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LETTER FROM THE EDITOR

Do not go where the path may lead, go instead where there is no path and leave a trail. –Ralph Waldo Emerson

It is clear to me that Weber State University is opulent with bright minds and inventive spirit. In my own time here at Weber, I've found that every academic step taken at this university—as individuals—is an act of interdisciplinary endeavor. To put it more potently: each student has something to bring to the table. Every piece of research, though specific in its scope, has a place among each of us as a new way to think and to learn.

It has been my incredible pleasure to be involved with ERGO this academic year, to share in the spirit of curiosity and exploration of ideas. All contributors to this journal have brought along something new from their respective field, in the effort of enriching themselves and the minds of others. As a graduating Wildcat, I am overwhelmed to see the research of others—from all disciplines—present in this book. I hope that you, dear reader, become as thrilled as I am to enjoy the student work that is here, for you, on display. And to all of our contributors this year I say a proud "thank you!" and I wish you well in any-and-all academic pursuits—as you look to future.

Cheers,

Jacob D. Alvey

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Telitha E. Lindquist College of Arts & Humanities

Communicative Outcomes of Dialectical Tensions in Sheriff's Deputies

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Mentor: MICHAEL AULT

COMMUNICATION

ABSTRACT

This qualitative research explores the outcomes of dialectical tensions experienced by sheriff's deputies, based on ninety hours of observation and ten semi-structured interviews. After collecting the data, the constant comparative method was used to analyze the data. Consequently, the research identified three outcomes of dialectical tensions: 1) jadedness, 2) hypervigilance, and 3) dark humor. These outcomes have negative effects that can lead to unhealthy relations and mental health issues. The results of this research will help future researchers seeking these consequential patterns to identify techniques that can heal intrapersonal and interpersonal maladies.

INTRODUCTION

The purpose of this research is to understand some of the communicative patterns and negative outcomes of dialectical tensions experienced by law enforcement officers. Dialectical tensions are defined by Baxter and Montgomery (1996) as "dynamic interplay between unified opposites" that people experience in relationships. It has been used in the framework of interpersonal communication (Rawlins, 1992) and organizational communication (Lewis, Isbell, & Koschmann, 2010). "The need to enforce the law and the need to be a peace-keeper" is referenced briefly as a dialectical tension in the context of law enforcement by Giles (2002).

Other dialectical tensions discovered from the data collected in this research are the desire to serve and protect citizens yet their desire to protect themselves is more important, the enjoyment of high-excitement calls contrasted with the soporific sessions of paperwork, and dealing with vituperative or violent people vs. quotidian interactions with supportive and polite individuals. The capricious situations surrounding these dialectical tensions can elicit intense stress.

DATA COLLECTION

The author was allowed to conduct interviews and accompany and observe

deputies as they carried out their day-to-day duties for a total of ninety hours. Ten out of twelve formally consented; the other two, did not for fear of becoming targets of violence.

As the research unfurled, we found ourselves on roads as diverse as highways, freeways, park-and-ride lots, fields knee-deep with snow, and a national forest. Most observations and interviews were conducted in patrol cars during ride alongs. However, research took us to many places outside of the patrol car. It took us to places such as debriefing meetings, dinner meetings, medical calls into homes, nursing homes, assisted living homes, a Super Bowl party, a firehouse, a hospital, and a jail.

RESULTS

With the time spent observed from the deputies and consequent coding, three main themes burgeoned and have become the focus codes of continual exposure to dialectical tensions: 1) jadedness, 2) hypervigilance, and 3) dark humor. Interestingly, these three indications seem to be deployed as coping mechanisms and protective measures.

Jadedness

Jadedness is an intense cynicism that seems to infiltrate the lives of most of the deputies. It is a disparaging worldview that seems to latch on and intrapersonally harass any attempt to see the world in a positive light. This is not to say that these deputies enjoy being jaded—the contrary seems to be more accurate. One deputy suggested jadedness is an internal process affected by the surrounding environment. He said, "You need to be able to protect your own emotions, but still be able to talk to someone like they're a human, and have compassion." This illustrates the cognitive and social dissonance caused by fighting to balance a compassionate view with harshly regulating emotions. Another deputy confessed the difficulty in this endeavor:

After a while, [jadedness] just happens. It's human nature. I know for a fact I'm not the same person I was before this job. We are surrounded by criminals but we don't follow the same standards they do. We are inundated by the worst of society and so it just happens.

One less experienced deputy, who had recently finished field training, had a different but corroborative view. The tyro said he had not yet "hit that jadedness yet. [But] I would be in denial if I said I hadn't changed. [After] years on the job, you start seeing things differently." These two quotes from both participants expose their sense of how inevitable jadedness is—it is as if they cannot escape the outcome of that dialectical tensions. In their minds, it is impossible for the experiences and continual exposure to traumatic or difficult situations not to

change them.

Jadedness takes a mental toll on the participants, not only in seeing the world in a more negative light, but also individuals. One deputy's words exemplify how jadedness seems to inundate the deputies' thought patterns and communication:

It gets hard over time when you have an experience and that similar people are not the same. …If you see a white skinny motherfucker with a bunch of weird tattoos, I assume they're meth heads. So, I need to recognize that when I talk to them, and then after an investigation, see if they really are or not.

Jadedness also takes a mental toll on the deputies' wellbeing, as they try to cope with stress from dialectical tensions. "This is the kind of shit that makes me jaded," said a deputy, recalling a pedophilia case. "It makes me hate society. I don't want to think about it too much, it makes me depressed. Let's go find a car [to pull over] and make me happy." This conversation revealed the negative impacts of jadedness; the deputy was obviously distraught and sought emotional relief.

Hypervigilance

Hypervigilance is a constant state of alertness, even when deputies are not on duty. It is a mindset that produces an uneasiness regardless of the situation. For example, one deputy explained it by saying, "You have to be cautious, like don't take things too lightly. But you gotta be careful because you don't know when someone is going to turn on you." This communication suggests the always-ready-for-attack anxiety that seems to permeate every facet of their lives. It also reveals the mindset that the deputies see anyone as a potential threat. Another deputy admitted:

It's a very difficult thing to show an equal amount of respect to everybody. I think in today's social and political climate, it's all about equality but that goes against nature. Some people are more threatening than others…. If human beings don't understand that, then they are opening up themselves up to harm.

This represents the motivation that drives deputies to cultivate hypervigilance. The deputy continues to explain his rationale by providing this metaphor:

You have to stereotype people that might be bad guys, because it's a defense mechanism. It changes things. It has to. If you get a knock on your door, if you have a girl scout with a bag of cookies, would you be interested? If it's raining, and she says can I come in, how will you respond? A few minutes later, there's a knock on your door with a few tears tattoos on his face, duffle bag, and he says he's checking cable inspections, how would you respond? You have a defense

mechanism in you that says, this might be a threat. …Society kind of molds us, and our experiences teach us what those threats are; and day in day out as a law enforcement officer, we see a lot more things that are threatening than you do. … We deal with that all the time: every traffic stop you deal with one or the other. This anecdote uncovers a startling confession the deputy sees stereotyping as appropriate and as a defense mechanism. It also alludes to the perspective that the deputy believes society and experience build up the hypervigilant mindset, which in turn produces jadedness.

Another facet of this hypervigilance is a communicative sensation known as the what-if game. This is a mental preparedness exercise that involves intrapersonal communication, in that the person will imagine a negative event and then make plans on how to react to the hypothetical situation. One deputy explains how he plays what-if, saying "I have sat in a restaurant and have mentally killed fifty people. When someone comes in with a gun, how am I gonna respond?" This hope-for-the-best, prepare-for-the-worst mentality is difficult to turn off. One deputy said, "I even carry a gun in my own house" because of the mentality, illustrating the omnipresence of hypervigilance.

Dark Humor

Dark humor is an outwardly inappropriate hilarity that covers topics as gruesome as suicide to topics as controversial and taboo as mental illness. In the words of one deputy, dark humor is, "…being able to crack jokes about the suicide that just happened. Most people would go to situations that we do, and say, 'I can't believe you deal with this.' [Well], most of us deal with that with dark humor."

This reveals that this deputy, and most of his colleagues, use dark humor as a way to process or cope with the constant exposure to negative or difficult experiences.

This dark humor, according to another deputy, is a social and psychological necessity. "You can't survive this career field without some type of humor. We can't live our lives all super serious and miserable. We laugh at all the time," he said. In similar tones, another deputy called it a "stress relief." This implies that the deputies use the dark humor to relieve themselves, it is a cathartic communicative behavior for them.

Another deputy agrees. He said, "A lot of people develop a dark humor, almost as a defense mechanism. It's not meant to offend it's just what it is. I gotta be real careful around the family about what I say." This communication is fascinating in that the participant recognizes the uniqueness of dark humor, and using code-switching while navigating through the apparently very different cultures of his organization and family.

Within the organization, humor not only serves as an emotional analgesic but also a connective tissue that builds camaraderie. Some examples of these are the practical jokes that the deputies play on each other. One of the deputies, with a smile, shared two of his pranks:

We have a jelly with numbing properties, so put that on the car door handle [of other deputies' vehicles]." or "if they leave car unlocked, get a wet towel and leave it on their seat, soak up all the water for a few days."
This lightheartedness seems to provide some consolation to the deputies while building camaraderie, similar to mischievous sibling shenanigans.
"[We're] kinda like a big family," one of the deputies explained. "We all trust each other, and look after each other, no matter how much we drive each other nuts. It's kinda like siblings, ya know?" This humor builds the family-like bond that increases cohesiveness and generates a playful atmosphere that helps assuage negative experiences and dialectical tensions.

DISCUSSION

Important Findings

Identifying the three themes of jadedness, hypervigilance, and dark humor is important for future studies involving law enforcement and communication. It is also important in combating the current mental health crisis in the United States. Though these themes arising from dialectical tensions may not be inherently destructive, they can lead to intrapersonal and interpersonal catastrophe. For example, one study by Freeman et al (2000) linked hypervigilance to the development of symptoms common of stress disorders in college students and Kimble et al (2014) suggested hypervigilance is associated with generalized anxiety disorder. The author's data also holds that jadedness and continual exposure to dialectical tensions can lead to anhedonia and depression. Dark humor has negative consequences in that it offends those who are not part of the group, particularly the family. This in turn leads to some of the deputies attempting to compartmentalize their communicative patterns.

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Communication Strategies Utilized by Nonprofit Organizations in Recruiting and Retaining Quality Volunteers

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COMMUNICATION

ABSTRACT

This preliminary study was conducted to approach the question of how nonprofit organizations increase recruitment and retain quality volunteers through communicative strategies. Using purposive sampling, I conducted three semi-structured interviews with employees of local nonprofit organizations. Through structuring a constant comparative analysis of the interviews, qualitative data revealed nonprofit organizations can reach this goal by cultivating inspired networks through fostering synergetic relationships. This axial code is constructed of five focus codes: transcendent values, strategic partnering, appreciation tactics, community outreach and leadership development. In applying these collective ideals, nonprofit organizations can compare success stories and pin point progressive communication tactics through reflection. Allowing for shared ideas and better understanding, this research acts as a base for broader research to be conducted so that nonprofit organizations can achieve their goals and lift overall knowledge, mindfulness and outcome.

INTRODUCTION

The central focus of this study looks at how organizations successfully entice volunteers to engage with their cause and stay active in their mission. By interviewing three employees of local nonprofit organizations, I gathered a better idea of communication strategies used to recruit and retain volunteers by comparing success stories and pinpointing progressive communication tactics within these organizations. This research allows for shared ideas and reflection on successful tactics to gain better understanding of the way nonprofit organizations recruit and retain dynamic volunteers.

LITERATURE REVIEW

In the United States, volunteerism is an important aspect of most people's lives. On average, Americans donate four to five hours of their time per week to various nonprofit causes (Hooghe, 2003). Despite the importance of volunteer

work, most organizational literature has focused on for-profit organizations (Kramer, 2011), neglecting this crucial source of labor and community growth. Socialization of volunteer organizations has explored the difference between for-profit and nonprofit organizations in recruiting talent. Importantly, nonprofit organizations often do not have the resources available to compensate workers for their effort and must find alternative methods of incentivizing membership (Haski-Leventhal & Bargal, 2008). The Volunteer Functions Inventory identified thirty reasons an individual might be encouraged to volunteer and measures six primary functions: values, understanding, career, social, esteem, and protective (Clary, Snyder & Ridge, 1992).

The literature described above gives good insight into the importance of volunteer workers and their motivations for involvement. However, this study seeks to understand how non-profit organizations recruit and retain volunteers in best practice. Thus, this study fulfills a significant need in volunteer research regarding nonprofit organizational structure and defining applicable strategies for success. To this end, I propose the following research question.
RQ: What communication strategies do nonprofit organizations use to increase recruitment and retain quality volunteers?

METHODS

Participants

As this research was intended for a course assignment, I conducted a small, preliminary study through purposive sampling. Purposive sampling is best applied when the researcher seeks qualitative information from individuals selected based on educated judgement regarding the study and how they could be complimentary through experience and standing (Lindlof and Taylor, 2011). Via e-mail and personal network referrals, I reached out to three women who are employed by nonprofit organizations and have relevant responsibilities. The first, Carley, is an operations manager for a foundation seeking to promote active lifestyles. The second, Brandy, is a volunteer coordinator for a nonprofit organization that aids those in poverty. The third, Aly, is an assistant site coordinator for an afterschool program that serves underprivileged youth. Participant ages ranged from 22 to 58.

Data Collection

After completing consent forms and describing the nature of the study, in-depth semi-structured interviews were conducted with each subject, ranging from 11 minutes to 40 minutes. By choosing semi-structured interviewing, I was able to engage in follow up questions leading to a more natural movement of conversation, gaining rich insight of personal experience and best practice. An interview guide with six main questions and seven discussion points was

developed to retrieve qualitative information from each subject. Examples of questions asked are, "Can you tell me about a time your organization has a really good volunteer turn out for an event? and "How do you retain volunteers and keep them excited about your organization?" Each interview was recorded on a smartphone and stored under password protection. When conducting the interviews, settings were chosen by participants which allowed for a comfortable environment of their choice; including two coffee shops and an office.

DATA ANALYSIS

In qualitative research, data would normally be collected until saturation occurs; however, due to the study being a class assignment and preliminary in context, three, qualitative interviews were used to craft a constant comparative analysis. Constant comparative analysis was the chosen approach as it allows for representation of each participant's thoughts and allows me, as the researcher, an opportunity to build a theory from patterns found in the data. (Charmaz, 2006) A similar approach was taken by Ault and Van Gilder (2016).

After conducting all three interviews, I listened and re-listened to each audio recording in an iterative fashion and took basic notes of concepts that stood out to me. After listening to each interview audio recording a third time, I then went through the process of data reduction by erasing any information that was not pertinent to the research question itself. Data remaining was then re-analyzed to identify promising and frequent codes in the practice of open coding. Next, I created focused codes by comparing any overlap in the initial codes and arranging them into larger categories of emphasis. Lastly, by linking the focused codes and repetitively returning to the raw data to fully assess information collected, I recognized an axial code that correlated interrelationships between the categories.

RESULTS

This constant comparative analysis accounted for all data comprehensively and brought together a preliminary study that shares insight to communication strategies in recruiting and retaining quality volunteers for nonprofit organizations. In response to the research question, "What communication strategies do nonprofit organizations use to increase recruitment and retain quality volunteers?" The data suggests that nonprofit organizations achieve this goal by cultivating inspired networks through fostering synergetic relationships. This axial code is comprised of five focused codes: transcendent values, strategic partnering, appreciation tactics, community outreach, and leadership development. These codes lead to a progressive understanding of strategic communicative tactics operated by nonprofits to recruit and retain quality volunteers.

Transcendent Values

In nonprofit organizations, there is often no product to be sold or service to be purchased, instead, donors, volunteers, and community members must find alternative benefits from participation with non-profit organizations. Every nonprofit has a mission statement that shares their specific cause, goal, and impact. In presenting mission statements, nonprofits can influence volunteers' emotions and provide experiences for volunteers that go beyond distributing food or raising money and focus instead on the transcendent value of helping those who need them the most. One way the participants described focusing volunteers on larger transcendent values was by documenting the outcomes of their service through taking pictures at events or during programming so that the volunteer can connect their service to an actual individual that benefited from their contribution. Aly shared,

It is important for our volunteers to really feel the impact that they make through their contribution whether it is time or money. Therefore, we make sure to include photos, stories of individual kids, videos, or handmade cards from kids. When we do this, the volunteers feel connected to something bigger than themselves and want to come back to keep making positive attributions to our organizations cause.

This imagery tells a story how the volunteers' labor is affecting the community being served. In asking Carley why pictures aid in recruiting and retaining volunteers she mentioned, "whether its people holding each other crying and celebrating together at the finish line, or its volunteers clearly having an enjoyable time, those pictures bring out emotion." In this case, Carley identified emotion as a driving motivation for volunteers and used it to recruit and retain their services.

Strategic Partnering

Whether it is building relationships with organizations that can provide resources or collaborating with organizations that can provide a workforce, partnership is of the highest importance in nonprofit organizations. All participants in this study shared that they have positive relationships with the local University. Brandy mentioned, "they have a health program and we have a health fair coming up, so I emailed the department and asked if any students wanted to come and volunteer." In finding areas of programming that relate directly to departments on campus, nonprofit organizations can pull volunteers that are seeking degrees aligning with their cause and offer them an opportunity to gain career building experience through volunteer internships.

In addition to universities, many big corporations and local companies like to partner with nonprofit organizations to allow their employees paid volunteer hours. Companies can form a fundraiser, event, programming, or supply large

numbers of volunteers ready to help a nonprofit's mission. Carley shared that for one of their annual marathons, they allow big groups of volunteers to host their own water stations and have a little competition for the best one. She mentioned that a majority who turn out for this are local companies and businesses. She said, the winner this year was a call center that dressed head to toe in orange clothes, wigs, and paint. They did a huge tailgate football style water station with big trucks set up and grilled hot dogs for the runners. It was so creative, and they supplied about sixty volunteers.

Local businesses and companies can also aide in suppling resources that the nonprofit organization could not otherwise afford. For example, Carley said, whenever we have an event, we always serve a meal to our volunteers. Thanks to the relationships we have made with local café and restaurant owners, we are able to get all meals donated.

In addition, another line of strategic partnering to explore are nonprofit to nonprofit collaborative partnerships. When nonprofit organizations can mutually benefit from programming together, it is a perfect way of gaining resource or volunteers. For example, Carley's foundation, which focuses on promoting active lifestyles, works with Aly's youth program by teaching healthy habits, exercises and training the youth for community races.

Appreciation Tactics

People want to feel like the work they are doing is noticed, appreciated and valued. With nonprofit organizations, this is elevated as volunteers need to feel a sense of belonging and worth within the cause because other incentives, especially monetary, are minimal. Participants explained that their organizations engage in multiple methods of showing appreciation to their volunteers. Even small gifts, such as t-shirts, are appreciated and aid volunteer commitment. When asked about relationships with volunteers, Carley emphasized the importance of making personal connections with each volunteer. Carley shared The other day I ran into two of our volunteers at the grocery store, an older couple, and they were wearing their volunteer shirts. They came and said hello to me, and I think that is special, something I never expected to happen.
 The idea of remembering names, personalities, ties to community and level of involvement for each volunteer seems extensive, but plays a crucial factor in gaining trust, comfortability and relationships. Each participant expressed steps their organizations take to show their appreciation to volunteers.

"Communication is key" Carley said, "volunteers want to know what they are doing, how they are doing, and that they are making a difference. They want to be acknowledged and in the know of what is happening in the organization." Carley shared one example of this acknowledgment; she conducts volunteer spotlights once a month where the organization gathers a short biography of a

volunteer and shares it on their social media outlets with a note of appreciation. "Not only do they feel appreciated" said Carley, "but they share it with their friends and family, who then want to get involved too."

Community Outreach

To be a successful nonprofit organization that serves its community, a nonprofit must be present in the community. This can be done through creative tabling at fairs and events, attending local community meetings and having a representative sit on community committees. When people see representatives of a nonprofit organization, it sparks conversations and questions, often leading to connections and volunteers. Carley mentioned, "with working in nonprofit, you are always representing the organization. Even if you are not working, you are always networking and communicating your cause." All employees of nonprofit organizations are very passionate about what they do, Brandy said "I am always talking about it. We have to get the whole community rallied around helping each other- it takes everyone to alleviate poverty." Networking and building connections can come in many forms of individual employee time, energy and passion.

Leadership Development

Engaging volunteers in personal development opportunities for them to increase their own skill, knowledge and experience helps to gain volunteers and keep them contributing to the organization. If nonprofit organizations invest time and energy into their volunteers, the volunteers are more likely to reciprocate. "To get people to want to do something you have to appeal to whatever it is in them that makes them want to volunteer" Brandy shares, "for example, a student wants to learn and gain experience, so we take the time to talk about what they want to get out of working with us and define channels for volunteering that would best reflect their goals." Training and leadership development opportunities expand their experience and create a stronger connection while giving the organization volunteers that are well informed, trained and ready to serve. Carley explained that her organization appoints a volunteer to be an event lead and then gives them the contact information for a certain number of volunteers. Carley explained

We have sixteen water stations at the marathon. We assign a captain to each station, who usually represent a business or friend group, and the captains oversee their own station. This includes recruiting their own volunteers, training them, setting up stations, taking down materials and getting people excited. With this concept of offering leadership roles to active volunteers, nonprofits can alleviate the stress of some tasks and the volunteers are empowered to take ownership. Volunteers that are empowered feel like they are more involved in the organization and that they belong.

DISCUSSION

Nonprofit organizations face a prominent challenge; they need the same amount of work done as for-profit organizations but often do not have the funding to hire a large workforce. Thus, nonprofit organizations rely heavily on hard working, committed volunteers that believe in their missions and are willing to spend time and energy without building monetary profit. Moreover, volunteers must have experiences that form a profit of value, impact, and civic connection. Nonprofit organizations can efficaciously recruit and retain volunteers by cultivating inspired networks through fostering synergetic relationships. This preliminary study has offered ideas, insight and data on how nonprofits successfully recruit and retain high-quality volunteers. Despite the exploratory nature of this study, the information gathered from three distinctive organizations can be related to an array of nonprofits that espouse similar goals in recruiting and retaining quality volunteers. Tactics included in this study may be utilized differently for a broad array of causes. However, caution is needed in interpreting these results considering that this study did not achieve theoretical saturation. It is important to identify the specific target audience for an organization's cause in utilizing messages to carry out these strategies in best practice. Naturally, this research is only a starting point and future research is needed to confirm these findings and test the efficacy of these strategies.

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Postcolonial Adaptation: Shakespeare's Caliban and Ariel as Symbols of Anti-Colonial Resistance

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COMMUNICATION

ABSTRACT

In Anglophone literature nothing is quite as quintessentially canonical as Shakespeare's works. Critiquing this canon, postcolonial writers and theorists such as Aimé Césaire and Ngũgĩ wa Thiong'o have used Shakespeare's The Tempest to express and develop anti-colonial sentiments, particularly embracing the character of Caliban as a symbol of the colonized. This paper explores why postcolonial adaptations continue to focus on the Caliban, rather than Ariel, who is also subservient. I argue, that while Ariel is a spiritual being, who already possesses language and culture, Caliban is mobilized as a symbol of anti-colonial thought, because he is dispossessed of his island and re-appropriates the language taught to him by his oppressor. Caliban also actively resists Prospero, while Ariel is complicit in his plans. Postcolonial adaptations use Caliban's situation to expand upon specific historical examples, such as colonial Martinique and Brisbane, because Caliban's and Prospero's relationship echoes the colonized/colonizer dynamic found in many such settings.

INTRODUCTION

Shakespeare's *The Tempest* has been the subject of many postcolonial adaptations. The character of Caliban, and his relationship with Prospero, represent the colonizer/colonized relationship explored in postcolonial theory. Caliban, however, is not the only character in *The Tempest* subservient to Prospero. Ariel, the spirit, is bound to Prospero and carries out much of his bidding. Although Ariel has been used in postcolonial versions of the play, he is never used as an allegory for "the Other" as Caliban is. This is due to the different and unequal power dynamics operative in Prospero and Caliban's relationship, and Prospero and Ariel's. Caliban's attitude and his appropriation of Prospero's language more closely align with cultural imposition experienced by colonized people. Ariel shares similarities to Caliban, but ultimately Caliban's characteristics are more easily read through a postcolonial lens.

To better understand the reasons Caliban and Ariel are used in postcolonial interpretations, and the differences between them, we must examine the

relationships that both these characters have with Prospero, the dominant power in the play. Shakespeare's Caliban is an unsavory beast and Prospero treats him with scorn. However, it is revealed that Prospero once treated Caliban gently and taught him how to speak: "You taught me language; and my profit on't / Is I know how to curse" (Shakespeare, I.ii.437). It is widely believed that Caliban was created partially in response to Montaigne's essay "Of Cannibals," where Montaigne argues that civilization corrupts the natural man. This idea is explored but rejected, as Sidney Lamb notes: "Caliban is Prospero's experiment with Montaigne's idea of the noble savage, and it has been a failure" (39). Caliban learns to speak in Prospero's tongue, but only uses this language to curse. Caliban is a lesser type of man in the text and, subsequently, Prospero's eyes. Prospero's attempt to civilize Caliban was foiled by Caliban's baser nature.

This portrayal reflects the notion of "the Other" in postcolonial theory. According to Ashcroft, Griffiths, and Tiffin, "[t]he colonized subject is characterized as 'other' through discourses such as primitivism and cannibalism as a means of establishing the binary separation of the colonizer and colonized" (146). Caliban is Othered by the text and Prospero much the same way that indigenous peoples were reduced and dominated by colonizing forces. Ashcroft asserts that the "racial debasement and demonization of the colonized… are a justification for subjugation" (Ashcroft 81). It was easy for Prospero to take the island from Caliban's mother, Sycorax, for this very reason. He saw Sycorax and Caliban as lacking language and, therefore, lacking culture. Initially his goal was to educate Caliban but when this failed Caliban became his slave. This betrayal makes Caliban a sympathetic character for those who have experienced the imposition of colonial power structures.

Alternatively, there is little evidence of Prospero viewing Ariel as primitive. There are more differences between Caliban and Ariel than there are similarities. Although Ariel and Caliban are never referred to as human, "Ariel clearly possessed language, culture, and associate spirits before Prospero freed him from the torment of the tree" (Pesta). Ariel was not taught language by Prospero, which Shakespeare clearly links to being civilized. Ariel is also musical and sings in many parts of the play. The music and poetic lyrics tie Ariel to, what might be considered, a more "advanced "culture. Ariel and Caliban stand at opposing ends of humanity since "Ariel is beyond humanity at the superhuman or spiritual end of the scale" and "Caliban is beneath humanity at the animal end" (Lamb 31). Thus, Ariel is not viewed as the Other like Caliban.

Moreover, Ariel is described as a spiritual being. Ariel is associated with air and fire, as shown by his first words, "I come / To answer thy best pleasure; be't to fly, / To swim, to dive into the fire, to ride / On the curl'd clouds" (Shakespeare, I.ii.225-7). Fire and air were, to the Elizabethans, associated with "the higher, rarefied elements of nature" (Lamb 30). Ariel's connection to the spiritual plane enables Prospero to control the weather and create illusions.

Ariel is tasked with carrying out delicate parts of Prospero's plan rather than carrying firewood like Caliban. Ariel's attitude is also markedly positive compared to Caliban's, while he "is unequivocally not human," he is "working sympathetically toward the same ends as Prospero" (Pesta). Ariel readily agrees with Prospero and quickly adopts his goals as if they were his own. Indeed, Prospero confides in Ariel, revealing motives that he hides from his own daughter. Ariel does not possess a physical form and does not inhabit the island or rely on its resources. Nor does he claim sovereignty over the island but only desires freedom. Ariel is not an inhabitant of the land because he resides in a spiritual form. Ariel does not experience freedom in the same way Caliban would, because Ariel has no concept of sovereignty and inheritance.

