion of the study were affected negatively to the point where they had similar biomass and plant diameter data to the plants in soils treated with zinc only (T4) (Fig. 2 and Fig. 3). This could be due to non-optimal conditions for one or more of the VAM species in the mix, perhaps causing a competitive or parasitic relationship to develop with the plants (10). The BioAg VAM Endo-Mix™, however, had a positive effect on plant growth, in both the zinc treated and untreated soils, when compared to their respective controls. This supports data found, in earlier experiments, on the ability of various mycorrhizal species to prevent heavy metal stress in *L. sativa* (13). The fungal species composition and ratios differed between the two VAM mixes, which could help explain the different impacts on *L. sativa* plant growth. Excessive heat in the greenhouse may have also played a role, although visible signs of widespread plant heat stress (e.g., chlorosis, wilting) were not evident through the course of the experiment.

Plant tissue grown in zinc amended soil without VAM (T4) had very high levels of zinc (952 ppm) (Fig. 4). In contrast, T5 and T6, also grown in zinc amended soil but with inoculated VAM mixes, had

substantially lower levels of zinc compared to T4 (573 ppm and 476 ppm respectively) (Fig. 4). This suggests the VAM were either sequestering some of the zinc in their fungal tissues, or possibly had chelated the zinc through release of extracellular chelators. Although the zinc levels in the soil remained high (according to the standards followed by the A&L Great Lakes Laboratories) (Fig. 5), the quantity of zinc in the soil of T5 was substantially lower than in T4, and zinc levels in the T6 soil was also lower than T4 (Fig.5) . This suggests that with longer VAM treatment times zinc concentrations may be brought back to within a normal range, if the fungi were sequestering the zinc from the soil into their tissues. The soils of T5 and T6 may also be considered nontoxic according to some standards that report a 300 ppm toxic threshold of zinc and the T5 and T6 values are below that 300 ppm (19). While the average value of T5 and T6 did measure below 300 ppm, health hazards may still exist (2). It is possible that treatments of *L. sativa* only, without the addition of mycorrhizae, could bring the soil to acceptable levels of heavy metal concentrations. However, using mycorrhizae would be preferential, as the lettuce would likely experience less strain from zinc toxicity (3, 13). Quantities of zinc remaining in the soil and tissues of T5 and T6 were different from each other (though not dramatically so); this, along with the unusually low plant biomass seen in T3 (which also had VAM treatment), suggest that different VAM mixes and pairing with proper plant species should be formulated to accommodate specific heavy metal contaminations more appropriately. Choosing VAM species that readily form mycorrhizae with the desired plants is important for this process. In this study, visual evidence of mycorrhizae formation was confirmed (Fig. 6). The results from this study support other published findings that combinations of VAM and their symbiotic plant species can be effective at remediating polluted soils (18, 24). Further, we believe combined remediation techniques such as using specific plant species, tailored VAM mixes, beneficial bacteria, and addition of chelators could be a highly successful strategy for bioremediation of polluted environments (9, 14, 16, 20, 21).

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THE ART OF THE SCIENCES THROUGH THE LENS OF UNDERGRADUATE RESEARCH

Hannah Fluhler

INTRODUCTION

I have always been a year younger than my peers, desperate to prove I measured up to them, meaning anything that could serve as a competition—from memorizing the unit circle to mastering a combination of challenging tap dance rhythms—has naturally manifested as such in my brain. Academics, of course, were the foremost concern in my life when my twin and I both accelerated past the eighth grade into high school from seventh, and I was eager to challenge myself with tough high school math classes (my favorite) and advanced placement courses. From 8am to 3pm I was a student, working through my lunch breaks and free periods on all homework assignments so that once 4:15pm came around, I could dive head first into my dance classes that would last until nearly 10pm on weeknights. Then, I would go to bed and start all over the next morning. This drive to be my best self in all facets and the focus I gained from this rigorous schedule served to propel me through school and allow me to succeed when I put my mind to something.

At some point early in my sophomore year of high school, a friend introduced me to Music and Memory, a student organization at Ball State through which Burris Laboratory School students could also volunteer to provide music therapy to nursing home residents with Alzheimer's and dementia. This was my first exposure to creative arts therapies, and looking back, it was the primary catalyst that propelled me toward the current path I am on now.

