Name(s)
Jamil S. Ahmad

Project Number
S1601

Project Title
Knock Your Socks Off: Developing Nanocomposites from Socks for Versatile Water Disinfection

Abstract

Objectives/Goals
Water borne illnesses continue to proliferate in developing countries and areas without reliable water infrastructures. Many emerging materials have fallen short of practical criteria for water treatment because they are expensive, require special resources, or are not developed in an "eco-friendly" manner. My research aims to develop a filter that could disinfect water effectively using relatively common materials and a simple development process.

Methods/Materials
Recycled cotton antimicrobial socks were utilized, which commonly use nanosilver to inhibit bacterial growth in limited amounts. These relatively cheap cotton socks were paired with a coating of Reduced Graphene Oxide for synergistic improvement of antimicrobial effectiveness and increased long-term use. Graphene Oxide was developed using a modified Hummers' Method (from Graphite) and immobilized it onto cotton fabric by a dip-dry process. Then, the rGO was fixed onto the cotton textile by heating to 160 Celsius for 15 minutes. The antimicrobial properties of 3 concentrations of textile were evaluated using untreated water samples from San Joaquin River. Samples were treated at increments of 1, 5, and 10 minutes of immersion of the material. The percent reduction in the Most Probable Number (MPN) of total coliform per 100ml was evaluated based on two samples before and after each run. Silver leaching was also analyzed by measuring silver in ppb in water samples before and after each run. Control samples of the water were also evaluated for total metals (by ICP-MS analysis) and total coliform to corroborate the data.

Results
In a proof of concept run, nanosilver decorated rGO composite had a higher inactivation of total coliform than nanosilver in isolation. Overall, the material showed promising antimicrobial efficacy around log 1 to log 2 reductions in total coliform. At the highest concentration interval for 10 minutes, the material achieved >97% bacterial inactivation. Silver leaching was below the detectable limit of .53 ppb, and is safe by EPA secondary drinking water standards.

Conclusions/Discussion
This material has shown promising effectiveness in bacterial disinfection in water and has possible applications in easy emergency water treatment in areas of high need.

Summary Statement
I made socks that are coated with a composite of nanomaterials that safely and effectively disinfects bacteria in water

Help Received
I researched, planned, performed background work (e.g. sampling), and perfromed the experiment myself. I received some help from AAPL and BSK Labs in Fresno for the analysis of metals and total coliform.

Name(s)
Sabrina Asefi; Natalie Imeshev

Project Number
S1602

Project Title
Human Health: Quantitative Comparative Analysis of Lactobacillus Colony Forming Units in Probiotic Supplements

Abstract

Objectives/Goals
The objective was to compare the number of colony forming units (CFU) in differently priced probiotic supplements. Measured CFU number and growth rate were compared to the advertised ones. The overall goal was to determine which probiotic is best for the person to consume, based on CFU, price, and growth rate.

Methods/Materials
The independent variable was the five different probiotic supplements, ranging in price (UP4, Nature Way, Target, Culturelle, and Albertsons). The dependent variable was the number of CFU that grew on MRS agar plates for each supplement. Clinically tested Culturelle Daily Probiotic Formula was used as a positive control. Five probiotic supplements were plated in a series of five logarithmic dilutions. The number of CFU was counted per plate. Each supplement was tested five times. The plates were dyed with a Gram Staining kit. All was done in a sterile indoor environment.

Results
The average number of detected CFU per one pill of supplement is as follows: UP4 - 10^5, Nature Way - 10^9, Target - 10^7, Culturelle - 10^9, and Albertsons - 10^2. All tested supplements contained Lactobacilli and Bifidobacterium, with no detectable contamination. Different probiotics grew at different rates - UP4 strains grew twice as fast as Albertsons.

Conclusions/Discussion
The data showed significant differences in probiotic concentration between different supplements. These differences cannot be explained by measurement error or variability between samples of a supplement. The range between individual samples is within two magnitudes; the range between different supplements is within seven magnitudes, significantly bigger than the measured variability.
Price has little correlation with the number of CFU in a supplement. Although the cheapest supplement had the fewest CFU, the most expensive supplement did not have the most CFU. Culturelle and Nature Way supplements demonstrated the best value (probiotic bacteria per dollar).
The growth rate showed the supplement's vitality. UP4 grew fastest, so its bacteria were most active. A consumer should seek to buy an active probiotic supplement.
Each tested supplement contained fewer CFU than advertised. The current labeling system of probiotic supplements does not provide sufficient information for a consumer. This data highlights the lack of clear labeling guidelines for probiotic supplements.

Summary Statement
Probiotic supplements, sold over the counter to promote human health, demonstrated remarkably different concentrations of Lactobacillus, despite similar advertisement labels.

Help Received
None. We designed, performed, and analyzed the experiments ourselves.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Lesley Bedolla; Daniela Flores; Vincent Wongsuchit

Project Number
S1603

Project Title
Healing the World One Shrimp at a Time

Abstract

Objectives/Goals
With the current wildfire and injuries caused to animals, there has been an increase interest for a suitable Band-Aid that can be used to facilitate would healing due to burns. The feasibility of using sterilized tilapia skin has been used to facilitate burnt paws on bears. Such medical treatment has facilitated the healing time. Sterilized tilapia skin is used because is bio-medically compatible if eaten by accident. Sterilized tilapia skin must be stitched onto the injured burnt paws of bear to facilitate healing [1]. The search for suitable replacement has led to polymerized chitosan as a suitable replacement since it is made from shrimp shell and claims to inhibit bacterial growth.

Methods/Materials
I. Polymerizing chitin.
II. Culturing and isolating bacteria for testing.
III. Testing polymerized chitosan on bacteria.

Results
Results are captured in the form of pictures taken with camera. Each pictuure has detail descriptions to what was collected and what is shown.

Conclusions/Discussion
We surmise that polymerized chitosan is effective in preventing bacteria from growing based on our results. Preliminary results would suggest polymerized chitosan is a viable replacement for tilapia skin to help facilitate healing. It would be interesting to see if adding chitosan to liquid medium instead of solid medium can also prevent microbial growth. Further development and testing is also needed to investigate the properties of polymerized chitosan on skin cuts\burns.

