Investigating the interactions between residential microbes of the facial microbiome and C. acnes treatments

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Project Summary

Acne vulgaris is an incredibly common dermatologic condition affecting nearly every teenager around the world at some point. Not only can this condition be painful, but it can also cause psychological harm and decrease one's quality of life. Current treatment regimens, oftentimes a combination of various antibiotics, have proven to be effective in the short term. However, over a period of just a few months, efficacy is soon diminished; acne-causing bacteria quickly become resistant to the antibiotics and colonize within the skin leaving the individual worse off than before. Treatments also come with a myriad of other side effects and a hefty price tag: Accutane (isotretinoin), for example, is a last resort drug used to treat severe acne that hasn't responded to other treatments and has been associated with joint pain, frequent nose bleeds, and extremely dry skin (Bettoli et al. 2019). Breathing problems, pressure on the brain, sun sensitivity, and, ironically, more acne are also common side effects of this pricey medication (Pietrangelo 2018). This current cycle of treatment not only fails to address the optimal balance of the microorganisms in the cutaneous microbiome but also shows a lack of consideration to the increasingly prevalent issue of antibiotic resistance. A balanced microbiome is critical in the prevention of acne and yet nearly all current treatments are aimed at eliminating one or more of these essential microorganisms, furthering a dysbiosis of the microbiome. Thus, it is imperative for physicians and patients to advocate for a more holistic approach and consider probiotic treatments aimed at restoring the balance of the microbiome.

It is necessary, then, to gain a better understanding of the interactions between the residential microbes of the facial microbiome and acknowledge the role of dysbiosis in the pathogenesis of acne. The proposed research will test the hypotheses that 1) a disrupted balance of the microbiome influences antibiotic resistance in *Cutibacterium acnes* and 2) growth rates of residential microbes are negatively impacted by different states of imbalance (e.g. *C. acnes* and *S. aureus* grown together in the absence of *S. epidermidis* may result in uninhibited proliferation of *C. acnes*). Additionally, we will test a single treatment of either a topical ointment or a probiotic to reduce the growth of *C. acnes,* as well as test the efficacy of a dual treatment of tretinoin and probiotic (consisting of *L. acidophilus* and *L. plantarum*) in decreasing the growth of *C. acnes.* A more complete understanding of how these factors relate to the development of acne can lead to improved treatment options, ultimately resulting in enhanced life quality for those suffering from this condition.

> ***Intellectual Merit:*** The findings of this research will contribute to a better understanding of the microbial interactions within the cutaneous facial microbiome. Furthering this understanding may lead to the discovery of a successful holistic treatment and support future innovations of personalized acne treatment tailored to the unique qualities of an individual's skin. It will also encourage the implementation of the "one body, one soul" ideology in relation to treating diseases. Meaning, when treating one condition, it is

important to consider additional factors occurring throughout the body. Moreover, this research has the potential to lessen the dermatological field's contribution to antibiotic resistant bacteria and increase appreciation for the importance of holistic treatments. ***Broader Impacts:*** The knowledge gained from this research will raise awareness of alternative acne treatments by shining a light on the negative consequences of prolonged antibiotic use and how it relates to antibiotic resistance in acne-causing bacteria. It will also serve to educate the general public on the lesser-discussed causes of acne and how these issues can be addressed in a more holistic manner. Our research will enlighten the general public and researchers on the beneficial impacts of using probiotics to treat acne. Through our partnership with Perfectus Biomed, we hope to spread this message throughout Wyoming communities and beyond. As this research will be spearheaded by three undergraduate students, it will provide us with valuable skills including writing research proposals and conducting our own research.