The use of the arts in aiding the physical and mental healing process of adults was a new idea to me in high school, but once a friend pointed out that I could go on to pursue something similar to this music project called dance/movement therapy, it all clicked for me. Coming into Ball State as a freshman, I still was not quite sure how to combine my love for dance with my ever-present desire to care for others. I knew I could learn about dance/movement therapy through a Master's program after graduating, but I did not know what that would mean for these next few years. After several major changes and picking up a dance performance minor the day before classes started that fall, I somehow made my way to now, as a double major in Nursing and Pre-Dance/Movement Therapy (an Integrated Studies major I created through the Honors College—that's a story for another day).

I have been extremely blessed in being a recipient of several life-changing scholarships. I am now a college senior and the inaugural recipient of a named, paid fellowship. Through this fellowship, I have applied for and received permission after a full review from the local hospital's Institutional Review Board to begin a research study in the Neonatal Intensive Care Unit. This study is exploring the physiological safety of movement and music using a Mamaroo baby swing for newborns suffering from withdrawal due to maternal drug use, known as Neonatal Abstinence Syndrome. In addition, I have presented information about safe sleep and the use of movement and music to help similar infants to mothers in Meridian Health Services' Maternal Treatment Program alongside a Neonatal Nurse Practitioner, applied for grants to fund the donation of Mamaroo swings to these mothers, and attended conferences about Neonatal Abstinence Syndrome and the use of creative arts therapies with this population.

In my next phase of life, I will be moving to Melbourne, Australia, for 10 months to pursue much different research in the Genome Stability lab at St. Vincent's Medical Institute for Research through a Fulbright scholarship. I will be exploring new treatment methods on the biochemical level for a rare disease called Fanconi anemia (FA). Because the cellular processes of FA are so similar to various cancers, this research offers hope for furthering cancer research, too.

Needless to say, though I am truly just beginning the process of completing undergraduate research, and in a completely different light than microbiology, I have failed more than I have succeeded and am grateful for the opportunity to pass along what little knowledge I have gained from the hurdles along the way.

THE DOS AND DON'TS OF UNDERGRADUATE RESEARCH

First and foremost, the first and most important step I took to accelerate the research process was to find an excellent—not just great—mentor. A few things specifically ensured I did this:

- I reached out to potential mentors whose research interests me. Whether current or past pursuits, the wisdom these individuals can pass down through a short, simple face to face conversation is immense. So, send that email to the address you found online, or reach out to that person your nutty professor nudged you toward. The worst thing that could happen is they don't respond. And, if that happens, show up to their office. Be persistent about gaining the best background information on the subject or project you are most interested in—it can only help you be better.
- I became comfortable with casually bringing up my research ideas with anyone who would listen. I can't specifically remember how long it was between the first time I thought about newborns suffering through drug withdrawal and actually engaging my mentor for the first time, but I can recall the most receptive people who I bounced ideas off of in the mean time. Not everyone will understand your idea, especially for those in the microbiology field, but you never know who will ask the right questions or spark the "ah-ha" moment when you mention your most specific interest and its applications to broader topics. For me, it was my psychiatric and mental health nursing clinical instructor who stood with me several Wednesdays in a row and happened to nod her head at the right times and egg me on when I doubted that my idea of using movement with newborns could be viable as a research topic. That instructor ultimately pointed me toward my eventual mentor in this endeavor.

The worst mindset I had during the process of developing an undergraduate research project was that if I worked harder and faster for more hours than I already was, that the whole cycle would magically speed up. This was problematic simply because so many factors were out of my control along the way. From the beginning, I knew that however I went about this, it would require the collaboration and careful leadership of dozens of moving parts, beginning with putting together a research team. Although individuals working as lab assistants do not necessarily have to assemble their own team, learning how to navigate these environments with opinions and viewpoints of people vastly different than yourself is beyond necessary. From team members, to research boards approving your work, to advisors and sources of funding, learning how to communicate with and make decisions as a part of something larger than yourself is integral to the success of your project.

It could be argued that communication is one of the most important pieces of any endeavor, but when it comes to research, it is everything. Communication has a role in applying to be a part of the project, proposing the steps to the project or dissecting the instructions for the research work, and finally, of course, documenting and disseminating the work you have done. While I do not believe there is a one-size-fits-all recipe to effective communication, I do know that patience, conciseness, and knowing your audience can solve most problems faced.