Summary Statement
Project intent is to develop a band-aid from shrimp shell that inhibits growth of bacteria.

Help Received
Louis Luu, our science advisor guided us to design a band-aid and test it using the scientific process.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Trevor Cambron; Natalie Owens

Project Number
S1604

Project Title
Trails, Soil, and SOD

Abstract

Objectives/Goals
We are studying Phytophthora ramorum, a water mold which is killing tanoaks and causing a foliar infection on Bay laurels in Santa Cruz County. Sudden Oak Death threatens the survival of oaks, an important keystone species, and creates a significant fire danger because of the large amounts of dead trees. The pathogen produces infective spores in wet weather, which are then spread through wind-driven rain, runoff, plant material, and soil causing the disease Sudden Oak Death (SOD). In last years project, we noticed that most of the infected trees behind San Lorenzo Valley High School were close to a small trail, and this year we wanted to test the hypothesis that infections are indeed closer to the trail than deeper into the forest.

Methods/Materials
To test whether infections were more common closer to the trail, we laid out a 100 meter tape along the trail and laid a perpendicular transect at 5 randomly selected distances. We then collected tanoak leaf samples at 0, 5, 10, 50, and 100 meters from the trail. After testing with Agdia Immunostrips, culturing the leaf samples on VARP media given to us from UC Berkeley and microscopically identifying sporangia or chlamydospores, we observed a greater tanoak infection rate closer to the trail. Additionally, we re-evaluated our plot from last year, by measuring each of the 25 marked tanoaks' distance from the nearest trail, finding a more significant infection rate closer to the trail. Lastly, to test soil for P. ramorum we used aqueous leaf baiting. We put soil samples and distilled water in a bag and floated uninfected Bay laurel and Camellia leaves, which we then tested with Immunostrips.

Results
Therefore, our tests have supported our hypothesis that Sudden Oak Death infections are more common closer to the trail. We have also detected P. ramorum in the soil along and near our trail using aqueous leaf baiting.

Conclusions/Discussion
Our results show that Sudden Oak Death infections are more common closer to the trail. To prevent the further spread of the disease, we plan to construct a boot washing sanitation station at the trailhead behind our school and implement a public education program for its use. The boot wash station should help prevent pedestrians from taking the pathogen from the forest and infecting other trees

Summary Statement
In our project, we found that Sudden Oak Death infections are more common closer to trail than deeper in the forest and that the pathogen is present along the trail behind SLVHS; to limit the spread we built a boot washing station

Help Received
Dr. Michael Loik helped us to find the direction of our project and answered many logistical questions. Douglas Schmidt, Tina Popenuck, and Laura Sims at UC Berkeley donated culturing materials, showed us procedures and their labs.

Name(s)

Melanie Cervantes; Ana-Sofia Munoz; Samantha Redline

Project Number

S1605

Project Title

Testing and Comparing the Ability of Different Types of Cutting Boards to Withstand Bacterial Growth

Abstract

Objectives/Goals

The objective of this project is to determine which types of cutting board, out of four different materials, constitute the least and most bacterial growth.

Methods/Materials

6 metal cutting boards, 6 wooden cutting boards, 6 plastic cutting boards, 6 bamboo cutting boards, raw chicken, 1.5 L of LB broth, 130 LB agar plates, 130 disposable 10 mL test tubes, knives, biosafety cabinet, incubator. Collected bacteria from cutting boards after use, determined and compared the concentration of bacterial growth from each board.

Results

The average concentration for each type of cutting board, derived using the 1:1000 serial dilution direct plate count, was used to determine which cutting board material harbored the most and least bacterial. Our results revealed that metal cutting boards produced the least bacterial growth concentration, with an average range of 0 - 8333.333, while wooden cutting boards produced the most, with an average range of 450.333 - 397,833.333.

Conclusions/Discussion

After comparing the average concentration of bacteria for each type of cutting board, it was revealed that metal produced the least bacterial growth, and wood produced the most. Following metal, plastic produced the second least bacterial growth, followed by bamboo. It can be concluded from this that in general, it is better to use metal or plastic cutting boards in the kitchen as they constitute less bacterial growth, even after being left out for a period of time.

Summary Statement

Our group collected and compared the bacterial growth concentration of different types of cutting boards to determine that metal produced the least bacterial growth, while wood produced the most.

Help Received

Our group#s biomedical science teacher and project advisor, Mr. Han, taught us how to make the media used in the project (LB broth and LB agar), how to determine concentration of bacteria using serial dilutions, and provided us with the materials and tools needed to carry out the project.

Name(s)
Seung Ah Annabelle Choi

Project Number
S1606

Project Title
Modeling a Bioluminescent LED that Converts the Light of Luminescent Bactera to Electricity via Dye-Sensitized Plate

Abstract

Objectives/Goals
The problem with current similar products, such as bioluminescent lamps is that it is made by simply putting luminescent bacteria inside a container with food. Thus, it is not very applicable because the light is dark and colored; one can only use it as night or party light. My goal is to model a bio-luminescent LED (bLED) that is more applicable than the currently existing bio-luminescent products by combining Photobacterium Phosphoreum with a dye-sensitized solar cell(DSSC). I also aim to enhance the light of the bLED by optimizing culture conditions of the luminescent bacteria and the DSSC.

Methods/Materials
First, I determined optimized culture conditions of Photobacterium Phosphoreum regarding Br concentration (Belousov-Zhabotinsky reaction) and the number of carbon nanotubes (Rayleigh scattering) by measuring the growth rate of bacteria after each reaction using a spectrophotometer. Second, I increased the efficiency of the DSSC by changing the dye and the amount of material used in the current DSSC (Gratzel Cell). Last, I quantified the efficiency by calculating how much power[V] the final bLED can save.