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Project Description

Statement of Problem Significance

Acne vulgaris, which is commonly referred to simply as acne, is the second most common dermatological condition worldwide, affecting nearly 85% of people at some point in their lives (Xu 2019). While the full extent of knowledge pertaining to the pathogenesis of acne is still incomplete, it has recently come to light that *Cutibacterium acnes* plays a major role in the mechanism. Within the pilosebaceous unit, *C. acnes* is by far the most abundant microorganism, making up nearly 90% of the microbiome (Xu 2019). Interestingly though, *C. acnes* is found in almost equal amounts in both healthy and acne skin (Dréno et al. 2018). Research now shows that it is often a dysbiosis of the cutaneous microbiome that triggers acne. Current acne treatments frequently include the use of antibiotics; however, *C. acnes*, as well as other resident microbial inhabitants, are particularly susceptible to mutations and therefore quickly become resistant to treatment (Byrd et al. 2018). Not only does this result in ineffective acne therapies after prolonged use, but on a larger scale, it also presents adverse implications for the ever growing list of antibiotic resistant pathogens. Additional investigation into the composition of the cutaneous microbiome and its dysbiosis is necessary for a better understanding of the pathophysiology of acne and other dermatological conditions. Furthermore, it is imperative that research be conducted on alternative acne treatments aimed at restoring the equilibrium of the microbiome in order to prevent the expansion of antibiotic resistant microbes. Probiotics, specifically of the *Lactobacillus* order, are a promising proposed alternative but aren't standardized or often even used in skin care. In order to accomplish effective future treatments, we must first better understand the interactions between the various microbes of the cutaneous facial microbiome.

Relevant Literature

Over the last two decades, our understanding of the human skin microbiome has expanded substantially. More than 40 different bacterial genera have been identified on the human skin, and we learn more about their various interactions and unique roles each day. The facial microbiome is a hot area of research, especially in the last few years, due in part to the lack of understanding of various biological processes. One such area of study is that of acne vulgaris; though its pathogenesis is not completely understood, *Cutibacterium acnes* is known to play a significant part. Recently, it has been shown that while certain strains of *C. acnes* regulate skin homeostasis and prevent the colonization of harmful pathogens, others act as opportunistic pathogens which most commonly result in the development of acne (Dréno et al. 2018). It has been demonstrated that some of the other most common microorganisms present in the pilosebaceous unit affect which *C. acnes* strains are most prevalent. *Staphylococcus epidermidis*, for example, can inhibit the over-colonization of acne-causing *C. acnes* strains through the production of antimicrobial peptides (Lee et al. 2019). *Staphylococcus aureus* and *Malassezia*,

on the other hand, exacerbate acne causing strains and, when present in abundance, contribute to various other dermatological problems (Byrd et al. 2018). This dysbiosis of the cutaneous microbiome is a primary source of acne.

Current regimens aimed at treating acne often involve the use of several classes of antibiotics. While this treatment is initially effective for most patients, efficacy soon wanes and the bacteria become resistant. First-line therapies include macrolides (such as erythromycin and azithromycin), clindamycin (belonging to the lincosamides), and tetracyclines (including tetracycline and doxycycline); all have been documented to facilitate the increase of resistant *C. acnes* (Xu 2019). From 1999 to 2016, clindamycin-resistant *C. acnes* rose drastically from 4% to 90.4% (Xu 2019). Similarly, recent studies have shown the resistance of *C. acnes* to erythromycin and azithromycin to be over 50% and between 82%-100%, respectively (Platsidaki 2018). Tetracyclines appear to produce the least amount of antibiotic resistance, though data differs by nearly 30% in various studies (Xu 2019). These commonly prescribed antibiotics also contribute to resistance in other skin bacteria and thus further results in a dysbiosis of the cutaneous microbiome. According to the Centers for Diseases Control and Prevention (CDC), antibiotic resistance is “one of the biggest public health challenges of our time”. Consequently, it is essential that we explore alternative treatments to acne and continue research into the skin microbiome. A better understanding of the interactions between the most prevalent microbes will glean insight as to how an imbalanced facial microbiome contributes to antibiotic resistance.

