

Name(s)

Yusuf Ahmad

Project Number

S1601

Project Title

Citrus in Jeopardy: Phase I, Determination of Presence and Typology of CTV Using ELISA, DTBIA, PCR, and Real-Time PCR

Objectives/Goals

The purpose of this experiment is to ascertain the validity of the aforementioned lab techniques in assessing the presence as well as the typology of Citrus Tristeza Virus strains among suspect plants.

Methods/Materials

Plant tissue gathered from 8 suspect samples along with a healthy control were homogenized and used throughout the experiments. ELISA used antibodies to capture viral antigen particles and substrate used for measurement of virus concentrations in a spectrophotometer to determine virus presence. The nitrocellulose membrane in DTBIA was treated with antibody and presence of virus was determined through a light microscope. PCR used the virus' coat protein cDNA (generated through a thermocycler machine) in gel electrophoresis with banding patterns used to indicate the presence of the virus. Real-time PCR consisted of the use of various probes to detect the presence of virus as well as to characterize the strain of the virus using the BioRad Lightcycler software to actively measure PCR.

Results

ELISA, DTBIA, PCR, and Real-Time PCR all showed matching results between plants tested positive for CTV in those infected with Real-Time PCR also characterizing different strains of the virus.

Conclusions/Discussion

ELISA, DTBIA, PCR, and Real-Time PCR were indicated to reliably test for CTV in infected plants with Real-Time PCR additionally characterizing the strain of the virus. Mild virus strains which were detected to be present in certain suspect plants using ELISA, DTBIA, and PCR were differentiated from harmful strains of the CTV virus using Real-Time PCR.

Summary Statement

ELISA, DTBIA, PCR, and Real-Time PCR all detected presence of the CTV virus with Real-Time PCR also differentiating between harmful and harmless strains of the virus.

Help Received

The procedure for all the aforementioned techniques were given by the USDA laboratory with lab equipment at the facility also being used. All the experiments were conducted under the supervision of Dr. Yokomi, Dr. Seleveraj, and Dr. Maheshwari.



Name(s)

Catherine Aitken; Dana Jian

Project Number

S1602

Project Title

The Effect of the Wavelength of Light on the Growth Rate of Cyanobacteria and Its Survival In a Martian Atmosphere

Objectives/Goals Abstract

The purpose of this experiment was to test in what wavelength the cyanobacteria Cylindrospermum (Substitute for Chroococcidiopsis) grows best and whether these wavelengths had an effect on the survival rate of cyanobacteria in a Martian atmosphere. The Martian atmosphere aspect was introduced because of a NASA statement that mentioned that Chroococcidiopsis could be important in the colonization of Mars.

Methods/Materials

The experiment consisted of two parts, Experiment A and Experiment B. Experiment A consisted of cell culture placed in incubators under six colored LED lights and incubated. After incubation, cell counts were recorded using a hemocytometer. Experiment B was a follow through of Experiment A that used the wavelength with the best growth rate to prolong the survival of the bacteria in a Martian atmosphere. The control was a culture left in Earth conditions while two other cultures (sunlight and LED) were placed in two airtight plexiglass cubes and introduced to a ratio of gases that matched the Martian atmosphere. Materials: Cell culture, Alga-Gro Medium, Erlenmeyer flasks, incubators, LED bulbs, microfuge tubes, microscope, hemocytometer, plexiglass sheets, and vacuum pumps.

Results

The results of Experiment A showed that the cyanobacteria samples exposed to longer wavelengths grew faster than samples placed under short wavelengths. The results of Experiment B showed that although the LED light increased initial survival compared to cell sample beneath the sunlight, this was followed by a rapid death due to lack of resources in the closed cubes. The results indicated a positive effect of longer wavelengths of light on growth rate, but that those same wavelengths reduced the survivability in a Martian atmosphere as it increased rate of decay.

Conclusions/Discussion

The results of Experiment A supported the hypothesis that predicted an increase in growth rate underneath longer wavelengths of light. The results of Experiment B opposed the hypothesis that predicted that the longer wavelengths would prolong the bacteria#s survival as the results showed that long wavelengths increased the rate of decay. The results fulfilled the objective in terms of obtaining data on the effects of different wavelengths on growth rate/survivability. The information furthers the knowledge of growth patterns in cyanobacteria and this could be used to prolong survival of cyanobacteria on Mars.

Summary Statement

We found, by measuring the cell counts over time, that longer wavelengths increase the growth rate of the cyanobacteria, but also increased the rate of death of the cyanobacteria in a Martian atmosphere.

Help Received

We used the support and guidance of Dr. Rebecca Zadroga at Hennepin County Medical Center in Minnesota, US. We also used the financial aid of the Jack Kent Cooke Foundation, who helped cover the cost of certain materials, including the Cylindrospermum cell culture.