In postcolonial interpretations of *The Tempest*, Ariel's character is usually shown to be more complicit in interactions with Prospero. In Aimé Césaire's adaptation, "Une Tempête," Ariel is a mulatto slave who believes that he can work with Prospero to ensure his freedom. In an interesting exchange between Ariel and Caliban, one which never occurred in Shakespeare's version, Ariel comes to warn Caliban about Prospero's plan against him. Caliban is unafraid, but Ariel is pitying, telling Caliban, "Well, I've at least achieved one thing: he's promised me my freedom. In the distant future, of course, but it's the first time he's actually committed himself." To which Caliban responds, "Talk's cheap. He'll promise you a thousand times and take it back a thousand times. Anyway, tomorrow doesn't interest me. What I want is (shouting) Freedom Now!" (Césaire 26). Caliban's attitude toward Prospero is reflective of Césaire's anti-colonialist stance and support for a pan-Africanist identity, embracing black pride. For Césaire, Caliban, not Ariel, embodies the rebellion and fervor that he hoped to inspire with his work.

Rebellious and spiteful, Caliban furthers the postcolonial narrative of the subversive colonized subject by using the oppressor's language against him. Césaire, born in Martinique, applauds Caliban "resistance to Prospero's control over language". Césaire, Singh argues, "is clearly sensitive to the way in which the name Caliban/Cannibal appears in Shakespeare's play and in colonial history as a cultural stereotype for the natives of the New World" (Singh). Caliban embodies the voiceless colonized subject, forced to use his oppressor's language. However, Caliban chooses to use this language to curse his master, a sentiment that postcolonial thinkers use to further their own revolutionary agendas. George Lamming, the Barbadian novelist, for instance, "had situated himself as a Caliban figure who uses his colonial English education to raise his voice against colonial oppression" and "Roberto Fernandez Retamar appropriated Caliban as a symbol of the oppression as well as rebellion of the Americas against colonialism" (Loomba 163). Postcolonial writers, some of whom take issue with using colonial languages, appear sympathetic to the figure of Caliban because he appropriates the language learned from Prospero, using it against him, just as Césaire, Lamming, and Retamar use their own educations for anti-colonial ends.

This appropriation is developed further in the choice to use *The Tempest* itself and adapt it into an anti-colonial statement. Kenyan writer Ngũgĩ wa Thiong'o, uses Shakespeare to confront Western imperialism through his central character in *A Grain of Wheat*. Ngũgĩ links Joseph Conrad's character Kurtz and Shakespeare's Prospero in his own character of Thompson to show "an aggressive attempt to bring European assumptions of cultural superiority into unflattering contact with the history those assumptions have imposed on the culturally dispossessed" (Cartelli 95). By juxtaposing the idealism of Prospero against the realities of colonialization, Ngũgĩ attacks the "high-minded" attitudes that colonizers used to justify their oppressive actions. Prospero's ideology behind attempting to control Caliban is revealed to be fiction used by oppressors to Other and control another population.

Ariel's voice is his own and this is reflected in a more recent adaptation from, Brisbane- based theater company, Zen Zen Zo. In their adaptation Ariel is a storyteller and uses music as narration. Ariel is not representative of Aboriginal Australians; rather, as reviewer, Natalie Lazaroo suggests, Ariel is closer to the white settler Australians, since "both are subservient prisoners to powerful mentors and complicit in an oppression of less powerful subjects" (Lazaroo 382). This interpretation is like Césaire's mulatto Ariel, but is adapted to fit a specific historical context, where displaced white settlers found themselves in neither the role of the colonizer nor the colonized, but still somehow complicit.

Ultimately, the use of Caliban and Ariel in postcolonial discourse relies on its comparison to real-world examples of colonialism. Since the realities of colonialism differ across historical contexts, Caliban cannot be the ultimate example of the colonized subject. Ania Loomba claims that during the "heyday of anti-imperialist movements" the figure of Caliban was often molded to fit as a generalized symbol because of the similar political happenings. Yet, other postcolonial thinkers find that "Caliban is not an adequate symbol of their oppression or revolt" (Loomba 165). By focusing on only one type of colonialism it ignores and even eclipses other, equally valid, forms and experiences.

Caliban served as a symbol of anti-colonial thought because of his enslaved and dispossessed status. As I have shown, postcolonial thinkers and writers use his character, rather than Ariel's, because of the way he defiantly reclaimed Prospero's language and education. Caliban also represents the Other, who is created by the rule of colonial power. Ariel is shown as a creature on a higher plane, who affirms Shakespeare's ideas about spirituality, language, and culture. When Ariel is used in postcolonial adaptations it is as Prospero's accomplice. It is for this reason Caliban is more often used as a symbol of anti-colonialist thought.

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Quantitative Easing's Effect on the Housing Market

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ECONOMICS

ABSTRACT

In the shadow of the Financial Crisis, the Fed turned to an unconventional monetary policy called quantitative easing (QE). This paper focuses on QE's effects on housing prices and the housing market specifically, whereas the previous research either focuses on immediate market responses or asset prices as a whole. This study finds that, while the impact on housing prices was statistically insignificant, the Fed's use of QE did have a positive and significant effect on other measures of the housing market, i.e., new building permits, new housing starts, and new houses sold.

INTRODUCTION

In the months and years following the financial crisis, the Federal Open Market Committee (FOMC, or better known as the Fed) turned to an unconventional tool called quantitative easing (QE) to conduct monetary policy; since their traditional tool, open market operations (OMO), was no longer an option due to the Federal Funds Rate (FFR) reaching zero percent, or the zero lower bound (Fawley & Neely, 2013). This paper is intended to build upon the findings and research of Huston and Spencer (2016) who focused on QE's effect on asset prices as a whole, while this paper focuses more specifically on QE's effect on housing prices and the housing market specifically. Using vector auto regressions (VARs), this study finds QE has a significant impact on the housing market, and an insignificant impact on housing prices.

LITERATURE REVIEW

QE presents a few different challenges when trying to analyze its effects on the economy. First, since QE policies are still new, only recently enough data is available to gather the necessary sample size to run statistical analysis. Before this, economists used event studies to measure the effects of QE policies. As Krishnamrthy and Vissing-Jorgensen (2011) and Gurkaynak and Wright (2015) show us, event studies were a popular way of measuring the effects of QE policies before enough data was available to run statistical analysis. In event studies, an

event is taken (in the case of QE the event is usually an announcement from the Fed regarding some form of QE policy), then changes in different markets are measured and assessed.

While Gurkaynak and Wright (2015) say the methodology of using event studies could be applied to studying QE, they also note limitations holding this method back from giving us the entire picture. The two main limitations Gurkaynak & Wright (2013) point out are, that event studies measure market expectations rather than actual outcomes, and event studies rely on high-frequency data while the data on interest rates and asset prices are reported on a low-frequency basis.

Huston and Spencer (2016) took their study in a different direction than papers previously published regarding the effects of QE policies. They decided to stray from the event study platform and use a more rigorous statistical regression model known as Vector Auto Regression (VAR). The authors rely heavily on the findings of Gurkaynak and Wright (2013) in coming to the conclusion of using a VAR stating, "[A] VAR is well suited here because this is a situation with multiple endogenous variables, the lack of a sound structural model, and an unknown lag structure."

Huston and Spencer (2016) used this approach to analyze the effectiveness of the Fed's QE policies on increasing asset prices and ultimately enabling the wealth effect. They looked at the effects QE had on measurements of equity and housing prices. Huston and Spencer (2016) found that QE had statistically significant increases in equity prices.

This study takes the method and idea surrounding Huston and Spencer (2016) but narrows the approach to housing prices and the housing market, rather than asset prices as a whole. This paper builds upon the findings in Huston and Spencer (2016) by expanding the data used by more than a year, from March 2016 to June 2017.

METHODOLOGY

The data used in this paper is taken directly from the St. Louis Federal Bank's, Federal Reserve Economic Data (FRED). The data used runs from September 2008 through June 2017. September 2008 was decided as the starting point, per the study done by Huston and Spencer (2016), due to the "credit market events of that month and the Fed's responses to those events" (Huston & Spencer, 2016). The data being used is time series data, so a typical linear regression is not the ideal method to be used since we assume that variables are dependent on each other. Woolridge explains, "An issue with time series data is we can rarely assume the data is independent across time. A lot of care needs to be take[n] when attempting to create an economic model that uses standard econometric

techniques when time series data is being used" (Wooldridge, 2006). For this reason, a Vector Autoregression (VAR) is used to run analyses on the data (Gurkaynak & Wright, 2013; Huston & Spencer, 2016).

VAR models allow for the analysis of multiple, inter-dependent variables simultaneously. Each variable is dependent upon fluctuations of the other variables in the model, as well as upon its past values. In a macroeconomic setting, this is important since country-level variables tend to show some degree of momentum. To show these relationships, the variables must be "shocked" then the variables paths can be traced through the model. These paths are commonly referred to as impulse response functions.

Below is the VAR used in this paper:

$$X\_t = \beta_0 + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \beta_3 X_{t-3} + C\varepsilon_t{}^1$$

$$X_t = \begin{bmatrix} BASE \\ UNEMP \\ x \\ VIX \end{bmatrix}$$

Where 'x' will be defined as different measures of housing prices and the housing market.

$$\beta\_0 \sim 4\text{x}1 \text{ Matrix}$$
$$\beta\_1, \beta\_2, \beta\_3, \text{and } C \sim 4\text{x}4 \text{ Matrix}$$
$$\varepsilon\_t \sim 4\text{x}1 \text{ Matrix}$$

DATA AND ANALYSIS

As was stated, the data used for this paper was acquired from FRED and spans from September 2008 through June 2017. September 2008 was decided as the start of the sample based on the Huston and Spencer (2016) observation of "The dramatic credit market events of that month and the Fed's responses to those events." June 2017 was the last month of available data at the time work began on this paper.

As Huston and Spencer (2016) point out, "The traditional measure of monetary policy is the federal funds rate. However, from late 2008 to the present, the policy rate has been near zero, and thus not useful for our purposes." Since the traditional measure of monetary policy is useless over the time period being

tested, the monetary base (BASE), as used by Huston and Spencer (2016), will be the measure of monetary policy in this study. The civilian unemployment rate (UNEMP) is used as a first-order interpretation of the economy, and the index of the S&P 500 volatility (VIX) is used as a second-order interpretation of the economy or the variance from our first order. The S&P Case-Shiller U.S. National home price index (NATHOME) was used to measure the housing prices. To gain a better understanding of QE's impact on the housing market as a whole, VAR's were run with the following data used in place of (NATHOME): New private housing units authorized by building permits (PERMIT), new privately owned housing unit starts (NATSTART), and new one-family houses sold (NATSOLD).

FINDINGS

When a positive1 percent shock is made to the BASE, there is an insignificant impact on housing prices at the 95 percent confidence level over a 4-year window, as shown in Figure 1.

CONCLUSION

The research on the effectiveness of the Fed's QE policies during the financial crisis is still new and developing. Early event studies show the immediate response of equity markets and interest rates to the Fed's announcement of these policies (Krishnamurthy & Vissing-Jorgensen, 2011). More recently, researchers have shown the same QE policies to have a significant effect on increasing asset prices over time (Huston & Spencer, 2016). Using monthly data from FRED and VAR estimation, this study finds that, while the impact on prices was statistically insignificant, the Fed's use of QE did have a positive and significant effect on other measures of the housing market the number of new housing permits, new housing starts, as well as new houses sold.

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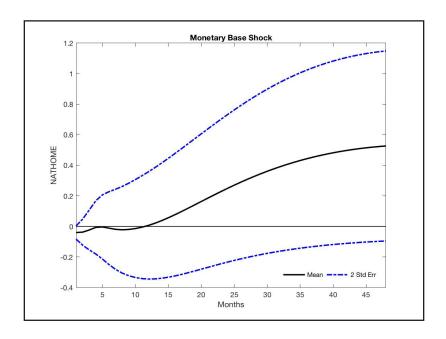
Figure 1 S&P Case-Shiller U.S. National Home Price Index response to the Monetary Base. Note: Choleski identification assumption used.
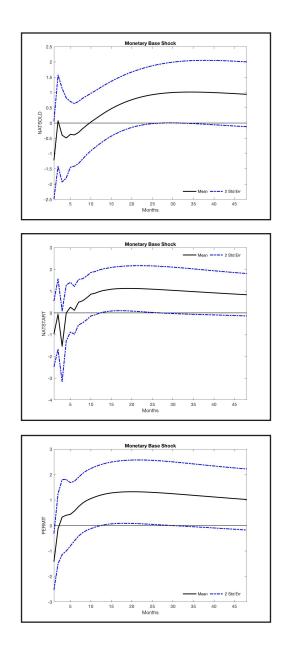
Figure 2 Key variables used to measure the housing market response to the Monetary Base. Note: Choleski identification assumption used.

An Analysis of two Measures of Human Capital

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ECONOMICS

ABSTRACT

This paper attempts to combine two growth models, the Solow Growth Model and the Romer Endogenous Model, by reconciling their assumptions to benefit more holistically from previous research contributions. The combined model is then used to test two different measures of human capital: the SCHOOL measure as used by Mankiw, Romer, Weil (1992) and the Human Capital Index (HCI), a relatively new statistic used by the World Economic Forum. A test for convergence is performed and found to be consistent with previous findings. An empirical test is then performed to determine which measure is better at predicting economic growth. Using the Davidson MacKinnon J-Test to empirically analyze the two models using sample data from 1980-2014, the result shows that the HCI measure is a better predictor of economic growth than the SCHOOL measure.

INTRODUCTION

Economists have been studying economic growth since the 19th century when National Accounts were first used to track country output statistics. Since then, governments interested in the welfare of their citizens and effectiveness of their economy have tracked important economic indicators, such as total national output or Gross Domestic Product (GDP), to see how their output and economy has changed over time.

The variety of theories and methods of measuring economic growth has led to different schools of thought, each making different assumptions about the economy. With so many differing opinions and methodologies, it becomes vital to find ways to reconcile and combine these theories to get a more inclusive, holistic understanding of economic growth.

This paper contributes to the knowledge already created by other researchers by attempting to combine two of the fundamental theories for economic growth: the Solow Model, which is an exogenous growth model, and the Romer Model, an endogenous growth model. It then tests two different measures of human

capital: the SCHOOL measure used by Mankiw, Romer, and Weil ("MRW" 1992) and a measure known as the Human Capital Index (HCI).

THEORETICAL ANALYSIS

There is a great deal of literature existing on the different ways to explain economic growth. These approaches differ significantly in their assumptions and have led to a number of different models. One of the most widely supported of these is the Solow Growth Model. Equation 1 shows the Solow Model using effective units of labor.

$$1.\ Y(t) = K(t)^a\ (A(t)L(t))^{(1-a)}$$

The notation for the terms is standard: Y is output measured as GDP, K is capital, L is labor, and A is the level of technology available to labor. In accordance with the Cobb-Douglas production function, a is the share of capital income. MRW found in their study that this factor also includes human capital, which should be measured independently for each country. Effective labor is assumed to account for the remaining share of the economy's income. The Cobb-Douglas function assumes constant returns to scale. If the log of the equation is then taken to measure percent changes in these factors, L and A will grow at rates of n and g, respectively. Since the model assumes that there are effective units of labor, the terms are combined in subsequent equations to grow at a rate equal to the sum of $n + g$.

The Solow model assumes that a constant fraction of output (s) is reinvested into the economy. It then becomes pertinent to change the production function to per effective units of labor, so that and, showing the average amount of capital available to each worker in the economy. This way, changes to small and large countries can be compared. Factors that contribute to changes in are shown by equation 2.

$$2.\ \Delta k = sy(t) - (n+g+\delta)k(t)$$

Following the Solow Model predictions, new investment tends to be equal in magnitude to the rate of capital depreciation, meaning that k will converge to a steady-state level and will not change in the long run, reaching a steady-state level of k^*.

$$3.\ k^* = [s/(n+g+\delta)]^{(1/(1-a))}$$

Because capital tends to reach a steady state, the Solow Mo=del focuses mainly on the impact of saving and population growth on per-capita income. MRW estimated the sum of the growth in technology and capital depreciation as 0.05 because data were not available for country-specific depreciation estimates.

However, newer datasets are now able which provide estimates for depreciation, so changes in the capital will be measured as a separate term, represented by $\ln(\delta)$.

The Solow Model's major drawback is that it assumes that the technology parameter, A, is the same for every country. However, using the same parameter for all countries means that technological growth (g) would be the same for developed countries, which tend to have more research capacity, as for underdeveloped or developing countries, which have a limited amount of resources and lower levels of direct investment. Empirical and qualitative data do not support the assumption that technological growth is the same for all countries. As argued by Jones (2014), some technologies are given patents that can only be used by the firms which have invested in it. Since some economies are heavily reliant on knowledge-based exports and copyrights, technological progress cannot be viewed as equal for all countries. It becomes pertinent, therefore, to be able to augment the Solow Model further to include changes in technology for each country.

The basic function for the Romer model is given in Equation 4.

$$4.\ \ \Delta A/A = g_A = z(L_A)$$

In the equation, z represents the productivity of labor measured as Total Factor Productivity (TFP). The model also assumes that there is a constant share of labor (L_a) employed in the Research and Development (R&D) sector of the economy. Romer showed that this share of workers in the economy tends to grow over time (Romer, 1994). However, for this research study, L_a is viewed as a steady fraction of labor consisting of the average share of labor employed in the R&D sector over the sample period. The share is estimated as shown in Equation 5.

$$5.\ L\_A = L - L\_y$$

Where L is the average number of effective units of labor and L_y is the share of labor not employed in the R&D sector of the economy. Equations 4 & 5 are a variation of the equation provided by Jones (1995). If equations 3 and 4 are substituted into equation 1, and equation 4 is substituted for the g variable, a log-linearized model can be produced:

$$6.\ \ln(Y_{2014}/AL) = b_0 + b_1\ (ln[y_{1980}]) + b_2\ (ln[s]) + b_3\ (ln[n+g]) + b_4\ ln(\delta)\ldots$$

This equation represents an augmented Solow Model with the addition of the Romer model to better explain technological progress for each country. The final parameter (shown in Equations 7 and 8, below) will then be added to this equation to test the different measures of human capital.

The first measure of human capital comes from MRW, which found that

adding an estimate for human capital resulted in a much better explanation of economic growth than using physical capital alone. MRW did this by using an estimate of the percentage of the total population enrolled in secondary education as a proxy.

Since that research was completed, newer estimates for human capital have been published which look at the returns to education rather than the percentage enrolled. One of the most popular of these statistics is the Human Capital Index (HCI) used by the World Economic Forum. The HCI measure considers the effectiveness of educational systems by looking at returns to education.

By comparing the older SCHOOL measure used by MRW and the newer HCI measure, it can be shown that one measure human capital will be better at explaining the sample data. The human capital parameters will be included as part of the regression analysis from equation 6.

$$7 \ \ldots + b_5 \ (ln[SCHOOL])$$
$$8 \ \ldots + b_5 \ (ln[HCI])$$

It was predicted that using HCI to measure human capital would better explain economic growth for the countries in my sample. It was assumed this would occur because HCI measures a country's ability to see returns from education and indexes them according to effectiveness.

EMPIRICAL TESTING

The hypothesis was tested by performing a regression analysis on the two versions of equation 6, followed by a J test. To answer the research question, data was gathered from various sources, including the Penn World data set, the World Bank, and the Barro-Lee dataset. Only the 150 largest countries by population were used to avoid outliers in the sample data, narrowing the sample size based on the countries which had the data available for the sample period. Out of the 150 countries, the sample size was narrowed down to 79 countries between 1980 and 2014 because of data holes. The results of the regression analysis for each model are given in Table 1.

Table 1 shows the regression results for conditional convergence. Both models were significant for explaining the data, implying that convergence held because Y_{1980}, the initial level of GDP has a negative coefficient. In other words, countries with higher initial levels of GDP can expect slower growth. These results are in line with the theoretical models and seem to better explain variation than the augmented MRW Model results.

Following the regression analysis of the individual models, the residual values (Yhat) from each model were taken and made it into an explanatory variable for

the other model. This is known as a Davidson-MacKinnon J test. The J test is not a new method for comparing different models and has been cited in hundreds of separate articles since it was introduced. Table 2 shows the results of the J test. The residuals from the HCI model are significant in predicting changes in economic growth for the SCHOOL model (see the third column of Table 2). The residuals from the SCHOOL model, however, are not significant in predicting changes in economic growth for the HCI model (see the fourth column of Table 2). This means that the null hypothesis is rejected at the standard p-value. It can be assumed, then, that the HCI measure is better at predicting economic growth than the SCHOOL measure.

CONCLUSION

The research question attempted to combine the Romer and Solow models and then to determine which, if either, of the measures of human capital, would be more effective in predicting economic growth.

According to the empirical results, the combined model was very effective for predicting economic growth. Both measures were able to explain over 90% of the data, making them overwhelmingly significant and showing that the combined model tended to perform very well. Combining the different schools of thought is a worthwhile and important endeavor for researchers of economic growth. While both measures were statistically significant for measuring human capital, it is important to determine which measure is better because of the policy implications of each. If the HCI measure were a better predictor, as was found to be the case in this empirical study, it would imply that countries should not focus on getting classrooms filled, but on getting higher returns from each student. If the SCHOOL measure were a better predictor, it would imply that the number of students seems to be more important than the returns, or at least that having more educated students regardless of returns tends to outweigh higher returns from fewer students.

According to the results of the J test, the former is true. Countries wanting to see an increase in economic growth should focus on getting higher returns from their students rather than on getting seats filled. Additionally, it means that researchers should use the HCI measure of measuring human capital as a proxy rather than the SCHOOL measure.

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Table 1: Test for Conditional Convergence		
Dependent Variable: log difference GDP per capita 1980-2014		
Sample:	150 largest countries by population	
Observations:	79	
	SCHOOL Model	HCI Model
Constant	4.877***	5.265***
	(0.87)	(0.889)
lny(1980)	-0.493***	-0.536***
	(0.077)	(0.080)
ln(s)	0.669***	0.744***
	(0.165)	(0.165)
ln(n+g)	0.213**	0.206*
	(0.106)	(0.111)
ln(δ)	1.070***	1.085***
	(0.248)	(0.253)
lnSCHOOL	0.375***	---
	(0.375)	---
lnHCI	---	1.057***
	---	(0.889)
R²	0.619	0.602

The table shows the coefficients and the standard errors (given in parethesis), for each explanatory variable. The statistical significance at the 0.1, 0.05, and 0.01 levels are represented by the symbols *,**, and *** respectively.

Table 2: Regression Results				
Dependent Variable: log GDP per capita 2014				
Sample:	150 largest countries by population			
Observations:	79			
	SCHOOL	**HCI**	**J-test(SCHOOL)**	**J-test(HCI)**
CONSTANT	6.272***	6.546***	1.848	3.533
	(1.056)	(1.035)	2.343	(2.577)
ln(s)	1.010***	1.027***	0.269	0.521
	(0.195)	(0.190)	(0.401)	(0.439)
ln(n+g)	0.710***	0.620***	0.181	0.287
	(0.093)	(0.102)	(0.267)	(0.280)
ln(δ)	0.747**	0.782***	0.211	0.414
	(0.303)	(0.298)	(0.391)	(0.414)
lnSCHOOL	0.374***	---	0.173	---
	(0.098)	---	(0.135)	---
lnHCI	---	1.230***	---	0.860
	---	(0.290)	---	(409)
Yhat--SCHOOL model	---	---	---	0.461
	---	---	---	(0.362)
Yhat--HCI model	---	---	0.699**	---
	---	---	(0.333)	---
R²	0.869	0.874	0.877	0.877

The table shows the coefficients and the standard errors (given in parethesis, for each explanatory variable.
*,**, and *** represent statistical significance at the 0.1, 0.05, and 0.01 levels, respectively.

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Expression of SLC6A3 Gene in Persistent ADHD

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ABSTRACT

Attention Deficit Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that begins in early childhood and is associated with impulsive behavior and inability to concentrate. Currently, ADHD diagnosis is based solely on survey responses by the patient. Without the definitive information provided by a physiological biomarker, ADHD may often be misdiagnosed. Overdiagnosis of ADHD has led to unwarranted over-prescription of the associated medications (U.S. Department of Justice, 2012). The aim of this study was to to identify a potential genomic biomarker for ADHD, which would allow healthcare providers to make more definitive diagnoses.

Mutations of the SLC6A3 (DAT1) gene have been suspected to have an association with persistent ADHD in adults. The DAT1 gene, also known as the dopamine transporter gene, codes for the DAT1 protein. The DAT1 protein facilitates the reuptake of dopamine in the neural synapse. Genetic analyses were performed on nucleated blood cells to evaluate the presence of a gene mutation in DAT1, specifically in repeat sequences found in the 3' UTR and Intron 8 loci. The expression of two repeat sequences, the 10-repeat on the 3' UTR and the 6-repeat within intron 8 was suspected to have an association with ADHD and its associated symptoms. Volunteers were provided with the ASRS-V1.1 survey and had two vials of whole blood collected. Volunteers' survey responses were compared with the resulting gene product yielded by PCR and gel electrophoresis. Cochran-Armitage and Odds Ratio analyses yielded no significant association with ADHD and the 3' UTR polymorphism.

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that begins in early childhood. ADHD is characterized by hyperactivity, inability to concentrate, and abnormally impulsive behavior. These symptoms often times persist into adulthood (Garnier-Dykstra et al., 2010). Currently, ADHD diagnosis is derived from survey-based criteria. Recent diagnostic trends of ADHD in the United States indicate an increased rate of prescription of the

associated medications between 2007 and 2011 (Matthews, M., Nigg, J. T., & Fair, D. A., 2013). The higher quantity of diagnoses requires more definitive diagnostic criteria. Identifying a genomic biomarker could aid in accurately diagnosing ADHD and could potentially decrease rates of subsequent abuse of the associated medications (Weyandt et al., 2014).

Several biomarkers for ADHD have been previously explored by researchers with limited success. A gene that has previously shown promise as a genomic biomarker for ADHD is the SLC6A3 (DAT1) gene, otherwise known as the dopamine transporter gene (Spencer et al., 2013; Tong et al., 2014). Dopamine is a neurotransmitter that has shown involvement in motor function, cognition and affect and is one of the main neurotransmitters of the nigrostriatal system. The DAT1 protein is specifically part of the sodium and chloride-dependent neurotransmitter transporter family. Irregularities of the expression of these proteins, along with additional genetic irregularities on the dopamine transporter gene, have long been associated with several neuropsychiatric disorders, including ADHD (Fuke, S. et al., 2001).

The aim of this study was to evaluate the 10-repeat allele of the 3' UTR VNTR and the 6-repeat allele of the intron 8 VNTR. The 10-repeat, in comparison to 9-repeat allele, has been associated with a lower total amount of dopamine transporter gene expressed and smaller brain volume in areas of the brain associated with ADHD. The 6-repeat allele found on intron 8, in comparison to the 5-repeat, has been indicated to have an association with slower cognition (Spencer et al., 2013). The aim of this study was to evaluate the individual and tandem expression of the 10 base-pair repeat on the 3'UTR and the 6 base-pair repeat on the intron 8 VNTR (10/6) of the DAT1 gene and was hypothesized to be associated with ADHD and its associated symptoms.

METHODS

Population

This study recruited a total of 84 participants using promotional materials placed around the campus of a local university. Classification of participants was based on their provided responses to the ADHD self-report scale survey (ASRS-V1.1). The ASRS-V1-1 survey asks the responder various questions, prompting them to rate themselves based on frequency of certain behaviors. Each participant provided a sample of EDTA whole blood for genomic analysis of both target sequences. The ASRV-V1.1, along with a consent form, was provided for completion by the participant prior to the blood draw. Participants were assigned a number that enabled correspondence between their blood and survey responses.

DNA Extraction & Amplification

DNA was extracted from white blood cells using the QiagenTM DNA extraction method. Amplification was performed using two separate Polymerase Chain Reactions (PCR), one for the DAT1 intron 8 VNTR and a second PCR reaction to analyze the DAT1 3' UTR. Primers of both regions were supplied by ThermofischerTM. The DAT1 intron 8 VNTR forward primer was 5' GCT TGG GGA AGG AAG GG 3' and the reverse was 5' TGT GTG CGT GCA TGT GG 3'. Initial PCR cycling conditions were taken from previous research for amplification of DAT1 intron 8 (Spencer et. al, 2013 & Tong et al., 2014). The reaction conditions for intron 8 were 95 oC incubation for 5 min followed by a 95 oC denaturation for 1 min, 65 oC elongation for 1 min, 72oC annealing for 1 min, and one hold cycle at 72 oC for 5 min, repeating for 35 cycles (Tong, et al., 2014). The DAT1 3' UTR forward primer sequence was 5' TGT GGT GTA GGG AAC GGC CTG AG 3' and the reverse primer utilized was 5' CTT CCT GGA GGT CAC GGC TCA AGG 3'. PCR amplification cycling conditions for 3' UTR VNTR were 95 oC for 5 min followed by 35 cycles of 95 oC denaturation for 30 seconds, 60 oC annealing for 30 seconds, 72 oC elongation for 1 min, and one hold cycle at 72 oC for 5 min (Tong, et al., 2014).