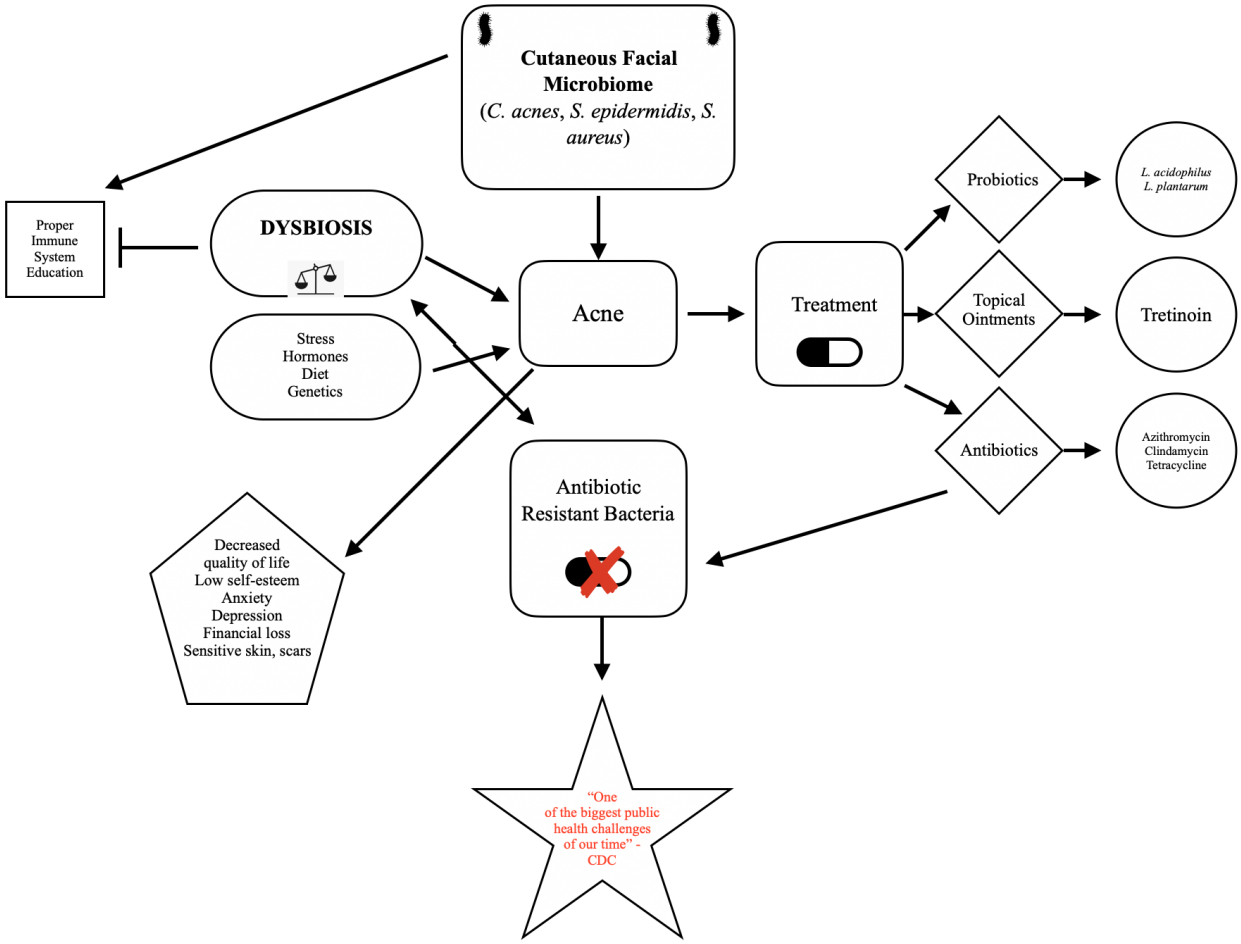
Antibiotic alternatives such as probiotics are well researched in the gut microbiome and have been found to impact overall human health. Recent studies have delved into microbe colonization across the body, focusing on *C. acnes* and *S. aureus* imbalances as a primary contributor to skin disorders like acne. To address these imbalances, they have also investigated the use of probiotics in both oral or topical forms (Dreno et al. 2016). Best explored is *Lactobacillus plantarum*. Currently, this probiotic is most commonly used in balancing the gut microbiome, but its effects on the skin microbiome are currently being researched. *L. plantarum* has been shown to increase collagen synthesis and assist in immunoregulation, thereby stabilizing the skin microbiome and contributing to acne healing. The information gathered from our research involving *L. plantarum* will provide a deeper understanding into its efficacy as a probiotic therapy for acne.

Preliminary Knowledge

Cutibacterium acnes is a facultative anaerobe requiring several days for growth and exhibiting extreme sensitivity to antibiotics. Because of this, current *C. acnes* models utilized by Perfectus Biomed, human skin donated from surgical procedures, present several difficulties. The donated skin is received with CHD soap and other antiseptics applied topically and therefore must be completely rinsed before it is viable for *C. acnes* growth. Not only is this a difficult process, but

it also leaves the skin void of its natural microbiome. All testing moving forward (including but not limited to: sensitization, cytokine analysis, and wound healing models) consequently lacks the major interactions between microbes that ultimately have an effect on how these tests would transpire *in vivo*.