Name(s)

Rohan Bhushan

Project Number

S1603

Project Title

Phototactic Analysis of the Effects of the Chemorepellent Nitrate on Taxis-Based Vertical Column Orientation in Protists

Objectives/Goals

Abstract

Nitrate pollution is a major issue around the world, and has contributed to much environmental damage. Many experiments have been conducted to explore the effects of nitrate on ecology, but none have fully explored the effects of nitrate on algae#s abilities. This experiment aimed to investigate the effects nitrate pollution has on algae#s motility to the surface of water, and the influence gravitaxis, phototaxis and chemotaxis has.

Methods/Materials

During my testing I analyzed three different variables: light, nitrate, and algal species, to properly isolate exactly which function of the algae the nitrate was impacting. Varying light helped isolate phototaxis, nitrate helped isolate chemotaxis, and the two different species of algae helped further display similar trends across species. I had a total of 12 tests, with three repetitions each. During my testing I isolated samples from the surface and depth of each vial and took two pictures at both 160x and 400x magnifications. These photos were later analyzed using an image analyzer called ImageJ, and these values were then substituted into two equations for cell concentration and size, which were developed by this project. I also measured glucose concentrations to analyze the algae#s dependence on glucose, therefore gaining insight into their dependence on photosynthesis as well.

Recults

Following the testing period, I analyzed my results. The main finding of this experiment was that due to nitrate concentrations, the algae tended to migrate deeper in the water. This was seen by comparing cell concentration values between tests with nitrate and without nitrate, and looking at surface and depth values. This migration to deeper levels also saw an increase in glucose metabolism, and concurrently, a decrease in photosynthesis. Tests without light also saw erratic behavior from cells, which led to the explanation that the nitrate was affecting gravitaxis as well, due to the fact that the cells were not able to migrate to the surface of the water in the absence of light.

Conclusions/Discussion

Nitrate is a major issue, and could be indirectly lowering carbon fixation rates by phytoplanktonic flagellates, which contribute to over ⅔ of the world#s carbon fixation. My results show what a huge issue excessive use of nitrate in agriculture is, and with recent rain causing severe runoff, this nitrate is leeching into our water sources and environment.

Summary Statement

This experiment isolated the effects of nitrate pollution on the ability of phytoplanktonic flagellates to maintain vertical column orientation for optimal photosynthetic output.

Help Received

Dr. Shannon Johnson and Dr. Josh Plant provided answers prior to experimentation for questions about methodology.



Name(s)

Titash Biswas

Project Number

S1604

Project Title

Pyrimidine Derivatives Conjugated to Gold Nanoparticles to Combat Antibiotic Resistance

Objectives/Goals

Abstract

Increased antibiotic resistance in bacteria has rendered many antibiotics useless; thus, the objective of this project was to develop a novel antimicrobial compound of pyrimidine derivatives conjugated to gold nanoparticles (AuNPs) to combat antibiotic resistance through varying mechanisms. Conjugated AuNPs were proposed to be self-therapeutic and to provide localized delivery of antibiotics. Combinations of the pyrimidine derivatives and common antibiotics may pose synergistic properties and demonstrate increased efficacies of the antibacterial agents.

Methods/Materials

Four pyrimidine derivatives with different functional groups attached to the rings and two antibiotics, ampicillin and gentamicin, were chosen for comparisons of antimicrobial properties against gram-negative Escherichia coli and gram-positive Bacillus subtilis. Each compound#s antimicrobial properties were determined by broth microdilution assays, which produced minimum inhibitory concentrations (MICs). Two pyrimidine derivatives with confirmed MICs were conjugated to 20nm gold nanoparticles using covalent conjugation chemistry and the conjugations were confirmed by spectrophotometry. In addition, the antibacterial properties of combinations of pyrimidine derivatives and antibiotics were analyzed using the fractional inhibitory concentration index.

Recults

Out of the four pyrimidine derivatives tested, the two with electron withdrawing groups, cytosine and 5-(4-chlorophenyl)pyrimidin-4-amine demonstrated strong antibacterial activity against E. coli and B. subtilis. After conjugation with the AuNPs, the two pyrimidines demonstrated enhanced efficacies against the bacteria and decreased MICs. Combinations of cytosine and the common antibiotics revealed synergistic relationships.

Conclusions/Discussion

The results of this project illustrated that the two pyrimidine derivatives with electron withdrawing functional groups possess antibacterial properties and, when conjugated to AuNPs, can deliver antibiotics more efficiently. The presence of a structure-activity and synergistic relationship between conjugated AuNPs and antibiotics was determined. These results can be utilized for new antibiotic design predictions in the long term battle against antibiotic resistant bacteria, a key issue in the healthcare industry.

Summary Statement

A compound of pyrimidine derivatives with electron withdrawing functional groups conjugated to gold nanoparticles is an effective antimicrobial agent to combat antibiotic resistance in bacteria.

Help Received

The experiment was conducted in TheLab Inc. and was supervised by Dr. Siddhartha Biswas.



Name(s)

Bronte N. Brazier

Project Number

S1605

Project Title

Azithromycin in Combination with Hamamelitannin as a Growth Inhibitor for Common Bacteria in Surgical Site Infection

Abstract

Objectives/Goals

The objective of this study is to see both the individual and synergistic antimicrobial effect of Azithromycin and Hamamelitannin on common bacteria in Surgical Site Infection. The bacteria labelled responsible for these infections include Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.

