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Edificio de Biología
Recinto Universitario de Mayagüez

Biología

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Undergraduate Science Program

¡Bienvenidos al Tercer Simposio de Investigación Subgraduada en Biología!

Por tercer año consecutivo el Departamento de Biología del Recinto Universitario de Mayagüez se une para celebrar y reconocer la diversidad y la labor investigativa de nuestros estudiantes subgraduados. Anualmente, nuestro departamento alberga unos 1,400 estudiantes, de los cuales aproximadamente el diez por ciento se envuelve en el diseño y la ejecución de proyectos científicos en diversas ramas de la biología. Con la divulgación de los resultados de sus investigaciones, dentro del contexto de la comunidad cívica y científica, nuestros estudiantes completan el método científico.

Este Tercer Simposio de Investigación Subgraduada tiene como propósito continuar proveyendo el espacio para consolidar el conocimiento adquirido durante la experimentación científica en el laboratorio y poner en práctica las destrezas de comunicación oral científica.

Como en años anteriores, el comité organizador agradece profundamente el apoyo del Departamento de Biología-Recinto Universitario de Mayagüez, Role Model-H.H.M.I. (subvención de Howard Hughes Medical Institute) y su Directora, Dra. Nanette Diffoot Carlo. También deseamos agradecer el apoyo y colaboración de los mentores de investigación de los estudiantes presentadores. Además, queremos agradecer profundamente a nuestros estudiantes graduados y a las asociaciones estudiantiles de nuestro Departamento por su ayuda durante la celebración del simposio. Finalmente, nos gustaría reconocer la ayuda de las señoras Mitzy Zavala, Brenda M. Soto Pérez y Alicia Collazo, secretarias de nuestro Departamento.

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SEARCH, ISOLATION AND CHARACTERIZATION OF RADIATION RESISTANT BIOPROSPECTS IN SOIL SAMPLES IN PUERTO RICO

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Geomicrobiology is an interdisciplinary field that studies how the microorganisms affect their geological environment, being some of these hostile due to physico-chemical factors like pH, high/low temperatures, salinity, desiccation, heavy metals and radiation. These conditions force the microorganisms to develop survival strategies such as specialized structures and physiological and biochemical activities. UV radiation has been link to serious health problems like skin cancer, immune system suppression and cataracts. That is why is important to identify activities and mechanisms that can help us fight the dangerous effects of UV. The main focus of this research is to isolate UVC radiation resistance (RR) microbes from soil samples in Puerto Rico. Soil samples from Cabo Rojo Salterns were serially diluted, replica plated and exposed to UVC at different times. The survivors were isolated from the master plate and growth curves were performed of *Escherichia coli* as negative control. Individual UVC expose to confirm resistant was performed, washing logarithmic growing cells with saline and diluted to 10-5. The diluted cells were plated and exposed to an UV lamp with 86μW/cm^2 at 1m. The exposure periods went from 15 sec to 5 minutes, increasing each period by 15 sec. A survival curve (SC) of E. coli to UV-C radiation was generated, showing 0 survival after 15 sec of exposure. A total of 29 RR bioprospects were isolated, and 10 were characterized in silico. The data suggested the presence of *Pseudomonas, Enterobacter, Shewanella*, and *Rhodopseudomonas*. Survival curves of the putative RR isolates are in progress.

EXPRESIÓN, CARACTERIZACIÓN Y PURIFICACIÓN DEL FACTOR DE CRECIMIENTO ENDOTELIAL VASCULAR 165 (VEGF165)