 A 2% agarose gel was prepared for electrophoretic migration and 5μL of ethidium bromide to stain the DNA. To enable visualization, samples were mixed with 5μL DNA loading dye. Following straining, 10μL of sample was loaded onto the agarose gel. A molecular weight standard was used to compare the size of the DNA and a water blank was added to control for contamination. The gel was placed in a 1X TEA buffer for ideal migration.

Under the mentioned PCR conditions, the desired Intron 8 region of the DAT1 gene did not yield any visible product on the gel electrophoresis. In attempts to obtain the 6-repeat product, the annealing and denaturation steps were modified. Annealing temperatures were adjusted from 65 oC to 60 oC, then to 62 oC on subsequent attempts. Each attempt failed to yield an adequate amount of DNA needed to yield observable product on the agarose gel. Despite the unfavorable results from the VNTR of DAT1, the 10 and 9 repeats of the 3'UTR did yield observable product and were still evaluated.

RESULTS

Population

According to DSM-IV criteria, participants with four or more grey responses in the 'A' section of the survey were categorized as ADHD positive while less than four responses in the 'A' category classified participants as ADHD negative. Utilizing these criteria, forty-two participants were categorized as "ADHD positive" the remaining 42 participants were categorized as "ADHD negative".

3' UTR

Statistical analysis of the 3'UTR was performed utilizing the Cochran Armitage Trend Test (CATT) (Table 1&2) and odd's ratio testing (Table 1&2). An odds ratio was chosen for this study to verify the association between ADHD positive participants and 10 repeat allele of the 3'UTR. Potential DNA product obtained included a homozygous expression of the 10-repeat allele, homozygous expression of the 9 base-pair repeat, or a heterozygous expression of the 10-repeat and 9-repeat. Separation and identification of genes was possible through gel electrophoresis (see Figure 1).

The Cochran Armitage Test enabled comparison of ADHD positive and ADHD negative participants and the genotypic variants expressed. The odd's ratio was also performed to examine the probability of each participant having ADHD given the product yielded by their sample obtained by gel electrophoresis. Using a 2X3 contingency table, a CATT score of 0.4615 was obtained with an Odds ratio of 0.9067. Thus, the odds of having persistent ADHD according to the ASRV survey are 0.9 times lower with the appearance of the 10-repeat allele on the 3'UTR (see Table 1&2). Since the p-value of this study is not below 0.05 the odds ratio calculated is not statistically significant. Both analyses and figures were obtained using R studio.

DISCUSSION

The obtained data indicate that no significant odds relationship exists between the 10-base pair repeat allele of the 3'UTR and ADHD positive individuals. Because the tandem expression may potentially yield significant results as yielded by Tong et. al, further attempts should be made to properly isolate and visualize the 6-repeat sequence through alterations of reaction temperature, along with primer and reagent concentrations.

A probable source of error in methodology may have arisen from how participants were categorized in this study, along with previous studies here mentioned. The main issue in identifying an accurate and precise biomarker for conditions related to ADHD arises from the means by which truly positive and negative samples are isolated. Researchers have to overcome the limitations associated with current diagnostic methods in order to properly categorize the population. Through the use of a self-report survey, participants may have under or overestimated their responses, which may have yielded false negative results for individuals with mild cases of ADHD and false positive results for individuals who tend to be more self-critical.

Moving forward, continued analysis of both of these polymorphisms must be conducted. To overcome the limitations of this study, future studies could use a semi-quantitative approach, categorizing participants based on severity as to evaluate the extent to which the 10/6 haplotype is expressed. Information about participants may also be obtained via interview conducted by a licensed behavioral health professional who can accurately categorize participants based on ADHD severity. Future studies may also analyze mature mRNA and translation products yielded by these polymorphisms to examine whether genomic and proteomic defects result post-transcriptionally that inhibit DAT binding.

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Genotypes of ADHD positive/negative individuals
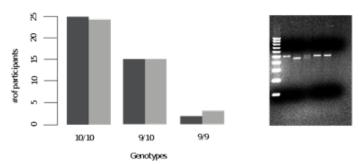
Figure 1: Y-axis is the amount of participants, X-axis is the allele present for each participant. Black represents ADHD negative patients and gray represents ADHD positive patients. Data was obtained from gel electrophoresis as illustrated above. Lane 1 adjacent to the protein ladders is the 10-repeat allele Lane 2 is the 9-repeat allele. Lane 3 is the heterozygous 10/9 allele.

Table 1: Odds Ratio analysis on the 10-repeat allele of the 3'UTR

Table 2: Data summary of all the patient samples collected. N represents the number of patients and (%) is the percent of participants in that specific category compared to the total amount of participants Categorization of ADHD (+) & (-) were based of responses to the ASRC-V.1 survery

Effects of 5-fluorouracil on Select Enteric Microbiota in Vitro

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ABSTRACT

Chemotherapy has been shown to disrupt intestinal bacterial populations leading to complications such as diarrhea, bacteremia, and septicemia. This research seeks to determine the effect of the common chemotherapeutic agent, 5-flurouracil (5-FU), on the survival rate of the normal intestinal organisms Bifidobacteria bifidum, *Lactobacillus acidophilus, Bacteroides fragilis,* and *Escherichia coli* in vitro. Initially, test organisms were exposed to four different 5-FU doses. At 24 hours, growth inhibition was not observed in any of the bacterial species tested. The experiment was repeated with much higher doses of 5-FU, and a dose dependent inhibition was observed over 24 hours in E. coli under aerobic conditions. The growth of *B. bifidum, L. acidophilus,* and *B. fragilis* did not show noteworthy inhibition.

INTRODUCTION

The Human Microbiome and Chemotherapy-Induced Gastrointestinal Mucositis

Most healthy individuals have unique and diverse intestinal microbiota. Low diversity in gut microbiota has been correlated with inflammatory bowel disease (IBD) (Huttenhower et al., 2012, p. 2). Chemotherapy-induced gastrointestinal mucositis or chemotherapy-induced mucositis (CD) produces symptoms that resemble IBD and also shows a reduced diversity in gut microbiota. The resulting multitude of symptoms including ulceration, diarrhea, nausea, vomiting, bloating, and constipation are associated with 5-FU treatment (van Vliet et al, 2010, p 1). Severe mucositis can result in chemotherapy treatment dosage reduction which presents a risk of decreased survival odds for the patient (Huttenhower et al., 2012, p. 2).

5-fluorouracil

5-FU is a chemotherapy drug that causes DNA and RNA damage resulting in inhibited DNA synthesis and p53 (tumor suppressor gene) activation (Sun et al, 2007, p. 8052). It is used to treat cancers including leukemia, ovarian, breast, and

intestinal cancers (How Chemotherapy Drugs Work, 2017). Studies have shown a decrease in intestinal Bifidobacteria spp., Lactobacillus spp., and Bacteroides spp. in chemotherapy patients taking 5-FU (Touchefeu et al., 2014, pp. 409,415).

METHODS

Estimation of Chemotherapy Dosage

The amount of drug present at the intestines after a patient receives infusion of 5-FU chemotherapy was not available from the drug manufacturer, Sigma Aldrich. Therefore, the drug concentration range to begin performing minimal inhibitory concentration (MIC) testing on the organisms was chosen based on the maximum infusion dose of 5-FU, 12 mg/kg of patient body weight (FLUOROURACIL - fluorouracil injection, solution, 2015).

Organism and Media Selection

B. bifidum (ATCC strain: 11863), *B. fragilis* (ATCC strain: 23745), *E. coli* (ATCC strain: 25922), and *L. acidophilus* (ATCC strain: 314) were chosen for experimentation because they are common normal flora in most individuals. *B. fragilis, B. bifidum, L. acidophilus* are strict anaerobes that have been found to decrease in patients with CD. *E. coli* has been shown to over-proliferate in many patients on 5-FU chemotherapy and can become pathogenic.

Thioglycolate broth, a common nutrient media that contains the reducing agent sodium thioglycolate, was used to grow anaerobic organisms in tubes. Hemin, a nutrient needed by slow growing and fastidious organisms, was added in a concentration of 0.005 g/L to produce the thioglycolate plus hemin broth media. (Kim et al., 2011, p 2).

Minimal Inhibitory Concentration (MIC) Testing

Initially, organisms were added to thioglycolate plus hemin broth to a .5 McFarland standard in 15 mL tubes with a range of 5-FU doses in ug/mL: 100, 50, 25, 12.5, 6.25, 3.125, 1.56. After 24 hours, a sheep blood agar (SBA) plate was streaked from the broth with a sterile 10 uL calibrated loop. The plates were incubated anaerobically for 24 hours and a colony count was performed for each plate.

To further explore the MIC of 5-FU of the test organisms, 5-FU was increased. Tests were performed in optical 96 micro well plates using thioglycolate plus hemin broth. 5-FU was added by serial dilution at the following concentrations in ug/mL: 75, 150, 300, and 540. Organisms grown in broth were added with a repeater pipette to a .5 McFarland standard in each micro well for a total volume of 200 uL. "Growth" wells were inoculated with organism in media, but did not

contain 5-FU, and "no growth" wells contained media that was not inoculated with organism. "Growth" and "no growth" on each plate were used for controls and comparison.

Incubation and Optical Density Reading

The strict anaerobes, *B. bifidum, L. acidophilus*, and *B. fragilis*, were incubated in anaerobic growth chambers using gas packs and methylene blue indicator strips to be sure an anaerobic environment was attained. *E. coli*, a facultative anaerobe that can grow in the presence of oxygen or anaerobically, was incubated anaerobically using the same method as the strict anaerobes and aerobically, to see if there was a difference in growth rate of *E. coli* based on environmental oxygen concentration.

The Epoch 2 plate reader was used to read the optical density (OD) of each well at 600 nm to measure growth at the following time points: 0, 6, 12, 18, 24, and 48 hours. The 18-hour time point was only used for aerobically incubated *E. coli* due to laboratory accessibility; aerobic incubation and agitation was automated, while this feature was not available for anaerobically incubated organisms. The 48-hour time point was not used for *E. coli* under aerobic conditions because of its speedy growth rate in the presence of oxygen. The strict anaerobes and anaerobically incubated *E. coli* are more fastidious and slow growing, so the 48-hour time point was used.

Statistical Analysis

A multivariate mixed-effects linear regression analyses was used to determine the regression coefficients for the mean difference in optical density over time compared to the growth control. Each model contained all parameters listed as well as the following time points in hours: 0, 6, 12, 18, 24, 48.

RESULTS

First round of MIC testing

Growth inhibition was not observed in *E. coli, B. bifidum, L. acidophilus,* and *B. fragilis* after 24-hour incubation. All organisms at all concentrations of 5-FU used showed >100,000 CFU/mL on SBA plates. It was determined that further studies must be done with higher concentrations of 5-FU to determine in vitro susceptibility of organisms to 5-FU.

Second round of MIC testing

After 48 hours of incubation, growth of *E. coli* was inhibited by 5-FU

proportionally to the dose of the drug when incubated under aerobic and anaerobic conditions. *L. acidophilus* showed slight growth inhibition, but inhibition was not statistically significant after 48 hours of incubation. Inhibition of growth was not seen in *B. bifidum* or *B. fragilis* after 48 hours of incubation. See Table 1 for statistically significant organisms and 5-FU concentrations.

Explanation of graph inconsistencies

A normal growth curve peaks, plateaus, and as cell death occurs, optical density remains consistent (Maier, Pepper, and Garba, 2009, p. 37). Figure 1b shows the growth curve of *L. acidophilus*. This graph appears strange because growth peaks at 6 hours and then gradually falls. This is likely due to human error. The Epoch 2 plate reader measures optical density from the bottom of the clear micro well plate. After the *L. acidophilus* and *E. coli* plates had been run, it was noted to wipe bottom of the micro well plate with a Kim wipe and to remove the lid to ensure that no condensation, dirt, or smudges were present to interfere with the optical density reading. The inconsistencies in the graphs of *L. acidophilus* (Figure 1b) and *E. coli* (Figure 1d) could be due to condensation or other interferences on the lid or bottom of the plate before wiping the bottom and removing the lid were added to the procedure.

Statistics

A statistically significant decrease in mean growth, measured in optical density, over time when compared to the growth control was seen with aerobically incubated *E. coli* grown with the following 5-FU concentrations in ug/mL: 150, 300, and 540, and anaerobically incubated *E. coli* grown with the following 5-FU concentrations in ug/mL: 300 and 540. All other parameters did not show statistical significance when compared to the growth control over time. See Table 1 for statistically significant p-values (marked with a *) in mean optical density over time.

DISCUSSION

Decreased populations of *Bifidobacteria spp.*, *Lactobacillus spp.*, and *Bacteroides spp.* and increased populations of *Escherichia spp.* have been observed in patients taking 5-FU (Stringer et al., 2013), however, the opposite effect has been shown by this in vitro study. 5-FU inhibited the growth of *E. coli*, but the growth of *B. bifidum, L. acidophilus* and *B. fragilis* did not show significant inhibition.

Cell-signaling pathways unique to certain genera and species could contribute to differing reactions of in vitro testing to previous in vivo studies. In vivo, cell-signaling between bacterial species is significant for cell function and gene expression (Kaper and Sperandio, 2005, p. 1). Pooled stool cultures for in vitro testing to better mimic the microbial intestinal environment, and agitation of

anaerobically incubated organisms would help mimic the haustral churning of the large intestine. Further research into the mechanism behind the decrease in intestinal organisms during 5-FU and other chemotherapeutic treatment could lead to solutions to quell symptoms of chemotherapy-induced gastrointestinal mucositis.

LIMITATIONS

Due to building hours and the necessity for researchers to be present to read anaerobic organism plates, time points were limited for the strict anaerobic organisms. Cell cultures would be ideal for in vitro testing to better mimic the intestinal environment, but money was a limiting factor since cell cultures are expensive and difficult to keep viable.

ACKNOWLEDGMENTS

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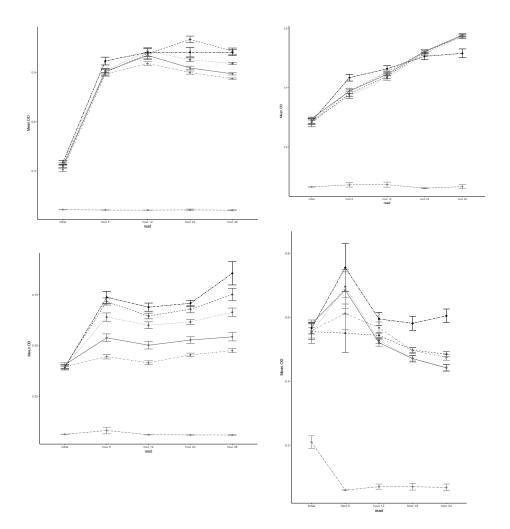
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Table 1. Growth Varies by 5-FU Concentration and Time		
Parameter	Mean difference in Optical Density (OD)	P-value
Bacteroides fragilis (anaerobic)		
75 ug/mL	-1.849×10^{-3}	NS
150 ug/mL	-3.262×10^{-2}	NS
300 ug/mL	-6.726×10^{-2}	NS
540 ug/mL	-9.538×10^{-2}	NS
Bifidobacteria bifidum (anaerobic)		
75 ug/mL	-1.525×10^{-3}	NS
150 ug/mL	-4.363×10^{-3}	NS
300 ug/mL	5.000×10^{-3}	NS
540 ug/mL	3.275×10^{-3}	NS
Escherichia coli (aerobic)		
75 ug/mL	6.092×10^{-2}	NS
150 ug/mL	1.470×10^{-1}	7.237×10^{-3}*
300 ug/mL	2.333×10^{-1}	1.42×10^{-4}*
540 ug/mL	3.079×10^{-1}	5.64×10^{-6}*
Escherichia coli (anaerobic)		
75 ug/mL	3.905×10^{-2}	NS
150 ug/mL	-9.3363×10^{-2}	NS
300 ug/mL	-1.734×10^{-1}	4.973×10^{-3}*
540 ug/mL	-2.391×10^{-1}	3.37×10^{-4}*
Lactobacillus acidophilus (anaerobic)		
75 ug/mL	-8.409×10^{-2}	NS
150 ug/mL	-8.264×10^{-2}	NS
300 ug/mL	-8.076×10^{-2}	NS
540 ug/mL	-7.879×10^{-2}	NS
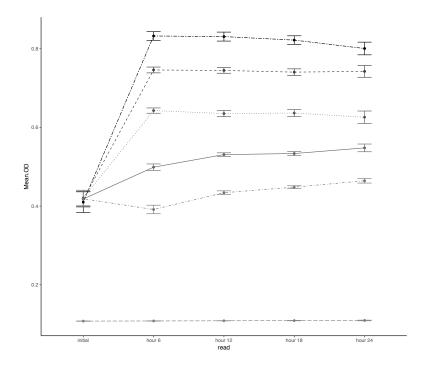
*Represents a statistically significant p-value.

Regression coefficients (mean difference in OD) are from a multivariate mixed-effects linear regression analyses. 5-FU concentrations tested; 75 ug/mL, 150 ug/mL, 300 ug/mL, and 540 ug/mL. Each model contained all parameters listed as well as time points in hours; 0, 6, 12, 18, 24, 48. 18-hour time point was not used for all organisms due to laboratory accessibility. NS = p > 0.05.

Figure 1: Legend: growth: black dash-dot-dash line, 75: black dash line, 150: gray dot line, 300: solid gray line, 540: dash-dot-dash gray line, no growth: gray dash line. 1A: B. fragilis growth curve over 48 hours under anaerobic conditions. 1B: B. bifidum growth curve over 48 hours under anaerobic conditions. 1C: E. coli growth curve over 48 hours under anaerobic conditions. 1D: *L. acidophilus* growth curve over 48 hours under anaerobic conditions.

Figure 2: Legend: growth: black dash-dot-dash line, 75: black dash line, 150: gray dot line, 300: solid gray line, 540: dash-dot-dash gray line, no growth: gray dash line. *E. coli* growth curve over 24 hours under aerobic conditions.

CRISPR-dCas9 Suppression of mecA Gene in MRSA

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ABSTRACT

Antimicrobial therapies have seen a decrease in effectiveness as microorganisms have developed a resistance to them. Creating methods to combat pathogens is an increasing area of research, as virulent strains have become more resistance to traditional treatment methods. Through CRISPR (clustered regularly interspaced short palindromic repeats) gene editing technology, it has become possible to create sequence-specific targeting mechanisms for virulence genes. The specific gene targeted was the mecA gene, which caused a strong resistance towards a wide variety of beta-lactam drugs. *Staphylococcus aureus* was targeted with a plasmid that contained a CRISPR/Cas9 system that affected the gene sequence responsible for the beta-lactam drug resistance and repressed expression. This resulted in a decreased growth of *Staphylococcus aureus* colonies due to the increased susceptibility to beta-lactam drugs, which were previously ineffective. The CRISPR/Cas9 plasmid also contained a resistance gene to chloramphenicol. Successful integration of the plasmid was displayed by the organism gaining a resistance to chloramphenicol, a formerly effective antimicrobial. The efficacy of the CRISPR system was measured by CLSI standard antimicrobial susceptibility testing on the transformed organism. This study opened doors in further areas of research in CRISPR/Cas9 therapy and treatment options for antibiotic resistant bacteria.

INTRODUCTION

Antimicrobials have been used for the last 70 years to combat infectious diseases, with great success. However, due to excessive use, infectious organisms that were once killed by these drugs have developed resistance to them. Each year in the United States, there are at least 2 million people that become infected with drug resistant bacteria. 23,000 people die as a direct result from these infections (CDC, 2017). Methicillin-Resistant *Staphylococcus aureus* (MRSA) caused over 70,000 infections in 2014 (CDC, 2016). MRSA causes a wide range of infections, including soft tissue infections, bone infections, toxic-shock syndrome, and pneumonia. (Monecke et al., 2011) MRSA is resistant to a wide range of beta-lactam antibiotics. The resistance is due to the organism's ability

to produce a modified penicillin binding protein (PB2A), a protein that disrupts the function of beta-lactam antibiotics. This protein is encoded in the *mecA gene* (Jevons, 1961, p.1924).

Clustered regularly interspaced short palindromic repeats (CRISPR) is a form of nucleic acid based adaptive immunity for prokaryotic organisms. Using small RNAs, prokaryotic organisms can use sequence-specific detection and elimination of foreign nucleic acids, such as phages and plasmids (Qi et al., 2013, p.1173). There are various steps to the CRISPR mechanism. First, the organism integrates short sequences of foreign genetic material into the repeating genetic elements known as CRISPR arrays. These CRISPR arrays are transcribed and processed by Cas proteins into small RNAs (crRNA). Lastly, a Cas protein complex made up of crRNA and pCas9 endonucleases targets and interferes with specific-sequences on the foreign nucleic acids (Wiedenheft, 2012, p.331). By modifying the Cas9 protein, which eliminates the cleaving activity of the endonuclease, you get a CRISPR/dCas9 system. Still forming a stable complex with the foreign nucleic acid, the CRISPR/dCas9 system prevents RNA polymerase from binding to the promoter region on the targeted sequence (Richter, 2012, p.2291). This approach allows for regulation of gene expression (Bikard, 2013, p.7429). CRISPR/dCas9 systems was used in this study to suppress beta-lactam resistance of MRSA. The CRISPR/dCas9 plasmids were designed to target specific sequences on the *mecA gene*. To test the plasmids effect on MRSA's susceptibility to beta-lactam based antibiotics, a Minimum Inhibitory Concentration (MIC) test was done. Through treatment of the CRISPR/dCas9 system, *mecA gene* expression reduced significantly. This is shown by a 90% decrease in bacterial growth when in the presence of a beta-lactam antibiotic.

METHODS AND MATERIALS

Plasmid Design

The genome sequence for MRSA ATCC 43300 was retrieved from GenBank, at the National Center for Biotechnology Information's website. The sequence was then sent to the University of Utah HSC Cores Department where it was scanned for potential CRISPR-dCas9 binding sites. The two best sites identified were site 43 and site 46, on the coding strand and non-coding strand respectively. Plasmids were then created which included CRISPR-dCas9 systems which target sites 43 and 46. A third plasmid was created which contained a CRISPR-dCas9 that had no programmed target site. The plasmids, named S43, S46, and Pdcas9, also contained a chloramphenicol resistance gene. This portion allowed the organisms to grow in agar and broth containing chloramphenicol. It also caused the plasmid to be expressed when in the presence of chloramphenicol. These plasmids were stored frozen at -80°C until electroporation.

Making SAB30 E. coli Electrocompetent

Electroporation was the method of cell transformation used. Cloning of the plasmids was performed by electroporating them into Escherichia coli SAB30 cells. SAB30 cells were grown on tryptic soy agar for 24 hours at 37°C. The cells were subcultured into ~40mL of Hanahan's broth (SOB) within two 50mL falcon tubes, and placed in a shaking incubator for another 24 hours at 37°C. While on ice, the falcon tubes were centrifuged to pellet the cells. The supernatant was replaced with ice cold sterile water. The cells were pelleted and resuspended in ice cold 10% glycerol. The suspension was aliquoted into 18 microcentrifuge tubes with volumes of ~2mL each. The cells were centrifuged with ice cold glycerin four additional times in a Sorvall Legend Micro 17R refrigerated microcentrifuge at 4700 rpm and 2°C for 10 minutes. After centrifugation and decantation, the contents of the microcentrifuge tubes were combined. This was repeated until one tube was remaining, with a volume of ~1mL of concentrated cells. This solution was transferred into ten 100μL aliquots and frozen at -20°C. The process of making electrocompetent cells was also performed on MRSA ATCC 43300 cells.

SAB30 E. coli Electroporation and Plasmid Extractionz

The electrocompetent cells and plasmids were thawed and kept on ice. 5μL of plasmid were combined with 80μL of SAB30 cells in 0.1cm Gene Pulser electroporation cuvettes. The cuvettes were electroporated in a Gene Pulser electroporation system. The settings were: 1700V, 25μF, and 200Ω for 2-3msec. 1.2mL of SOC Broth was immediately added to the mixtures post electroporation. The mixtures were incubated for an hour at 37°C for the cells to recover. The contents of the cuvettes were streaked onto tryptic soy agar plates containing 10% chloramphenicol, and incubated for 48 hours at 37°C. A Qiaprep Spin Miniprep Kit was used to isolate the cloned plasmids from the SAB30 cells. An Epoch 2 Microplate Spectrophotometer quantified the plasmids. 20μL of each plasmid were isolated, varying from 40 - 60ng/μL of high purity plasmids. The isolated plasmids were frozen at -80°C.

MRSA ATCC 43300 Electroporation

The electrocompetent cells and plasmids were thawed and kept on ice. The cells were washed one last time in 10% glycerol to ensure proper function of the electroporator. 2μg (~15-20μL) of plasmids were added to 85μL of washed cells in a 0.1cm electroporation cuvette. The cuvette was electroporated at: 2300V, 25μF, 200Ω for 4-5msec. Immediately following electroporation, SOC broth was added to the cuvette for the cells to recover. The solutions were incubated at 37°C for two hours before being poured onto tryptic soy agar containing 10% chloramphenicol. The agar plates were incubated at 37 for 48 hours.

TESTING METHODS

Four different MRSA cell populations were created; one with S43, one with S46, one with S43 and S46, and one with Pdcas9. A microtiter plate MIC panel was set up with dilutions of 32, 16, 8, 4, 2, 1 and 0.5μg/mL of oxacillin and inoculated with 0.5 McFarland Standards of each MRSA population in triplicate. Control wells were created for each organism that contained no oxacillin, to ensure that the organism was still viable for testing. The MIC panel was incubated at 37°C for 24 hours and was read by the Epoch 2 Microplate Spectrophotometer. Mueller Hinton agar plates were cultured with each cell population and a cefoxitin disk placed in the center. These were incubated at 37°C for 24 hours before being analyzed.

RESULTS

The MIC panel for oxacillin susceptibility was read at 600 nm and the absorbance recorded for each concentration of antimicrobial. The mean absorbances were compared to the absorbances of the control wells with no antimicrobial for each cell population. CLSI susceptibility testing standards of ≤ 2 μg/mL as susceptible and ≥ 4 μg/mL as resistant were used to evaluate susceptibility.

Results showed that the S43 population was more susceptible to oxacillin than the S46 population, which closely resembled the pdcas9 cells. The MRSA that had a combination of S43 and S46 had the greatest susceptibility of the groups tested (Figure 1). The cefoxitin disk diffusion test results were inconclusive. The test was repeated twice once on Mueller Hinton and once on TSA plates. Results for the repeated tests were also inconclusive.

DISCUSSION

Results showed that the S43 population showed an increase in susceptibility over the pdcas9 control, but combining it with the S46 proved to be the most effective at inactivating the mecA gene. This effect was associated with the fact that the cas9 endonucleases had multiple targets, one on the coding strand and one on the non-coding strand of DNA, allowing for inactivation of the mecA gene in multiple ways. A 90% decrease in growth was shown in the presence of oxacillin at 4 μg/mL, the standard concentration for determining susceptibility of MRSA. While testing showed the CRISPR-cas9 system effective for increasing susceptibility, further testing of this system in mammalian hosts would be required to show this as an effective alternative to traditional antibiotics. Sequence specific targeting of bacteria with CRISPR-cas9 antimicrobial therapies offer unique strategies for treatments. The bacteria can be targeted for killing or suppression of resistance or virulence genes. Phagemid delivery systems for CRISPR-cas9 antimicrobials have shown promising results in preliminary studies (Bikard, 2014, p.1146). The specificity of this form of treatment could prove to be a more effective therapy for those bacteria, like MRSA, that are harder to treat with traditional methods.