Results
At first, I decided to use the enzyme reaction as the light source, but could not do so due to instability, so I changed my light source to the bacteria. I determined that an optimal Br concentration and amount of carbon nanotubes exists for culturing conditions. I figured the light absorbance from the DSSC is highest when a small amount of sulfuric acid and starch is used, and the paste is not too thick or viscous. The results were put together to make a finalized bLED. The brightness was 89.5[lx], and the power saved for 30 LEDs was 58.2[W]. The error was 0.11[W].

Conclusions/Discussion
The bLED can emit continuous light because the DSSC can re-emit energy as brighter light when necessary. Theoretically, it also can be an indefinite cycle if the growth rate of bacteria is controlled properly. Thus, to maintain the growth rate I found optimal culturing conditions that is not previously determined. In the future I will attempt to minimize project constraints and do correlated research to increase the efficiency of DSSC. If I do succeed initiating the luciferin-luciferase reaction, I will pursue my goal to model a bioluminescent LED in vitro by determining optical conditions for firefly luciferase enzyme.

Summary Statement
I designed a Bioluminescent LED that converts the luminescent light of Photobacterium Phosphoreum to electricity via optimized dye-sensitized plates of optimum bacteria culture conditions (regarding B-Z reaction and Rayleigh Scattering).

Help Received
I designed the experiments methods myself after internet research. All experimentation was done under my mentor's supervision. My school biology teacher reviewed the results.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)

Daniel Feng

Project Number

S1607

Project Title

Discovering Antibacterial Properties of "Old" Cultural Herbs Using a New Simulation-Aided Approach

Abstract

Objectives/Goals

The identification of new antibiotics and new methods for antibiotic testing can fight the antibiotic resistance crisis. The goals of this project are to (1) find antibacterial effects among traditional cultural herbs, and (2) develop a new and efficient approach to quantify the antibacterial strength of these herbs and identify synergistic combinations.

Methods/Materials

I identified promising antibacterial activity in traditional herbs from Chinese and Southern Californian Native American cultures. By combining experimental data with a computer simulation, I developed a new method to quantify antibacterial strength and synergies. First, I tested 13 traditional herbs to identify those with the most promising antibacterial properties. Aqueous extracts from them were tested against E. coli using a disk diffusion assay. I then wrote a Python computer program to simulate the diffusion of extracts on a petri dish. By combining this simulation with experimental data, I found the MIC (Minimum Inhibitory Concentration) of each. Lastly, I tested the antibacterial strength of herb combinations by pairing a double-disk diffusion assay with my simulation. This new method achieves in one assay what would require ~64-100 separate tests using conventional methods.

Results

Of the 13 herbs tested individually, Huang Lian, Huang Qin, Ishwish, and garlic showed strong signs of antibacterial activity. The identification of Ishwish is exciting, as the antibacterial properties of this Native American herb have never been determined previously in the literature. Huang Lian and Huang Qin had the strongest effect, with MICs at 4.8 mg (herb)/mL. Other antibacterial herbs had MICs ranging from 9.0 to 31.0 mg/mL. Finally, I found that all tested combinations had FICs (Fractional Inhibitory Concentrations) ranging from 1 to 2.5, classifying all of them as "indifferent" or "additive."

Conclusions/Discussion

Overall, I identified several cultural medicinal herbs with antibacterial properties, with Ishwish being a new result never recorded before. I then quantified the antibacterial strength of these herbs and combinations of herbs by developing a novel method that quantifies the antibacterial strength of a drug with much more efficiency than conventional methods. With further research, these herbs and this simulation aided-approach may be used to find new medicines to fight against antibiotic-resistant bacteria.

Summary Statement

This project identified traditional herbal medicines with antibacterial properties and developed a novel, very low-resource method to quantify the antibacterial strength of substances and find synergistic combinations.

Help Received

I received help in bacteria culturing and conducted disk-diffusion assays at UC Irvine under the supervision of graduate student Tae Il Kim. Thanks to the UCI Arboretum and the "Tongva Hidden Garden" (Pitzer College) for permission to gather Native American plants.

Name(s)
Chen E. Filler

Project Number
S1608

Project Title
The Role of Tetraspanins in the Uptake of the Fungus Candida albicans by Host Cells

Abstract

Objectives/Goals
My hypothesis is that specific endothelial cell membrane proteins called tetraspanins are necessary for C. albicans to invade endothelial cells, and my goal was to discover which tetraspanins were important for invasion.

Methods/Materials
The HUVEC-TERT endothelial cell line was grown in tissue culture. The tetraspanins, CD9, CD63, and CD151 were detected by indirect immuno-fluorescence using specific mouse monoclonal antibodies. Each mouse antibody was detected with a fluorescent labeled goat-anti mouse antibody. Internalized organisms were identified by staining the endothelial cells with Alexafluor-labeled phalloidin which labels the endothelial cell actin microfilaments that are respon-sible for the endocytosis of the organism. The cells were imaged by confocal microscopy. The capacity of the anti-tetraspanin antibodies to inhibit the uptake of C. albicans by endothelial cells was determined using a differential fluorescence assay.

Results
By confocal microscopy, it was found that all three tetraspanins, CD9, CD63, and CD151, accumulated around C. albicans hyphae that were invading endothelial cells. Using blocking monoclonal antibodies, it was determined that only CD9 and CD63, but not CD151 were required for C. albicans to invade endothelial cells.

Conclusions/Discussion
The results indicate that the tetraspanins, CD9 and CD63 are required for C. albicans to invade endothelial cells, thus supporting my hypothesis. Blocking CD9 or CD63 is a potential approach to prevent C. albicans infections in patients.

Summary Statement
I found that the tetraspanins CD9 and CD63 were required for endothelial cells to endocytose Candida albicans.

Help Received
Dr. Hong Liu helped me design the experiments and taught me the experimental techniques. I performed all the experiments by myself.