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El Factor de Crecimiento Endotelial Vascular (VEGF, por sus siglas en inglés) es una glicoproteína implicada en el proceso llamado angiogénesis, del cual se forman nuevos vasos sanguíneos a partir de los ya preexistentes. VEGF, en conjunto al aptámero de DNA llamado VEa4, podrían actuar como un dispositivo de señalización para la detección temprana de algunas enfermedades tales como el cáncer. Por lo tanto, nuestro objetivo principal es el llevar a cabo estudios preliminares sobre la interacción entre la proteína VEGF165 y el aptámero VEa4. Este mecanismo proteína-aptámero, de ser exitoso, podría servir como un biosensor para detectar así los niveles en aumento de VEGF165 en el plasma debido al crecimiento desmedido de células. El trabajo que se realiza actualmente consiste en el aislamiento, la purificación y la caracterización de la proteína recombinante humana VEGF165 a partir de un sistema de expresión en células competentes de una cepa de *Escherichia coli* llamada KRX. Este proyecto de investigación es apoyado en parte con fondos del programa NIH-MARC.

CAN HAPTOGLOBIN ATTENUATE HEMOGLOBIN-INDUCED VASCULAR DAMAGE?

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Hemolytic diseases and infections can lead to intravascular hemolysis, which causes increased plasma hemoglobin (Hb) concentration. Over time, this "cell free Hb" is toxic to the blood vessels, exerting oxidative damage, nitric oxide (NO) scavenging, or inflammatory responses within the vasculature. The exposure of increased Hb on the lung tissue can lead to pulmonary hypertension (PH), which is high blood pressure in the pulmonary arteries due to changes in arterial wall. Haptaglobin is an acute phase protein responsible for binding and clearance of Hb. We hypothesized that haptoglobin (Hp) can attenuate PH secondarily to vascular hemoglobin exposure. To test our hypothesis, Hb was chronically administered to rats at physiologically relevant concentrations as observed in the sickle cell disease (SCD) (0.2 μ;mole/day) for 5 weeks. To further mimic the SCD process, rats were placed in hypobaric hypoxic chambers (~17,000 ft). Animals were divided into 4 groups; (1) Control (sham), (2) Hb infusion, (3) Hp injections (biweekly), and (4) Hb: Hp (chronically infused with Hb treated biweekly with Hp). At the end of the 5 weeks, we evaluated pulmonary blood pressure, cardiac output, and pulmonary vascular wall thickness. Additionally, lung analysis for indicators of endothelial nitric oxide synthase (eNOS) and intercellular adhesion molecule 1 (ICAM-1) expression were performed. Preliminary data demonstrates that groups infused with haptoglobin had lower pulmonary blood pressure, decreased vessel wall thickness, and decreased ICAM-1 expression. We observed no difference in eNOS expression between groups. This suggests that Hb induced inflammation may be attenuated by Hp.

ANALIZANDO LAS MUTACIONES DEL GEN FGG: DESCUBRIENDO SUS IMPLICACIONES EN LA SALUD Y SU FRECUENCIA EN LA POBLACIÓN PUERTORRIQUEÑA

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El proyecto de Adopta un Gen consiste en el estudio de genes cuyos polimorfismos han sido asociados al desarrollo de enfermedades. El propósito es descubrir la frecuencia de estos polimorfismos en la población puertorriqueña. Nuestro estudio trata sobre el gen FGG que codifica para una de las subunidades de fibrinógeno. Esta proteína juega un papel importante en el proceso de coagulación. Mutaciones en FGG pueden afectar la velocidad de la coagulación y aumentar el riesgo de padecer de trombosis venoso. Para lograr las metas del estudio, primeramente estudiamos los 55 individuos puertorriqueños secuenciados en el estudio de Mil Genomas. En relación al resto del genoma, donde la ascendencia europea es predominante y la ascendencia indígena es la menor, el gen FGG muestra un alza en la ascendencia indígena y una disminución en la africana. Se identificaron 38 haplotipos distribuidos en cuatro familias que se distinguieron por tres polimorfismos de los cuales ninguno se encontró en la región codificante del gen. Entre ellos rs1800792, en la región promotora del gen, ha sido asociado a linfoma no-Hodgkin, a un contaje reducido de plaquetas en mexico-americanos, y, en mujeres, influye sobre el nivel de las lipoproteínas de alta densidad y el índice de masa corporal. Además, rs1049636, en la región 3' no traducida, ha sido asociado a bajos niveles de fibrinógeno gamma. La frecuencia de estos tres polimorfismos y de las familias de haplotipos que distinguen está siendo investigada en las muestras del "Local Genome Diversity Project" correspondientes al pueblo de Lares.