Conceptual Model



Justification of Methods

Our research involves the co-culturing of three bacterial species followed by the isolation of *Cutibacterium acnes*. Co-culturing methods derived from Spittaels et al. will be utilized. Because *Staphylococci* are known for their resilience and ability to grow under most conditions, it is necessary to use a selective media for *C. acnes*. Tryptone yeast extract agar is a sufficient medium for the growth of *C. acnes*. When supplemented with furazolidone, an antibiotic active against *Staphylococci*, it will allow us to isolate our bacteria of interest and prevent the growth of the other bacteria being used (Marito et al. 2021). This research project also includes testing for phenotypic antibiotic resistance. The Kirby-Bauer test for antibiotic susceptibility was

standardized by the World Health Organization in 1961 and has been successfully utilized ever since to determine the sensitivity or resistance of bacteria to specific antibiotics. Probiotics are a common holistic method to treat microbiome dysbiosis; *L. plantarum* and *L. acidophilus* are primarily grown on De Man Rogosa Sharpe (MRS) agar/broth. Both strains have been extensively researched and proven to be effective as probiotics.

Research Plan

Objectives

The overall objective of this research project is to gain a better understanding of how the residential microbes of the facial microbiome interact under typical circumstances as well as how they behave in altered states. Each project has additional, unique goals.

Project 1

- To further understand the relationship between *S. epidermidis*, *C. acnes,* and *S. aureus* and their role as commensal and opportunistic microbes.
- To provide more information on *L. plantarum*'s effect on bacteria within the skin microbiome (specifically *S. epidermidis*, *C. acnes,* and *S. aureus*).
- To further the data gathered on novel probiotic therapies for acne as well as community knowledge of holistic approaches.

Project 2

- To understand how *S. epidermidis* and *S. aureus* affect the growth of *C. acnes* when co-cultured in broth.
- To understand how *S. epidermidis* and *S. aureus* impact the antibiotic resistance of *C. acnes*.
- To suggest alternative therapies to antibiotics for the treatment of acne vulgaris such as probiotics aimed at restoring the equilibrium of the cutaneous facial microbiome.

Project 3

- To propose a better treatment for *C. acnes* based on its relationship to *S. aureus* and *S. epidermidis.*
- To understand if a single treatment of either a topical ointment or a probiotic decreases the presence of *C. acnes* when it is co-cultured with two *Staphylococcus* species.
- To understand if a dual treatment consisting of tretinoin and a probiotic decreases the presence of *C. acnes* when it is co-cultured with two *Staphylococcus* species.

Hypotheses

Project 1

H_1: When *S. epidermidis* is co-cultured with *C. acnes* and *S. aureus*, the titer of both *C. acnes* and *S. aureus* will decrease as compared to the control.

H_2: *L. plantarum* will have inhibitory effects on *C. acnes* and *S. aureus* growth.

H_3: *S. epidermidis* will be more impactful than *L. plantarum* at controlling growth and decreasing present colonies.

H_4: *S. epidermidis* and *L. plantarum* will be more effective at controlling growth and decreasing present colonies when combined.

Project 2

H_1: *Cutibacterium acnes* will show growth that does not differ from the control when cultured in broth with *Staphylococcus epidermidis* and *Staphylococcus aureus.*

H_2: *C. acnes* will show reduced growth as compared to the control when cultured in broth with *S. epidermidis*.

H_3: *C. acnes* will show increased growth as compared to the control when cultured in broth with *S. aureus*.

H_4: *C. acnes* grown in conditions that mimic a dysbiosis of the microbiome will exhibit more antibiotic resistance than *C. acnes* grown in conditions that mimic a balanced microbiome.

Project 3

H_1: When applying a single treatment of a topical ointment and a probiotic, there will be less evidence of decreased growth of *C. acnes* than compared to the dual treatment.

H_2: When applying a dual treatment of tretinoin and a probiotic (*L. acidophilus and/or L. plantarum*), the growth of *C. acnes* on TSA will decrease.

Specific Aims

Project 1

- We will establish the effects of the probiotic *L. plantarum* on residential microbes found on skin. *L. plantarum* will be inoculated onto media with *C. acnes* and *S. aureus*; visual as well as quantitative analysis will be made on the colonies after a growth period. Possible biochemical outputs as well as colony size and growth will be measured.
- We will create a better understanding of relationships between residential skin microbes *S. epidermidis*, *C. acnes,* and *S. aureus.* All three will be inoculated onto a plate, and biochemical outputs in response to neighboring bacteria and colony changes will be documented - similar to specific aim one.
- We will combine *L. plantarum* and *S. epidermidis* in an attempt to produce a more productive probiotic therapy than currently exists against *C. acnes* and *S. aureus*. Equal levels of *L. plantarum* and *S. epidermidis* will be combined into a lotion (oil/water emulsification) and then applied onto a media with *C. acnes* and *S. aureus.* Microscopic analysis of the lotion will confirm the presence of *L. plantarum* and *S. epidermidis*. Visual changes in population via microscopic analysis and titrations will provide quantitative data.