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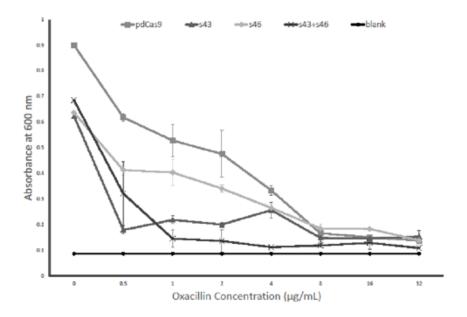
Figure 1. Oxacillin MIC panel result: Measurements of Optical Density (OD) of each modified MRSA strain in increasing concentrations of oxacillin (µg/mL). Pdcas9 (square) had no effect on its growth. S43 (triangle) growth dropped dramatically in the presence of oxacillin, indicating increased susceptibility. S46 (diamond) was moderately effective in increasing susceptibility. S43 + S46 (cross) had the most substantial increase in susceptibility, as growth was significantly reduced at 1µg/mL onward. The bottom line is a measurement of no growth, where no organism was initially inoculated

Linking Depression and Diabetes Through BDNF and Myeloperoxidase

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ABSTRACT

Diabetes-induced depression has become an important point of research because a difference between the rate of depression in diabetics (people who have a Hemoglobin A1C >6.5% and also more commonly have a higher Body Mass Index (BMI).) and "healthy" people has become apparent. A point of concern is that diabetics may have a physiological difference that makes them more susceptible to depression. Two biomarkers that have been found to be consistent with depression and diabetes in separate studies are Brain Derived Neurotrophic Factor (BDNF) and Myeloperoxidase (MPO). These biomarkers were tested for in twenty-six participants that were split into two groups: a "non-depressed" group and a "depressed" group. The depression level of the participant was determined according to their responses to the questions of the Beck Depression Inventory Questionnaire (BDIQ). Between both groups, pre-diabetic and diabetic participants (with an A1C >5.7 %) were dispersed into either group depending on their BDIQ answers. These two groups were compared to show correlation between the values of BDNF, MPO, A1C, and the BDIQ. BDNF and MPO were measured through participant's blood samples using ELISA kits to look for physiological signs of depression. The results of the BDNF, MPO, A1C, and the BDIQ were analyzes statistically using R studio. Through this statistical analysis, the results of this study showed that there was a minor correlation between low BDIQ scores and low BDNF levels ($p = 0.0004425$) as well as a high BMI and high BDNF levels ($p = 0.03693$). The correlations, although minor, are significant enough to afford further research into this topic, especially concerning high BMI and high BDNF levels.

INTRODUCTION

Through previous research, it has been determined that the diabetic population experiences a surprisingly high rate of depression (Bilello et al., 2015). Significant research has gone into developing a way to objectively diagnose just depression, typically through the detection of biomarkers in patient samples. Through the process of research there have been specific biomarkers that have been found to rise and fall with the presence or absence depression and have had specific links

to diabetes as well. The two biomarkers that this research project determined to be the most promising as links between diabetes and depression are BDNF and MPO (Gebel, 2015). In previous studies, BDNF and MPO have been tied to depression, and in others BDNF and MPO have been tied to diabetes, but very little research, thus far, has been done to determine if there is a link between diabetes, depression, and the biomarkers, BDNF and MPO.

BDNF is a protein that acts on neurons, vital in the process of communication in the brain, within the central nervous system and the peripheral nervous system. Its purpose is to support the survival of neurons and stimulate the growth and differentiation of new neurons and synapses in the brain. Depression has been shown to decrease the expression of BDNF, which can lead to atrophy of the hippocampus, a part of the brain thought to regulate emotion, memory, and other functions of the body that we do not voluntarily control. It has also been shown that those who have been diagnosed with diabetes have expressed lower amounts of BDNF and other studies have suggested that BDNF may play a role in insulin resistance, a common issue for those with Type 2 diabetes or adult onset Type 1 diabetes, where cells do not respond correctly to insulin which is a hormone that plays a major role in the body's process of blood sugar regulation (Krabbe, Nielsen, & Krogh-Madsen, 2007).

MPO is an enzyme that is found abundantly in white blood cells, a part of the immune system that is responsible for protecting the body against foreign bodies, and is found to be an important inflammatory enzyme in depression (Talarowska, Szemraj, & Galecki, 2015). In various studies it was found that those who were part of the depressed population had significantly higher levels of MPO. It has also been found that higher levels of activation of MPO in adipose tissue has played a part in obesity and insulin resistance, and thus diabetes (Wang et al, 2016).

This study attempted to find a way to help objectively diagnose depression and find a possible cause for the increased levels of depression in diabetics. To do this it looked to bridge the previous gap between diabetes studies and depression studies, and look at BDNF, MPO, depression, and diabetes all at once. The research that was performed found that there was a correlation between low depression levels and low BDNF as well as high BMI and high BDNF.

METHODS

Twenty-six participants were recruited for this study from the Weber State University student and faculty population. Each participant was asked to sign a form of consent, a health survey, the *Beck Depression Inventory Questionnaire*, and donate two tubes of blood.

The health survey contained questions about the participant's gender, age,

ethnicity, height and weight (for BMI calculation), possible diagnosis of diabetes, and possible diagnosis of depression. Two questions about possible cardiovascular disease and recent infection were asked because either condition could possibly affect the BDNF and MPO results.

The *Beck Depression Inventory Questionnaire* is a survey that is used by the American Psychological Association to help diagnose depression and was used in this study to determine the depression level of each of the participants. The BDIQ contains 21 questions that pinpoint common factors in depression and is scored from 0-63. 0-13 indicated minimal depression, 14-19 indicated mild depression, 20-28 indicated moderate depression, and 29-63 indicated severe depression.

The blood from the participants was drawn into two tubes—one EDTA and one serum separator tube (SST). The EDTA blood was tested on the Mindray BS-200 used to determine the participant's A1C level. The SST tubes were spun at 6000 rpm for 10 minutes and the serum was used to perform the ELISA testing of BDNF and MPO.

The procedures for the BDNF ELISA kit and the MPO ELISA kit were the same with the only differences being the sample preparation and dilution, and the antigens in each 96 micro-well plate. The antigens included in each 96 micro-well plate were the BDNF antigen and the MPO antigen respectively. Both the standards and the patient's samples were run in triplicate.

For the BDNF ELISA kit, Assay Diluent B was diluted by a factor of five with deionized water. Next, Assay Diluent C was used to dilute the participant's serum samples by a factor of ten. The BDNF standard was prepared by adding 400 uL of Assay Diluent C to a lyophilized standard vial, which creates a 50 ng/mL standard. 30 uL of the BDNF standard and lyophilized standard mixture is then mixed with 470 uL of Assay Diluent in a new tube to prepare a 3000 pg/mL standard. Through serial dilutions of 200 uL, 1200, 480, 192, 76.80, 30.72, 12.29, and 0 pg/mL standards are made.

For the MPO ELISA kit, Assay Diluent B was diluted by a factor of 5 with deionized water. Next, Assay Diluent C was used to dilute the participant's serum samples by a factor of two. The MPO standard was prepared by adding 400 uL of Assay Diluent C to a lyophilized standard vial, which creates a 400 ng/mL standard. 180 uL of the MPO standard from the lyophilized standard should then be added to a tube with 300 uL of Assay Diluent C, which prepares a 150 ng/mL standard. The 150 ng/mL standard solution is then used to create a dilution series by adding 200 uL of the standard serially. This creates 150, 60, 24, 9.6, 3.84, 1.54, 0.61, and 0 ng/mL standards.

The BDNF ELISA kit and the MPO ELISA kit were both read using the EPOCH

microplate reader. Both kits were read at 450nm and 550nm with the absorbance at 550nm being subtracted from 450nm to make the standard curve from which the results were obtained.

The results from this experiment were obtained using R Studio software, a program used for statistical comparison of research data. Through this software graphs were used to check for normality and graphed comparisons were used to check for the ability to use a linear regression on the available data.

RESULTS

The first step to obtaining results was to check the normality of the obtained data and to check for normal distribution of the sample data. Any data that is not normally distributed would suggest to the researchers that the data was not obtained from a random sampling of the available population, and there may be some bias to the results.

BDIQ Scores:
The scores for the BDIQ were not normally distributed, suggesting extreme bias on the lower end of scoring for our samples. Most of the participants sampled fell on the lower end of the spectrum of the BDIQ, suggesting a good deal of our participants were not depressed to minimally depressed.

BDNF Scores:
The scores for BDNF were normally distributed, but slightly skewed to the left end of the graph. This would suggest that the participants sampled typically had lower levels of BDNF.

MPO Scores:
The scores for MPO were normally distributed, but slightly skewed to the left end of the graph. This would suggest that the participants sampled typically had lower levels of BDNF.

BMI Scores:
The scores for BMI were normally distributed, but slightly skewed to the left end of the graph. This would suggest that the participants sampled typically had lower BMIs.

Hemoglobin A1C:
The scores for Hemoglobin A1C were normally distributed, but slightly skewed towards the lower end of the range of data. This would suggest that most of the participants sampled for this research study had lower Hemoglobin A1Cs.

h a linear regression (a p-value that was considered significant for this

MPO vs. Depression reported a p-value of 0.3163, BDNF vs. MPO reported a p-value of 0.7337, BDNF vs. A1C reported a p-value of 0.5928, MPO vs. A1C reported a p-value of 0.7307, BMI vs. MPO reported a p-value of 0.4868, A1C vs. Depression reported a p-value of 0.7897, A1C vs. BMI reported a p-value of 0.1369, and BMI vs. Depression reported a p-value of 0.4342, all of which are not considered significant for this research study.

DISCUSSION AND LIMITATIONS

The main hypothesis for this study was that there could be a correlation between depression and diabetes through biomarkers BDNF and MPO. Based on the data that was gathered, there was statistical correlation between BDNF and Depression as well as a correlation between BDNF and BMI. However, there was no significant statistical correlation between depression and diabetes through BDNF and MPO. The results of the BDIQ were between 0-45, with some of the participants being prescribed anti-depressants. The A1C results were between 4.1-6.9%, with some of the participants being prescribed diabetes medication. The participant's BMIs ranged from 18.1-42.8 (normal = 18.5-24.9). The MPO levels had a range of 2-144 pg/mL (normal = 0-2450 pg/mL). It is possible that no correlation could be made due to the low sample size collected. Future research projects should consider gathering more samples because other studies have been able to find a correlation after they were able to obtain a larger sample size and have a greater variety of participants. Most of the participants, for this study, were young college students and diabetes is most often seen in an older population.

Future projects may also want to consider obtaining a fasting glucose sample to know the severity of the diabetes as well as know the type of diabetes each participant has for grouping purposes. These added groups may have allowed the researchers to see a trend along the increasing severity of diabetes in correlation with the biomarkers.

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Variables	p-Value	Variables	p-Value
BDNF vs. Depression	0.0004425	MPO vs. A1C	0.7307
BMI vs. BDNF	0.03693	BMI vs. MPO	0.4868
MPO vs. Depression	0.3163	A1C vs. Depression	0.7897
BDNF vs. MPO	0.7337	A1C vs. BMI	0.1369
BDNF vs. A1C	0.5928	BMI vs. Depression	0.4342

Figure 1: After the data had been assessed for normality, the researchers were able to then able to look at the data in terms of comparisons. Comparisons that had a significant p-value were then assessed with a linear regression (a p-value that was considered significant for this research study was any p-value less than 0.05).
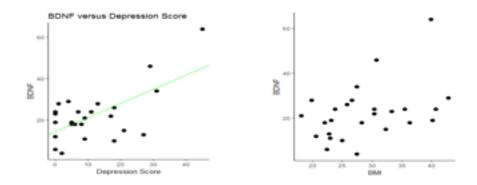
Figure 2a (left): BDNF vs. Depression had a p-value of 0.0004425. The results of the linear regression suggested that there was a minor correlation between BDNF and Depression, though the results correlated in the inverse of what was initially expected. As depression increased, it was suspected that levels of BDNF would decrease, here the researchers could see as depression increased in the sampled data, the levels of BDNF increased.

Figure 2b (right): BMI vs. BDNF had a p-value of 0.03693. With the p-value not being significant enough to qualify for a linear regression, a scatter plot of the data could still be formed to assess for correlations in the data. As the BMI increased in the sampled data, the BDNF levels increased. While the correlation is minor, the information may be found important in later testing. The two graphs in comparison may suggest, with the similar scatter of data, that the BMI scores perhaps may correlate with the Depression scores. This would require further testing, but may be a point of interest to determine if the Depression scoring is impacted by BMI.

The Effects of Various Stilbenoids on Mortility, Growth, Biofilm, Formation and Quorum Sensing of *Escherichia Coli 0157:H7*

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ABSTRACT

Quorum sensing is a bacterial communication system that controls the expression of genes in response to population density. This gene expression can lead to increased pathogenicity in humans, making quorum sensing an interesting target for the development of new antimicrobial compounds. This study's goal is to determine and compare the ability of the stilbenoids resveratrol, combrestatin, viniferin and polydatin to inhibit quorum sensing, growth, biofilm formation and swarming motility in *Escherichia coli O157:H7* (EHEC). N-(3-oxo- octanoyl)-L- homoserine lactone (AHL), the quorum sensing ligand in Gram negative bacteria, was used to induce SdiA in EHEC. Swarming motility was determined on motility agar plates in the presence of the various stilbenoids and AHL. Inhibition of growth was compared with optical densities at 600 nm. Biofilm formation was measured by staining adhered cells in the presence of the various stilbenoids and AHL with crystal violet. Resveratrol and viniferin were found to inhibit growth and motility whereas polydatin enhanced these characteristics. The findings of this study could potentially lead to the development of new routes of treatment for EHEC.

INTRODUCTION

EHEC is an aggressive human pathogen found in contaminated food and water that colonizes the large intestine where it produces potent Shiga-like toxins that cause anemia, hemolytic uremic syndrome (HUS), kidney failure and can result in death. According to the Centers for Disease Control (CDC), there are 73,000 illnesses and 60 deaths due to this pathogen annually. The most susceptible populations are elderly and children under 5 years old. Treatment of EHEC with trimethoprimsulfamethoxazole and quinolone antibiotics is problematic and causes induction of the "SOS" response, triggering the phage lytic cycle leading to increased Shiga-like toxin production and increased risk of developing HUS. The symptoms of HUS include thrombocytopenia, hemolytic anemia and nephropathy. Diarrheal illness is frequently treated before the cause is known, potentially increasing risk to EHEC patients. Antibiotic-mediated prophage induction in animal models has shown the possibility of horizontal gene

transfer of the STX-containing plasmid to other Enterobacteriaceae (Pacheco & Sperandio, 2012; Xiaoping et al., 2000). EHEC infections have become a serious public health concern due to the spread of antibiotic-resistant strains making the development of new treatment methods necessary (Selma et al., 2012). Quorum sensing is a cell-to-cell signaling mechanism used by bacteria to regulate gene expression at higher population densities. In EHEC, this process is controlled by the gene sdiA in response to exogenous AHL. There is little research available on which genes are regulated through the SdiA system and it is poorly understood.

Plants produce phytochemical stilbenoids in response to pathogens, suggesting that they may have antimicrobial properties (Chong, Poutaraud, & Hugueney, 2009). Recent research has shown stilbenoids are effective in inhibiting quorum sensing, swarming motility, and pyocyanin production in *Pseudomonas aeruginosa* and swarming motility and virulence factor production in *Proteus mirabilis*, suggesting that they may be a promising target for new drug development (Sheng, Chen, Tan, Chen, & Jia, 2015; Wang et al., 2006).

MATERIALS AND METHODS

Minimal Inhibitory Concentration Determination and Growth Assay Resveratrol

(Resver.), combrestatin (Combr.), viniferin (Vinif.) and polydatin (Polyd. solutions were made by dissolving 10,000 ug/ml in DMSO. Sub inhibitory (sMIC) concentrations and growth inhibition for the stilbenoids was determined using an overnight culture of E. coli O157:H7 in LB broth inoculated in a 96 well plate. Stilbenoids were added at concentrations of 50, 100, 200, 300, and 400 ug/ml with AHL added at 75 ug/ml. EHEC was added to wells that contained DMSO at the same concentrations as a control. The plates were incubated in an EPOCH 2 microplate reader at 370C for 18 hours. Readings were taken at 600 nm every 2 hours with a 15 minute agitation before each reading.

Swarming Motility Analysis

To determine the inhibition of swarming motility, EHEC was seeded onto LB plates containing 0.3% agar and 0.05% triphenyltetrazolium chloride with and without the stilbenoid compounds at a concentration of 50ug/ml. The agar plates were incubated at 37OC for 24 and 48 hours. After incubation, the motility halo was measured and the results compared to the EHEC control plate.

Biofilm Formation Analysis

To detect biofilm formation, a 0.5 McFarland standard culture was inoculated into 200 ul of LB broth with and without the stilbenoid compounds in a 96 well-plate. The plates were incubated at 37OC without shaking for 24 and 48 hours. The cell cultures were washed 3 times with water to remove all non-adhered

cells and stained with crystal violet for 20 minutes. The stained cells were rinsed 3 times with water and then extracted using 95% ethanol. To quantify biofilm formation, the absorbance was measured at 570 nm (Kim, Lee, Kim, Baek, & Lee, 69 2015).

RESULTS

The DMSO concentration decreased growth when compared to the EHEC control (Figure1). The stilbenoids were suspended in DMSO, so further studies included a DMSO control to compensate for its effects on growth. AHL suspended in DMSO showed no further effects on the growth rate of EHEC (Figure 1).

Resveratrol reduced the growth of EHEC at 100ug/ml and completely inhibited growth at 200ug/ml when compared to the DMSO control. Addition of AHL and resveratrol increased the growth reduction at 100ug/ml but allowed for more growth at 200ug/ml when compared to resveratrol alone. Combrestatin reduced the growth of EHEC at concentrations above 200 ug/ml when compared to the DMSO control. AHL and combrestatin did not have any effect when compared to combrestatin alone. Polydatin had no effect on growth at 100, 200, and 300ug/ml when compared to the DMSO control. At 400ug/ml of polydatin, an increased growth was observed when compared to the DMSO control. AHL and combrestatin had no effect compared to combrestatin alone. Viniferin reduced growth at concentrations above 200 ug/ml compared to the DMSO control. AHL and viniferin increased growth compared to viniferin alone (Table 1, Figure 2).

The addition of AHL at 75ug/ml in motility agar plates decreased motility compared to the EHEC control. Resveratrol and viniferin decreased motility compared to the EHEC control at 24 and 48 hours, with resveratrol having the greatest effect. Polydatin had minimal effect on motility at 24 hours but reduced motility at 48 hours compared to the EHEC control. Combrestatin increased motility at 24 hours and decreased motility at 48 hours compared to the EHEC control. The addition of AHL to combrestatin and polydatin increased growth compared to combrestatin and polydatin alone (Table 2, Figure 3).

DISCUSSION

The activation of the SdiA quorum sensing system by the addition AHL decreased motility. This role of SdiA in suppressing motility is supported by research in which a SdiA mutant strain had increased motility (Sharma, Bearson, & Bearson, 2010). It is unknown how the polydatin and combrestatin are interacting with SdiA or AHL to increase motility in plates that contain AHL in combination with polydatin or combrestatin. It is possible that these compounds are inactivating SdiA or interfering with the sensing of AHL by SdiA. The activation of SdiA by the addition of AHL had little to no effect on growth. It is

unknown why at 100ug/mL AHL and resveratrol had greater effect on growth than resveratrol alone while at 200ug/mL the reverse is true. This may be due to technical error or some competitive effect between resveratrol and AHL. Further research is needed to understand the effects of SdiA quorum sensing in EHEC. No biofilm was observed for EHEC under any of the growth conditions tested. It may be that a different media composition or additional requirement is necessary to trigger biofilm formation. Further research will be necessary to understand the effects of stilbenoids and activation of SdiA on biofilm formation. Resveratrol and viniferin had the most effect on growth and motility and could be promising targets for new drug development for EHEC. It is interesting to note that viniferin is the polymerized form of resveratrol. Combrestatin and polydatin, the methylated and glycosylated versions of resveratrol respectively, did not have the desired effect on either growth or motility. Combrestatin had little effect on growth or motility while polydatin enhanced both characteristics; more so with AHL. This information would be beneficial in drug modification of resveratrol to increase its effectiveness against EHEC.

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	100	200	300	400
DMSO	0.502	0.478	0.479	0.433
AHL	0.598	0.492	0.555	0.364
Resver.	0.353	0	0	0
Resver+AHL	0.198	0.108	0	0
Combr.	0.432	0.273	0. 336	0.227
Combr.+AHL	0.489	0.247	0.227	0.222
Polyd.	0.527	0.429	0.479	0.467
Polyd.+AHL	0.504	0.445	0. 553	0.428
Vinif.	0.456	0.162	0.162	0.162
Vinif.+AHL	0.352	0.193	0.309	0.271

Table 1: Growth Inhibition Assay. The values represent the corrected OD600 after 18 hours of incubation. The corrected OD was figured by subtracting the starting OD from the final OD.
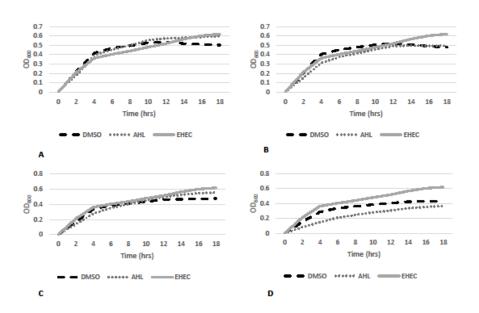
Figure 1: Growth Curve Comparison Between EHEC, DMSO and AHL. A: 0.5% DMSO compared to 100ug/ml of AHL. B: 1% DMSO compared to 200ug/ml AHL C: 1.5% DMSO compared to 300ug/ml AHL D: 2% DMSO compared to 400ug/ml AHL; background levels of E. coli present in all wells.
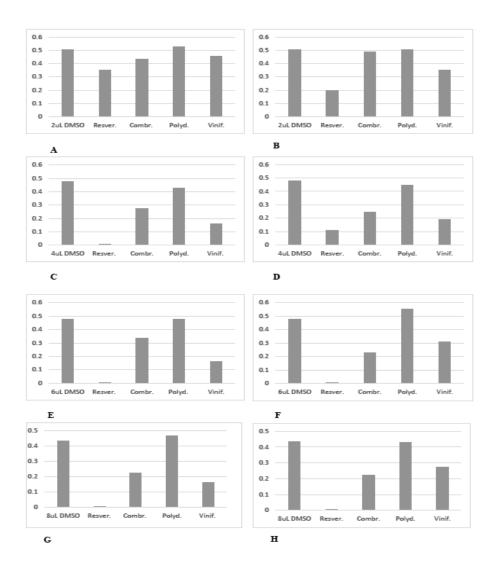
Figure 2: Growth Inhibition by Various Stilbenoids. Numbers represent the OD600 after 18 hours of incubation. A) 100ug/mL concentrations added; B) 100ug/mL concentrations added with AHL; C) 200ug/mL concentrations added; D) 200ug/mL concentrations added with AHL; E) 300ug/mL concentrations added; F) 300ug/ml concentrations added with AHL; G) 400ug/ml concentrations added; H) 400ug/ml concentrations added with AHL.

	Control	Combrestatin	Resveratrol	Viniferin	Polydatin
24 hrs	90	100	50	70	90
24 hrs w/ AHL	60	100	50	70	110
48 hrs	280	260	210	200	210
48 hrs w/ AHL	220	260	190	270	580

Table 2: Motility plates were measured in mm at 24 and 48 hrs of incubation. Values were taken as an average of two measurements from the inoculum origin to the motility front. The stilbenoid compounds were added at a concentration of 50ug/ml.

A

B

C

D
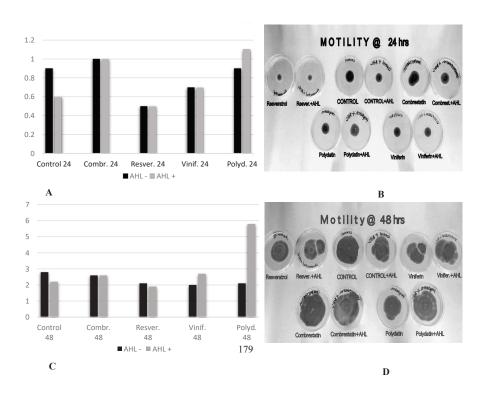
Figure 3: Motility Assay. A) Measurement from edge of inoculation to motility front in cm at 24 hrs; B) Swarming agar plates at 24 hrs; C) Measurement from edge of inoculation to motility front in cm at 48 hrs; D) Swarming agar plates at 48 hrs.

College
of Science

Determining if GSL Microbialite Inhabitants can Facilitate Calcite Precipitation

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MICROBIOLOGY

ABSTRACT

Microbialites are sedimentary structures that are formed through the binding and trapping of grains or precipitation of minerals by members of the microbial communities associated with the microbialite. Expansive microbialite deposits composed mostly of carbonate minerals exist in the Great Salt Lake (GSL), but the exact mechanism of their formation is not known. We hypothesize that one mechanism could be microbial urease activity. The enzyme urease breaks down urea into ammonia and carbonate (CO_3^{2-}), which in turn can react to form carbonate minerals. Bacteria cultured from the microbialite and lakewater were found to be positive for urease activity using a urease broth test (Brink, 2010). Demonstrating urease activity in living microbial communities collected from GSL microbialites would provide strong support for the assumption that the structures are built, at least in part, by microbial activity.

INTRODUCTION

Microbialites—rocks formed by the actions of microorganisms—are useful tools for understanding environmental conditions of the past. We live less than an hour's drive away from putative modern microbialites in Great Salt Lake, Utah (GSL), yet it has never been conclusively demonstrated that the structures in GSL are actually built by microorganisms. The purpose of this research is to determine whether or not the halophiles (microbes that thrive in high salt concentrations) inhabiting GSL microbialites have the enzyme urease, whose action can facilitate the precipitation of carbonate minerals, which are found in the microbialites.

The microbialites of the GSL are assumed to be biogenic, i.e., built by life. This is largely based on the observation that the surfaces of microbialites in the South Arm of GSL host active photosynthetic communities of microorganisms and algae (e.g., Lindsay et al., 2016). However, determining if the surface photosynthetic community is actually capable of precipitating the different carbonate minerals that compose the microbialites has never been demonstrated. One means by which microorganisms can facilitate carbonate

mineral precipitation is via the activity of the enzyme urease, which breaks down urea (excreted by animals such as birds and brine shrimp in GSL) into inorganic carbon and ammonia and increases the pH of the environment (Webba, Jella, & Baker, 1999).

METHODS

Microbialite samples were collected near the shore of Ladyfinger Point on Antelope Island in GSL (41.0611 N, -112.2477 W) using a shovel to carefully extract an intact, submerged microbialite and maintain orientation. The microbialite was pulled apart from its adjacent ooid sand and cut in half using the shovel, and subsamples were obtained immediately. Four subsample locations were chosen (Figure 1; described in more detail below): two from the exterior and two from the interior of the microbialite with two samples collected from each location. In addition, samples were collected of the lake water at the location where the microbialite was sampled. One set of subsamples was moistened with lake water and stored at 4°C for one week prior to culturing and urease tests, the other set was flash-frozen in liquid nitrogen in cryogenic storage tubes for later DNA extraction and sequencing.

The interior of the microbialite was much more loosely compacted and easier to sample than the sediment on the exterior. The two exterior sites were chosen based on color, the upper dark green layer (samples EG1 & EG2, ~1.5mm thick) and just below it, the peach-colored layer (samples EP1 & EP2, ~12 mm below the dark green layer). This second layer also showed evidence of pink-pigmented colonies, potentially purple sulfur bacteria. These were included in DNA sample EP2 and urease test sample EP1. The interior 2 sites were labeled IU1 & IU2 (~25mm below the peach layer) as well as IL1 & IL2,(~35mm below the IU site). (See Figure 1 and Table 2 for details.)

Culture Growth

Microbialite community samples were inoculated in modified nutrient broth (MNB) consisting of 3 g Difco nutrient broth, 20 g urea, 10 g ammonium chloride, 2.1 g sodium bicarbonate, and 2.8 g anhydrous calcium chloride in 1 L of distilled water (pH of 8.41), and filter-sterilized (Corning 250 mL filter, 0.22 μm pore size) prior to inoculation. Twenty-six autoclaved 13mm test tubes were filled with 9 mL of GSL UNB and 1 mL of culture sample (8 samples in triplicate plus two negative controls with no sample added). Growth was monitored via optical density measured at 600 nm (OD600) with a spectrophotometer.