PURIFICATION AND CHARACTERIZATION OF AN ALPHA-GLUCOSIDASE (MALTASE) FROM THE HALOPHILIC ARCHAEON *HALOQUADRATUM WALSBYI*

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Recently, with the discovery and genome availability of halophilic Archaea, an interest has arisen in isolating and studying their enzymes. Enzymes from extremophiles possess unique properties useful to develop novel biotechnological applications. They can perform in industrial processes under conditions that are detrimental to the conventional proteins used. This study focuses on the characterization of a maltase from *Haloquadratum walsbyi*. The gene, followed by a his-tag, was cloned into E. coli for faster growth and production. The recombinant protein is extracted by breaking the cells with a sonicator, and then renaturing of the enzyme is achieved by reconstituting in Tris-HCl buffer with high NaCl concentration. Specific activity is measured using colorimetric enzyme and protein assays. Purification is performed using a Ni-NTA column to which the histidine residues in the his-tag bind. After washing a few times, elution is obtained with a high concentration of imidazole. Optimization of this process is required in order to obtain a high yield of protein that maintains its activity. Proteins in the different eluting solutions are analyzed using SDS-page. Preliminary data measuring activity without complete purification shows optimum conditions of the recombinant enzyme at 40°C, 15% NaCl w/v and a pH of 6.0. The goal is to achieve complete purification and detailed characterization of the enzyme and determine its possible use in the industry and research.

DOMINANCE AND DISCOVERY OF AN INVASIVE ANT SPECIES, *WASMANNIA AUROPUNCTATA*, WITH POTENTIAL COMPETITORS WITHIN ITS NATIVE RANGE

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The little fire ant, *Wasmannia auropunctata*, is an invasive species throughout the Caribbean, the Pacific Islands and the Middle East. We want to know if *Wasmannia*'s domination in the places where it is invasive has to do with the richness and abundance of biodiversity in such places. To test this hypothesis we went to southern Mexico, a place where *W. auropunctata* is native, less abundant, and not considered a problem for agriculture or biodiversity decrease. The results we obtain for these experiments are going to be compared with the same kind of experiments that will be carried out in the island of Puerto Rico, a place where *W. auropunctata* is considered invasive, it is very abundant, and also considered an agricultural pest. We found in the field experiment that *W. auropunctata* is not a good competitor against two of the species that we chose for these experiments. The lab experiments show that *W. auropunctata* is a better competitor than *Solenopsis picea*. We could suggest that *Ph. synanthropica* may be contributing to the control of *W. auropunctata*'s spread.

INTERMEDIATE DISTURBANCE HYPOTHESIS: DO BENTHIC FORAMINIFERA FOLLOW THE TREND?

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Benthic foraminifera are known to be useful indicators of heavy metal pollution, paleoceanographic dynamics and water quality. Due to the latter, a FORAM Index (2003) was designed to classify habitats based on their degree of disturbance. The general trend is high diversity and abundance of foraminifera in mesotrophic waters in accordance with the Intermediate Disturbance Hypothesis. However, there is scarce information regarding the validation of the index, particularly for Puerto Rico. For example, the southwest have high nutrient inputs and geomorphological transformations due to coastal urban development. Therefore, coastal sands under intermediate levels of disturbance should have higher abundance and diversity of foraminifera than coastal sands under low and high disturbance regimes. Diversity and abundance were estimated from random surface sediments under three different regimes of disturbance in the Buye Beach. Based on the FORAM Index and the Intermediate Disturbance Hypothesis, higher abundance and diversity of foraminifera should be found under the intermediate disturbance regime. Our preliminary data, on the contrary, suggests higher abundances in the site under high disturbance. Possible explanations for this include the influence of sediment grain size as well as the history of past disturbances.