Project 2

- We will measure phenotypic antibiotic resistance of *C. acnes* against the three most commonly prescribed acne antibiotics (macrolides, lincosamides, and tetracyclines) using a Kirby-Bauer assay.
- We will measure the growth rate of *C. acnes* after being co-cultured with *S. epidermidis* and *S. aureus* by plating *C. acnes* on a selective media and manually counting the number of isolated colonies.

Project 3

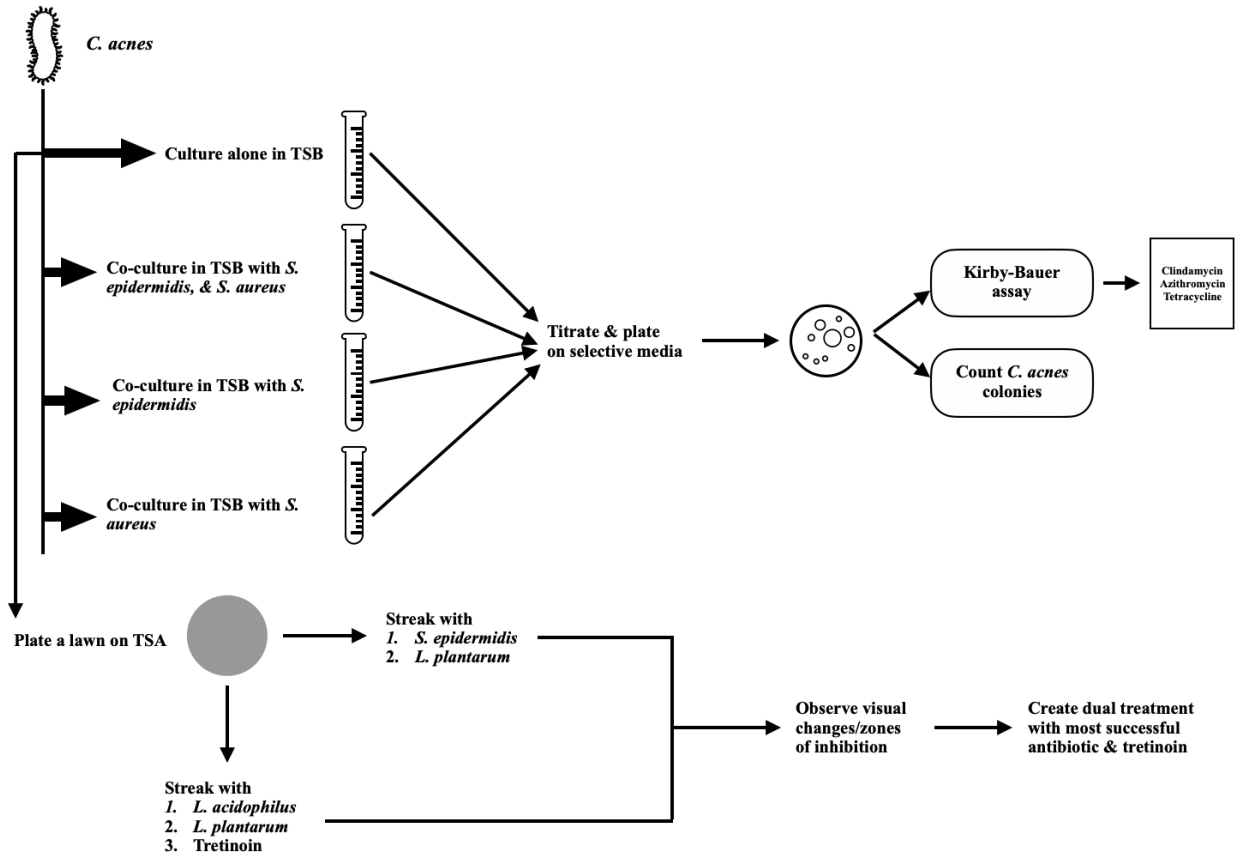
- We will establish a baseline for which topical ointment and a probiotic work best in decreasing the presence of *C acnes.*
- Using evidence from aim one, we will examine whether the dual treatment, consisting of a topical ointment and the best determined probiotic, is more effective in decreasing the presence of *C. acnes* than using a singular treatment.