Methods/Materials

The Kirby-Bauer diffusion assay was used to see growth inhibition caused by both antibiotics at different concentrations combined and individually against S. epidermidis.

Results

The results indicate that by combining 1.0 micrograms Azithromycin/ mL H(2)O and 10 micrograms Hamamelitannin/ mL H(2)O, the growth of S. Epidermidis can be inhibited 25% or more effectively than either antibiotic at any concentration individually.

Conclusions/Discussion

This study shows that the combination of Azithromycin and Hamamelitannin can effectively prevent Staphylococcal infection. It also suggests that the combined antibiotic has the potential to contribute to the treatment and prevention of Surgical Site Infection.

Note: For the County Fair I only had time to run the antibiotic against Staphylococcus epidermidis, however for State the process was repeated with the other three.

Summary Statement

This study shows both the individual and synergistic antimicrobial effect of Azithromycin and Hamamelitannin against common bacteria in surgical site infection.

Help Received

I conducted and designed my experiment individually, however, I was given advice on lab technique and sterility by my AP Research teachers and peers. My State experiment was completed at CSUCI, where I learned how college labs are organized and received advice for efficiency in the lab.



Name(s)

Cielo Capistrano; Rawan Ghanim

Project Number

S1606

Project Title

Competitive Exclusion between Isolated Probiotics and **Pathogen-Behaving Strains**

Objectives/Goals

Determine the competitiveness between probiotics and pathogenic-behaving organisms. Our hypothesis was that the Staphylococcus epidermidis will prohibit the growth of the probiotic, Bacillus subtilis, and the yogurt. The research will be used to confirm if isolated organisms will have a stronger reaction individually or in a naturally occurring edible form such as yogurt.

Abstract

Methods/Materials

The probiotics that were used were Bacillus subtilis and plain yogurt with live cultured bacteria. Staphylococcus epidermidis was the bacterium used to replace the pathogenic strain, Staphylococcus aureus. Both organisms were given the same amount of time to grow together, as the two were split into two groups. Each group had S. epidermidis with a probiotic. The sterile streaking method was first employed to plate Staphylococcus epidermidis on all nutrient agar plates. Then, the various probiotics were placed at the center of the plates.

Results

The majority of the plates were observed to include both present specimens. Of the samples, not one organism was taking over because it inhabited a part of the agar plate and took what is needed from the nutrient agar. Bacillus subtilis plates demonstrated a clear, barrier-like zone of inhibition which showed growth of S. epidermidis was prohibited. In the other group, the yogurt coexisted with S. epidermidis and grew normally where they had been streaked on the plate.

Conclusions/Discussion

This study found that probiotic, Bacillus subtilis can, in fact, through the competitive consumption of nearby nutrients in the environment, interrupt the growth of the pathogenic-like organisms, Staphylococcus epidermidis. Pure Bacillus subtilis isolates showed the strongest response. These results suggest, that even the weaker responses represent the ability of probiotics to challenge the dominant species in the digestive tract. Instead, yogurt attempts to support a more balanced population of organisms in the gut, helping to prevent a disease state. This proves the hypothesis wrong. This zone was the gap between the streak of S. epidermidis and B. Subtilis colony supports the statement that probiotics can potentially help prevent further growth of pathogenic bacteria.

Summary Statement

Through plating the probiotic Bacillus subtilis and yogurt cultures with the strain Staphylococcus epidermidis, we found that probiotics could potentially combat pathogen-like organisms.

Help Received

My medical biology teacher helped in assisting me and my partner with the knowledge needed to design the experiment and using the right equipment to collect our data.



Name(s)

Maggie S. Chen

Project Number

S1607

Project Title

Cell Membrane-Coated Nanodevice for Anti-Virulence Therapy against Antibiotic Resistant Bacteria

Objectives/Goals

Abstract

With the growing issue of antibiotic resistant bacteria, anti-virulence therapies have emerged as an attractive and effective treatment regimen against pathogenic proliferation, which does not induce resistance in the bacteria as antibiotics do. Furthermore, removing the secretory toxins of these bacteria facilitates immune clearance of the toxin-secreted bacteria, without interference by drug molecules. I aimed to develop a hydrogel tubular nanostructure with through channels to promote blood flow, and encapsulated cell membrane-coated nanoparticles within the structure for detoxification. Thus, this nanodevice has the ability to absorb bacterial toxins for combating antibiotic resistant bacteria without using antibiotics, in a patient specific and effective manner.

Methods/Materials

First, I designed the hydrogel tubular nanostructure incorporating through channels in the shapes of stars, circles, or triangles. The red blood cell (RBC) membrane coated nanoparticles were synthesized through self-assembly methods, and were then incorporated into the hydrogel monomer solution. This solution was polymerized layer-by-layer by a 3D bioprinting method, creating the hydrogel tubular device with through channels and embedded with RBC membrane coated nanoparticles.