MASTERING THE ART OF SCIENCE

As an undergraduate, I have found power in connecting my interests across the lines of various classes and subject matters. From jazz dance technique class to my pediatric nursing courses, and even throughout my 5-week summer abnormal psychology course, I found that I learned so much more when I became confident enough to inquire about my interests, and to approach my peers or my professors who may help me connect the dots I am seeing. I was first urged to apply for a named, paid fellowship by a friend who recently graduated from BSU who is also a recipient of a scholarship which pays for full tuition, room, and board, and instantly knew I wanted to take advantage of the opportunity by at least applying. For several weeks, I struggled to choose which of my pursuits would best fit this fellowship. It wasn't until nearly two weeks before the application deadline that I knew what I would write a 10-page proposal about, and it

connected almost directly to the major I had created for myself: exploring a very physiological problem through an artistic approach.

I know a lot of people who are undergraduates and took years to decide what single major they would pursue. For me, I always knew I would need more than one major, but that it was likely it would be best if I was able to combine several. Dozens of my friends had played a sport or had a relatively "artsy" extracurricular activity that took up most of their time in high school, but most of them had all but abandoned this main love of theirs for a more typical career path by the end of our freshman year. Even my best friend had told her dad she wanted to be a teacher, but was faced with the statement that it wouldn't make enough money, so she should choose another path. While this may seem like an either-or decision for her, I was not so willing to give up my biggest pull—dance—to help people as a health care professional. So, I sought out the circumstances necessary to create my own path, which of course came with its own set of challenges.

My background as a competitive dancer taught me what I believe a lot of scientists, even undergraduate research lab assistants, should know. Pushing yourself out of your comfort zone while remaining open to constructive criticism, and then using that feedback to continue to grow, can be a game-changer in an environment as self-driven as research. Whether in a kinesiology, microbiology, or creative arts-based pursuit, being able to continuously make adjustments to your thoughts, actions, and words can be the difference between completing a project with your head barely on straight or reaching the finish line with a successful result and fresh energy to pursue the next set of experiments. Shaping yourself to best fit your passions, your vision for your future, and the requirements for a project you desperately want to be a part of is vital to research. I had never completed my own literature review before this research, nor had I presented my ideas in front of a board of community professionals with the hope that they would give me the green light instead of stopping my project in its tracks. These were two of the biggest growing experiences for me. Along with the task of forming the research team, these experiences will help me endlessly in my future. If I had not adjusted my mindset going into this project, knowing that I would have to do hard things I had never even tried before, I would not have gained approval. I can imagine that lab work is similar—whether you have executed an experiment before or not, it is nearly guaranteed that you will be a professional at the research task at hand when completed. All it takes is to have the courage and drive to begin, and the passion to continue to see it to the end.

FINDING YOUR FOCUS: THROUGH YOUR LENS

One of my outstanding literature classes in high school taught me that everyone sees life in general, as well as individual conversations, through a certain lens. Whether this lens is one of gender, race, class, or even profession, we all have a certain lens through which we automatically view most things. We all also have the potential to switch our lenses to match that of someone else or of a new perspective, theory, or ideology of our choosing. I know there is extreme value in viewing one thing through several different lenses, and that this can offer a new perspective otherwise unseen through our initial lens. I have also learned lately that focusing on your passions and pursuits is impossible without the consistency of a personal lens.

For example, a white, snowy background can turn red or blue with different pairs of sunglasses on, but you wouldn't want to wear a different pair of glasses to attempt to perform different parts of a single experiment on the snow, as this could cause skewed results and inaccurate data collection. For the same reason, I have tried my best to gather all of the information available to me about newborns withdrawing from various drugs and the causes and treatments used for them. I began from day one of my undergraduate career forming my own individual lens through which I have been able to take into consideration the psychological and physiological causes of addiction in the mothers. Addiction competes with a mother's drive to do her best to keep her baby healthy, in order to best empathize with her when asking for her consent to enroll her three-day-old child in a research study. If I were to listen to one physician's biased opinion that all of these mothers, like his first patient who came in high to visit her baby, are simply addicts who have no hope of providing a good life for their child, I would not see another mother's sobriety. If I were to listen to one counselor's viewpoint that every mother with an addiction can be helped with an opioid agonist, I would not hear another mother's opinion that it did not work for her. If I were to simply believe that the

current line of treatment for babies with Neonatal Abstinence Syndrome—treating their symptoms with morphine— is the best and only option there will ever be, I would be cheating babies with mild symptoms of a drug-free neonatal period. If I were to take any one biased opinion and take it as truth, use it as my lens, I would not be able to contribute to literature on these topics to hopefully improve the treatment and outcomes of these mothers and their children.