Name(s)
Alicia N. Hans

Project Number
S1609

Project Title
Fertilizer vs. Fungi, Part II: How Nitrogen Fertilizers Affect Beneficial Mycorrhizal Fungi

Abstract

Objectives/Goals
The objective of this study was to determine whether exposure to nitrogen fertilizer will decrease the number of beneficial mycorrhizal fungi that grow into the root systems of California grassland plants. The fungi assist their host plants in water and nutrient uptake and provide protection from pathogens.

Methods/Materials
Collected experimental and control soil samples, extracted and rinsed plant roots, stained plant roots using 2.5% potassium hydroxide, 1% hydrochloric acid, an acidic glycerol/trypan blue mixture, and acidic glycerol. Made permanent slides and counted the fungi using a compound light microscope. Extracted nitrate from soil using 2.5% potassium chloride, then performed nitrate tests using cadmium powder and a nitrate testing kit.

Results
I counted beneficial mycorrhizal fungi for plant roots with and without exposure to nitrogen fertilizer. The plant roots not exposed to fertilizer showed more mycorrhizal fungi than those exposed to fertilizer. Nitrate levels in the soil with and without added nitrogen fertilizer were tested. The soil with fertilizer added had a higher level of nitrate than the soil without fertilizer added.

Conclusions/Discussion
The plant roots exposed to nitrogen fertilizer had fewer beneficial mycorrhizal fungi than those not exposed to nitrogen fertilizer. I concluded that nitrogen fertilizers can lower the number of beneficial mycorrhizal fungi in plant root systems. This indicates a potential harmful side effect of the use of chemical fertilizers.

Summary Statement
I found that the addition of nitrogen fertilizer can lower the number of beneficial fungi in plant root systems.

Help Received
Dr. Kathleen Treseder of the University of California, Irvine, allowed me to work in her laboratory, provided all the materials and equipment, and explained all the procedures.

Name(s)
Philippe Hansen-Estruch

Project Number
S1610

Project Title
A Microbiome Approach to Treat Galactosemia, a Life Threatening Genetic Disorder

Abstract

Objectives/Goals
There are no drugs to treat galactosemia, a life-threatening genetic disease in which patients can't metabolize galactose, a sugar found in food. As a result, galactose builds up to toxic levels in the blood and organs causing serious brain and liver damage and intellectual impairment. The only approach to manage galactosemia is a highly restrictive low-galactose diet which is not sustainable overtime since most foods like milk, fruits, and vegetables contain galactose. Therefore, there is a need for a long-term cost-effective solution. The aim of this study was to develop a yeast strain able to detoxify galactose in the gut before its absorption by intestinal cells and its toxic accumulation in blood.

Methods/Materials
The galactose degradation capabilities of multiple yeast strains was evaluated by measuring (1) their growth kinetics on galactose containing medium and (2) the remaining galactose concentration in the spent medium using an enzymatic test. The performance of the lead candidate, designated Y10, was further improved by adaptive evolution. Its stability/survival rate to harsh gut environment was investigated by exposure to simulated gastric and simulated intestine fluids in presence of enzyme/low pH. Y10's ability to establish itself in the gut was evaluated by measuring its adherence to human intestinal cells.

Results
We successfully developed a yeast strain Y10 by adaptive evolution which can degrade galactose in the gut environment. Y10 degraded by 50% in just 2 hours the galactose present in milk, food with the highest amount of galactose (2.5 g/100 mL). Y10 survived simulated gastrointestinal conditions and it also exhibited adherence to human intestinal cells suggesting that it can establish itself where it needs to be most active. A dose of 10e9 CFU was estimated to be sufficient for delivery of viable and active yeast cells to the gastro-intestinal tract; this dose can be achieved with current oral delivery formulation technologies.

Conclusions/Discussion
Yeast strain Y10 developed in this study could potentially be used to treat patients allowing them to ingest a more normal galactose-containing diet and minimizing the onset of symptoms associated with galactosemia.

Summary Statement
I created an efficient yeast strain to detoxify galactose in food for patients with galactosemia.

Help Received
I designed the experiments and created the yeast strain. My Science Fair coordinator teacher, Mr. Haas, supported me with encouragement. I used a bench at the laboratory of Vetica Labs to perform the experiments. I connected with the Rare Science organization to network with the rare disease community.

Name(s)
Vivek V. Kamarshi

Project Number
S1611

Project Title
Tacrolimus-Induced Changes in Fusogenicity of Varicella Zoster Virus

Abstract

Objectives/Goals

Varicella Zoster Virus (VZV) causes chicken pox and shingles. Cells infected with VZV undergo cell-to-cell membrane fusion mediated by viral fusion proteins. The rate of cell-cell fusion has been found important to VZV pathology; increased fusion hurts virus propagation.

In a screening assay, the drug tacrolimus increased fusion by 300%. My project: Through which cellular pathways does tacrolimus increase cell-cell fusion?

Methods/Materials

To quantify fusion, a cell-based model of virus infection: viral fusion proteins are transiently expressed in reporter melanoma cells, which glow upon fusing.

Tacrolimus and other "macrolide" drugs bind FKBP proteins in the cell. This binding inhibits FKBPs' natural activity, but FKBP-drug complex also has cellular interactions. I used a shRNA system to knock down FKBP1A in melanoma cells. I generated three different shRNA cell lines, with 95%, 82%, and 88% decreases in cellular FKBP1A levels.

Results

I found that in the absence of VZV fusion proteins, no macrolide caused cell fusion. Tacrolimus has a dose-dependent effect on fusion: 10uM drug causes fusion increase ($P<0.0001$), 0.1uM drug has an insignificant effect. Thus, tacrolimus' effect on fusion occurs through clinically relevant pathways. Out of the macrolides, drugs tacrolimus and pimecrolimus elevate fusion ($P<0.0001$ at 2.5 and 5uM drug), while everolimus and sirolimus decrease it ($P=0.0229$, 0.0581 at 1.25uM). Decrease of cellular FKBP1A correlated with less elevated cell-cell fusion from pimecrolimus. The drug cyclosporin increased fusion ($P=0.001$ at 2.5uM).