Results

Through extensive testing and positive results, I found that my nanodevice was extremely effective in absorbing a wide variety of secretory toxins from antibiotic resistant bacteria. The thorough channels in the hydrogel structure allowed for uninhibited blood flow without any blockage, and enhanced interaction between the toxins within the blood and the structure itself.

Conclusions/Discussion

My nanodevice displays nanoparticle retention and toxin absorption abilities, is both time and cost efficient, and allows for patient specific shapes, sizes, and designs. The personalization of treatment discourages immunosuppression, and the detoxification ability facilitates immune clearance of antibiotic resistant bacteria without the usage of antibiotics.

Summary Statement

I created a cell membrane coated nanodevice, the first of its kind combining 3D bioprinting with nanotherapeutics, to eliminate antibiotic resistant bacteria without the usage of antibiotics through anti-virulence therapy.

Help Received

Used the lab equipment of Dr. Liangfang Zhang at the University of California, San Diego



Name(s)

Ryan K. Ebrahimy

Project Number

S1608

Project Title

Combating Antibiotic Resistance among Gram-Negative Bacteria through Inhibition of AHL-mediated Quorum Sensing

Objectives/Goals

Abstract

The experiment sought to determine a novel method for combating antibiotic resistance among gram-negative strains by targeting the quorum sensing signaling pathway responsible for biofilm formation, which was done through identifying an analogue to the N-acyl homoserine lactone (AHL) signaling molecule. Three analogues - bergamottin, cinnamaldehyde, and proanthocyanidin - were observed for their effect on biofilm development.

Methods/Materials

Materials included 100 mm (diameter) x 15 mm (depth) sterile petri dishes, nutrient agar, 4 dead California market squid (Doryteuthis opalescens), pure cinnamaldehyde, bergamot oil derived from bergamot oranges, and grape seed extract with 95% proanthocyanidins. When prepared in synthetic seawater, the exterior mantle of the squid provided a growth site for the gram-negative bacterium Vibrio phosphoreum. Four groups of nutrient agar culture media, including a control group and three experimental groups incorporating an AHL analogue, were prepared and inoculated with the experimental strain, and the petri dishes were cultivated for four days until visible colony forming units (CFUs) developed.

Results

Culture media that included nutrient agar and bergamottin had a mean CFU count that was 35.8 CFUs fewer than the mean of the control group, with agar media containing cinnamaldehyde and proanthocyanidin having 30.8 and 10.3 fewer CFUs than the control, respectively. Furthermore, bergamottin had the lowest dispersion value of visible colonies with 0.89 CFUs/cm².

Conclusions/Discussion

Bergamottin was indicated as the most effective AHL analogue as biofilm development was reduced the most significantly compared to the other compounds, which is a result of mimicking binding patterns between AHLs and the active site of the LuxR-type receptor. Competitive inhibition and disruption of the quorum sensing pathway can reduce cell aggregation so as to prevent horizontal gene transfer and other mechanisms that contribute to resistance.

Summary Statement

I identified an effective analogue to the AHL signaling molecule capable of disrupting the quorum sensing pathway necessary for biofilm formation, allowing for gram-negative strains to exhibit increased susceptibility towards antibiotics.

Help Received

I devised the experimental design myself and conducted the experiment independently. My AP Biology teacher Mr. David Knight gave constructive criticism regarding my final report, and my Chemistry teacher Mrs. Julie Pomerleau allotted space in her laboratory for culturing the bacteria.



Name(s)

Aarzu Gupta; Maya Shukla

Project Number

S1609

Project Title

Efficacy of Combinations of Plant-Based Antimicrobial Agents in Inhibiting the Growth of Antibiotic Resistant Bacteria

Abstract

Objectives/Goals

According to the US Centers for Disease Control and Prevention, if antibiotic-resistant bacteria continue to impact the human population, by 2050, 10 million people will start dying each year from the impact of

superbugs. We hypothesized that combinations of goldenseal and garlic with plant extracts will be more effective than individual agents in inhibiting the growth of bacteria, in the fight against antibiotic-resistance.

Methods/Materials

We applied combinations of the pure extracts of five natural antimicrobial agents (Turmeric, Ginger, Cinnamon, Goldenseal, and Garlic) on four different bacteria (Escherichia coli, Citrobacter freundii, Staphylococcus epidermidis, and Bacillus coagulans), two gram- and two gram+ to determine the efficacy. We used the Kirby-Bauer disk-diffusion method to find out if certain bacteria can colonize when tested with combinations of these extracts. We measured the diameter of the zone of inhibition around each of the disks to determine whether the antimicrobial agent was effective in killing the bacteria.

Results

Statistical analysis of the data revealed that while not all combinations of garlic and goldenseal were more effective than the individual substances, many did have increased efficacy. On both gram-positive and gram-negative bacteria, garlic in combination with ginger or turmeric, as well as goldenseal and turmeric, had significant improvements in inhibiting bacterial growth when compared to individual efficacy levels.

Conclusions/Discussion

Many combinations of plant-based antimicrobial agents are more effective than single agents in inhibiting bacterial growth, particularly combinations of those that have limited individual antimicrobial properties. Turmeric and ginger are the most promising agents to pursue for further research against antibiotic-resistant bacteria. Understanding that not all chemical combinations have synergistic effects is also crucial to selecting and designing future combination therapy experiments.