MICROSCOPIC AND MOLECULAR CHARACTERIZATION OF ACTINOBACTERIA ASSOCIATED WITH *NASUTITERMES COSTALIS* (ISOPTERA: TERMITIDAE) NESTS

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Nasutitermes costalis are fully social insects that live in colonies. These insects create their nest with soil, wood, fecal secretions and saliva, and above soil level on trunks and tree branches. *Nasutitermes costalis* shows uncertain relationships with Actinobacteria, repeatedly isolated from its nests. Actinobacteria are Gram positive genera that are found mostly in soil as degraders of organic matter and are also known for antibiotic production. Actinobacteria are morphologically complex, constantly changing their morphology throughout their life cycle, making their characterization difficult. In addition, phylogenetic analysis based on 16SrRNA fails to separate groups into specific clades. Given these difficulties, a microscopy analysis based on spore structure and molecular fingerprinting (BOX-PCR) techniques were implemented. Samples were taken from three distinct nests in a tropical forest from Mayaguez. We obtained 39 morphotypes of Actinobacteria, which clustered in 5 groups that concur with the characterization previously established from spore structure. These results indicate that these two techniques can be utilized in conjunction to obtain a more accurate identification at species level.

THE DOMINANT POPULATION OF ENTEROCOCCI IN FECAL SAMPLES OF HEALTHY HUMANS IS EQUIPPED WITH THE SAME VIRULENCE FACTORS PRESENT IN DISEASE-CAUSING ENTEROCOCCAL STRAINS

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Enterococci are commonly found in the intestinal microflora of humans and animals, on the surface of plants, and in dairy products. In the last decade, Enterococci have become one of the leading causes of nosocomial bacteremias, surgical wound and urinary tract infections. The aim of this study was to determine the dominant Enterococci in the gastrointestinal (GI) tract of healthy humans and the abundance and frequency of virulence factors. Isolates obtained from clinical samples and human feces from healthy Puerto Ricans were characterized with respect to species status and virulence factors by PCR analyses, while resistance to antibiotics was done by Kirby-Bauer. The dominant enterococci population was represented by *E. faecalis* and *E. faecium* in both, human feces (86%) and clinical samples (100%). The clinical samples showed highest resistance to rifampicin (44%), followed by vancomycin (36%) and piperacillin (10%). In contrast, in the feces of healthy humans the enterococci were most resistant to vancomycin (45%) followed by rifampicin (6%) while none were resistance to piperacillin. In healthy humans, 62% of the isolates contain at least one virulence factor (gelE, asa1, esp or cylA) the remaining 38% did not contain any virulence factors. All of the clinical isolates at least contain one virulence factor. The most abundant virulence factor in both populations was gelE, the dominant genotype with more than one factor was asa1/gelE in clinical samples while in human feces was asa1/gelE/cylA. Our preliminary data suggest that healthy humans contain Enterococci that carry the same virulent traits than disease-causing Enterococci.

EXPERIMENTAL VALIDATION AND SEARCH FOR POLYMORPHISMS OF EXONIC AND PROMOTER INSERTIONS AND DELETIONS FROM PRIMATE GENOME COMPARISONS

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Comparisons between human and primate genomes are important to understand the evolution of our own species. Indels discovered by sequence alignments among closely related species or individuals are common in these comparisons. The study of insertions and deletions (indels) in the coding and promoter regions is of primordial importance because these seem to play the key role in primate evolution. We conducted a computational pairwise comparison between five reference genomes: humans (*Homo sapiens*), chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla*), rhesus macaque (*Macaca mulatta*), and orangutan (*Pongo abelii*). This comparison revealed a total of 33,479 indels >10bp; 153 are found in coding regions while 63 of these indels are in promoter regions up to 1,000 bp upstream from the transcription initiation sites. So far, we have validated the existence of the polymorphic indels in the coding regions in vitro by designing degenerate primers and using PCR amplification of interrogated segments for these five primate species. Indels that were validated between the species were tested in two African populations and in a Human Genome Diversity Panel (HGDP). From the 153 genes containing candidate indels in the coding regions, all have been tested in the laboratory. Of these, only 30% have not been validated from human to chimpanzee and 15% from human to rhesus comparisons. Validation results suggest that computational analysis alone is not sufficient for the reliable discovery of indels, and additional laboratory testing is required. Further analysis is required to understand the precise impact of these indels on gene expression.