Timeline

Project	Week	Tasks
General Preparation	1 (Sept 27-Oct 1)	- Plate *C. acnes* from Perfectus sample - Create *C. acnes* freezedown - Plate penicillin-resistant *S. aureus* - Plate *S. epidermidis*
	2 (Oct 4-8)	- Make TSA and TSB - Broth tube prep - Inoculate with *S. aureus*, *S. epidermidis*, & *C. acnes*
Project One	3 (Oct 11-15)	- Treatment plates - Plate growth checks - Grow *C. acnes*
	6 (Nov 1-5)	- Titrations → plating - Check growth of *C. acnes* plates/combine with staph strains in broth tubes - *Lactobacillus* testing - Analysis
Project Two	4 (Oct 18-22)	- Create the *C. acnes*-selective media (TYG + F) - Inoculate TSB with *C. acnes* & allow for ~4 days of growth
	5 (Oct 25-29)	- Inoculate *C. acnes* tubes with *S. aureus*/*S. epidermidis* & allow for 2 days of growth
	6 (Nov 1-5)	- Titrations → plating - Plate titrated samples on selective media & allow for 4 days of growth - Kirby-Bauer assay

Project Three	3 (Oct 11-15)	- Grow *C. acnes*
	6 (Nov 1-5)	- Titrations → plating - Check growth of *C. acnes* plates/combine with staph strains in broth tubes - Treatment plates - Check plate growth
General Allocation	7 (Nov 8-12)	- Allocated time for retesting
	8 (Nov 15-19)	- Allocated time for retesting - Data analysis - Lab cleanup
	9 (Nov 22-26)	THANKSGIVING BREAK
	10 (Nov 29-Dec 3)	- Data analysis - Poster creation
	11 (Dec 6-10)	- Poster presentation

Research Schematic



Materials and Methods

C. acnes will be cultured in a tryptic soy broth (TSB) and grown for seven days at 37℃; there will be four groups total, three test groups and one control. After the *C. acnes* has had a chance to grow, TSB tubes from group two will be inoculated with *S. epidermidis* and *S. aureus*. Tubes from group three will be inoculated with only *S. epidermidis*, and tubes from group four will be inoculated with only *S. aureus*. Groups three and four are intended to mimic a dysbiosis of the facial microbiome while group two will mimic a balanced microbiome. Group one will serve as the control and provide insight into the behavior of *C. acnes* when cultured alone. Due to the invasive nature of *Staphylococcus*, these co-cultures will only be grown for two days.

After this time period, a series of titrations will be performed on all tubes. A sample from the fifth dilution of each tube will be plated on a tryptone yeast extract agar (TYG) supplemented with furazolidone to isolate *C. acnes*. These plates will be grown at 37℃ for no less than four days. Isolated colonies of *C. acnes* can then be manually counted.

Because *C. acnes* is an anaerobic organism, gas chambers and anaero packs will be utilized to maintain a low oxygen concentration during all cycles of growth mentioned above and below.

Project 1

A sample of *C. acnes* from a pure broth culture will be plated on tryptic soy agar using a sterile cotton swab to create a lawn. Then, *S. epidermidis* will be streaked directly from a broth tube onto the center of the *C. acnes* plate with a sterilized inoculation loop. The above process will be repeated using *L. plantarum* instead of *S. epidermidis*. These plates will be incubated at 37°C for no less than four days. Growth can then be observed and documented.

Project 2

Using a sterile swab, samples from each tube (pre-titration) will be evenly spread over the same selective media mentioned above (TYG + F). A Kirby-Bauer assay will be conducted using discs of azithromycin, clindamycin, and tetracycline to test the *C. acnes* for antibiotic resistance. Each antibiotic disc will be placed in its own one of three sectors of each plate. After allowing for four days of growth at 37°C, zones of inhibition and resistance can be measured.