DNA Extraction

DNA was extracted from the frozen samples and water samples using the DNeasy Powersoil DNA extraction kit (Quiagen, Germantown, MD) and

the corresponding protocol. The extracted DNA was quantified using spectrophotometry on a NanoDrop 2000 (ThermoFisher Scientific).

Urease Test

After seven days incubation in GSL UNB at room with natural light, samples were transferred to a urease test medium. The medium consisted of 100 mL of Difco urea broth medium, which was filter-sterilized (Becton Dickenson syringe filter, 0.22 μm pore size) prior to transferring 3 mL into each of 26 sterile screw-cap 10mL tubes. Sample cultures were transferred into the urease test medium using a sterile inoculation loop. Urease tubes were checked regularly for 11 days for appearance of the fuchsia color indicative of a pH increase due to ammonia production as a result of urease activity. It should be noted that the first tube in which the media was added, IL1A, as well as the two blanks, had a faded pink coloration at the onset of incubation (as compared to the expected orange observed in all other tubes), probably due to oxidation and not indicative of premature denaturing or a positive result of the broth.

In addition, an attempt was made to isolate organisms from the microbialite samples on modified nutrient agar (MNA) (30mL plates). The agar was produced by adding 15 g per liter of MNB without urea added; the broth was autoclaved, and urea powder was added in a biological safety cabinet once the agar mix was partially cooled. Streak plates of each site sample were prepared and allowed to grow for five days at room temperature (20-22°C). Additional MNA streak plates of culture from each sample site were incubated anaerobically in anaerobic jars for one week at 21.7 .

RESULTS

Culture Growth

Growth was observed in the enrichment media cultures after five days of incubation at room temperature (20-22 °C). The absorbance values had increased (Figure 2), and an abundance of microbial cells were observed by microscopy of wet mounts and gram stains of the cultures. The cultures consisted of both gram-positive cocci and gram-negative and gram-positive rods (Figure 3).

When the samples were plated normally (aerobically), microscopy revealed that only the cocci had proliferated. In contrast, one of the anaerobic plates had gram-negative filaments (that would be expected with Cyanobacteria) in addition to gram-positive rods and cocci.

DNA Extraction

Concentrations of extracted DNA varied substantially in the different samples (Figure 4), with the highest concentration yields obtained from the exterior green layer, followed by the exterior peach layer. Substantially less, but measurable, DNA was obtained from the microbialite interior. DNA purity was quantified and deemed acceptable, with A260/A280 values determined using the NanoDrop 2000 greater than 1.8 for all samples.

Urease Test

After 24 hours, none of the urease tubes showed any fuchsia color to indicate urease activity, and the faded pink tubes were still faded pink. After a week of incubation at room temperature (20-22 °C) positive results were observed in the majority of the samples from both the exterior and interior communities, some more gradually manifested than others. Figure 5 illustrates the gradient of results exhibited by the urease test while Table 1 summarizes the urease results from each tube. In general, the exterior showed a faster response to the urease test, but both the exterior and interior samples gave equal number of tubes showing positive test results.

DISCUSSION

The results of the DNA extraction, and growth of the samples, suggest that microbial life is distributed throughout the microbialites. Although the greatest quantities of DNA were obtained from the green (oxygenic photosynthetic) exterior layer, which has been subject to most of the work to date on understanding the microbialite community and is thought to be the location in building the microbialites (e.g., Lindsay, et al. 2016), the results of these DNA extractions and culturing tests suggest there is a significant community in the interior of the microbialite. This complements the findings of Pace et. al. (2016), who suggested that some of the mineralization occurring in the GSL microbialites could be due to active sulfate reducing communities in the interior of the GSL microbialites. Urease results suggest an additional potential mineralization mechanism: although the fastest urease activity was found in the exterior samples, both the exterior and the interior show urease activity. Urease activity can facilitate carbonate precipitation, which is a mechanism that had not previously been proposed for the GSL microbialites. Together, these results indicate that the microbial community is very likely capable of producing carbonate minerals in both the exterior and the interior of the microbialites, adding support to the hypothesis that the structures are biogenic, and suggesting an additional formation mechanism.

Future work will focus on determining which organisms are responsible for the urease activity as well as establishing their relative abundance in the overall microbialite community. Extracted DNA will allow sequencing and analysis of the metagenomes of microbialite samples and allow analysis for the urease synthesis genes ureABC, ureD, and ureEF (Connolly, 2013). DNA extracted from enrichment culture isolates will allow us to determine whether organisms isolated from microbialite samples are representative of the overall microbialite communities. Isolates that exhibit urease activity can also be tested for carbonate precipitation, which could link precipitation (and potentially specific minerals) to the minerals seen in the microbialites.

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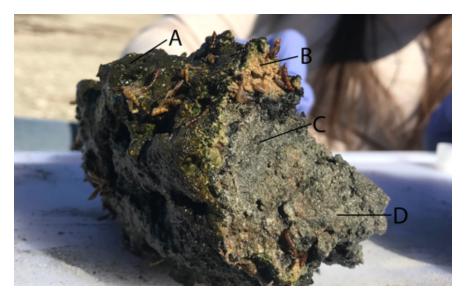
Figure 1. Sampled microbialite from the GSL. (A) Exterior Green Layer (EG1 & EG2); (B) Exterior Peach Layer (EP1 & EP2); (C) Interior Upper Layer (IU1 & IU2); (D) Interior Lower Layer (IL1 & IL2).
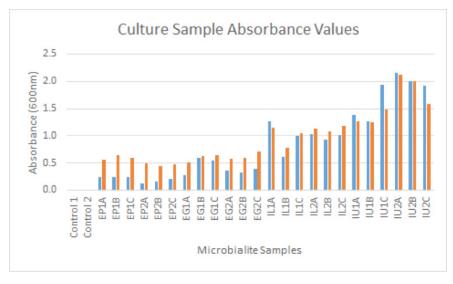
Figure 2. Absorbance values, (600nm), of the culture samples in nutrient-rich broth. Initial OD's (blue) are shown in comparison with values after five days of growth (orange). It is unknown as to why some have decreased in their absorbance, but one hypothesis is that more of the cells and/or constituents of the broth may have settled out.
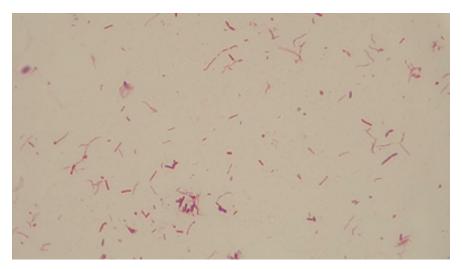
Figure 3: Photomicrograph of inoculated broth sample (EG1A), photographed at 1000x magnification, showing gram-positive cocci and rods as well as gram-negative rods. This photo was captured using the AirLab app and a Leica DM750 microscope.
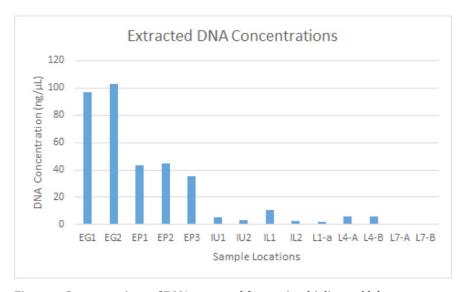
Figure 4. Concentrations of DNA extracted from microbialite and lake water samples quantified using a NanoDrop spectrophotometer at 260 nm.
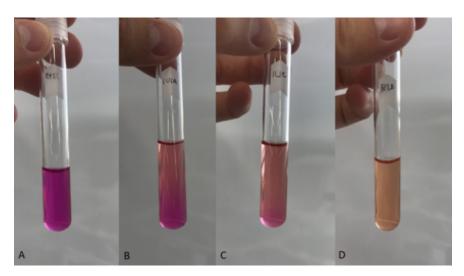
Figure 5. Results of the urease test and the labeling key for Table 1. (A) Very positive for urease activity, (B) & (C) Partial positive for urease, (D) Negative for urease activity.

	Exterior Green Layer			Exterior Peach Layer			Interior Upper Layer			Interior Lower Layer			ks
	EG1 A	EG1 B	EG1 C	EP1A	EP1B	EP1C	IU1A	IU1B	IU1C	IL1A	IL1B	IL1C	Blank 1
11/11	-	-	-	-	-	-	-	-	-	-	-	-	-
11/17	+	++	+	++	-	-	+	+	+	-	+	-	-
11/20	+	++	++	++	-	+	+	+	+	-	+	+	-
	EG2 A	EG2 B	EG2 C	EP2A	EP2B	EP2C	IU2A	IU2B	IU2C	IL2A	IL2B	IL2C	Blank 2
11/11	-	-	-	-	-	-	-	-	-	-	-	-	-
11/17	++	-	++	+	-	++	+	+	++	+	-	-	-
11/20	++	+	++	++	-	++	+	+	++	+	+	-	-

Table 1. Results of urease test. (++) Positive for urease activity (fuchsia), (+) Partial positive for urease (gradient from orange top to pink bottom, possible obligate anaerobe), (-) Negative for urease activity.

Location	Short Name	DNA Concentration	Max Urease Result
Exterior Green	EG1	96.8	++
	EG2	102.8	++
Exterior Peach	EP1	43.4	++
	EP2	44.6	++
Interior Upper	IU1	5.2	+
	IU2	2.9	++
Interior Lower	IL1	10.6	+
	IL2	2.5	+

Table 2. A summary of the sample site locations, short names, DNA concentration (ng/µL), and maximum urease test results.

Effect of Organic Acids on Suppressing Growth of *Lactobacillus wasatchensis*

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Mentor: CRAIG OBERG

MICROBIOLOGY

ABSTRACT

Lactobacillus wasatchensis is a slow growing, non-starter lactic acid bacterium (NSLAB) causing late gas formation in aging cheese, which results in significant economic losses to the producer. During cheese aging, organic acids can be produced by other NSLAB cultures or purposefully added to cheese during manufacture. Organic acids are often used as preservatives, occur naturally in foods, generally don't affect flavor or product quality, and, under acidic conditions, enter bacterial cells altering the cell's proton motive force. Selected organic acids, in their natural concentration range (2.5-560 mM) in Cheddar cheese, were investigated for their ability to inhibit *Lb. wasatchensis* (WDC04). Five organic acids (lactic, formic, propionic, citric and acetic) that can be produced by NSLAB organisms were selected for this study, they were added at their minimum, median, and maximum concentrations as found naturally in aged Cheddar cheese to individual wells of a 48 well plate containing MRS broth with 1% ribose (MRS + R) inoculated with WDC04. Once interesting results were discovered in the initial MRS+R broth (pH 7.0) the tests were run again at pH 5.1 to determine if the chosen organic acids would be more effective at a pH similar to that found in aging Cheddar cheese. Growth rates were determined on a Tecan Infinite 200 PRO spectrophotometer over 40 hours with results graphed on Excel. Both formic and citric acid showed significant inhibition of *Lb. wasatchensis.* As formic acid concentrations increased, the inhibitory effect also increased. The maximum concentration (100 mM) showed the most inhibition, but the median concentration (63.15 mM) and the minimum concentration (26.3 mM) also caused observable inhibition. The addition of citric acid at the minimum (12 mM) and median (13.5 mM) concentrations showed similar inhibition. The use of selected organic acids at concentrations normally found in Cheddar cheese is a potential antimicrobial measure to prevent or reduce late blowing in aging cheese.

INTRODUCTION

The microflora of fermented food products play a key role in flavor profile and development, quality of product, and spoilage over time. Dairy products, specifically Cheddar cheese, require a very specific microflora to achieve desirable product. While some bacteria that make up the Cheddar cheese microflora are added during cheesemaking (starter lactic acid bacteria, SLAB), others (non-starter lactic acid bacteria, NSLAB) are not intentionally introduced yet become the predominant bacteria by the end of the aging process. NSLAB contribute significantly to the cheese taste, quality, and defects, sometimes eventually replacing SLAB cultures initially added by the manufacturer. *Lactobacillus wasatchensis* is a novel, slow growing, NSLAB that was found to cause late gas formation in aging cheese (Oberg et al., 2016). This particular organism is one of great concern throughout the dairy industry because it makes the cheese a consistency that is difficult to cut and may be undesirable to the consumer resulting in significant economic loss (Ortacki, Broadbent, Oberg, McMahon, 2015). While these effects have been observed for some time without a specific microorganism to blame, identification of *L. wasatchensis* opened the door to, subsequently, identify antimicrobial measures that can be used to reduce the incidence of late gas blowing.

The use of organic acids produced by NSLAB in order to inhibit the growth of other NSLAB cultures is a new application in controlling aging defects. Due to the classification of LAB as organisms generally recognized as safe (GRAS), the ability to utilize compounds produced by these organisms in concentrations naturally found in a food environment means less regulation and higher consumer approval than using a synthesized chemical that has the same function. The effectiveness of an organic acid as an antimicrobial compound depends on the pH of the food, temperature, and the pK of the acid. Organic acids can alter the internal environment of the cell in several ways depending on if they are dissociated or become undissociated. These acids reduce the pH of the food as a whole which can inhibit some bacteria, but can also interfere with nutrient transport and energy generation, damage the macromolecules of cells and, alter the ionic environment of spores (Ray, 2005).

Lactic, propionic, formic, acetic, citric, butyric, hippuric, orotic, and pyruvic acids are all found at various concentrations in Cheddar cheese as it ages (McMahon et. al., 2014). The organic acids selected (lactic, propionic, formic, acetic, citric) were those that naturally occur at the highest concentrations in the control Cheddar cheese. These acids were then added to inoculated MRS+R broth containing *Lb. wasatchensis* (WDC04) at their minimum, 50% and maximum concentration (McMahon et al, 2014) to determine their antimicrobial effect of each, on *Lb. wastachensis* at either pH 7.0 or pH 5.1 (cheese pH).

MATERIALS AND METHODS

Bacterium and Growth

Lactobacillus wasatchensis WDC04 (Oberg et al, 2015) was propagated by inoculating a frozen stock culture (stored at -80°C) into Difco Lactobacilli de Man, Rogosa, and Sharpe broth (Becton Dickinson Inc., Sandy, UT) with 1% ribose (Bioenergy Life Science, Inc., Minneapolis, MN) (MRS+R) and incubated for 40 hours at 25°C. This culture was subsequently added to 200 mL of MRS+R broth and incubated for another 40 hours at 25°C. The resulting culture was washed with 0.2% sterile peptone and back diluted with MRS+R to an absorbance of 0.2-0.3 (read at A_{600}). MRS+R was inoculated at a pH 7.0 and also at a pH 5.1 (cheese pH: altered by addition of HCl and use of a pH meter).

Organic Acids

Lactic, propionic, acetic (Avantor Performance Materials, LLC., Center Valley, PA), citric (Fisher Scientific International, Inc., Pittsburgh, PA)and formic (Mallinckrodt Pharmaceuticals, St. Louis, MO) acids were selected due to their significant concentrations found naturally in Cheddar cheese in comparison to all other organic acids found in aged Cheddar cheese (McMahon et al, 2014). The minimum, 50%, and maximum concentrations were calculated to produce a 10X stock solution of each acid. Each organic acid solution was filtered through a 0.20 µm sterile syringe filter (Corning, Inc., Corning, NY).

Plate Reader

A Falcon 48 well plate (Corning, Inc., Corning, NY) was inoculated with MRS+R containing the WDC04 culture and the appropriate amount of organic acid stock solution to achieve the desired final acid concentration in triplicate adjacent wells. Water was added to uninoculated broth controls to ensure each well had a total volume of 1 mL. The plate was inserted into the Infinite 200 PRO spectrophotometer (Tecan Trading AG, Switzerland) and absorbance (A_{600}) was measured in 30 minute kinetic intervals over 40 hours with standard deviation calculated every 10 hours.

RESULTS AND DISCUSSION

Control

Figure 1 shows the resulting absorbance values of MRS+R in the presence and absence of inoculum prior to the addition of organic acid. Both uninoculated growth curves show a constant value for the entirety of the 40 hour run. The inoculated MRS+R (pH 7.0) showed the end of lag phase, the entirety of the log phase, and part of the stationary phase of WDC04 with a difference in

absorbance values of 0.59 from start to finish. The other inoculated pH 5.1 MRS+R showed the similar growth phases with the exception of the log phase taking less time and a difference of 0.68 in absorbance values over the course of 40 hours.

Formic Acid

Figure 2 depicts the product absorbance values of uninoculated, minimum, 50%, and maximum concentrations of acetic acid added to a culture of WDC04 in MRS+R broth. For formic acid both uninoculated growth curves remain fairly constant over 40 hours. The maximum concentration showed the most inhibition (difference of 0.08-0.1), followed by the 50% (difference of 0.15-0.2), and finally the minimum concentration of formic acid (0.43-0.5 difference) had absorbance values indicating the most growth.

Citric Acid

The absorbance readings of the uninoculated wells stayed fairly constant through the entirety of the run as did the minimum and 50% readings of the MRS+R (pH 5.1) with differences in absorbance values less than 0.1. The minimum and 50% acid concentrations of MRS+R (pH 7.0) with WDC04 showed a slight change in absorbance over time (0.25 or less after 40 hours). The maximum citric acid concentration at both pH 7.0 and pH 5.1 resulted in the highest levels of growth over time (differences of 0.51 and 0.25, respectively) (Figure 3).

Lactic Acid

The uninoculated lactic acid absorbance values over time showed a slight decrease in absorbance at pH 5.1 (0.12 difference) and a slight increase in absorbance values at pH 7.0 (difference of 0.2). The addition of lactic acid at any concentration when MRS+R had a pH of 5.1 showed minimal growth (differences in absorbance less than 0.1). At pH 7.0, the difference absorbance values of minimum and 50% concentrations was about 0.2 (0.21 and 0.23). The maximum concentration of lactic acid at neutral pH showed a difference of absorbance values of 0.37, meaning it showed the highest levels of growth.

Acetic Acid

At pH 7.0, the minimum, 50% and maximum all showed similar differences in absorbance over time: 0.63, 0.69, and 0.64 respectively (Figure 5). The pH 5.1 absorbance readings showed a slight decrease in absorbance with increasing acid concentration (minimum=0.72, 50%=0.73, maximum=0.50). Both uninoculated controls remained fairly constant over the course of 40 hours (Figure 5).

Propionic Acid

Growth curves for WDC04 at varying levels of propionic acid concentration and different MRS+R acidity showed a decrease in WDC04 growth as acid concentrations increase, especially at pH 5.1 in Figure 6. The difference in absorbance at neutral pH is highest for 50% (0.72), followed by maximum (0.69), minimum (0.49), and finally, uninoculated (0.04). At the pH closer to that of cheese, the difference in absorbance is greatest at minimum (0.73), followed by 50% (0.61), maximum (0.19), then uninoculated (0.04).

CONCLUSION

The desired results were inhibition of WDC04 growth in media at Cheddar cheese pH (5.1) with increasing concentrations of acid. The controls showed that MRS+R broth without WDC04 didn't show a change in absorbance and the growth curve of WDC04 without organic acids (figure 1). Citric acid resulted in similar differences in absorbance at minimum and 50% concentrations and highest difference at maximum acid concentrations. This pattern was also followed by lactic acid, but only at pH 7.0. Acetic acid was increasingly inhibitory at pH of 5.1, but at pH 7.0, showed similar absorbance differences regardless of concentration. Formic acid was more inhibitory of WDC04 at all concentrations, especially at the highest concentrations. While propionic acid didn't inhibit WDC04 at the increasing concentrations at pH 7.0, it did follow the correct pattern of increasing inhibition with increased concentration at MRS+R that was altered to have a pH of 5.1

Difficulties in comparing the absorbance readings exactly occurred in the data due to an inability to start the growth at the exact same absorbance in all 48 wells for every single run on the plate reader. A way that was found to be easier was looking at the difference in absorbance from start to finish as well as identifying if the growth curve graphed is one that was expected. In addition to the research done, future research on the inhibitory affects of these organic acids could attempt to make the environment of each well into one as close to the complex cheese environment as possible. There are also a few more organic acids (sorbic, butyric, etc) that were not investigated due to their low concentrations in Cheddar cheese in comparison to the five that were tested that may necessitate further research.

ACKNOWLEDGMENTS

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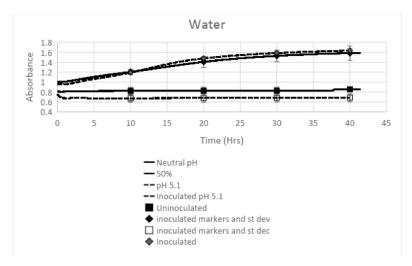
Figure 1. Change in absorbance of MRS+R inoculated (with WDC04) or uninoculated over 40 hours. pH 7.0 is indicated with closed markers, pH 5.1 is indicated with open markers.
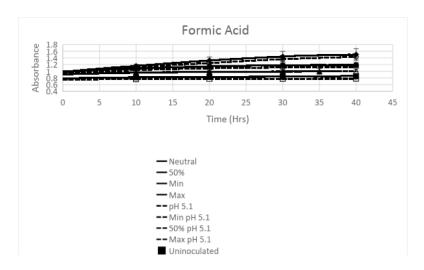
Figure 2. Change in absorbance at minimum (26.3 mM), 50% (63.2 mM), and maximum (100 mM) formic acid concentrations in MRS+R (pH 7.0 or pH 5.1) with or without WDC04 over a period of 40 hours. pH 7.0 is indicated with closed markers, pH 5.1 is indicated with closed markers.
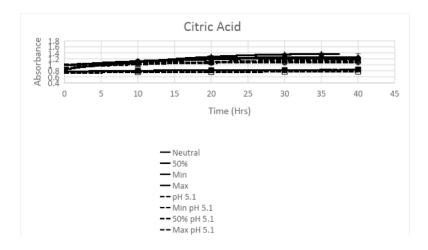
Figure 3. Change in absorbance at minimum (12 mM), 50% (13.5mM), and maximum (15 mM) citric acid concentrations in MRS+R (pH 7.0 or pH 5.1) with or without WDC04 over a period of 40 hours. pH 7 markers are closed and pH 5.1 markers are opened.
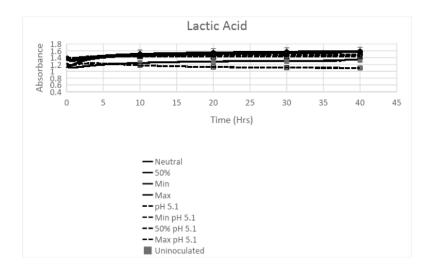
Figure 4. Change in absorbance at minimum (450 mM), 50% (505 mM), and maximum (560 mM) lactic acid concentrations in MRS+R (pH 7.0 or pH 5.1) with or without WDC04 over a period of 40 hours. pH 5.1 is indicated with open markers, pH 7.0 is indicated with closed markers.

Figure 5. Change in absorbance at minimum (6 mM), 50% (38 mM), and maximum (70 mM) propionic acid concentrations in MRS+R (pH 7.0 or pH 5.1) with or without WDC04 over a period of 40 hours. pH 7.0 lines have closed markers, pH 5.1 dashed lines have open markers.

Figure 6. Change in absorbance at minimum (2.5 mM), 50% (9.75 mM), and maximum (17 mM) acetic acid concentrations in MRS+R (pH 7.0 or pH 5.1) with or without WDC04 over a period of 40 hours. pH 7.0 is indicated with closed markers, pH 5.1 is indicated with open markers.
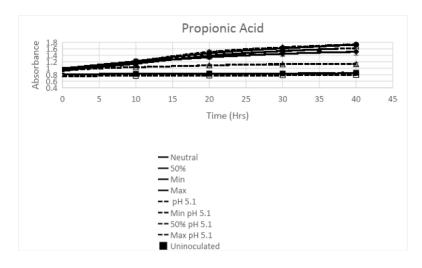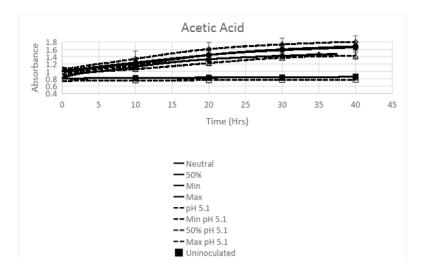
Antibiotic Resistance and Hemolysins of Lactic Acid Bacteria Isolated from Over the Counter Probiotic Products

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MICROBIOLOGY

ABSTRACT

Many studies have characterized laboratory strains of lactic acid bacteria (LAB) noting their potential health promoting features which have encouraged their use as probiotics. However, it is known that some LAB strains have traits, such as antibiotic resistance, that may have a negative impact on one's health. This study's purpose was to characterize the antibiotic resistance and hemolytic activity of 8 strains of LAB, isolated from probiotic products purchased in Utah stores. The LAB strains were tested using the disc diffusion assay in which all 8 LAB strains exhibited antibiotic resistance to vancomycin, oxacillin and bacitracin—five were also resistant to cefoxitin and 3 were resistant to ciprofloxacin. The minimum inhibitory concentration of cefoxitin, oxacillin, and tetracycline of the 8 LAB strains was determined using E-test strips. Antibiotic sensitivity results of the E-test were consistent with the disc diffusion assay when Mann Rogosa Sharp agar (MRS) was used for both assays. This was in contrast to E-test results that differed from the disc diffusion test results when, following the manufacturer's directions, sheep blood agar (SBA) and a higher concentration of the LAB strains was used instead. Interestingly, as a result of using SBA, five of the LAB strains were hemolytic after 48 to 72 hours of anaerobic incubation at 37°C. This is surprising since hemolysis may be an indication of the potential for pathogenicity yet probiotic LAB strains are considered harmless. This study is one of a few that characterized probiotic strains obtained from products readily available to the consumer and which indicated the potential for adverse outcomes from the use of probiotics.

INTRODUCTION

Probiotics are defined as living microorganisms that have the potential to benefit the health of an individual when ingested (Degnan, 2008). The three most commonly used and studied probiotics in animals and humans are *Lactobacillus, Bifidobacterium, and Saccharomyces* (Yan & Polk, 2011). These common probiotic strains are generally part of the normal human microbiota, and can be found in various foods and over the counter supplements.

Probiotics provide various beneficial effects. They do this by blocking pathogenic bacterial effects—by producing substances that are bactericidal and compete against pathogens for space on the intestinal epithelium. This promotes intestinal epithelial cell survival, contributing to an enhanced barrier function, stimulating protective responses from intestinal epithelial cells, and enhancing innate immunity (Yan & Polk, 2011). Probiotics have been shown to control irritable bowel syndrome and inflammatory bowel disease, lower serum cholesterol levels, alleviate food allergy symptoms in infants, and improve lactose tolerance (Parvez et al., 2006). For these reasons and more, probiotics are the subject of various medical research projects and are the source of a multi-billion dollar industry (Mackowiak, 2013). Even with all the known benefits of probiotics, there are still safety concerns and many unknowns surrounding them. Some things that are a cause of concern regarding probiotics are: their potential to cause infections in patients with an immunodeficiency, the possibility of transfer of their antibiotic resistance genes to normal microbiota or pathogens, issues related to genetic stability of the probiotic over time, and deleterious metabolic activity (Sanders et al., 2010). The purpose of this study was to identify and characterize antibiotic resistance and hemolytic ability as potential risks present in probiotic products readily available in Utah stores.

MATERIALS AND METHODS

Isolation of LAB strains from Over-The-Counter Probiotic Products

Eight probiotic products were purchased in the Ogden, Utah area (Table 1). Contents of each probiotic capsule were placed in sterile saline and vortexed for 10 minutes. A loopful of this mixture was placed onto MRS (Mann Rogosa Sharpe) agar and streaked for isolation. Plates were incubated for 48 hours at 30 °C anaerobically. Isolates were transferred to MRS broth, incubated at 30 °C for 24 hours, and tested for purity by streaking onto MRS agar plates (which were incubated as previously described). Gram stains indicated that all the 8 isolates were gram positive rods of varying lengths.