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I would love to thank Kelsey Davis for asking me to write this perspective piece and Professor John McKillip for fostering my first spurs of interest in undergraduate research during my time in *Fine Focus*. Many thanks to Dr. Renee Twibell for being the most incredible mentor I could imagine, and to each and every one of my professors and clinical instructors for the constant encouragement and questioning about my impossible quest to graduate with several majors in seemingly unrelated fields. Finally, thank you to Sweet Aria for being the brightest star in the sky and to her mother, Rachael, for the continuous inspiration to keep fighting the good fight to improve the lives of the most complex pediatric patients.

POSITION ANNOUNCEMENTS

Dean, College of Arts and Sciences

Indiana Tech University

At Indiana Tech, we excel at educating students from all walks of life, for all kinds of meaningful work. We're a truly inclusive community of learners, ensuring that students are supported, inspired, and empowered to maximize their professional prospects, advance in their careers, and improve their lives and the lives of others.

We are a thriving, independent, teaching-oriented university where students learn best when they meaningfully engage with a real-world professor. With an alum. With a cause. With a team. With a peer. Simply put, our model is hands-on.

Indiana Tech's focus on career/professional programs includes offerings in business, engineering, information technology and computer science, criminal justice, life sciences, human services and many other concentrations. Indiana Tech enrolls over 1,500 students in its traditional programs on itspicturesque main campus in Fort Wayne, and nearly 6,000 students in its College of Professional Studies programs at 18 regional locations and online. Degrees are offered at the associate, bachelor's, masters and doctoral levels.

Over 48% of our students are either globally or domestically diverse; this contributes to our unique, vibrant and inclusive campus community. All academic programs are designed to provide students with relevant and practical experiences, including an emphasis on internships. The university is accredited by the Higher Learning Commission, and holds numerous discipline-specific accreditations.

The Indiana Tech community recently engaged in a comprehensive strategic planning process, designed to develop a roadmap for continued growth and development leading up to our centennial year in 2030. Learn more about our compelling plans for our future at Indiana Tech Strategic Plan.

The Dean of Indiana Tech's College of Arts and Sciences will join this special community to provide leadership and effective management in all matters pertaining to the College of Arts and Sciences, including administration and continuous improvement of its academic and co-curricular programs, program and institution-wide assessments, faculty development, and the recruitment, success, and retention of students.

The Dean will initiate and manage collaborative relationships with supporting and associated departments, lead college faculty and others as needed to build, launch, and continuously improve the college's programs and courses, and ensure the relevance of the College's programs to practitioners and the linkage of each to the standards of its respective field. Additionally, fostering academic innovation and consistently outstanding teaching in all modalities, supporting, developing, representing, and advocating for faculty in teaching, service and scholarship initiatives, and participating in the search for, and approving the hiring of outstanding faculty for the College are essential duties. Evaluating faculty service and performance and teaching one course per semester are requirements of the role.

Indiana Tech's new Dean of Arts and Sciences will participate in the development and implementation of the university strategic plan, team with other Deans, the Vice President for Academic Affairs and others to lead the academic initiatives of the univer-

sity, plan and manage the College's budget, and support fundraising and alumni efforts. The Dean is expected to develop and utilize alumni/practitioner advisory boards, develop and maintain a presence in the local community, and identify and arrange meaningful co-curricular opportunities for students in the College.

The Dean chosen will hold an earned doctorate in a discipline relevant to Indiana Tech's College of Arts and Sciences and a minimum of five years' successful leadership experience in an academic setting, with past experience as a dean preferred.A minimum of five years' successful college teaching experience is required, with online teaching experience preferred. Evidence of creation, launch, and management of new academic courses and programs is required. The candidate will have familiarity with the professorial rank, tenure, and promotion process with earned tenure, preferred.