Project 3

A sample of *C. acnes* from a pure broth culture will be plated on tryptic soy agar using a sterile cotton swab to create a lawn. A sterilized inoculation loop will then be used to streak tretinoin, *L. acidophilus*, and *L. plantarum* onto one of four quadrants. The fourth quadrant of the plate will not be streaked with anything and left as the control. After four days of growth at 37°C, the probiotic showing the most success (inhibition of C. acnes)

will be used in a dual treatment with tretinoin on a lawn of co-cultured C. acnes, S. aureus, and S. epidermidis, and results will be measured again after four days.

Analysis and Expected Results

Culture titers (or cell counts) of *C. acnes* will be measured by manually counting isolated *C. acnes* colonies. We will test for antibiotic resistance through the use of Kirby-Bauer Assays. We expect to find that growth of *C. acnes* differs from the control when grown in imbalanced cultures with *S. epidermidis* and *S. aureus* as these are intended to mimic a state of dysbiosis. More specifically, we expect that growth will be increased when *C. acnes* is co-cultured with *S. aureus* and decreased when co-cultured with *S. epidermidis*. Furthermore, we expect that growth will not differ from the control when *C. acnes* is co-cultured with both *S. epidermidis* and *S. aureus* as this is intended to mimic a balanced microbiome. Similarly, we expect that the phenotypic antibiotic resistance of *C. acnes* will not differ from the control when co-cultured with both *S. epidermidis* and *S. aureus*. We expect that the antibiotic resistance of *C. acnes* will be increased as compared to the control when it is co-cultured individually with *S. epidermidis* and *S. aureus*.

We also expect that when *S. epidermidis* is inoculated onto a plate with a large population of *C. acnes* and *S. aureus,* the imbalance will cause *S. epidermidis* to release antimicrobial peptides and therefore decrease growth of the surrounding bacteria. After counting the *C. acnes* colonies manually and doing titrations, we anticipate to conclude that *C. acnes* colonies decreased in the presence of tretinoin. For the treatment of *C. acnes* with the probiotic *L. acidophilus* and *L. plantarum,* we expect to have decreased growth.

Our process and the data collected throughout our research can be tracked in our laboratory notebooks and will be stored on a Google Excel Sheet.

References & Annotations

Bettoli, V., Guerra-Tapia, A., Herane, M. I., & Piquero-Martín, J. 2019. Challenges and Solutions in Oral Isotretinoin in Acne: Reflections on 35 Years of Experience. *Clinical, cosmetic and investigational dermatology*, *12*, 943–951. https://doi.org/10.2147/CCID.S234231

Accessed via Pubmed database. Reviews the benefits and side effects of using Accutane. Possible side effects: altered skin barrier function causing photosensitivity and sensitivity to environmental allergens, anomalies in liver transaminases/triglycerides present in blood, dryness and desquamation that can affect the lips and lead to painful fissures.

Blanchet-Réthoré, S., Bourdès, V., Mercenier, A., Haddar, C. H., Verhoeven, P. O., & Andres, P. 2017. Effect of a lotion containing the heat-treated probiotic strain *Lactobacillus johnsonii* NCC 533 on *Staphylococcus aureus* colonization in atopic dermatitis. *Clinical, cosmetic and investigational dermatology*, *10*, 249–257. https://doi.org/10.2147/CCID.S135529

Accessed via Pubmed database. Mechanisms of HT La1 - antimicrobial, immunomodulating, epithelial cell attachment. Lotion creation - oil in water emulsion. HT La1 effects on the microbiome as a whole is analyzed.

Byrd, A., Belkaid, Y. & Segre, J. 2018. The human skin microbiome. *Nat Rev Microbiol* 16, 143–155. https://doi.org/10.1038/nrmicro.2017.157

Accessed via the NCBI database. This article provides an overview of the human skin microbiome, roles and interactions of the various microorganisms, sequencing techniques, and various skin disease states.

Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A. and Roques, C. 2018. *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates. J Eur Acad Dermatol Venereol, 32: 5-14. https://doi.org/10.1111/jdv.15043

Accessed via the NCBI database. This article contains updated information from recent studies on *Cutibacterium acnes* and acne vulgaris. It provides an in-depth explanation of the role of the immune system in the pathogenesis of acne and differentiated between a multitude of different *C. acnes* phylotypes and their actions.

Dréno, B., Dagnelie, M. A., Khammari, A., & Corvec, S. 2020. The Skin Microbiome: A New Actor in Inflammatory Acne. *American journal of clinical dermatology*, *21*(Suppl 1), 18–24. https://doi.org/10.1007/s40257-020-00531-1

Accessed via the NCBI database. This article also outlines the role of the immune system in relation to acne. Additionally, it provides a medical perspective on the consequences of dysbiosis.