Summary Statement

Combinations of certain plant-based agents are effective treatments against bacterial antibiotic-resistance.

Help Received

The Harker School (lab facility access); Dr. Gary Blickenstaff (scientific mentor); Dr. David Casso (scientific mentor); Mr. Chris Spenner (presentation mentor)



Name(s)

Priyansh Gupta

Project Number

S1610

Project Title

Quantifying CRISPR-Cas9 as a Method for Preventing Geminivirus Replication through Virus Replication Site Mutation

Abstract

Objectives/Goals

This experiment aims to assess the feasibility of using the CRISPR-Cas9 system to generate Geminivirus resistance in plants by targeting a site in the viral genome that is essential for viral rolling circle replication. If the viral replication is inhibited, the virus will be unable to spread throughout the plant, rendering the plant free of disease.

Methods/Materials

Agrobacterium cells were transformed with plasmids containing the gRNA and the virus. Nicotiana benthamiana plants were grown and agroinfiltrated with a derivative of the Bean Yellow Dwarf Virus and a CRISPR-Cas9 cassette targeting the Ori of said virus. DNA was extracted from plant tissue samples after agroinfiltration of both the cassette and the virus using Qiagen's Plant DNeasy Mini Kit. The efficiency at which replication was inhibited was qualified by quantitative, real-time PCR. For qPCR, a ten-fold dilution series was permformed to determine the needed concentration for flourescent detection. A ribosomal DNA standard was used for comparison with the samples.

Results

I did not find any significant difference between the viral DNA levels in between the control and gRNA containing agroinfiltrated samples in the two and three days post infiltration samples. I currently believe that the reason CRISPR-Cas9 did not work is due to too little time between agroinfiltration and DNA extraction. More samples are being run as of now with hopes that viral titer is inhibited.

Conclusions/Discussion

Repeated qPCR runs of the two and three days post infiltration samples show no statistical difference between the viral titer with presence of the gRNA and without the presence of the gRNA. As of now, it is concluded that CRISPR-Cas9 was not able to effectively cleave the virus replication site.

Summary Statement

I tested the efficiency of CRISPR-Cas9 to attenuate the spread of Geminiviruses through targeted mutation of the viral origin of replication.

Help Received

Dr. Steve Jacobsen provided access to lab facilities. Dr. Basudev Ghoshal taught me certain protocols such as Agroinfiltration, DNA extraction, and qPCR operation. Dr. Bob Sandrock assisted me with the technical aspects of the project, including the design of the gRNA.



Name(s)

Shayle S. Gupta

Project Number

S1611

Project Title

Bioadhesive Particles Add Durability to Sunblocks and Maintain Efficacy

Objectives/Goals

Abstract

Skin cancer rates continue to rise in America and are primarily due to UV exposure. Zinc oxide was previously found to be the most effective barrier. This experiment evaluates the effectiveness and duration of action of a novel skin protectant product developed from the mixture of Zinc oxide and a bioadhesive. This experiment will employ a common, inexpensive, edible bioadhesive to encapsulate a physical block to create a durable, effective sun protectant.

Methods/Materials

Petri dishes with agar were inoculated with sun sensitive bacteria and allowed to grow for one week in the dark. A colony count was performed and one of the UV protectants was applied. The protected bacteria were then exposed to UV radiation and colony counts of the bacteria were made at seven days following UV exposure. The second stage of the experiment assessed the duration of the protection conferred by measuring UV irradiance with a UV meter hourly under protected pig skin.

Results

In all four treatment groups, bacteria grew as expected prior to colony count I. Groups without the bacteria plated also behaved in the expected fashion with very little bacterial growth and change between colony counts. In the No Protection group, sun exposure produced the expected nearly complete bacterial elimination by the second colony count. In the Zinc oxide group with bacteria and sun exposure, the colony count nearly doubled. The bioadhesive alone did not provide any protection as nearly all of the bacteria died when exposed to the sun. The Zinc oxide plus bioadhesive group demonstrated strong protection of the bacteria as the colony count doubled in the sun exposure experimental group. The data from the irradiance measurements revealed that bioadhesive alone was not protective at all. Zinc oxide was protective for five hours, and then began to lose its shielding effect. The addition of the bioadhesive to Zinc oxide maintained the irradiance below the threshold of 30 W/m2) until the nine hour mark.

Conclusions/Discussion

This project determined that combining the most effective sun protective with a bioadhesive produced a more effective and durable protection. The addition of the bioadhesive allows the product to persist and provide reflectiveness all day. Extending this experiment to cultured human skin will confirm these findings and potentially reduce skin cancer rates.

Summary Statement

A skin protective product made by combining Zinc oxide and a bioadhesive provides effective sun protection and extends the duration of the protection.

Help Received

My parents helped with the photography and layout of this board. My father also helped confirm the bacterial colony counts.