Conclusions/Discussion

Increased fusion due to tacrolimus-related drugs was shown to depend on presence of FKBP. Therefore, the downstream interactions of the FKBP-macrolide complex result in the observed change in fusion.

Note: tacrolimus or pimecrolimus complexes inhibit Calcineurin phosphatase [CaN] and everolimus or sirolimus complexes inhibit mTOR kinase. Thus, the findings that only tacrolimus and pimecrolimus increased fusion, and that (alternate-mechanism) CaN inhibitor cyclosporin also increased fusion, suggest that inhibition of CaN increases cell-cell fusion.

As in vivo immunosuppresants, CaN-inhibitors are not ideal anti-VZV drugs. However, if the fusion-related downstream step of this pathway (potentially one of the proteins which CaN dephosphorylates) can be identified, this would create the target for a new fusion-increasing, anti-VZV drug.

Summary Statement

I validated that tacrolimus mediates an increase in varicella zoster virus-caused cell-cell fusion, and traced the effect to inhibition of Calcineurin by a tacrolimus-protein complex; this creates a new target for anti-VZV drug research.

Help Received

I would like to thank Drs. Stefan Oliver and Marvin Sommer (Stanford Pediatrics Infectious Diseases, Arvin Lab), who allowed me to use their lab facilities and gave mentorship on experimental design and data analysis throughout the project. Thanks to Ms Renee Fallon for presentation advice.

Name(s)
Achyutha Kodavatikanti; Advait Thaploo

Project Number
S1612

Project Title
Saving Lives with Viruses: How Bacteriophages Can Kill Harmful E. coli Bacteria

Abstract

Objectives/Goals
The problem we sought to solve with this project is food poisoning caused by E. Coli bacteria. Our objective was to demonstrate how T4r bacteriophage can reduce the number of E. Coli cells in inoculated tryptic soy broth and beef broth.

Methods/Materials
Materials:
2-mL Tubes of Soft Agar (6), 9-mL dilution tubes (24), 1- x 0.01 mL sterile pipettes (24), Sterile Petri Dishes (12), Bacterial Incubator, 37°C
Beef Broth

Methods:
Day 1:
Start the E. coli Culture.
Day 2:
Add only E. Coli B to one of the tubes (Amount varies depending on dilution on next day)
Add E. Coli B and T4r Phage to one of the tubes (Amount varies depending on dilution next day)
Shake tubes and put them back in the incubator for use the next day.
Day 3:
Now start the phage dilutions.
Plaques should form 10-24 hours after plating.
Day 4:
Make a data table, with the dilutions you used in one of the columns.

Results
Our results show a 99.99% reduction in the number of E. Coli cells in tryptic soy broth and a 94.39% reduction in beef broth. This proves a strong correlation between the addition of T4r phage and reduction in the number of E. Coli cells.

Conclusions/Discussion
The findings of this project demonstrate that bacteriophages reduce the number of E. Coli colonies in inoculated beef broth by a factor of 94.39%. This affirms our hypothesis that the E. Coli population will diminish once the bacteriophage is added to the beef broth. Our results are highly applicable to process of meat sanitation; addition of phage to the meat would greatly reduce the chance of contamination.

Summary Statement
We showed that adding the T4r bacteriophage to E. Coli-inoculated beef broth reduced the number of E. Coli cells by 94.39%.

Help Received
My partner and I both came up with the idea, and got help from Dr. Srividhya Ramamoorthy, the Lab Manager at the Sacramento Regional County Sanitation District in order to complete the experiment and interpret our results.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Jillian M. Labador

Project Number
S1613

Project Title
CRISPR Interference Knockdown of Ampicillin Resistance Gene in Escherichia coli

Abstract

Objectives/Goals
Synthesis of a CRISPRi plasmid to silence the ampicillin resistance gene in Escherichia Coli.

Methods/Materials
Constructed a gRNA scaffold inside the CRISPRi plasmid encoding for a 20bp sequence of the ampicillin resistance gene, transformed E. coli with the plasmid through heat-shock, plated bacteria onto chloramphenicol LB agar plates (plasmid contained a chloramphenicol resistance gene), swabbed bacteria onto new LB agar plates and placed one ampicillin disc onto each plate, measured the zone of inhibition around each disc after a 24-hour growth period in incubator.

Results
The measurements of the zones of inhibition surrounding the ampicillin disc on the plate containing the transformed E. coli showed susceptibility to the antibiotic while there was no zone of inhibition on the E. coli not transformed with the CRISPRi plasmid which demonstrated a resistance to the antibiotic.

Conclusions/Discussion
The constructed CRISPRi plasmid showed efficacy to the suppression of the ampicillin resistance gene in Escherichia Coli. This demonstrated then value of utilizing CRISPR or CRISPRi in the plasmid form to modify/regulate gene sequences and expressions in organisms, including the repression of antimicrobial resistance genes in bacteria which can help treat infectious diseases with more efficiency.

Summary Statement
I constructed a CRISPRi plasmid that suppressed the ampicillin resistance gene in Escherichia Coli.

Help Received
The backbone of my plasmid was provided by addgene and the gRNA scaffold was synthesized at GeneWiz with my instructions. My biology teacher helped understand the basic mechanism and function of CRISPR.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Charlotta G. Lebedenko

Project Number
S1614

Project Title
Evaluating the Effects of Artificial Sweeteners and Caffeine on Human Gut Microbiota to Prevent Glucose Intolerance

Abstract

Objectives/Goals
Non-caloric artificial sweeteners (NAS) are among the most commonly used food additives in the world. These artificial sweeteners can actually lead to obesity indirectly by affecting the plethora of microbiota in the human gut and affecting glucose intolerance. Instead of testing on living subjects, this research uses an in-vitro approach in testing the direct effects of sweetener on the microbiota. In addition to NAS, caffeine is often consumed along with these additives. If NAS inhibits bacteria growth and caffeine promotes it, this research examines if the two food additives could possibly cancel out the effects of each other.