Antibiotic Resistance Using Disc Diffusion Assay

This assay was performed and interpreted as per CLSI standards (CLSI, 2015a; CLSI, 2015b). Discs containing antibiotics were acquired from Hardy Diagnostics (USA): bacitracin 10 units, cefoxitin 30 μg, ciprofloxacin 5 μg, erythromycin 15 μg, novobiocin 30 μg, oxacillin 1 μg, tetracycline 30 μg, and vancomycin 30 μg. Measurements of the diameter of the zone of inhibition around each disc were taken at 24 and 48 hours of incubation (Figure 1).

E-test Assay to Determine Minimal Inhibitory Concentration (MIC)

LAB strains were grown for 24 hours anaerobically at 37 °C in TSB. Cultures were diluted to 0.5 McFarland and 1.0 McFarland standard. Cultures standardized at 0.5 McFarland standard were swabbed onto MRS agar plates as per CLSI standards. Cultures standardized at 1.0 McFarland standard were swabbed onto SBA agar plates as per CLSI standards. After 5-15 minutes, an E-test strip (Biomerieux, USA) containing dilutions of one antibiotic was placed on the swabbed plate. Plates were incubated anaerobically for 24 hours at 37 °C. The lowest concentration of antibiotic that inhibits growth of the organism was recorded (Figure 2). Plates were incubated another 24 hours and the results of inhibition at 48 hours were also recorded. Each LAB isolate was tested with E-test strips, with each strip containing either cefoxitin, oxacillin, or tetracycline.

Hemolysis

Overnight cultures of the LAB strains were grown in sterile MRS broth incubated at 37 °C for 24 hours. Each LAB strain was quadrant streaked onto sheep blood agar (SBA) and incubated anaerobically using gas packs at 37°C. Presence of hemolysis was noted by observing the plates with backlighting at 24, 48, and 72 hours.

RESULTS

The LAB strains were tested using the disc diffusion assay in which all 8 LAB strains exhibited antibiotic resistance to vancomycin, oxacillin and bacitracin (Tables 2 and 3). Five strains were also resistant to cefoxitin and three were resistant to ciprofloxacin. Results were similar when read at 24 and 48 hours (Tables 2 and 3). Only three changes were observed when comparing results from 24 to 48 hours, as noted in Table 2.

The results of the minimum inhibitory concentration (MIC) of cefoxitin, oxacillin, and tetracycline for the 8 LAB strains were determined using E-test strips. The measurements from the E-test were converted to sensitivity results, which are consistent with the results of the disc diffusion assay of LAB strains using MRS agar incubated for 24 hours (Table 4). The only exception to this was for strain GX with the antibiotic cefoxitin (Table 4). There were more changes observed when results were read at 48 hours, showing that longer incubation made comparisons between the 2 tests less consistent (data not shown). The results of the antibiotic disc diffusion assay and the MIC from the E-test are similar when testing LAB strains on MRS agar at a concentration of ~0.5 McFarland standard (Table 4), but not consistent using LAB concentrations of ~1.0 McFarland standard on SBA (data not shown).

Probiotic LAB strains were tested for hemolysis using sheep blood agar (SBA) plates which were incubated for 24, 48, and 72 hours at 37°C anaerobically. All 8 strains showed no detectable hemolysis at 24 hours. Partial hemolysis, appearing as a greening of the blood agar, occurred for 5 of the 8 strains by 48 hours which progressed to beta hemolysis (i.e. complete lysis of the red blood cells) by 72 hours of incubation (Table 5).

DISCUSSION

These results indicate that some LAB strains can hemolyze sheep red blood cells when incubated anaerobically at 37°C, for 48 to 72 hours. While previous studies of LAB strains found no beta hemolytic LAB strains, these blood agar plates were incubated only 24 to 48 hours at 37°C (Jose et al., 2015; Vesterlund et al., 2007). However, when plates were incubated for up to 72 hours at 37°C, our results revealed beta hemolysis. Our results are similar to the results of other researchers, who observed that an occasional *Lactobacillus* strain could hemolyze blood and whom identified secreted surfactants as the cause of that hemolysis (Gudina et al., 2010; Sharma & Saharan, 2016). Further research is required to determine if the hemolytic activity found in this study is due to accumulation of organic acids or other metabolic end products (e.g. proteases, hemolysins, surfactants etc.). It should be noted that even though bacteria exhibit hemolytic activity as observed in this study, its actual role in human disease is still unproven without further study (Gudina et al., 2010; Sharma & Saharan, 2016). Since probiotic use is increasing worldwide, testing for virulence factors including hemolysins in LAB strains should be a consideration to ensure safety of these products.

Antibiotic disc diffusion results were most consistent with the E-test results when the concentration of the LAB strains and the medium used were the same, as noted by other researchers (Mayrhofer, et al., 2008). Based on our study, measurements should be taken at 24 hours for more consistent results. Measurements are more difficult and more prone to error when LAB strains are grown on sheep blood agar due to the opacity of the intact red blood cells at 24 hours of incubation (data not shown). Antibiotic resistance of these probiotic strains is consistent with the reports of other researchers. It has been reported that *Lactobacillus sp.* are intrinsically resistant to vancomycin and as such that trait is likely not transferable to other bacteria (Sharma et al., 2014). However, all of our isolates were also resistant to oxacillin and bacitracin, and a few strains were resistant to ciprofloxacin and cefoxitin. Further research is needed to determine if the genes for resistance to these antibiotics in our strains are transferable. Taxonomic analysis using 16S rRNA gene analysis of these isolates is underway to determine their genus and species. Further testing of media, timing of readings and concentration of the test organisms is needed to optimize conditions for antibiotic testing.

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Probiotic Source	Abbr.	Probiotic Strain(s) Listed on Label	Gram Stain of Isolate Tested
Culturelle lot#15260C4GB	C1	*Lactobacillus GG* 10 billion CFUs	Gram Positive Rod
Culturelle lot#16021C4GW	C2	*Lactobacillus GG* 10 billion CFUs	Gram Positive Rod
Nature's Bounty Probiotic GX lot#990124-01	GX	*Lactobacillus plantarum* Lp229v 20 billion CFUs	Gram Positive Rod
Culturelle KIDS lot#161334C3M11	KID	*Lactobacillus GG* 5 billion CFUs	Gram Positive Rod
Renew Life Women's Care Ultimate Flora Probiotic lot#09151023	RE	*Lactobacillus acidophilus* *Lactobacillus gasseri* *Lactobacillus brevis* *Lactobacillus casei* *Lactobacillus paracasei* *Lactobacillus plantarum* *Lactobacillus rhamnosus* *Lactobacillus salivarius* 15 billion CFUs	Gram Positive Rod
Trunature Digestive Probiotic lot#611815367	TRU	*Lactobacillus acidophilus* *Bifidobacterium lactis* *Lactobacillus rhamnosus GG* 10 bilion CFUs	Gram Positive Rod
VSL #3 The Living Shield Probiotic Medical Food lot#604094	VSL	*Streptococcus thermophilus* *Bifidobacterium breve* *Bifidobacterium longum* *Lactobacillus acidophilus* *Lactobacillus plantarum* *Lactobacillus paracasei* *Lactobacillus delbrueckii subsp. Bulgaricus* 450 billion CFUs	Gram Positive Rod
Walgreens Super Probiotic lot#50409937	WAL	*Lactobacillus acidophilus* *Bifidobacterium lactis* 20 billion CFUs	Gram Positive Rod

Table 1. Source of Lactic Acid Bacteria Characterized

LAB Isolates	Cefoxitin		Oxacillin		Tetracycline	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
C1	R	R	R	R	S	S
C2	R	R	R	R	S	S
GX	S	S	R	R	S	S
KID	R	R	R	R	S	S
RE	I	I	R	R	S	I* (4mm)
TRU	R	R	R	R	S	S
VSL	I	S* (1mm)	R	R	S	I* (3mm)
WAL	R	R	R	R	S	S
Total Resistant	5/8	5/8	8/8	8/8	0/8	0/8

Table 2. Disc diffusion assay results at both 24 hours and 48 hours. R=Resistant, I=Intermediate Resistance, S=Susceptible as determined by measuring the diameters of the zones of inhibition and as interpreted by CLSI standards (CLSI 2015a and CLSI 2015b). Changes in the measurements of the zone of inhibition that occurred over time are shown in parentheses.

LAB Isolates	Bacitracin	Ciprofloxacin	Erythromycin	Novobiocin	Vancomycin
C1	R	I	S	S	R
C2	R	I	S	S	R
GX	R	R	S	S	R
KID	R	I	S	S	R
RE	R	R	S	S	R
TRU	R	I	S	S	R
VSL	R	R	S	S	R
WAL	R	I	S	S	R
Total Resistant	8/8	3/8	0/8	0/8	8/8

Table 3. Disc diffusion assay results at 48 hours for 5 antibiotics (no changes in results between 24 and 48 hours). R=Resistant, I=Intermediate Resistance, S=Susceptible as determined by measuring the diameters of the zones of inhibition and as interpreted by CLSI standards (CLSI 2015a and CLSI 2015b).

Isolate	Cefoxitin		Oxacillin		Tetracycline	
	Disc Diffusion	E-Test	Disc Diffusion	E-Test	Disc Diffusion	E-Test
C1	R	R	R	R	S	S
C2	R	R	R	R	S	S
GX	S	I	R	R	S	S
KID	R	R	R	R	S	S
RE	I	I	R	R	S	S
TRU	R	R	R	R	S	S
VSL	I	I	R	R	S	S
WAL	R	R	R	R	S	S
Total Resistant	5/8	5/8	8/8	8/8	0/8	0/8

Table 4. Side-by-side results comparing Antibiotic Disc Assay and E-Test results at 24 hours. Bacteria were standardized at a 0.5 McFarland Standard when applied to the agar surface.

	24 hrs.	48 hrs.	72 hrs.
C1	Negative	Alpha	Beta
C2	Negative	Alpha	Beta
GX	Negative	Negative	Negative
KID	Negative	Alpha	Beta
RE	Negative	Negative	Negative
TRU	Negative	Alpha	Beta
VSL	Negative	Negative	Negative
WAL	Negative	Alpha	Beta

Table 5. Hemolysis Assay using Sheep Blood Agar incubated anaerobically at 37°C. Negative = no hemolysis, Alpha = partial hemolysis indicated by a greening of the blood agar, Beta = complete hemolysis of the red blood cells resulting in a clearing of the blood cells from the agar.
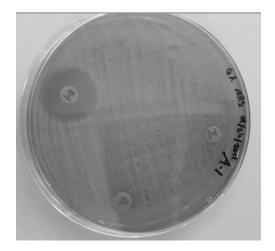
Figure 1. Antibiotic Disc Assay in which the diameter of the zone of inhibition is measured around the antibiotic containing disc.

Figure 2. E-test strips are placed on an agar plate that had been swabbed with a probiotic bacteria. After incubation, the lowest concentration of antibiotic that can inhibit bacteria is measured (Represented by an arrow in the figure on the left). The right agar plate indicates LAB resistance to the antibiotic cefoxitin.

Microbial Degradation of Art-Waste Solvents

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MICROBIOLOGY

ABSTRACT

Paint and solvents used in acrylic and oil painting generates wastes resistant to chemical breakdown, requiring expensive disposal fees, and causing health hazards during storage. Containers holding these painting byproducts were found to have bacteria growing in them that could be degrading the waste. Microbial degradation of three solvents, linseed oil, bestine, and turpenoid, by bacteria isolated from paint waste containers was investigated. In addition, bacterial strains isolated from jet fuel contaminated soil were tested for their ability to degrade paint waste. All bacterial isolates were propagated in M9 minimal media containing each solvent. Eight of the 16 isolates have been identified by 16S rRNA sequencing and taxonomic analysis of remaining individual isolates is still underway. Identified isolates that degrade paint wastes include *Pseudomonas zhaodongensis, Planococcus citreus,* and *Planococcus rifletoensis*. Gas chromatography mass spectrometry (GC/MS) was used to measure microbial degradation of the solvents. GC/MS results indicate six bacterial isolates degrade both bestine and oleic acid, a selected component of turpenoid, as a number of new peaks (breakdown products) were detected and the initial solvent peak areas decreased over time. Results show that bacterial strains isolated from the paint waste and from jet fuel contaminated soil have the ability to degrade paint waste solvents. Future work to optimize growth conditions (pH, oxygen, temperature) is currently underway to maximize solvent biodegradation. Once the most efficient bacterial strains and their optimum growth parameters are identified, they could be inoculated into waste containers to degrade paint waste, potentially reducing disposal fees and health risks.

INTRODUCTION

Art studios use paint and solvents requiring regulated disposal. Many paints contain metals like lead, arsenic, copper, zinc, along with other heavy metals. Solvents commonly used are often highly flammable materials, for example turpenoid, linseed oil, and bestine (Pratt Institute [n.d.]). These paints and solvents are toxic, dangerous to the environment, and a health hazard to those

who handle them. Art studios that use these paints and solvents are required by law to have toxic waste bins to hold these materials (Substances of the Toxic Substance Control Act, 2016). These bins are periodically emptied with their contents taken to hazardous waste disposal facilities. These chemicals are resistant to degradation, so they long term storage. This can, and has, led to waste leaks into the environment, which could result in health or related issues (EPA). The goal of this research project was to find microorganisms that could possibly speed up the process of biodegradation of these materials, which are often very resistant to breakdown. Organisms tested for biodegradative potential were isolated from paint waste containers and from jet fuel contaminated soil.

MATERIALS AND METHODS

Isolation of Microorganisms from the Solvent Wastes

Samples were taken from the paint and solvent waste bins at the Weber State University Art Department using sterile swabs and collection containers. Samples were inoculated into M9 minimal media supplemented with different individual solvents. Seven flasks of M9 broth were inoculated with various samples from the waste bins. One of three solvents, turpenoid, bestine, or linseed oil, used by the art department painters was added to each flask. A flask of M9 media not inoculated with a paint bin sample was used as the control. Each solvent was tested at either a 0.3% or 0.6% solvent concentration with each bin sample. All seven flasks were incubated at 24ºC with shaking for up to four weeks, until biofilms became visible on the sides of the flasks.
Biofilms from the enrichment flasks were sampled by transferring a small amount of the biofilm to a nutrient agar plate. Unique colonies were transferred to isolate individual organisms from the mixture of colonies using the quadrant streak plate method. Isolates were grown at 24ºC for 14 days. Isolates were examined for colony and cell morphology, and Gram reaction. Individual isolates were transferred to nutrient broth for DNA analysis.

16S rRNA Gene Sequence Analysis

DNA was extracted from the isolates using the MoBio Ultra Clean Microbial DNA Extraction Kit (MoBio, Carlsbad, CA). The 16S rRNA gene was amplified using bacteria specific primers (27F 5' AGA GTT TGA TCM TGG CTC AG 3' / 1492R 5' ACG GYT ACC TTG TTA CGA CTT 3'). The reaction mixture contained 200 nM of each primer, 200 µm of the dNTPs, 1U Taq DNA Polymerase and the diluted reaction buffer (Promega Corp., Madison, WI). Amplification parameters were 94˚C for 3 min., followed by 25 cycles of 94˚C for 45 sec., 50˚C for 1 min., 72˚C for 2 min., and a final extension step at 72˚C for 7 min. Sequencing was done by the Idaho State University Molecular Research Core Facility (Pocatello, ID). Sequences were compared to the GenBank database using the BLAST search tool.

Solvent Degradation by Isolates

Isolates were inoculated into M9 minimal media broth with either 0.2%, 0.6%, or 1.3% of heptane or oleic acid, which are specific components of the paint solvents. Gas chromatography mass spectrometry (GC/MS) was used to determine which solvents were being broken down by different bacterial isolates. Heptane samples were run through the GC/MS with peak areas compared to the control standard for heptane, searching for new peaks and peak area changes over time. Oleic acid samples were derivatized using a standard method and analyzed by GC/MS for degradation and metabolite generation.

RESULTS

All of the samples from the art department waste bins enriched in solvent-containing medium produced thick, colorful biofilms at the interface of the solvent and medium (Figure 1 and Table 2). A collection of individual isolates were cultured from the biofilms, which had diverse colony and cell morphologies (Figure 2). An interesting observation of the isolates was that as they were transferred to new plates, from time to time the pigmentation would disappear and, with further transferring, the pigment would return. This suggests an interesting characteristic of gene activation and trait selection for these organisms. Characteristics of the isolates selected for further analysis are summarized in Table 1. After further analysis was conducted, isolates sequenced for identification using 16S rRNA analysis (Table 3).

Six isolates tested were found to break down oleic acid and heptane. As determined by changes in the peak area of GC/MS essays. Biofilms were also observed growing in the tubes during the change in composition of the solvents (Figure 5). Figure 6 shows the differences observed between the control standards compared to one of the isolates tested, with the other isolates exhibiting similar results. Peak area changes were observed as well as new compounds showing up as new peaks. For example, in Figure 6, isolate TL8 for oleic acid showed a retention time difference in the main peaks of 6.676, 7.506, and 8.352 comapred to 6.737, 7.543, and 8.375. Although small, changes in these times show different compounds coming off at those times. Isolate TL8, when incubated with heptane, showed a difference between the uninoculated standard with only three main peaks, and heptane inoculated with TL8 of eight additional peaks.

DISCUSSION AND CONCLUSION

Isolates 2, 3, and 6, were strongly related to *Pseudomonas zhaodongensis*, which has been found in soil and saline environments in Zhaodong City, Heilongjiang Province, China (Zhang, 2015). Isolates 7 and 9 were strongly related to *Planococcus citreus* and *Planococcus rifietoensis*, which have been found to be

proline producers, moderate halophiles, and similar to some bacilli (Galinski, 1993). Some other closely related strains appear to be moderately halophilic, and halo-alkaline tolerant (Meng, 2014). This information provides a clearer understanding of the type of bacteria found in art waste bins that may be able to degrade art waste.

Data gathered from the GC/MS shows that selected isolates degrade solvents found in the art waste bins. Knowing what compounds are being formed by the isolates helps to understand the metabolic abilities of each isolate. From the data gathered, a lot has been determined about how each of the organisms grow, with further tests required to determine if these organisms could be used to inoculate waste bins to degrade the solvents inside.

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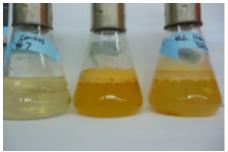
Figure 1. Enrichment of microorganisms from art waste containers. Panel 1: Far left flask: control, no solvent added and not inoculated. Middle flask: 0.3% Linseed Oil, inoculated with biofilm from sample from art department. Far right flask: 0.6% Linseed Oil, inoculated with biofilm from sample from art department. Panel 2: Far left tube: 1-5 Control, not inoculated. Middle left tube: 1-6, 2.5 mL Linseed Oil, inoculated with biofilm from enrichment flask 6. Middle right tube: 1-3, 2.0 mL Linseed Oil, inoculated with biofilm from enrichment flask 3. Far right tube: 3A1, 2.0 mL linseed Oil, inoculated from enrichment flask 3 quadrant streak plate 1.
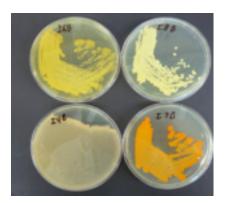

Figure 2. Examples of quadrant streak plates of isolates from the enrichment culture biofilms. Left Panel 1: Top left: Isolate 6B. Top right: Isolate 8B, Bottom left: Isolate 4B, Bottom right: Isolate 7B. Right Panel 2: Top left: Isolate 1B, Top right: Isolate 2B, Bottom left: Isolate 3B, Bottom right: Isolate 5B.

Table 1. Colony Morphology and Gram Reaction of Isolates

Isolate	Colony Morphology	Gram Reaction and Cell Morphology
1A1	Irregular, umbonate, curled, Pigment-Orange, rough	Negative, rod
1A2	Irregular, raised, erose, Pigment-tan, rough	Positive, Rod
1A3	Irregular, umbonate, curled, Pigment-tan, smooth	Positive,Rod
3A1	Irregular, umbonate, curled, Pigment-white, rough	Negative, Rod
4A1	Irregular, umbonate, curled, Pigment-light pink, rough	Negative, Rod
5A1	Circular, convex, entire, Pigment-Yellow, smooth	Positive, Rod
5A2	Irregular, Pulvinate, entire, Pigment-Orange, smooth	Positive, Cocci
6A1	circular, raised, entire, Pigment-Yellow, smooth	Positive, Cocci
6A2	circular, convex, entire, Pigment-Orange	Positive, Cocci

Table 2. Growth of Isolates on Turpenoid, Bestine, and Linseed oil

Isolate		1A1	1A2	1A3	4A1	1-1	1-4
Solvent	Concentration	Growth					
Turpenoid	0.8%	++ (B)	+++ (B)	++ (B)		++ (B)	
	1.0%				++ (B)		++ (B)

Isolate		2A1	5A1	5A2	1-2	1-5	
Solvent	Concentration	Growth					
Bestine	0.8%	+ (B)			+ (B)		
	1.0%		++ (B)	+ (-)		+ (B)	

Isolate		3A1	6A1	6A2	1-3	1-6	
Solvent	Concentration	Growth					
Linseed Oil	0.8%	++++ (T)*			++++ (T)*		
	1%		++++ (T)*	++++ (T)*		++++ (T)*	

Control		7A1	7A2				
		No Growth					

* (T) growth on media solvent interface

Table 3. Identification of Isolates based on 16s rRNA sequencing.

Isolate	BLAST MATCH	Percent Similarity	Accession Number
1A1	poor sequence		
1A2	*Pseudomonas zhaodongensis* NEAU-ST5021	100%	NR_134795
1A3	*Pseudomonas zhaodongensis* NEAU-ST5021	98%	NR_134795
5A1	*Pseudomonas zhaodongensis* NEAU-ST5021	100%	NR_134795
5A2	*Planococcus citreus strain* NBRC 15849	98%	
6A2	*Planococcus citreus* strain NBRC 15849	98%	

Figure 5. Biofilm growth on Heptane ane Oleic Acid.
Left picture: Isolate (6A2) inoculated into three different concentrations
of heptane. Right picture: Isolate (6A2) inoculated into three different
concentrations of Oleic Acid. Each of these samples were run through GC/MS.

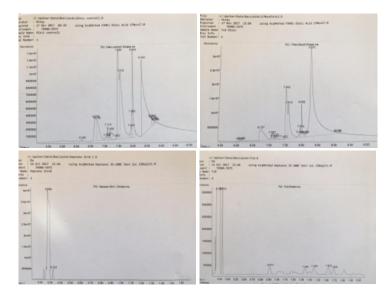|	Notes about Match (Environment)	Second BLAST MATCH	Percent Similarity
	saline and alkaline soils		
	saline and alkaline soils		
	saline and alkaline soils		
	firmicutes	*Planococcus rifietoensis* strain M8 - algal mat collected from a sulfurous spring	98%
	firmicutes	*Planococcus rifietoensis* strain M8 - algal mat collected from a sulfurous spring	98%

Figure 6. GC/MS Peaks for Oleic Acid and Heptane inoculated with isolate TL8.
Top Left: Oleic acid control from GC/MS. Top Right: TL8 GC/MS results
Bottom Left: Heptane control from GC/MS. Bottom Right: TL8 GC/MS results.

Bacteriocin Production by Bio-protective LAB Cultures that Inhibit Lactobacillus wasatchensis

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MICROBIOLOGY

ABSTRACT

Late gas defects in aging cheese result in significant losses to the manufacturer. *Lactobacillus wasatchensis*, a novel non-starter lactic acid bacteria (NSLAB), was recently identified as an important cause of late gas defect. Controlling growth of this unwanted NSLAB may be possible by incorporation of bio-protective LAB cultures (BP-LAB) into the cheese during manufacture, which could inhibit its growth during cheese aging. Previous research has shown several BP-LAB cultures inhibit *Lb. wasatchensis* to varying degrees but the extent and exact mode of inhibition was not determined. Quantification of inhibition between BP-LAB cultures and *Lb. wasatchensis* was done using the spot test with the agar-flip method then measuring inhibition zones over time. MRS agar with 1% ribose (MRS-R) was inoculated with each BP-LAB and incubated anaerobically at 35°C for 48 h to form a spot colony. Inoculated agar was flipped over and a Lb. wasatchensis strain swabbed on the exposed surface then incubated anaerobically at 25°C for up to 72 h. The five most inhibitory BP-LAB cultures were *Lactobacillus rhamnosus* LB3, *Lactobacillus paracasei* P-210, *Lactobacillus brevis* ATCC 13648, *Lactobacillus casei* F19, and *Lactobacillus paracasei Lila*. In addition, potential synergistic quantification of inhibition by co-BP-LAB strains was tested by taking 1 mL each of two different BP-LAB strains mixing them together, and then following the previous described protocol. Four different co-cultures were tested LB3/ P-210, LB3/P-220, P-200/P-210, and P- 200/P-220. No significant increases in inhibition zones were observed when BP-LAB cultures were paired versus individual strains. Results confirm selected BP-LAB strains can inhibit growth of *Lb. wasatchensis*. Initial results also suggest BP-LAB cultures may be producing bacteriocins that inhibit *Lb. wasatchensis*, so we are currently isolating potential bacteriocins produced by these BP-LAB.

INTRODUCTION

During cheesemaking there are two groups of lactic acid bacteria that drive both flavor development and maturation of the cheese. Starter lactic acid bacteria (SLAB), added directly to the cheese during manufacture and non-starter lactic acid bacteria (NSLAB), which are notadded to the cheese but dominate

during cheese ripening (Settanni and Moschetti, 2010). NSLAB are part of the milk flora and also enter the cheese from processing equipment and the plant environment. The obligatory heterofermentative (OHF) group of NSLAB are of interest during cheese ripening because they can cause gas formation resulting in body defects such as slits and cracks (Banks and Williams, 2004). A novel OHF NSLAB, *Lactobacillus wasatchensis*, has recently been identified as an important cause of late gas defect in aging cheddar (Ortakci etal., 2015). Gas formation in Cheddar cheese has recently become a recurrent and wide spread problem in the dairy industry. Gas defects in aging cheese can cause cheese to be downgraded and crumble when being sliced, which results in economic losses to cheese manufacturers. Previous research has shown that bio-protective lactic acid bacteria (BP-LAB) may have potential to inhibit the growth of unwanted NSLAB (Leroy and Vuyst, 2010).

The purpose of this study was to determine what BP-LAB cultures could best inhibit the growth of *Lb. wasatchensis* and to quantify their level of inhibition.

MATERIALS & METHODS

Media & Bacterial Propagation

Lactobacillus wasatchensis WDC04 was grown in de Man, Rogosa, and Sharpe (MRS) broth supplemented with 1% ribose (MRS-R) at 25º C. Bio-protective non-starter lactic acid bacteria (BP-NSLAB) were grown in MRS broth at 30º C. Agar flip test plates and agar protein spot test plates were made using MRS-R broth with the addition of 1.5% agar. Agar plates used for the flip test method contained 20 mL of media.

Spot Tests of Bio-protective Cultures

The 10 bio-protective cultures (BP-LAB) used for spot testing were *Lactobacillus rhamnosus* LB3, *Lb. rhamnosus* P-200, *Lb. paracasei* P-210, *Lb. paracasei* P-220, *Lb. fermentum* 23271, *Lb. brevis* 13648 ATCC, *Lb. casei* F19, *Lb. paracasei* Lila, *Lb. brevis* ATCC 367, and *Lb. helveticus* 32. The BP-LAB cultures were grown in MRS-R broth for 48 hours at 35 o C. After 48 hours 20 µL of each culture were inoculated onto the center of a MRS-R plate and grown anaerobically in a GasPak EZ. After 48 hours, plates were flipped into the lid of the petri dish using a sterile spatula and the newly exposed surface swabbed with *Lb. wasatchensis* WDC04. After swabbing, plates were incubated at 25 o C for 48 hours anaerobically. Any clearing zones were photographed and recorded. All tests were done in triplicate.

Spot Tests of Bioprotective Co-Cultures

The four BP-LAB cultures tested for cooperative bio-protection were *Lb. rhamnosus* LB3, *Lb. rhamnosus* P-200, *Lb. paracasei* P-210, and *Lb. paracasei*

P-220. The BP-LAB cultures were grown in MRS broth for 24 hours at 35 o C. After 24 hours, 1 mL of a BP-LAB culture was combined with 1 mL of another BP-LAB culture and vortexed, then 20 µL of BP-LAB co-culture were plated onto the center of MRS-R plates and incubated at 35 o C for 48 hours anaerobically. After 48 hours, the plates were flipped into the petri dish using a sterile spatula and then were swabbed with *Lb. wasatchensis* WDC04. After swabbing, the plates were incubated at 25 o C for 48 hours anaerobically and results photographed and recorded. All tests were done in triplicate.