Name(s)

Riana Karim; Sharon Truong

Project Number

S1612

Project Title

The Effect of Different Probiotic Bacterial Strains on Ammonia (NH3) Production Levels

Objectives/Goals

Abstract

The chief objective of this experiment is to distinguish which probiotic bacterial strain, Bifidobacterium longum, Lactobacillus bulgaricus, and Streptococcus thermophilus, has the greatest capacity to decrease the most ammonia (NH3). We hypothesized that Lactobacillus bulgaricus will have the greatest capacity to decrease ammonia because of its ability to foster natural antibiotics and acids to neutralize the toxin.

Methods/Materials

50 billion CFU Custom Probiotics Strain Bifidobacterium longum, 50 billion CFU Custom Probiotics Strain Lactobacillus bulgaricus, 50 billion CFU Custom Probiotics Strain Streptococcus thermophilus, MRS Broth Powder, 0.1 Mol Ammonia Solution, 800 mL Distilled Water, Ammonia Test Paper (0.0-6.0 ppm), Incubator

We prepared a solution of 50 billion CFUs for each live probiotic bacterial strain dissolved in 10 mL of MRS broth in test tubes to grow in an incubator at 37°C. After 24 hours, we added 3 mL of 10 ppm ammonia solution to each solution of live probiotics. For three 24 hour intervals, we used test strips to measure the amount of ammonia in each test tube solution.

Results

After analysis of the data, the strain Streptococcus thermophilus possesses the best ammonia reduction abilities with an averaged 6.66 ppm ammonia reduction over the course of 48 hours, a 66.66% decrease. Lactobacillus bulgaricus broke down an average of 6.34 ppm of ammonia after 2 days (a 63.4%). The probiotic strain Bifidobacterium longum reduced the least amount of ammonia at 6 ppm or 60% after 2 days.

Conclusions/Discussion

All three probiotic strains exemplified an ambient ammonia reduction of over 60%, revealing its alleviating abilities for ammonia toxicity levels. By conducting our experiment in the human body temperature (37°C), Streptococcus thermophilus performed in its optimal temperature, followed by Lactobacillus bulgaricus, whose optimal temperature is 48°C to 46°C, and finally Bifidobacterium longum. Streptococcus thermophilus portrays its detoxifying enzymes that break down ammonia into urea and amino acids, but all strains portray similarly strong properties of ammonia reduction. Our experiment opens up new opportunities for investigations into ammonia toxicity therapy for liver diseases like hepatic encephalopathy.

Summary Statement

This experiment investigates the capacity of probiotic bacterial strains on reducing environmental ammonia levels and exemplifies the mitigating abilities of probiotics on ammonia toxicity.

Help Received

My partner and I set up and designed the experiment ourselves, including the selection of the probiotic strains to test. Our mentor, Mrs. Yi, provided us with the laboratory materials to conduct our experiment (graduated cylinders, Erlenmeyer flasks, electronic balance, incubator shaker and other basic materials).



Name(s)

Hangyul Lyna Kim; Marcos Perez

Project Number

S1613

Project Title

Comparing Antimicrobial Properties of Propolis from Different Subspecies of Honey Bees

Abstract

Objectives/Goals

Submitted by Partner

Methods/Materials

Submitted by Partner

Results

Submitted by Partner

Conclusions/Discussion

Submitted by Partner

Summary Statement

We used the growth of E.Coli to show that flavonoid content may not directly impact the antimicrobial properties of propolis.

Help Received

Dr. Shauna Bennett showed us how to work with E.Coli. We designed and performed the experiment. Dr. Cory Tobin provided us with access to the lab.



Name(s)

Prashant P. Naidu

Project Number

S1614

Project Title

Treating with Turmeric: An Alternative in Treating Foodborne Illness-Causing Bacteria

Abstract

Objectives/Goals

To determine if various concentrations of a turmeric solution would inhibit the growth of bacteria that are responsible for causing foodborne illnesses.

Methods/Materials

Gram-negative bacteria, Escherichia coli O157:H7, Shigella, and Salmonella enterica, were tested for susceptibility with a full strength, half strength, and quarter strength turmeric solution and compared to the zone of inhibitions when the bacteria were treated with ampicillin. The initial or full strength solution consisted of 10 grams of powdered turmeric combined with 250 mL of saline. An additional 250 mL of saline was added to obtain the half strength solution, then an additional 250 mL was added to obtain the quarter-strength solution. All three concentrations of the solution were applied to both filter paper diffusion disks and Vancomycin disks. Disks were placed on agar plates streaked with a bacteria and incubated as part of the in vitro method. Zone of inhibitions were recorded by measuring the diameter in millimeters and compared to a chart to determine if the solution was susceptible, intermediate, or resistance to each bacterium. The experiment was completed twice, with Vancomycin disks saturated with the various concentrations of solution only used in the second round.