Goers, L., Freemont, P., & Polizzi, K. M. 2014. Co-culture systems and technologies: taking synthetic biology to the next level. *Journal of the Royal Society, Interface*, *11*(96), 20140065. https://doi.org/10.1098/rsif.2014.0065

Accessed via Web of Science. This article provides information on co-culturing

methods.

Kober, M. M., & Bowe, W. P. 2015. The effect of probiotics on immune regulation, acne, and photoaging. *International journal of women's dermatology*, *1*(2), 85–89. https://doi.org/10.1016/j.ijwd.2015.02.001

Accessed via NCBI database. Five percent extract of *L. plantarum* applied topically reduces overall symptoms of acne and preserves procollagen.

Lee, Y. B., Byun, E. J., & Kim, H. S. 2019. Potential Role of the Microbiome in Acne: A Comprehensive Review. *Journal of clinical medicine*, *8*(7), 987. https://doi.org/10.3390/jcm8070987

Accessed via NCBI database. *Staphylococcus epidermidis* and *C. acnes* relationship on microbiome. *S. epidermidis* has antimicrobial properties (releases succinic acid) and can inhibit *C. acnes* growth. Antibiotics can lead to increased *Pseudomonas* infections post treatment. Table of 'potentially beneficial microbes' including *Lactobacillus*.

Marito, S., Keshari, S., Traisaeng, S., *et al*. Electricity-producing *Staphylococcus epidermidis* counteracts *Cutibacterium acnes*. *Sci Rep* 11, 12001 (2021). https://doi.org/10/1038/s41598-021-91398-7

Accessed via Nature. This article provided justification for the use of furazolidone in isolated *C. acnes*. It also provided useful data on working concentrations of the antibiotic.

Paetzold, B., Willis, J. R., Pereira de Lima, J., Knödlseder, N., Brüggemann, H., Quist, S. R., Gabaldón, T., & Güell, M. 2019. Skin microbiome modulation induced by probiotic solutions. *Microbiome*, *7*(1), 95. https://doi.org/10.1186/s40168-019-0709-3

Accessed via PubMed database. *Cutibacterium acnes* strains determine pathogenicity. Specific data surrounding acne/sebaceous areas of the body's microbiome before and after probiotic cream (microbiomes from people with healthy skin applied to those with acne).

Platsidaki, E., & Dessinioti, C. 2018. Recent advances in understanding *Propionibacterium acnes* (*Cutibacterium acnes*) in acne. *F1000Research*, *7*, F1000 Faculty Rev-1953. https://doi.org/10.12688/f1000research.15659.1

Accessed via the NCBI database. This article explains the effect of antibiotics on *Cutibacterium acnes* as well as the other commensal and pathogenic bacteria part of the skin microbiome.

Spittaels, K. J., Ongena, R., Zouboulis, C. C., Crabbé, A., & Coenye, T. (2020). *Cutibacterium acnes* Phylotype I and II Strains Interact Differently With Human Skin Cells. *Frontiers in cellular and infection microbiology*, *10*, 575164. https://doi.org/10.3389/fcimb.2020.575164

Accessed via PubMed. This article provides a succinct justification of the co-culturing methods utilized in this experiment. It is particularly useful because it involves the co-culture of *Cutibacterium acnes*.

Tsai, W. H., Chou, C. H., Chiang, Y. J., Lin, C. G., & Lee, C. H. 2021. Regulatory effects of *Lactobacillus plantarum*-GMNL6 on human skin health by improving skin microbiome. *International journal of medical sciences*, *18*(5), 1114–1120. https://doi.org/10.7150/ijms.51545

Accessed via the NCBI database. Discussed is *L. plantarum* and the inhibitory effects on *C. acnes* growth. Specific biochemical mechanisms are thoroughly discussed between both bacteria as well as *L. plantarum*'s beneficial effects on skin.

Xu, H., & Li, H. 2019. Acne, the Skin Microbiome, and Antibiotic Treatment. *American journal of clinical dermatology*, *20*(3), 335–344. https://doi.org/10.1007/s40257-018-00417-3

Accessed via the NCBI database. This article summarizes the effects of antibiotic treatments for acne, the consequences of antibiotic resistance in a clinical sense, and looks at recent studies pertaining to the composition of the skin microbiome.