Bacteriocin Protein Spot Test

The four different cultures being used for protein spot testing were *Lb. paracasei* P-210, *Lb. brevis* ATCC 13648, *Lb. casei* F19, and *Lb. paracasei* Lila. MRS broth was inoculated and protein extraction was done on days 3, 4, and 5. On the given day of incubation, each sample was placed into a 15 mL centrifuge tube avoiding the cell material at the bottom of the broth tube. Samples were centrifuged for 15 minutes at 4500 rpm, then filtered through 0.2 µm micronfilters. Two tests were done using the cell free extract (CFE) protein filtrate. The first test was done by spotting protein filtrate onto MRS-R plates that were swabbed with *Lb. wasatchensis* WDC04 diluted to 10^{-1} , 10^{-2} , and 10^{-3} . Each plate was inoculated with four separate volumes of filtrate (20 µL, 10 µL, 5 µL, and 2 µL). The second test used 13 mm diameter Taxo TM blank paper disks containing 50µL of filtrate placed onto MRS-R plates swabbed with *Lb. wasatchensis* WDC04.

RESULTS

Spot Tests for Bioprotective Cultures

Initially all ten BP-LAB cultures were tested on their level of inhibitory effects on *Lb. wasatchensis* WDC04 and *Lb. wasatchensis* CGL04. The six BP-LAB cultures with strong inhibitory effects were *Lb. rhamnosus* LB3, *Lb. paracasei* P-200, *Lb. paracasei* P-210, *Lb. brevis* ATCC 13648, *Lb. casei* F19, and *Lb. paracasei* Lila (Table 1). Following the initial screening all flip test was conducted with the 6 BP-LAB so the zones of inhibition against *Lb. wasatchensis* could be quantified and standard deviations calculated (Figure 1). The zones of inhibition for BP-LAB cultures were all similar. *Lactobacillus brevis* ATCC 13648 produced a zone with the mean of 3.4, which was the largest of all cultures tested.

Spot Test of Bioprotective Co-cultures

Co-cultures of BP-LAB were tested by spot test comparing the zones of inhibition for two cultures grown together to the zones of inhibition for each culture grown by itself. *Lactobacillus rhamnosus* P-200, *Lb. paracasei* P-210, and *Lb. paracasei* P-220 grown individually produced similar zones of inhibition when compared to *Lb. rhamnosus* P-200 grown with Lb. P-210 and *Lb. rhamnosus* P-200 grown

with *Lb. paracasei* P-220 (Figure 2). *Lactobacillus rhamnosus* LB3, *Lb. paracasei* P-210, *Lb. paracasei* P-220 produced slightly smaller zones of inhibition when compared to *Lb. rhamnosus* LB3 grown with *Lb. paracasei* P-210 and *Lb. rhamnosus* LB3 grown with Lb. P-220 (Figure 3). However, the slight increase of inhibition produced by the *Lb. rhamnosus* LB3 was not significant.

Bacteriocin Protein Spot Test

The protein spot tests done with the BP-LAB CFE showed minimal zones of inhibition for day 3, 4, or 5 for any of the four cultures tested. The protein spot tests using blank paper disks showed no zones of inhibition for any of the four BP-LAB cultures tested.

DISCUSSION & CONCLUSION

The growth of *Lb. wasatchensis*, a recently isolated heterofermentative nonstarter lactic acid bacterium that causes late gas defect in aging Cheddar cheese can be inhibited through the utilization of BP-LAB. While a number of BP-LAB showed some inhibition, the four BP-LAB cultures with the greatest inhibitory effects were *Lb. paracasei* P-210, *Lb. brevis* ATCC 13648, *Lb. casei* F19, and *Lb. paracasei* Lila. When BP-LAB cultures were grown together, *Lb. wasatchensis* was inhibited but the level of inhibition did not increase significantly when compared to using a single culture. Results suggest that the BP-LAB cultures may be producing bacteriocins that inhibit the growth of *Lb. wasatchensis*. Creating a cell free extract to isolate potential bacteriocins from the four best BP-LAB cultures has yet to show any significant inhibition against *Lb. wasatchensis*. This may be occurring because the bacteriocins are too dilute in the CFE or the bacteriocin proteins might be binding to other targets deactivating their inhibitory effects. Currently, we are trying to isolate potential bacteriocins produced by the BP- LAB cultures.

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Bioprotective Culture	*Lactobacillus wasatchensis* WDC04	*Lactobacillus wasatchensis* WDC04
Lactobacillus rhamnosus LB3	+++	++
Lactobacillus rhamnosus P-200	+++	++
Lactobacillus paracasei P-210	+++	++
Lactobacillus paracasei P-220	++	++
Lactobacillus fermentum 23271	+	+
Lactobacillus brevis 13648 ATCC	++	+++
Lactobacillus casei F19	+++	+++
Lactobacillus paracasei Lila	+++	+++
Lactobacillus brevis ATCC 367	X	X
Lactobacillus helveticus 32	+	+

Table 1. Inhibition by potential bio-protective LAB cultures against Lactobacillus wasatchensis. The +++ denotes significant amount of clearing, ++ denotes moderate clearing, + denotes minimal clearing, and X denotes no zone of clearing.
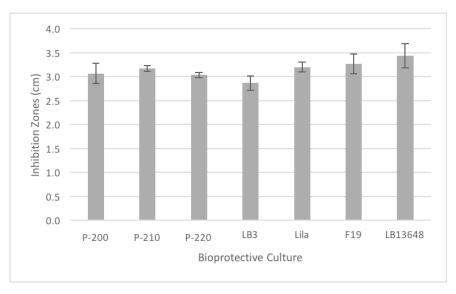
Figure 1. Inhibition by bio-protective LAB cultures against *Lactobacillus wasatchensis*. Measurements were taken in centimeters. The error bars show the standard deviation of the plate measurements taken in triplicate.
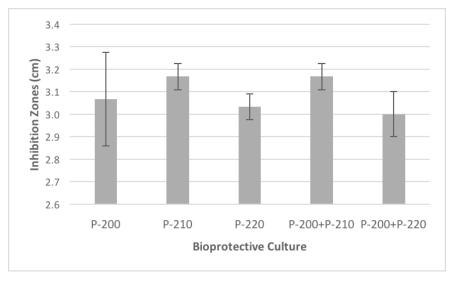

Figure 2. Inhibition by bio-protective co-cultures using *Lactobacillus rhamnosus* P-200. Measurements were taken in centimeters. The error bars show the standard deviation of the plate measurements taken in triplicate.
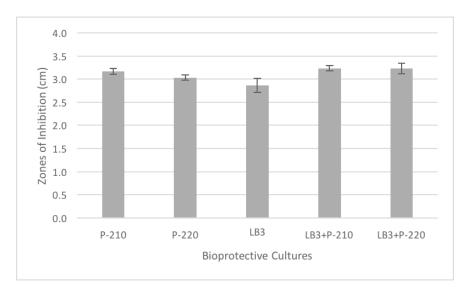
Figure 3. Inhibition by bio-protective co-cultures using *Lactobacillus rhamnosus* LB3. Measurements were taken in centimeters. The error bars show the standard deviation of the plate measurements taken in triplicate.

College
of Social &
Behavioral
Sciences

Embodied Cognition: Effects of Social Exclusion and Consuming Warm and Cold Beverages

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PSYCHOLOGY

ABSTRACT

Embodied cognition researchers have found physical warmth can help alleviate feelings of social coldness. The current study investigated whether physical warmth was effective in reducing feelings of ostracism. The researchers hypothesized consuming a warm beverage would lessen effects of social exclusion. The virtual ball-tossing game Cyberball was used to produce feelings of social ostracism. Study 1 results showed excluded participants had non-significantly lower loneliness scores than included participants. However, contrary to hypothesis, participants who consumed a warm beverage had higher loneliness scores than those who consumed a cold beverage. In study two, with a lowered beverage temperature, results showed consuming a warm beverage was associated with non-significantly lower loneliness scores, but was not related to participation in the exclusion group or inclusion group.

INTRODUCTION

Embodied cognition is the concept of a bilateral interaction between mind and body. Within this area, several researchers have documented the parallels between social warmth and physical warmth (Bruno, Melnyk, & Volckner, 2016; Murphy & Standing, 2014) finding the same brain regions, the anterior and middle insula, appear to process both physical warmth and social warmth (Inagaki & Eisenberger, 2013). When people are emotionally cold, they perceive physically cold stimuli less favorably (Bruno et al., 2016).

Studies have shown that physical warmth moderates the effects of social exclusion (Murphy & Standing, 2014), however, there has been a lack of studies investigating whether consuming warm food or beverage can moderate these effects. Choi (2013) found physical warmth conveyed through spice level of food could reduce social exclusion effects. Consumption of spicy soup led to increased feelings of belongingness, but only after several minutes had passed. Immediately after consumption, participants' mood had deteriorated. Because spice level is confounded with physical temperature in this study, the question of whether consuming food or beverage at different temperatures can alleviate the

effects of social exclusion remains unanswered.

The current study attempts to answer this question by manipulating both beverage temperature and social exclusion.

METHODS & MATERIALS

Social warmth, defined as social inclusion or social exclusion, was manipulated through the virtual ball-tossing game Cyberball (Williams, Yeager, Cheung, & Choi, 2012). Participants believed they were interacting with other people in the game. However, they were only interacting with the computer program. Participants in the inclusion condition received the ball 6 times out of 30 throws, compared to the exclusion condition where participants received the ball 1 time out of 30 throws.

A Keurig single-serve beverage maker was used to make beverages for the warm condition (brew temperature of 187° Fahrenheit Study 1, 125º F Study 2) and canned beverages are used for the cold condition (stored at 38° F). Beverage choices consisted of three options for warm beverages (coffee, green tea, and hot chocolate) and three options for cold beverages (lemonade, water, and Sprite). Loneliness was assessed with the UCLA loneliness scale (Russell, Peplau, & Ferguson, 1978). This survey consisted of 20 questions about mood, companionship, social relationships, and isolation. Five additional questions about exercise and sugar intake were added to mask the true nature of the study. For each survey item, participants indicated how often they engaged in a particular thought or behavior (Often, Sometimes, Rarely, Never). Answers were assigned numeric values and summed to get a final score (Often = 3, Sometimes = 2, Rarely = 1, Never = 0). A lower score indicated lower levels of loneliness. Answers to the five filler questions created by the researchers are not included in this calculation.

PROCEDURE

The study utilized a 2 (warm beverage vs. cold beverage) x 2 (exclusion vs. inclusion) between-subjects design. Participants were randomly assigned to one of the four groups. Upon arrival, participants chose a beverage to consume based on their randomly assigned condition (i.e., warm, cold). If participants declined a beverage, they were still allowed to participate, although their data was discarded. Participants consumed their drink while playing Cyberball, and were specifically instructed to complete the majority of their beverage during the game (approximately 5 minutes).

After completing the game and finishing their drink, participants completed the UCLA loneliness scale. After completion of the survey, participants were

debriefed and thanked for their participation. In Study 2, room temperature was recorded immediately after each participant completed the study.

Participants

Undergraduate students enrolled in Introductory Psychology volunteered to participate in exchange for partial course credit (Study 1 n = 43, Study 2 n = 62).

Study 1 Results

Loneliness scores from 35 participants were subjected to a factorial ANOVA. Results of analyses revealed no statistical significance. Contrary to hypothesis, there was no interaction between beverage temperature and social warmth, F(1, 31) = 0.01, p = .93, η2 = .001. Participants in the inclusion/warm condition (M = 22.8, SE = 3.08) and inclusion/cold condition (M = 18.17, SE = 3.98) reported loneliness scores similar to participants in the exclusion/warm condition (M = 28.43, SE = 3.68) and exclusion/cold condition (M = 24.42, SE = 2.81).

Additionally, there was no main effect of beverage temperature; participants consuming a warm beverage (M = 25.61, SE = 2.43) reported non-significantly higher loneliness levels than those consuming a cold beverage (M = 21.29, SE = 2.4), F(1,31) = 1.60, p = .22, 2 = .05. These data can be seen in Figure 1. There was, however, a marginal main effect of social warmth showing that participants who were excluded (M = 26.42, SE = 2.32) had higher loneliness scores than participants who were included (M = 20.48, SE = 2.5), F(1,31) = 3.02, p = .09, η2 = .09. Although this effect was not statistically significant, there was an observed difference between the group means (see Figure 2). The difference between these groups was present, and there was a moderate effect size, so the lack of significance may have been due to a power issue (1 − β = .34).

Study 2 Results

Loneliness scores from 59 students were subjected to a factorial ANOVA and correlation. Results of the analyses revealed no statistical significance. A correlation was conducted to see if room temperature was related to loneliness score. The results showed no correlation coefficient, so room temperature was excluded from all subsequent analyses, r(57) = .01, p = .94.

Results of the ANOVA suggested no interaction between social warmth condition and beverage condition, F(1, 55) = 0.15, p = .71, η2 = .003. Participants in the inclusion/warm condition (M = 21.83, SE = 2.97) and exclusion/warm condition (M = 23.06, SE = 2.50) reported loneliness levels similar to participants in the inclusion/cold condition (M = 25.71, SE = 3.89) and exclusion/cold condition (M = 24.70, SE = 2.15).

As in Study 1, there was no significant main effect of beverage temperature. However, the pattern of results was opposite; participants consuming a warm beverage (M = 22.45, SE = 1.94) reported non-significantly lower loneliness levels than those who consumed a cold beverage (M = 25.21, SE = 2.22), $F(1,55) = .88$, $p = .35$, $\eta2 = .02$.

The lack of main effect of social warmth showed that participants who were excluded (M = 23.77, SE = 2.45) reported loneliness scores virtually identical to participants who were included (M = 23.88, SE = 1.65), $F(1,55) = .001$, $p = .97$ $\eta2 = .001$.

DISCUSSION

The results of Study 1 showed that participants in the warm beverage condition reported slightly higher loneliness scores. Cyberball program appeared to be effective in creating feelings of social ostracism, but a warm beverage was not effective in relieving those feelings. This finding was not statistically significant having only a small effect size, but it is of interest because this was opposite of what the researchers hypothesized.

A possible explanation for this finding comes from the temperature of the room used for data collection, which was unusually warm for an indoor space. It is also possible that the warm beverages were too hot and caused pain. Physical pain can cause a person to feel more socially isolated (Chen, Poon, & Dewall, 2015; Choi, 2013). Participants were not able to consume the majority of their beverage because of the excessively hot temperature and drinking the beverage likely caused pain, which may have accounted for higher loneliness scores.

A second study addressed the confounds of room temperature and beverage temperature with results showing room temperature was not correlated with loneliness score. The results of Study 2 showed that participants who consumed a warm beverage reported loneliness levels slightly lower than participants who consumed a warm beverage, but not significantly. Lowering the temperature of the warm beverages appeared to be successful in reversing the unexpected effect seen in Study 1. This strengthens the researchers' claim that pain caused increased loneliness scores in the warm beverage condition of Study 1 from excessively hot beverage temperatures.

An interesting difference between the two studies is the lack of effect that Cyberball condition had in Study 2. Study 1 illustrated that social exclusion in Cyberball is associated with higher levels of reported loneliness. This same pattern was not found in Study 2. A trivial difference of .11 points separated mean loneliness scores of the inclusion and exclusion group.

A possible explanation comes from the programming of throws in the inclusion condition. Throws to the participant were clustered toward the beginning of the game, rather than evenly spread throughout the 30 throws. During the debriefing, several participants in the inclusion condition reported feeling excluded. The programming of throws in the inclusion condition may not have fully conveyed social inclusion. However, this does not explain why an effect was observed in Study 1.

It is also possible this difference may be attributed to unintentional research bias. In Study 1, researchers may have inadvertently emphasized Cyberball more than the beverage, causing participants to focus on how the game made them feel rather than the effect of the drink. In the second study, the researchers had altered the temperature of the warm beverage so that more emphasis may have been placed on the beverage instead of the game. This could help explain why Cyberball condition affected loneliness scores in Study 1, but not Study 2. The results of this study should not be taken to mean physical warmth does not influence feelings of social warmth; many studies have documented this finding. Instead, this study should be taken as a reminder of the importance of replication in psychology and the need for more replication-based studies in the future.

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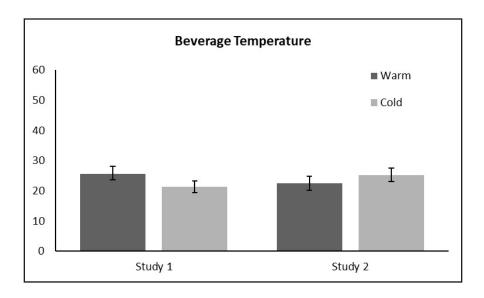
Figure 1. Mean loneliness score based on beverage temperature. Error bars represent standard errors.
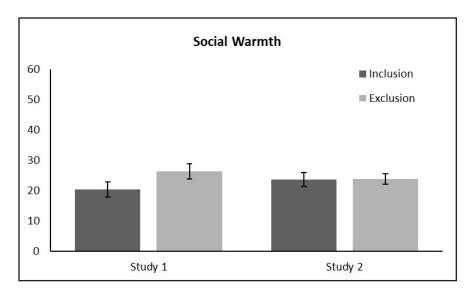

Figure 2. Mean loneliness score based on social warmth condition in Cyberball. Error bars represent standard errors.

A New Black Image: A Journey through Migration, Rebirth, and Civil Rights, 1916-1968

Author: SARA KAMPI
Mentor: BRANDEN LITTLE

HISTORY

After the American Civil War, newly emancipated blacks were frustrated because pervasive racial discrimination and violence undercut their new- found freedoms. The sharp rise of Jim Crow segregation and racial violence after Reconstruction suggested conditions for blacks were deteriorating. The *Houston Informer* summed conditions up for African Americans: "It appears that quite the number of Southern communities not only do not know that slavery has been abolished in this country but on the contrary, they are maintaining a species of peonage far worse than anything conceived or practiced during the period of human bondage." African Americans wanted genuine equality and sought to abolish racial stereotypes that oppressed them by perpetuating ideas that blacks were mere laborers. Many former slaves remained trapped by sharecropping, a form of agrarian bondage that resembled slavery. Working in the fields owned by their former owners did not promote independence or equal treatment. Frustrated by endemic racism, 1.2 million African Americans migrated north after WWI. They hoped for a better life and better opportunities. This "great migration" was called an "exodus" by many black observers who linked the imagery of a Biblical exodus of an enslaved people into freedom to their own difficult experience.

The migration led, moreover, to a cultural explosion known as the Harlem Renaissance that triggered an explosion and expansion of black intellectual strengths and creativity. The philosopher Alain Locke ignited this rebirth of blackness and cultural consciousness through a celebration of the "New Negro." The "New Negro" awakened a black separatist movement in the 1910s and 1920s led by Marcus Garvey. And it inspired the civil rights activists of Stokely Carmichael in the 1960s. Though Garvey and Locke, wanted different outcomes, Locke, Garvey, and Carmichael strived for the greater good for blacks in America. Their advocacy created a new black image free of the stigma of slavery and fully endowed with the power of freedom. From Harlem, Alain Locke inspired the "New Negro" movement. Locke was born nearly twenty years after the Civil War had ended, in 1886, in Philadelphia. A talented scholar, Locke graduated from the Harvard University in 1918. He was one of the first African Americans to be a recipient of the Rhodes scholarship, and he continued his studies in England and Germany. Alain Locke taught English at Howard University before returning

to Harvard to earn his Ph.D. in Philosophy. Locke was a brilliant journalist, a philosopher, and a teacher, but still suffered from racial discrimination. Locke challenged African Americans to redefine their place in a predominantly white America, and in 1925, Locke published a series of essays titled *Enter the New Negro*, which soon became the manifesto of the Harlem Renaissance. It awakened a new "consciousness" among African Americans. The Harlem Renaissance ignited a cultural explosion that lasted until the 1930s. Artists, poets, musicians, photographers, dancers, and writers expressed their creative energies in Harlem. This cultural movement began to separate the American Negro from derogatory white stereotypes. Blacks, moreover, wanted to break away from the paternalistic and condescending ideas upon which whites would base their racist beliefs.

In the midst of this transformative era, Locke became the father of this identity revolution by articulating his vision of the "new Negro." The reincarnated Negro, Locke insisted, would independently forge their own identity," and be "liberated from racist limitations and boundaries." "The Negro has been more of a formula than a human being," Locke wrote, "a something to be argued about, condemned, or defended, to be kept down or in his place, or helped up, to be worried with or worried over, harassed or patronized, a social bogey or a social burden." The old Negro was a slave. Slaves were tools. They were property. Their every move was watched, they were not allowed to think, read, or write, and they were intended to always be controlled or kept in a submissive state. The Old Negro was surely but slowly becoming a myth in the eyes of Locke. The New Negro, he envisioned, would overthrow those shackles and pursue his endeavors and aspirations. The key to the New Negroes' future would be their assertiveness. Locke hoped that a new American attitude toward blacks would result in which racism would disappear.

 He hoped *Enter the New Negro* would awaken the black community. Slavery had done a significant amount of damage to African Americans. He felt the echoes of slavery trapped many blacks with the mentality of a slave. Locke wanted African Americans to become politically conscious, to embrace the beauty of their rich culture, and to fight inequality in America. Locke believed Negro spirituals could inspire black pride and alert Americans to their vibrant heritage. He wanted Americans to know that blacks could be so much more than just a sharecropper or a house cleaner. "The day of aunties and uncles and mammies is equally gone. Uncle Tom and Sambo have passed on, and even The Colonel and George play barnstorm roles from which they escape with relief when the public spotlight is off." Despite the pervasive legacy of slavery, Locke nevertheless, believed in the American dream and did not want conflict between White and Blacks. He wanted blacks to be an essential part of the fabric of American life. However, his was not the only voice in the Harlem Renaissance.

A contemporary of Locke's, Marcus Garvey was a Jamaican African who

organized one of the first and most important Black Nationalist Movements. After living in Central America and London for a brief period, Garvey returned home to Jamaica where in 1914, he created the Universal Negro Improvement and Conservation Association and African Communities League. Unlike Locke's advocacy for integration, Garvey's stance was separatism. Garvey, however, did not have much of a following in Jamaica, so he moved his organization to Harlem in 1916, where his ideas met an enthusiastic reception. Garvey gained as many as 2,000,000 followers. He, too, spoke of a new Negro and the pride of being black. Garvey's newspaper *Negro World* emphasized the richness of African culture. Before Garveyism, as the movement came to be called, no movements existed in black America that encouraged African Americans to think internationally. Garvey, however, promoted the idea of creating a separate black nation in Africa for former slaves and their descendants in the Americas. He also insisted that the only way blacks would be respected in the white man's world was if they were economically powerful. "Until you produce what the white man has produced," he claimed, "you will not be his equal."

Garvey practiced what he preached. He founded the Negro Factories Corporation and gave many blacks the opportunity to purchase stocks. He also opened a chain of restaurants and grocery stores, hotels, laundries, printing presses, and The Black Star Line, a steamship company. He firmly believed in black economic independence.

Garvey was well known for stating that "black was beautiful" before it became popular in the 1960s. He wanted African Americans to know that they, too, were members of a special race. "We must canonize our own saints, create our own martyrs, and elevate to positions of fame and honor black men and women who have made their distinct contributions to our racial history." He encouraged black parents to let their children play with dolls that looked like them and to believe that they were equal to any white man. He believed that African Americans were arbitrators of their own destiny. White oppression could no longer stand in their way. Garvey believed that God made blacks who they were for a reason. He encouraged teaching black youths to embrace their cultural power and to reject oppression "Let the sky and God be our limit, and Eternity our measurement. There is no height to which we cannot climb by using the active intelligence of our own minds. Mind creates, and as much as we desire in Nature we can have through the creation of our own minds." Black power as Garvey's notion of black power inspired the subsequent civil rights activism in Stokely Carmichael.

Stokely Carmichael emerged as a civil rights activist who popularized the Black Nationalist slogan, "Black Power." He was born in Trinidad, but he and his family immigrated to America in 1952. Carmichael was raised in an ethnically and racially diverse Bronx community but was still aware of racial hierarchies. He did not become involved with rights for African Americans until he became

a student at Howard University, where he joined the Student Nonviolent Coordinating Committee and other organizations dedicated to social justice. At nineteen years old, Carmichael was jailed for participating in the freedom rides of 1961. He was also the youngest person that received jail time for attempting to integrate an all-white cafeteria in Jackson, Mississippi. Although Carmichael was a strong believer in the nonviolence movement that was fashioned by Martin Luther King Jr., he departed from its precepts. No matter how peaceful and respectful protestors were, they still found themselves mistreated and victims of brutality. Carmichael coauthored a Manifesto for radical black youths and called it "Black Power." In this speech, Carmichael argued nonviolence would not eliminate discrimination. Previous civil rights leaders had softened the tone of the movement for equality championed since the Harlem Renaissance, but he felt as though Black Nationalism would reinvigorate the movement.

The Phrase "black power" was the fire the movement needed to keep going. It became a rallying cry for the younger members of the movement. Black Power also became the battle cry for the fight against European Imperialism across Africa. In 1968, Carmichael wrote a book titled *Black Power*. "It is a call for black people in this country to unite, to recognize their heritage, to build a sense of community. It is a call for black people to define their own goals, to lead their own organizations." Black Power thrust the Civil Rights away from its nonviolent roots. The Black Power movement can also be associated back to the teachings of Garveyism. Both Carmichael and Garvey seemed to approve of black separatism. Black Power meant challenging a system that was created to keep an entire race of people enslaved or virtually so. They needed to shake white America, and reclaim a Nation that was built on the backs of slave labor. They needed the respect that was long overdue. There was no use in standing around and waiting for their rights to be given to them because that would never happen. "Now, then, to understand white supremacy, we must dismiss the fallacious notion that white people can give anybody their freedom. No man can give anybody his freedom. A man is born free."

Blacks wanted to destroy the racial stereotypes that oppressed them. As Locke, Garvey, and Carmichael struggled to conceptualize a place for blacks in a hostile world of entrenched racism, they awakened African Americans to embrace a new identity. This rebirth followed the mass migration from the south, the cultural explosion known as the Harlem Renaissance, and the powerful ideas of Blackness and cultural consciousness. Locke inspired people of his generation to stand with him like Garvey, to civil rights activist of the future Carmichael. Though Garvey and Locke, wanted different outcomes, Locke, Garvey, and Carmichael strived for the greater good for blacks in America. Their pioneering efforts remain a vibrant part of American history and the urgent imperative to combat racism today.

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ABSTRACTS

Irene Ryan Competition At The Kennedy Center

Author: KATELYNN BILLS
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Attending the Kennedy Center American College Theatre Festival, would benefit me in many was as an actor. Through attending professional productions of new works and exciting theatre, I will become more well-rounded and educated about theatre today. By attending workshops, I will improve my skills and learn completely new ones. I hope to participate in many different workshops that focus on skills that I already have, and developing skills that I have either never had the opportunity to pursue, or skills that do not come easily to me. By attending dance classes, I hope to increase my ability to remember choreography and learn it more quickly, as well as improve on my technique.

By performing for The Cabaret available at KCACTF, I will gain feedback from fellow students and network myself into the world of theatre outside of college. I will also learn what it is like to perform in professional settings. Competing in the Irene Ryan Competition, will allow me to grow through insight from other professors from all over the nation and work to improve my performance with my partner each round of the competition.

At the festival, I will be able to attend performances of new and exciting theatre which will broaden my horizons and educate me as an actor and member of the theatre community. In all that I do at KCACTF, I will be representing Weber State University. As a representative of Weber State, I will strive to show a spirit of professionalism, creativity, collaboration, devotion, and passion.

Realized Set Design for *Deluge*

Author: BRINDLE BRUNDAGE
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Experiencing the Kennedy Center American College Theatre Festival firsthand will provide me with opportunities to learn from some of the most talented and experienced minds in the country. A wide variety of workshops will help me learn new skills and hone existing ones, while the sheer size of the event guarantees that I will be afforded chances for networking that are absolutely vital for students of theatre. In attending the festival, I will be able to improve myself as an actress, director, and, most importantly of all, designer. The chance to hear feedback from some of the region's preeminent professionals, particularly their critiques of my work, will provide me with perspectives I had never considered before and assist me on the path to creating something uniquely magnificent. I hope to represent myself as a true ambassador of Weber State University, both as an individual and a designer, so that I can share with fellow students the creativity, enthusiasm, and professionalism that embody our school.