Methods/Materials
Using a standardized in-vitro digestion method, the sweeteners and caffeine are digested and then exposed to three types of bacteria found in abundance in the gut: Escherichia Coli, Staphylococcus Epidermidis, and Enterobacter Aerogenes. The growth curves of these cultures are plotted using absorption spectroscopy, and the curves are compared to a control curve. This experiment consists of nine experimental groups. The nine groups are as follows: control, sucrose, saccharin, sucralose, aspartame, two different concentrations of caffeine, a combination of aspartame and caffeine, and a combination of sucralose and caffeine. Each trial is done for each type of bacteria and repeated three times for statistical analysis.

Results
Aspartame increases bacteria growth for all three species while saccharin and sucralose inhibit growth. Caffeine consistently increases growth even with lower concentrations. When combined, aspartame and caffeine result in an even higher growth rate and the combination of sucralose and caffeine balance out bacteria growth towards the control. The standard deviations for each trial are plotted on the graphs as error bars and ANOVA tests were run on each set of data to assure statistical significance.

Conclusions/Discussion
The combination of aspartame and caffeine is so common in America, yet it could be causing significant changes to the composition of the gut. However, since the effects of sucralose and caffeine balance each other out, that combination could be less of a health hazard than previously assumed. Further studies on the effects of aspartame versus sucralose in the context of the entire mixture being consumed could help the food industry make wiser choices about their sweeteners.

Summary Statement
This research tests the effects of artificial sweeteners and caffeine on human gut bacteria to evaluate the negative changes in bacteria populations in order to prevent consumers from developing glucose intolerance and diabetes.

Help Received
The advanced science research class at Los Gatos High School taught by Catherine Messenger.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)

Christina Ngo; Nikki Nguyen; Christina Tran

Project Number

S1615

Project Title

Investigating the Effect of Makeup Primer Bases on the Growth of Staphylococcus epidermidis and Escherichia coli

Abstract

Objectives/Goals

The objective of this study is to determine which makeup primer base (oil, silicone and water) will cause an increased growth rate in bacteria (S.epidermidis and E.coli).

Methods/Materials

A single bacterial colony of S. epidermidis/E.coli was picked and placed into 2 separate test tubes containing LB broth. A 3rd tube was used as the control, containing only 2 mL of broth. The test tubes were incubated for an hour at 37 C. While the tubes were incubating, 6 different makeup primers were each spread onto 3 plates each. Distilled water was spread onto 3 plates and used as another control group. When the tubes finished incubating, the bacteria (S. epidermidis/E.coli) and control culture were spread onto 7 separate plates. Each culture was spread onto a different makeup primer/control plate once, The plates were incubated for 48 hours. After 48 hours, our data was quantified by counting colonies. The experiment was repeated 2 more times, for a total of 3 biological replicates and 63 plates.

Results

All the primers except for the Benefit Cosmetics (silicone-based) primer had approximately increased the S. epidermidis bacterial colony growth by 1,200-4,000 when compared to the control culture. All of the primers increased the amount of E. coli bacterial colony growth by approximately 200 colonies when compared to the control culture. The Physician's Formula primer (oil-based) grew the most colonies compared to other primers when cultured with both, S.epidermidis and E.coli. The primer grew 4,000 more colonies than the S.epidermidis control culture and 300 more colonies than the E.coli control culture.

Conclusions/Discussion

After identifying that the oil-based primers grew the most colonies of S. epidermidis and E. coli, our hypothesis was supported because it meant the oil-based primers produced more bacterial colonies than the water and oil based primers. We concluded that oil, whether it is found naturally on our face or derived from a plant, is a factor in bacterial growth because opportunistic bacteria like S.epidermidis and E.coli like to grow within oily conditions in order to easily cause inflammation/infection in our skin. Inflammation/infection in our skin occurs due to the overproduction of oil, so opportunistic bacteria like S.epidermidis and E.coli like to grow within these oily conditions.

Summary Statement

Our project was finding out which makeup primer base (oil, water and silicone) would produce the most bacterial growth when cultured with S.epidermidis and E.coli.

Help Received

We were mentored by a Stanford graduate student (Auora) that helped us turn our ideas into a testable research question.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Angelynn H. Nguyen

Project Number
S1616

Project Title
Determining if the Acidity of the Growth Medium Affects Candida albicans' Biofilm's Mechanism

Abstract

Objectives/Goals
Candida albicans have been recognized as the most prevalent fungal species in humans. When C. albicans form a biofilm they become resistant to typical antifungal treatments. This project is an exploration as to which concentration of antifungals, can effectively target and eliminate C. albicans in suspension and with a biofilm in an environment of pH 6 and pH 5.

Methods/Materials
During the suspension experiment, different concentrations of Amphotericin B and Clotrimazole were added to a solution of C. albicans and YPD media, with HCl acid added to four groups. One group was the negative control. The test tubes were placed on a shaker. Then the absorbance level of the fungus solution was determined. During the biofilm experiments, silicon were weighed then placed in a well dish. Next, C. albicans and YPD was added to the well dishes and treated with the conditions from part one. The well dishes were placed on a shaker, then the YPD media was extracted. The silicon was weighed again to determine the mass of the fungus. The absorbance level and the fungus weight was converted to number of C. albicans cells. The experiments were repeated five times to reduce random errors.

Results
After experimentation, it was determined that the fungus cells with the presence of a biofilm had a significantly larger amount of C. albicans cells in comparison to the fungus in suspension. Both tests show that for the 2 µL Clotrimazole condition, the environment of pH 6 was more effective, while the acidic environment was for the 20 µL condition. As for the Amphotericin B, at 2 µL, the acidic environment was more effective, while the antifungal at 20 µL was more effective in the environment of pH 6.

Conclusions/Discussion
The data indicates that Clotrimazole does not stimulate the ERG11 gene as expected, hence the antifungal would eliminate the cells. Amphotericin B more efficiently killed the C. albicans cells in decreased concentrations, which is possibly due to HCl degrading the cell's biofilm. Ultimately, Clotrimazole is more effective than Amphotericin B, therefore it is recommended to use Clotrimazole in the concentration of 2 µL or apply acid (vinegar) before adding the antifungal. This research could transform the field of science by providing a more efficient way of eliminating fungus, which would benefit many patients that suffer from fungus infections.