The Dean must have a strong commitment to the role of the College of Arts and Sciences in a university focused on preparing students for professional careers and lives of significance and worth;to providing educational experiences delivered in varied term lengths, locations, and modalities; and to engaging with students and leading faculty to deliver a consistently outstanding student experience. The candidate chosen must have a record of scholarship and the ability to mentor and support faculty and students in scholarly pursuits, and have evidence of a commitment to shared governance. The ideal candidate will be able to demonstrate outstanding communication and interpersonal skill, show evidence of effective team leadership and a history of working well in collaboration with others, speak to the ability to build consensus in the pursuit of shared goals, and confirm a commitment to developing and supporting diverse groups of faculty, staff, and students.

A complete job description is available to qualified candidates.

Indiana Tech embraces diversity and equal opportunity intentionally. We are committed to building a team that represents a variety of backgrounds, perspectives, and skills. We believe that diversity andinclusion among our employees is critical to our success with respect to educating our students to become global citizens. We are an equal opportunity employer.

Application materials must include a cover letter, vitae, and transcripts.For preferential review of materials please apply by November 20, 2019.

Assistant Professor of Biology

Ball State University

The Department of Biology at Ball State University invites applications for a tenure-track faculty position in the area of Immunology. The successful candidate will be able to teach undergraduate Immunology and Introductory Biology. Other teaching assignments may include courses in the applicant's area of expertise. The candidate's research program should involve undergraduate and Master's students in novel avenues of investigation in thefield of Immunology while complementing and collaborating with current faculty in the Department of Biology and/or the BSU Center for Medical Education (Indiana University School of Medicine-Muncie Campus).

The strongest candidates will have a record of accomplished teaching at the undergraduate and graduate level; demonstrate evidence of outstanding scholarship in the form of peer-reviewed publications, and potential for external funding. The Department of Biology benefits from strong institutional support, promotes collaborations amongst faculty, and shares core research and teaching equipment.

Minimum qualifications: Earned doctorate in Immunology, Biology, Molecular Biology or related field or M.D. /Ph.D. from an accredited college or university.Employer will consider sponsorship.

Preferred qualifications: Demonstrated teaching ability in Immunology, Introductory Biology, or related disciplines using student-centered learning techniques; evidence of scholarly activity, such as postdoctoral experience, publications, and successful procurement of external grants; established research agenda that complements existing departmental research programs; collaborative potential with existing faculty at Ball State University. Effective written and oral communication skills; commitment to excellence in teaching; and competency in current research concepts and approaches.

Apply online at:http://bsu.peopleadmin.com/postings/19211. Include the following documents with your application: Cover letter addressed to Dr. Doug Bernstein, Curriculum Vitae, Teaching Statement/Portfolio, Research Statement/Papers and Teaching Evaluations. The option to upload transcripts is available. Original, official transcripts showing the highest related degree earned is required at the time of hire (even if obtained at BSU). Degree verification will be conducted. Review of applications will begin immediately and will be accepted through November 28, 2019.

Ball State University is located in Muncie, Indiana, approximately 45 miles northeast of Indianapolis. Approximately 21,000 undergraduate and graduate students enroll each year in diverse academic programs on and off campus. Our students come from all Indiana counties, all 50 states and 68 countries to pursue knowledge in seven academic colleges offering 190 undergraduate majors, 130 undergraduate minors, 140 graduate programs and 200 study abroad programs.

The Ball State way is rooted in the Beneficence Pledge – a commitment to excellence in teaching and scholarship, honesty and integrity, social responsibility, gratitude and valuing the intrinsic worth of each member of our community. Ball State students, faculty and staff are empowered in a culture that believes in them and demands they believe in themselves. They are partners in an innovative, immersive approach to education. They are supported by living and learning facilities that enable intellectual curiosity. We graduate scholars who are changing the world, and we've dedicated our University to do the same.

The university offers an excellent wellness program and extensive benefits offerings to include a generous paid time off package and paid parental leave. For further information regarding benefits please visit: https://cms.bsu.edu/About/AdministrativeOffices/HumanResources/Jobs/Benefits-and-Community/Faculty

Ball State University is an Equal Opportunity/Affirmative Action employer that is strongly and actively committed to diversity within its community. Women, minorities, individuals with disabilities and protected veterans are strongly encouraged to apply. All qualified applicants will receive equal consideration foremployment without regard to race, color, religion, sex, national origin, age, disability, protected veteran status or any other legally protected status.