Results

The outcome for both rounds was positive. The full strength or initial turmeric solution was most effective in inhibiting the growth of each bacterium. The ZOI (Zone of Inhibitions) for the bacteria, when treated with ampicillin, had an average diameter of 16.5 mm. The ZOI of the bacteria, when treated with the full strength solution, had an average diameter of 15.25 mm. This presented the fact that the full strength solution was as equally effective as using ampicillin to treat Escherichia coli O157:H7, Shigella, and Salmonella enterica

Conclusions/Discussion

When foodborne illness-causing bacteria were exposed to the full strength concentration of a turmeric solution, it inhibited bacterial growth effectively, therefore turmeric may be used as an antibiotic in the future.

Summary Statement

I was able to create various concentrations of a turmeric solution that were applied to both diffusion disks and Vancoymin disks to see if they would inhibit gram-negative bacterial growth as effectively as ampicillin.

Help Received

Dr. Mahendra Poudel, MD, Salinas Valley Memorial Hospital; Mr. Donald Harris, BS in Microbiology, Salinas Valley Memorial Hospital; Ms. Kacee Fujinami, BS, Biology Teacher Salinas High School; Dr. Dharma Naidu, PharmD, Community Hospital of the Monterey Peninsula; Mrs. Wendy Naidu, RN, BSN.



Name(s)

Laura Noronha

Project Number

S1615

Project Title

Role of Intravenous Fluids in Treating Gram Negative Sepsis

Abstract

Objectives/Goals

To study the effect of 3% saline on gram negative bacterial solutions in vitro and to see whether this effect is additive/synergistic with the antibiotic Meropenem.

Methods/Materials

Using known controls for Pseudomonas aeruginosa, Klebsiella pneumoniae carbapenemase producing bacteria (KPC), Klebsiella pneumoniae, and Escherichia coli (E. coli) and the Prompt Inoculation System-D, a standardized bacterial suspension of each bacterium was prepared. 20 µl of each bacterial suspension was added to 2 tubes each containing 3 ml of sterile inoculum water. One tube was used as a control for each bacterium. 3 ml of 3% saline was added to the second tube for each bacterium. After mixing the solutions well, 100 µl of each bacterial solution was plated on to 2 blood agar plates each. A 10 µg Meropenem disc was placed on one of the plates for each bacterium. 100 µl of each bacteria + 3% saline solution was plated on to 2 blood agar plates each. A 10 µg Meropenem disc was placed on one of the plates for each bacterium. After incubating overnight at 37° C with 5% CO2, the number of colonies on each plate was counted and the zone of clearing around the Meropenem disc was measured.

Results

For KPC, average colony counts were 435.5 for the control, 184 for KPC with 3% saline, 269 for KPC + meropenem and 182.5 for KPC + 3% saline + meropenem. For E. Coli, average colony counts were 245.5 for the control, 82.5 for E. Coli with 3% saline, 85 for E. Coli + meropenem and 36.5 for E. Coli + 3% saline + meropenem. For Pseudomonas aeruginosa (PA), average colony counts were 67 for the control, 52.5 for PA with 3% saline, 39 for PA + meropenem and 29.5 for PA + 3% saline + meropenem. For Klebsiella pneumoniae (KP), average colony counts were 326 for the control, 157 for KP with 3% saline, 231.5 for KP + meropenem and 56 for KP + 3% saline + meropenem. Average zones of clearing with Meropenem disc were 21 mm for KPC, 24 mm for KPC with 3% saline, 44.5 mm for E. Coli, 47.5 mm for E. Coli with 3% saline, 37 mm for PA, 41.5 mm for PA with 3% saline, 39 mm for KP, and 43 mm for KP with 3% saline.

Conclusions/Discussion

The combined effect of 3% saline and Meropenem was greater than if either one was used independently, as demonstrated by both colony counts and zone of clearing. This shows that using hypertonic solutions like 3% saline with antibiotics may be a better choice for patients with sepsis due to gram negative bacteria.

Summary Statement

I showed that hypertonic IV solutions like 3% saline increased the effect of the antibiotic Meropenem against gram negative bacteria.

Help Received

Ms. Victoria Go mentored me and helped me conduct the experiments.



Name(s)

Stephen T. Ott

Project Number

S1616

Project Title

Using Tardigrade DNA to Make Bacteria Resistant to Radiation

Objectives/Goals

Abstract

How are astronauts going to even survive getting to Mars with all the radiation they would be rendered helpless against? Assuming that humans do find a way to get to Mars safely in the next few decades, then what? How can we prevent skin cancer that many people around the world contract just because they have to be in the sun a lot? These are all problems that could be easily abridged by human cells or plants cells having a higher resistance to radiation.

Methods/Materials

Research showed that bacteria may be able to resist higher doses of radiation if given the gene to manufacture a protein called Dsup, short for damage suppressor. Following that line of thought, how hard is it to give bacteria these genes? Turns out, this can be achieved through a bacterial transformation with a plasmid with the gene for Dsup and resistance to the antibiotic ampicillin.

Results

While this research was unable to determine if our transformed bacteria actually were radiation-resistant, the bacterial transformation was a success, as evidenced by the simple fact that bacteria were growing on the petri dishes with ampicillin.