Summary Statement
This research tested how antimicrobials, specifically Amphotericin B and Clotrimazole, target Candida albicans in suspension and with the presence of a biofilm, in environments of pH 5 and pH 6.

Help Received
My mentor helped by supplying materials for the experiment and reviewing my conclusions.

Name(s)
Lekha Pillarisetti

Project Number
S1617

Project Title
Assessment of Effects of Nutrient Runoff Levels and Temperature on Accelerated Algal Growth and Eutrophication

Abstract

Objectives/Goals
Algal blooms cause major environmental damage. The bloom zones have increased rapidly in recent years. Total number of dead zones in the world went from 4 in 1910 to 400 in 2008 (last 100 in 20 years). They now cover 95,000 sq. miles of the water in world. Each of these zones is increasing in area - Gulf of Mexico dead zone, largest in the world, increased from 5000 sq. miles to 8000 sq. miles in 2 years and soon predicted to be 10,000 sq. miles. While factors that contribute to these blooms are known, cause of the accelerated growth is not fully known. My objective is to evaluate relative impact of different factors on algal growth to identify the factor(s) that are contributing to this accelerated algal growth.

Methods/Materials
4 different water bodies (lake and marine) were sampled for surface water using phytoplankton net. Samples were subject to different nutrient runoff levels simulated using 15.5% Nitrogen solution of H4CaN2O3 and 52% Phosphorous solution of KH2PO4 in a simulated environment. The samples were also subjected to different temperatures (20 & 22 deg C). Some samples were then treated with Alum to evaluate reduction in effect of nutrient runoffs. Algal growth was measured in all 144 samples using Colony Forming Unit (CFU) count, Surface Area and Biomass using optical density. A 1000x microscope was used to measure the algal cell sizes.

Results
Algal growth increased by 36%-107% at different runoffs and 44% with just temperature increase (2 deg C warmer). However, when there is both runoff and temperature increase the algal growth (as measured in CFU count) accelerated to 241% and 302% increase. This trend was seen in measures like surface area and biomass and also in water from all locations. When Alum was added to the water, the increase in growth was 120% and at higher Alum it was actually lower by 39%.

Conclusions/Discussion
Nutrient runoffs and temperature increase algal growth. But combined effect of these two factors is the key factor in the accelerated growth. Temperature change acts as a powerful stimulant and increases the nutrient effect on algae and phytoplankton growth by 3x. Reducing warming effect alone can greatly reduce algal growth even with runoffs. Also reversing the effect of nutrient runoff is possible through treatments. However, this study does not evaluate the impact of the additives on the marine life. This needs to be studied and evaluated in a follow up study.

Summary Statement
In a water body with nutrient runoffs, I showed that a temperature increase of 2 deg C acts as a catalyst that increases algal growth by 3 times.

Help Received
Ms.Amulya, Principal Microbiologist and Ms.Basu, biology teacher gave professional guidance on incubation techniques. Mr.Pillarisetti, parent, gave overall guidance and drove me to all locations. I did the sampling, experiments and analysis by myself.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Kate L. Popky

Project Number
S1618

Project Title
The Effects of Temperature on the Responsiveness of Escherichia coli to Ampicillin

Abstract

Objectives/Goals
The study will be conducted to evaluate how temperature affects E. Coli responsiveness to ampicillin. The key variable of this experiment is the temperature of the environment in which the E. Coli is incubated. Using the Bauer-Kirby Disk Susceptibility Test, the study results will suggest whether treating fever in the setting of a bacterial infection is helpful or harmful.

Methods/Materials
Four incubators were set to their assigned temperatures and E. coli was grown in petri dishes in the presence of ampicillin disks. A second round of incubation was then performed that followed the same procedures, using the surviving E. coli from each incubator.

Results
After the first round of incubation, the 37ºC petri dishes had an average zone of inhibition of 25mm, the 38ºC petri dishes had an average zone of 24.7mm, the 39ºC petri dishes had an average zone of 23mm, and the varied temperature (37-39ºC) petri dishes had an average zone of 23.3mm.
After the second round of incubation, the 37ºC petri dishes had an average zone of inhibition of 24mm, the 38ºC petri dishes had an average zone of 23.7mm, the 39ºC petri dishes had an average zone of 22.7mm, and the varied temperature (37-39ºC) petri dishes had an average zone of 23.3mm.

Conclusions/Discussion
The results do not support the original hypothesis that using ampicillin to treat E. Coli at the average human body temperature (37ºC) will allow for the least E. Coli growth, compared to E. Coli incubated at higher temperatures. Although the antibiotic was less effective in the presence of higher temperatures, its effectiveness did not decline in the second round of incubation as much as in the 37ºC environment. In the varied temperature environment, designed to simulate the varying body temperature of a person with fever, the E. coli's level of responsiveness to ampicillin remained the same over the two stages of incubation. At higher temperatures, the E. coli was less responsive to ampicillin than in the incubators with lower temperatures. However, the responsiveness remained more constant at these higher temperatures. The environment that had the least decline in responsiveness between the two stages was in the incubator with a varied temperature.

Summary Statement
Through measuring the zones of inhibition of petri dishes of E. coli placed under different temperatures, I found that having a fever could be beneficial in the presence of an E. coli infection.

Help Received
My biology teacher, Mrs. Kiest, helped to review my writing, and my dad helped me with placing E. coli onto petri dishes.

CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

Name(s)
Yolanda A. Shen

Project Number
S1619

Project Title
Using Penium margaritaceum to Investigate Cytokinesis Conservation

Abstract

Objectives/Goals
I chemically inhibited the cytokinesis of algae to characterize both the growth and the cell wall components of the algae.

Methods/Materials
Applied Endosidin 7 (chemical) to P. margaritaceum, then utilized monoclonal antibody JIM5 and secondary antibody Alexafluor 488 to label. Observed under confocal microscope and compared to Dimethyl sulfoxide (DMSO; control)-treated algae. Repeated process with secondary antibody TRITC.