Lighting Design for Off-Broadway Play *Memoriam at 59E59 Theaters*

Author: TIFFANY CAMPBELL
Mentor: JESSICA GREENBERG

PERFORMING ARTS
MEMORIAM at 59E59 Theaters
New York City, New York | November 11–18, 2017

Tiffany Campbell is traveling with Mentor Jessica Greenberg to work on Memoriam at the 59E59 Theaters in New York. Tiffany will be creating a 3-D model of the theater. She will also be performing duties such as hanging and focusing the lights with another student. She will be broadening her resume, and this opportunity will allow her to work with a variety of professionals working in New York City. It will give her experience working at a professional level, and make connections with people who can help her grow.

Projection Design for *Where Words Once Were*

Author: TIFFANY CAMPBELL
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Tiffany Campbell is attending the Kennedy Center for the Arts College Theatre Festival to present her projection design for Where Words Once Were. She will be competing against other students with similar design projects to win grants and scholarships. While attending this festival Tiffany will be honing her presentation skills and making contacts for future work in the field.

Coeur D'Alene Symphony National Concerto Competition

Author: YIAN CHI
Mentor: VIKTOR UZUR

PERFORMING ARTS
Coeur D'Alene Symphony National Concerto Competition
Spokane, Washington | January 4-7, 2018

My name is Yi An Chi and I am a junior majoring in cello performance. On Nov. 15, 2017, I turned in my concerto recording with my pianist, Jonathan McDonald, to the Coeur D'Alene Symphony Orchestra National Young Artists' Concerto Competition. On December 8, I received the news that I had been selected as a finalist in the competition, which will be held at Whitworth University in Spokane, Washington on January 6, 2018.I will be performing a concerto written by Edward Elgar.

This proposal seeks funds for the cellist Yi An Chi, and her accompanist Jonathan McDonald to participate in the National Coeur D'Alene Symphony Orchestra Young Artists' Concerto Competition. It is an honor to represent Weber State University to compete in the final round of the competition in Spokane.

Lighting Design Submission for *We Foxes* to KCACTF

Author: DANIEL GARNER
Mentor: CATHERINE ZUBLIN

I am a student of technical theater in the Department of Performing Arts here at Weber State University. I specialize in Lighting Design and Stage Management. I am presenting my lighting design of *We Foxes* at the Kennedy Center American College Theater Festival. I am so proud of this production and I would love to share what I experienced with my colleagues and peers.

Among the many students in our department, I'm honored that I have this opportunity for many reasons. First, the work I have done and will be presenting will allow me the chance to receive feedback from industry professionals who do this kind of work every day for a living. Second, I will be able to learn from others who are also presenting at this festival and receive inspiration for future projects. Third, I will be able to build brand new professional networks I can use in my career. Finally, this is an incredible experience that is very rare among undergraduates. I know that this trip to KCACTF will only help to increase my skills and make work even harder during my college stay here at Weber.

Kennedy Center American College Theatre Festival

Author: PATRICK KIBBIE
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Attending the Kennedy Center American College Theatre Festival is an opportunity to learn from professors, actors, and theatre technicians from all over the country. This allows students to gain experience from individuals and professionals in the world of theatre, that they might not get otherwise. By attending the Kennedy Center American College Theatre Festival I will have the opportunity to learn from professionals in different areas of acting/devised theatre/playwriting, and so much more. This can only enhance my abilities as a theatre professional and allow me to become a more well-rounded actor as a whole.

Participating as an actor in the Irene Ryan Scholarship awards as a representative from Weber State University would allow me the unique opportunity to gain insight and critiques from theatre professionals from all over the country. As well as allow me to learn from my fellow actors competing for the awards. Constructive feedback is the best way to learn and grow and I plan to take full advantage of this to better myself as an actor.

As a participant in the Kennedy Center American Theatre Festival I will have the opportunity to take classes, attend workshops, and actively participate in the making of live theatre. This is an amazing opportunity to show off the skills I have learned at Weber and to showcase myself as an ambassador for the Weber State Theatre Department, and Weber State College as a whole.

Costume Designs for *Deluge*

Author: ALICIA KONDRICK
Mentor: CATHERINE ZUBLIN

Attending the Kennedy Center American College Theatre Festival is an opportunity to learn from professors, designers and production teams from all over the country. The festival is an educational but also competitive setting in which students can experience what it is like to take workshops, present work, and perform and in return they gain feedback and critiques they normally don't get the opportunity to hear or learn from. Critiques is what students use to better their performances, research or designs for better results and present better work in the future. If I attend KCACTF, I will learn new ideas on technical theatre, ideas on how to present costume designs in a professional manner and explain the process that goes into designing a full show. I will have the chance to attend workshops that I am both comfortable in taking but id also love to take some in which I don't know a lot and have the chance to let myself learn new skills and step outside my comfort zone. Ill also get to meet and work with fellow designers from schools all over the country.

Gaining a new experience of critiques from other professionals and peers from differing schools will allow me to gain new ideas about what theatre means to others. I hope to gain as much experience as possible within the few days. I plan to put together a visually comprehensive and attractive board show casing the work I did on our student written and designed piece Deluge. I will present my work through oral presentation and learn from other students and designer's presentations as well. I hope to gain insightful and educational critiques and feedback that I am given, which in turn will help me become a better educated and practiced designer. I will then take the notes I am given and apply it to my presentation for any future presentations as well as future designs for future shows. Overall, I plan to present myself with dignity and professionalism. I want to represent myself   as having the creativity, passion and drive that our students have at Weber State University.

Technical Direction For *Deluge* Presentation

Author: BENJAMIN READING
Mentor: CATHERINE ZUBLIN

For every artistic endeavor, there prevails the "nuts and bolts" of transmuting concept into construct. In the industry of live theatrical production, the specialist charged with facilitating and coordinating the realization of artistic concepts into tangible works upon an open stage ,is referred to as the "Technical Director". For this project I have an opportunity for a unique experience, particularly as an undergraduate. I have been selected by my peers and mentoring faculty to be the Technical Director of a completely student produced, student written, new work. This is not an opportunity normally available to undergraduate students in almost any theatre arts program across the United States . I will be working with a production team of my peers to meet the needs of the script (written by Riley French). It is my task to physically produce a full sized production of new play, by leading other student, on a professional level, and to do it safely. I will be expanding, and testing my body of knowledge, through practical experience, non-theoretical research, and real-world problem solving, using the skills and practices I have learned over the last 3 years of my college career.

Costume Designs for *We Foxes*

Author: VICTORIA SNOW
Mentor: CATHERINE ZUBLIN

I have been invited to present my ideas and design process at the Kennedy Center American Theater Festival Region VII. This festival is an opportunity to present my work and attend workshops taught by some of the most talented technicians and teachers in our region. Getting critiques and learning from these professionals is invaluable because they have skills and specialization that they are willing to share with me in my future career. Attending this festival will be incredibly important to me because it will help me to present myself professionally and to gain indispensable knowledge in my field.

At this festival, I will discuss my costume design process of We Foxes, my analysis of the script, how I collaborated with other designers on the team, and how my concepts went from ideas to production. I will compete with other designers for the opportunity to present my work in the finals round at the Kennedy Center in Washington D.C.

The 2017-18 school year is the season of new works for the theater department. I was honored to be involved in *We Foxes,* a musical written by Ryan Scott Oliver, as the costume designer. The musical tells the story of Willa, a plucky young orphan in 1945, who experiences strife with her adopted family while she waits for her brother, George, to return from the war. While the director still had a larger vision of the show, the rest of design process was placed in the hands of students with faculty mentorship.

This particular festival will be exciting because, since this is the first time *We Foxes* has been fully produced, it also means that I am the first person to design the costumes for the show. That also gives me the chance to present my work to an unbiased jury. My success and failure will depend on my material and explanations instead of prior knowledge about the Musical. Last year, I presented my work at KCACTF and I was a regional finalist. I was also awarded a scholarship to the Stagecraft Institute of Las Vegas' summer program. With the knowledge that I gained last year, I know I can do even better this year..

Irene Ryan Nominee for *Where Words Once Were*

Author: LANDRY THOMAS
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Through Weber State University, I was nominated for an IRene Ryan award for my work in the play *Where Words Once Were* as the character Eila. I have been invited to audition in Mesa, Arizona at The Kennedy Center American College Theater Festival to showcase my skills as an actress. Having this opportunity through the performing arts program, I have to prepare two contrasting scenes with a partner and one monologue for the audition. While in Arizona at this event, I will have other opportunities like meeting Directors, playwrights, and even getting to receive guidance on how to become a better actor. Finally, this is also an opportunity that my peers and I will have at receiving a scholarship.

Irene Ryan Nominee for *We Foxes*

Author: HAILEY WEEKS
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

The Kennedy Center American College Theatre Festival is where the annual Irene Ryan competition is held. I was nominated for the Irene Ryan competition for my work on the production We Foxes in the role of Vesta Quimby. I would like to attend said festival to compete in the Irene Ryan competition for a chance at scholarships, and to learn and grow as a performer and actress. This opportunity is a wonderful experience to learn from educators that I would normally never get the chance to work with, work with fellow peers, and further my craft. I would like the opportunity to take workshop classes and perform to expand my knowledge, and to represent Weber State University's performing arts department.

Irene Ryan Competition

Author: CASSIDY WIXON
Mentor: CATHERINE ZUBLIN

PERFORMING ARTS
Kennedy Center American College Theatre Festival
Mesa, Arizona | February 12-18, 2018

Various numbers of actors throughout time have boasted of the skills and qualities learned from working with others in their field. I, too, have found an unmeasurable resource in those whom I have shared a stage and the gifted directors I have had the pleasure to work with. There is great power in understanding and embracing whatever role you are given.

I will travel to the KCACTF's regional festival with my partner to audition and compete in the scholarship program. The opportunity of attending the Irene Ryan competition furthers my study of others in their individual roles and allows me to draw from their performances any skills that I may use to further my career in theatre. I also look forward to meeting new friends and networking with students from other universities across the country.

The award-winning actor Ewan McGregor once stated of acting, "The beautiful thing about it is that no two directors or actors work the same way. You also learn not to be afraid of discussion and conflict."

Influence of Meal Caloric Distribution in Metabolic Syndrome Parameters Among College Students

Authors: ASHLEY PETITTA, ALEXIS ELINKOWSKI
Mentor: DAVID AGUILAR-ALVAREZ

MPurpose/hypothesis We investigated the influence of meal calorie distribution on metabolic syndrome parameters in Weber State students. We hypothesize that variance in the percentage of calories eaten at each meal will affect MetS parameters in both males and females. Methodology We assessed MetS parameters in 168 Weber State University student participants, ages 18-54 years. Two-day diet records for each participant were collected and analyzed using Diet and Wellness Plus. Participants were separated by gender (Male:53; Female:115) and by meal calorie distribution. Groups included high, medium and low percentage of calories in breakfast, lunch, dinner, and snacks. ANOVA tests were used to determine mean differences in MetS parameters between meal calorie distribution groups. Results Our results show that women in the high calorie breakfast distribution group presented lower systolic blood pressure than women in the low or medium breakfast group (μ= 107.3 mm/Hg, μ= 113.3 mm/Hg, μ= 115.01 mm/Hg, p ≤ 0.05). Men in the high snack consumer group presented higher HDL-C (μ= 41.3 mg/dL, μ= 35.1 mg/dL, μ= 32.3 mg/dL, p ≤ 0.01) as well as higher blood glucose (μ= 97.8 mg/dL, μ= 92.1 mg/dL, μ= 91.5 mg/dL, p ≤ 0.05) than the low and moderate snack groups. Conclusion In accordance with our results observed in the female subgroup, previous studies have shown that skipping breakfast increases cortisol levels, which may result in higher blood pressure. Furthermore, many breakfast associated foods have shown to exert blood pressure lowering effects. The differences observed on male HDL- C may be due to HDL-C promoting foods consumed as snacks. High snack consumption is linked to increased exercise which is known to increase HDL-C. This study suggests that meal calorie distribution affects MetS parameters differently among genders.

Honey, Did You Remember the Milk? Metacognitive Insight and Accuracy in Couples

Author: SABRINA BADALI
Mentor: SHANNON MCGILLIVRAY

Couples often divide and conquer when it comes to what information each person remembers. For example, maybe one person is good at remembering directions, so the other does not need to. Despite how common this behavior is among couples, an important question is, do we really know what our partner will remember? Studies of metacognition often require participants make judgments of learning (JOLs) about what they will later remember, and accuracy of these JOLs can be assessed. A recent meta-analysis (Rhodes & Tauber, 2011) found that individuals have a moderate ability to predict their own memory performance. However, very little is known about the ability to predict your partner's memory performance. The current study recruited romantic adult couples who have been co-habiting at least 6 months. Each person in the couple was given a variety of memory tasks meant to reflect real-life scenarios that one would likely encounter, such as remembering directions for navigating an unfamiliar city, remembering bill amounts and due dates, and items grocery list. For each set of materials, individuals gave predictions (i.e., JOLs) for their own and their partner's performance. These predictions were compared to actual performance and the data revealed that participants were roughly as accurate at predicting their own performance as they were their partner's performance.

The Collaborative Process: *How law enforcement agencies and universities research together*

Author: STEINER HOUSTON
Mentor: MONICA WILLIAMS

The current project is a collaboration between an official at a local law enforcement agency (LEA) and undergraduate students and faculty at a regional university. Although the main focus of the study is on disproportionate minority contact, this presentation will focus on the collaborative efforts of those involved in collecting and analyzing the data. In an effort to receive available grants and other funding, many LEAs have sought out partnerships with local institutions of higher education to conduct research on various topics. The willingness of LEAs to seek out such research shows a desire to look inside their organizations and proactively address any issues that may be discovered. As part of the current collaboration, the undergraduate students have maintained constant contact with the law enforcement official and have been given the opportunity to counsel with various record keeping and information technology personnel. This further collaboration has aided the student researchers by providing a means of obtaining redacted information on individuals who have previously contacted or been contacted by local officers. The redacted information allows the researchers to more quickly process and analyze the collected data. Some challenges have resulted from utilizing the data stored in the LEA's records management system. Various systems have been used to collect data over the years and not all data has been transferred to the updated system. There is also some variation in how officers collect and interpret data. A final challenge that may be faced by all undergraduate research students wishing to analyze topics related to race and ethnicity is the manner in which various racial backgrounds are classified. Because previous research has rarely provided an in depth look at the collaborative process, this study will be a great resource for future researchers and community partners who wish to enter the collaborative process together.

Attitudes Towards E-Cigs and Vaping: *Comparing non-smokers and nicotine users*

Author: CAYLA LYNCH
Mentor: TODD HILLHOUSE

In a ten-year period (2005-2015), there has been a 5% decrease in traditional cigarette smokers and a significant increase in the number of people using Electronic nicotine delivery systems (ENDs) and Electronic-cigarettes (E-cigs) in the United States. Approximately, 50% of college age students have reported using any illicit drug during the same period. Several systematic reviews have suggested that ENDS serve as a "gateway" to other tobacco use, and thus, it is possible that ENDS can serve as a "gateway" to illicit drug use. The aim of this study was 1) evaluate the perceptions of ENDS across various demographics and 2) compare nicotine poly-drug use trends among ENDS and traditional cigarette users. Study 1 compared the perceived health and safety risks as well as the research needs for ENDS and traditional cigarettes. Participants were recruited through flyers and include college students and members of the community (i.e. non-college students). Participants were classified into four demographics: A) non-smokers, B) traditional cigarette smokers, C) ENDS/E-cig users, and D) those who had switched from traditional cigarettes to ENDS/E-cigs. Study 2 surveyed a sample of college students on their nicotine and drug use.   Study 1: In general, traditional cigarettes were perceived more negatively or harmful as compared to ENDS or E-cigs. All of the groups (except for cigarette users) agreed that ENDS were safer than cigarettes and there is a need for more research. Interestingly, cigarette users perceived ENDS and traditional cigarettes the same for all questions. Study 2: College students self-reported drug use is as follows: ENDS or E-cigs 15%; cigarettes 4.3%; cigars and cigarillos 4.3%; Alcohol 34.5%; and marijuana 11%. We found greater ENDS poly-drug use with alcohol and marijuana as compared to traditional cigarettes. For example, 27% of the participants used alcohol in combination with ENDS compared to 18% that use the combination of alcohol and traditional cigarettes. Additionally, we found a significant proportion of the population that used the combination of alcohol and marijuana. Taken together, the positive or safer perceptions of ENDS may lead to the greater poly-drug use trends as compared to traditional cigarettes. Due to the high prevalence of ENDS/E-cig use in combination with alcohol and marijuana, further research needs to evaluate the physiological effects of these drug combinations.

An Analysis of and Reaction to Utah v. Strieff

Author: TIMOTHY MASERANG
Mentor: MARK DENNISTON

Evidence suppression has long been a controversial and fascinating area of both federal and state constitutional law. This paper explores the history of the United States Supreme Court's recent decision in Utah v. Strieff, 136 S. Ct. 2056 (2016) as well as provide an analysis of that decision, a decision which brings the evidence suppression debate into the 21 st century and expands upon the classical interpretation of the attenuation doctrine. This paper will also analyze federal circuit and state appellate court reactions to and interpretations of Utah v Strieff.

Electronic Nicotine Delivery Systems (ENDS) and Electronic Cigarettes (E-cigs)

Author: MAKENZIE PETERSON
Mentor: TODD HILLHOUSE

Electronic nicotine delivery systems (ENDS) and electronic cigarettes (E-cigs) have been introduced to the market as a safer alternative to traditional cigarettes. The research community is still investigating the short-and long-term effects of ENDS on many psychological and biological systems. For example, ENDS have been shown to reduce withdrawal effects in current cigarette users; however, minimal research has evaluated the withdrawal effects of daily ENDS users that are first time nicotine users. The present sought to extend the findings of ENDs on both psychological (e.g. usage patterns, cravings, dependence, etc.) and biological (withdrawal symptoms, biomarkers, etc.) health. In study 1, a sample of college students were surveyed on their current and past nicotine use. In study 2, subjects participated in a withdrawal study that was composed of two experimental sessions. For the first experimental session, we asked the participants to smoke their ENDS or traditional cigarettes ad lib (preferably within 30 mins of the study). The second experimental session, required the participants to abstain from nicotine use for 12 hours. Several physiological measures were taken during both sessions that included carbon monoxide (CO) concentration, blood pressure, heart rate, and blood was collected via venipuncture. Approximately, 15% of the college students reported using ENDS; whereas, only 4.3% reported using traditional cigarettes. Sixty-two percent of the students using ENDS were new nicotine users. For the students that switched from cigarettes to ENDS, 23% switched because they viewed ENDS as safer and 15% switched to ENDS as a smoking cessation. Participants report using E-juice solutions with 7.86 ± 1.08 mg/mL of nicotine when they started using ENDs; whereas, now the students have decreased their nicotine concentrations to 4.51 ± 0.51 mg/mL. These results suggest that college students may have a higher rate of ENDS use (15%) as compared to the general population (~10%). Moreover, ENDS may be a successful smoking cessation or a mechanism to reduce nicotine intake as the students reported a significant decrease in e-juice nicotine concentration.

Disproportionate Minority Contact in Policing:
Where does the bias lie?

Author: CARRIE STONE
Mentor: MONICA WILLIAMS

The current project involves a collaboration between a local police department and undergraduate students and faculty at a regional university to examine a heretofore underexplored explanation for disproportionate minority contact (DMC). Previous researchers have explained DMC by utilizing two hypotheses. The first, differential treatment, is the theory that variations exist in policing whereby officers disproportionately focus on minority groups. The second, differential offense, is the theory that variations exist in patterns of offending whereby minority groups disproportionately place themselves within police focus. Utilizing reports provided by a local police department, we analyze a third hypothesis that we call differential civilian response. Differential civilian response is the theory that civilians disproportionately place minorities within police focus. After controlling for offense rates, we will examine the extent to which biases within police departments and among civilians contribute to DMC between officers and community members. By examining an underexplored explanation for DMC rates, findings from this study have the potential to enhance community education efforts, influence police training practices, and aid future researchers in understanding how civilian bias impacts rates of DMC within communities.

Engaging Students in Family Life Education Throughout Undergraduate programs: *On Campus Engagement*

Author: GRACE BINGHAM
Mentor: PAMELA PAYNE

Child and Family Studies Student Association (CFSSA) at Weber State University uses active involvement methods (e.g., play doh, prize wheels) to engage prospective & current students in events and departmental activities. Events hosted immerse students in Family Life Education (FLE). Professional development focused events include Pathway events (e.g., work, graduate school, networking) and employment socials. Additional events promote the program and collaboration between students and faculty through breakfast bars, service opportunities, field experiences and teaching opportunities. This presentation discusses various events that increase engagement and visibility of both the Family Science program and our student

Determining Kinetic Data for the APEH and ACY Pathway

Author: CARSON COLE
Mentor: TRACY COVEY

Metabolism is critical for proper the growth and function of cells. Cells contain numerous metabolic pathways all with unique functions. Malfunctioning metabolic pathways have been implicated in numerous disease processes. This study examines one such metabolic pathway. The APEH/ACY pathway, named for the two enzymes involved, is part of cellular catabolism. It functions to break down small proteins into their constituent amino acids. These building blocks can then be used to make an extensive array of protein products that are necessary to the proper growth and function of the cell. The mechanism of this process is well understood and has been extensively studied, however, much less is known about its overall kinetics. Through using gas chromatography and mass spectroscopy it is possible to examine the flux of these two enzymes. This process was able to show different rates in strains of HELA and A549 cancer cells, as well as in normal red blood cells otherwise known as erythrocytes.

The Refiner's Fire: Removing Barriers in Education for Marginalized Community Members

Author: CHEYENNE ESPINOSA
Mentor: GINA SHELLEY

Since ancient times, the process of refining silver or gold has been known to mankind. It requires a craftsman to sit patiently next to a burning, hot fire with temperatures more than 1000 degrees Celsius while stirring and skimming the metal to remove its impurities that would rise to the surface. When a piece of gold or silver is initially pulled from the filthy dirt or the mud of a river, it is impossible to see inside of the nugget to know its full worth until it is refined. Only then, is it ready to be crafted into a beautiful work of art. Building upon this analogy, many students that our future teachers will encounter will be from homes where their parents are going through different stages of their refining process. The effects of unreliable parenting on children transfer over into the school environment, which places added challenges for teaching and learning.

Project Literacy Instruction to Further Education (Project LIFE) is a non-profit program that is sponsored by Weber State University, located in an urban community. This adult education class was designed to build literacy skills and provide GED and college placement exam tutoring for individuals in the criminal justice system, or for those who are in poverty, in family court, or are dealing with other issues of marginalization. The program invites teacher education students to volunteer as tutors to work with the participants once a week. For over two years, this program has successfully helped several community members to complete their GEDs as well as help others gain acceptance and begin programs at Weber State or at local technical schools. Project LIFE program was designed using service-learning principles of social justice, community building, sustained organizational commitment, active training, and an opportunity for all participants to critically reflect upon their experiences. Each semester, the program director recruits primarily eight to ten teacher education students to volunteer as tutors for the entire semester. This adult education program reported huge benefits for both the participants and the university students who were their tutors.

ACTNow: A Transition Discussion Tool
DCDT Conference

Author: MARIE MCFADDEN
Mentor: SHIRLEY DAWSON

Evaluation of a Tier 3 Interagency Communication Intervention (ACTNow) for Collaborative Transition Intervention: ACTNow is a systematic Tier 3 approach to improve self-awareness/self-knowledge in students with high incident disabilities. The tool supports transition planning for high school students. The intended outcome is increased transition engagement in post K-12 settings through an improved ability to self-determine appropriate and needed disability supports. Additional outcomes are improved communication about need for support, and increased application for post K-12 work or education. Purpose: The evaluation investigates whether ACTNow influences self-determination for high school students, though its function as a standardized shared interagency communication tool, to increases student engagement with practitioners who work directly with students with disabilities in post K-12 settings. Setting: High school and post K-12 urban and suburban settings in Davis and Weber Counties in northern Utah. Population/Sample: Student participants, have high incident disabilities, are preparing to transition to post K-12, and likely need support in future academic or employment settings. Sample is typical of students with disabilities compared to other populations in Utah. Research Design and Methods: Using a one-year regression discontinuity design, students were assigned intervention or treatment conditions based on pre-test SASK scores. Treatment is ACTNow and normal curriculum, Control classrooms have access to normal curriculum only. Key Measures: Two main sources of data are used, ACTNow data and student post-high school engagement, The tool provides student data on 16 factors linked to post high school success and consists of 26 items scored on a five-point scale, allowing for 130 different scoring combinations. Engagement is measured by the number of students in both the treatment and control groups who a) visited one of the transition institutions, or b) enrolled in a post-high setting. Data Analytic Strategy: To determine effectiveness of the ACTNow tool on self-determination, a pretest/posttest design is used. A series of two analyses of administrative data determined whether the ACTNow tool had an impact on student engagement after high school. The first analysis measured engagement through initiating contact with a transition institution. The second analysis measured enrollment in a transition institution.

Gendered Household Roles and their Impact on Relationship Outcomes

Author: ASHLEY VANDENBERG
Mentor: DANIEL HUBLER

Previous research shows that gender roles can have exert a large influence on many different aspects of an individual's life. The current dyadic study observes the impact of gender role ideologies on relationship outcomes with a specific focus on traditional and egalitarian roles. Additionally, the relationship between gender roles and life satisfaction, relationship satisfaction, and social support is also explored. Participants were 145 heterosexual romantic couples who reported being married or in long-term relationships. Respondents were invited through various online invitations and announcements. Results revealed a significant and positive relationship between flexible/egalitarian gender roles and life satisfaction, relationship satisfaction, and partner support levels. The results of this study point to the importance of gender role ideologies within romantic relationships and their impact on satisfaction levels and perceived support.

Synthetic Strategies for Zirconium Metal-Organic Frameworks

Author: JEHAD ABOURAHMA
Mentor: BRANDON BURNETT

Zirconium Metal-Organic Framework (MOFs) have shown to have difficulties in formation of single crystal MOFs. It was hypothesized that breaking up the synthesis into separate sequences, apposed to the the traditional one pot synthesis, would provide a greater control over the formation of a single crystal. Our studies focused on developing a new synthetic strategy for the synthesis of these MOFs. We report our successful application of Infrared Spectroscopy and X-Ray Diffraction for the quantification of single crystal Zirconium Metal-Organic Framework.

Processing of Lead Iodide Perovskite Thin Films for Solar Cell Applications

Author: HEATHER BARTON
Mentor: BRANDON BURNETT

We present two process methods for the construction of high purity, polycrystalline lead iodide perovskite films – dip method and chemical vapor deposition method. Furthermore, we compare the merits of both types of thin film processes including product purity, process cost, environmental impacts, and time commitment.

Ferritin Associations with Immune Cell Profile and Inflammatory Markers in Cross-Country Athletes

Authors-: BESS BAUER, BECKY ROSE
Mentor: DAVID AGUILAR-ALVAREZ

In this session, we will explore how prospective elementary teachers (PSTs) deepen their knowledge of fraction multiplication and division in an arithmetic for elementary teachers course. Historically, PSTs have had difficulties recalling and learning how to explain these topics and misconceptions still persist today. With regard to fraction multiplication, PSTs tend to believe the idea that multiplication always makes things bigger, misapply other procedures like multiplying the reciprocal, and complicate the process by finding a common denominator. Dividing fractions, whether procedurally or conceptually, has also been a challenge for PSTs. Much of the challenge is likely due to the way in which typical textbooks treat division of fractions. They simply state that dividing by a fraction is the same as multiplying by its reciprocal. There is little or no attention given to the meaning of fraction division and no connections are made between division with fractions and division with whole numbers. We will discuss the results of a study in which we used manipulatives, pictures, and real-life examples to fortify PSTs' knowledge of and confidence in multiplying and dividing fractions and how to apply them to real life.

Preservice Teachers' Learning of Fraction Multiplication and Division

Author: MARLISE WEYBURN
Mentor: SHAWN BRODERICK

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Author: BOSTON WORKMAN
Mentor: SHAWN BRODERICK

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