Conclusions/Discussion

This research had the goal of learning more about Dsup, how it protects DNA, and if this would lead to cells being able to therefore survive larger doses of radiation. While I was unable to ascertain if this was true experimentally, future experiments are planned to determine once and for all if there is in fact a way to make cells able to survive larger doses of radiation.

Summary Statement

I attempted to create bacteria that were resistant to radiation.

Help Received

My project adviser, having almost a decade of experience in biotechnology, advised me and helped to set up the experiment and provided materials, however the actual procedure was still executed by myself



Name(s)

Justine E. Sato

Project Number

S1617

Project Title

The Effect of 28 kHz Ultrasound Exposure on the Transformation Efficiency of pGLO Plasmids into E. coli

Objectives/Goals

Abstract

Low frequency ultrasound (US), has been rarely used for transformation of non-competent cells which begs the question: how does 28kHz ultrasound affect plasmids and their transformation efficiency into E. coli?

Methods/Materials

I created LB plates with ampicillin, and arabinose (LB:AMP:ARA), ensuring that fabrication temperature was below AMP and ARA denaturation, so that they would have desired thickness, translucence, and even distribution of AMP and ARA (indicators of transformation). I performed the experiment with 2 different procedures: I initially mixed E. coli colonies from a streak plate with CaCl2, exposed to US for various times while adding pGLO in last 5 sec, mixed it with LB broth, and spread onto an LB:AMP:ARA plate; I modified my protocol by mixing more E. coli colonies, CaCl2, and pGLO, exposing it to US, mixing it with LB broth thoroughly, and spreading it onto an LB:AMP:ARA plate. After E. coli grew into colonies, images of the petri dishes were collected and a MatLab script analyzed images, counted colonies, and calculated transformation efficiency.

Results

For plates with pGLO added in last 5 sec of US exposure (no US exposure for plasmids, but full exposure for E. coli), there were few colonies (little to no transformation efficiency) per plate and no correlation between exposure time and transformation efficiency; all colonies that grew were fully transformed. For plates with pGLO exposed to US (full US exposure to plasmids and E. coli), there were 500% more colonies than the number normally grown when thermal shock is used for transformation of non-competent cells.

Conclusions/Discussion

After using a modified protocol, it was found that as the exposure time increased, the transformation efficiency increased exponentially, but only for colonies expressing the phenotype of ampicillin-resistance and not glowing under UV light. However, the colonies expressing both phenotypes had no correlation between exposure time and transformation efficiency. Because some of the colonies did not express all expected phenotypes, it suggests that some bacteria were only partially transformed, while colonies that expressed all expected phenotypes suggests some bacteria were completely transformed.

Summary Statement

I developed a protocol that created 2 types of E. coli from a single source using US which controlled the transformation of E. coli with exposure time and increased transformation efficiency of plasmids by 500% (compared to thermal shock).

Help Received

Using lab space and equipment at Beryl Technologies



Name(s)

Perrin J.G. Turney

Project Number

S1618

Project Title

Effects of Climate Change (Increase in Temperature, Salinity and Pollutants) on Freshwater Microorganism Mortality Rates

Abstract

Objectives/Goals

Over the span of three years, I tested the effects of three aspects of climate change on the mortality rates of freshwater microorganisms at the Arcata Marsh: increases in salinity, increases in industrial and agricultural pollutants, and, this year, increases in temperature.

Methods/Materials

Ten 2.5 gallon tanks were filled with Arcata Marsh water from the freshwater Log Pond and were maintained at four temperature points representative of potential environmental temperature increases should climate change not be addressed and prevented. Using adjustable aquarium heaters, two tanks were kept at 65 degrees F, two tanks were kept at 70 degrees F, and two tanks were kept at 75 degrees F. The two control tanks were not heated. Samples of each tank were taken daily for 30 days and the microorganism populations were assessed for numbers of organisms, health, and appearance.

Results

In all temperature gradients, mortality rates for all observed microorganisms increased except for Hypotrichida, which manifested a population increase as the mortality rates of other organisms increased thereby increasing the food source for Hypotrichida. The tanks held at 10 degrees higher than the control had rapid mortality rate increases while the tanks maintained within 5 degrees of the control had a slower mortality rate increase.

Conclusions/Discussion

Climate change continues to threaten the planet and every one of its ecosystems. This three year study provides further support that freshwater wetlands are at a very real risk of depletion as ocean temperatures increase and the sea level rises and pushes the brackish and freshwater watersheds toward the mountains, narrowing those ecosystem habitats. My hypothesis was correct that the freshwater microorganisms at the Arcata Marsh are highly susceptible to environmental changes including increases in salinity, industrial and agricultural pollutants, and temperature due to further human growth and development without environmentally responsible alternatives for energy.

Summary Statement

This three-year study determines Arcata Marsh freshwater microorganism mortality rates in correlation with climate change: increase in temperature, increase in salinty, and increases in industrial and agricultural pollution.

Help Received

Greta Turney -- microobiologist and mom; helped to identify organisms, gather samples, set up tanks. Ed Woodcock, RVT -- Humboldt Veterinary Laboratory Owner and diagnostician; loaned me the counting chamber slide, gave me microscope slides and cover slips.