Results
The pectin labeled by JIM5 allowed for the visualization of the isthmus zone of the algae. The isthmus zone of the ES7 treated algae was greater than that of the DMSO by 150%. JIM5 pectin specks were still found in the isthmus zone, where there should be no immunofluorescence. This experiment resulted in no correlation with algae growth and chemical treatment, despite the quantification that the DMSO treated algae grew more than the experimental group by 47%.

Conclusions/Discussion
ES7 inhibits P. margaritaceum cytokinesis, as the elongated isthmus is due to the cells' bilateral expansion before attempting to divide. The immunofluorescence in the isthmus may indicate the algae's attempt to restabilize the forming cell plate to counter ES7's effects. Cell elongation cannot be correlated to treatment due to slightly de-synchronized cell division cycles of the algae. This experiment will contribute to further understanding of the evolution of the cell wall (ES7 used in complex land plants before) as well as the development of more efficient biofuel.

Summary Statement
I chemically inhibited the cytokinesis of algae to characterize both the growth and the cell wall components of the algae.

Help Received
I performed my experiment in the Drakakaki Lab at UC Davis under the guidance of graduate student Destiny Davis.

Name(s)
Eric T. Vuong

Project Number
S1620

Project Title
Assessing the Efficacy of Phytochemical Therapy for Biofilm Inhibition in vitro

Abstract

Objectives/Goals
Biofilms infecting orthopedic implants, catheters, and pacemakers pose a significant healthcare risk due to their resistance to antibiotics and phagocytosis. Every year, 1.7 million Americans acquire biofilm-related infections, and 100,000 die as a result. Currently, promising preventative measures include quorum sensing disruption and metal-coated implants. However, these measures may be hazardous to patients. Impregnating medical implants with non-toxic phytochemicals may be a viable solution for inhibiting or preventing biofilm formation following insertion. The objective of this project is to determine the efficacy of salicylic acid, methyl salicylate, thymol, and silver nitrate for inhibition of Staphylococcus epidermidis and Escherichia coli biofilms, which develop postoperatively at surgical sites.

Methods/Materials
Biofilms were grown in a 24-well microtiter plate. A crystal violet assay was used to determine the % biofilm inhibition of each chemical when 10, 25, 50, and 100 microliters were added. Silver nitrate was tested to compare the effectiveness of silver-coated implants with the phytochemicals. 3 trials were conducted for both species.

Results
In both species, thymol was the most effective phytochemical for inhibiting biofilm formation at higher concentrations, while methyl salicylate was more effective at lower concentrations. At all concentrations, silver nitrate inhibited the most growth. When 100 microliters of each respective chemical were added to the S. epidermidis wells, silver nitrate inhibited 84.9% biofilm growth, methyl salicylate inhibited 70.2%, thymol inhibited 75.7%, and salicylic acid inhibited 45%.

Conclusions/Discussion
The results indicate that methyl salicylate and thymol are effective in preventing biofilm growth when compared to silver nitrate. Although silver nitrate inhibited the most growth, its use is impractical due to the potential toxicity of elemental silver. Thus, phytochemical-impregnated implants are a viable alternative to silver-coated implants, which may be toxic to patients.

Summary Statement
The effects of salicylic acid, methyl salicylate, and thymol on biofilm formation were evaluated in comparison with silver nitrate to identify a solution for preventing postoperative biofilm growth.

Help Received
Clinical lab scientist Kimmie Long allowed me to conduct the experiment at the Antelope Valley Hospital laboratory. However, I designed and conducted the experiment independently. Only the incubator was used at the lab. My chemistry teacher lent me the LabQuest, temperature probe, and colorimeter.

Name(s)
Katherine F. Zhang

Project Number
S1621

Project Title
Deep Learning Analysis of Human Gut Microbiome Metagenomic Data with Applications in Geolocation and Disease Prediction

Abstract

Objectives/Goals
Previous studies of microbiome metagenomic datasets have relied on linear models (PCA, SVM, and RF, etc) and known species and biomarkers in reference databases and ignored about 50% of the reads. I used deep learning directly on DNA kmer abundances to study the human gut microbiomes.

Methods/Materials
DNA sequences from 1030 human microbiomes in four large microbiome metagenomic datasets (HMP, MetaHit, T2D, RA) were first preprocessed into 5-mer counts per sample and then L1 normalized into relative abundances, which were used as features for both unsupervised and supervised learning.

Autoencoder was used on HMP to find whether there is nonlinear structure in the kmer data by comparing best nonlinear model against the linear model.

For supervised learning, the kmer relative abundances were normalized to have zero mean and unit std across training samples. Then, autoencoder was used to pretrain the model, after which its decoding layers were replaced by the final softmax layer for classifying the microbiomes by continent, country, or diseased/healthy.

Results
Analysis of PCA and autoencoder modeling on the microbiome data clearly suggests that there is nonlinear structure. Additionally, supervised learning showed that using only DNA kmer relative abundances as features, we can predict with near-perfect Area Under the Curve (AUC) the continent (0.998) and country (0.989) origins of the microbiome samples while it was previously thought that differentiating between American and European samples would be difficult. The same supervised learning techniques also predicted IBD (0.947) and T2D (0.759) with AUCs exceeding state-of- the-art published results.

Conclusions/Discussion
Using deep learning directly on raw DNA kmer abundances in the microbiome is a very effective approach for studying the human microbiomes, and it can potentially enable scientists to take advantage of unknown organisms as well as new genotypes in the microbiome.

Summary Statement
I showed that deep learning on human gut microbiome metagenomic DNA kmers provided better predictions on both geolocation and diseases such as IBD and T2D than previously published results, which used only linear models on known organisms.

Help Received
Dr. Garud (UCSF Gladstone), Dr. Greenblum (Stanford Medicine), and Professor Pollard (UCSF Gladstone) provided the original ideas and advice. I implemented all the Python code and performed all the experiments as well as analysis.