TRACKING THE ANCESTRY OF SNPS IN GENES RESPONSIBLE FOR PEDIATRIC OBESITY IN PUERTO RICO: LEP, FTO, MC3R, AND MC4R

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Obesity is one of the leading causes of death in the US and Puerto Rico. Pediatric obesity [PO] has a debilitating effect through life and poor prognosis, and its incidence has increased significantly in recent years. Several genes have been associated to obesity, most notably FTO, LEP, MC3R, and MC4R. Single nucleotide polymorphisms (SNPs) in these genes may play a significant role in the outcome of obese phenotypes. In Puerto Rico, these SNPs may be related to ancestry, as obesity-risk alleles may have gained frequency in their original population under stress environments where gain weight in early life may have been advantageous. To test this hypothesis, the LEP, FTO, MC3R, and MC4R gene sequences of 51 Puerto Ricans were acquired from the 1000 Genomes database, all of known local ancestry (European, African, Native American). With this information and the use of linkage data and programs such as NETWORK and DNA ALIGNMENT, the Puerto Rican haplotype networks for LEP, FTO, MC3R, and MC4R were determined. Haplotype-family-informative SNPs were determined and SNPs discovered were compared to others believed to cause PO. SNPs rs17151922 and rs28959469 are informative for LEP and distinguish several ancestral haplotype families. SNPs rs17151922 and rs28959469 have been tested for in Puerto Rican samples in the laboratory at UPRM, providing interesting results. Future experiments include similar analyses of Puerto Rican pediatric samples for the haplotype-informative SNPs determined thus far for all genes.

ACTIVITY-DEPENDENT VESICLE CYCLING IN NEURONAL CANCER CELLS

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In neuroscience a reliable and practical model is needed for testing new drugs that target the synaptic function. Neurons are used as the current model but they are difficult to culture. Neuroblastoma cells may be suitable as a replacement model for neurons. In contrast to neurons, they proliferate quickly and, therefore, are readily available in large numbers. For neuroblastoma cells to qualify to become a candidate to replace the existing model they should be neuron-like, specifically they should be able to undergo vesicle recycling. We tested the neuroblastoma cells for such capabilities. Neuroblastoma cells were cultured, transfected with the pH sensitive green-fluorescent protein, SynaptopHluorine, and were studied for the presence of vesicles. Then we exposed the neuroblastoma cells to the fluorescent dye FM4-64 and studied whether activity stimulates its uptake and release. Using the SynaptopHluorine protein, we found the presence of vesicles within the cells. And the uptake and release of FM4-64 by the cells let us determine that neuroblastoma cells do recycle vesicles in an activity-dependent manner. The results suggest that neuroblastoma cells may be used as a model for high through put screening for drugs that target the synaptic vesicle cycle.

FUNCTIONAL CHARACTERIZATION OF CYSTEINE SYNTHASE, A NOVEL CASSAVA GENE INVOLVED IN CYSTEINE SYNTHESIS

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Cassava (*Manihot esculenta* Crantz) is a widely cultivated crop due to its high calorie content, high yields and growth in poor soils. Unfortunately cassava is a cyanogenic crop due to the presence on linamarin in the vacuoles of all cells. Improper processing of linamarin during food preperations can lead to several cyanide-related disorders as Konzo, Goiter, and Tropical Ataxic Neuropathy. Despite the fact that cassava has its own detoxification pathway, the different compartmentalization of the cyanogenic glycoside synthesis and breakdown pathways prevents the complete removal of toxic cyanogenics from raw cassava. Recently, two genes from the cassava cyanide detoxification pathway, Manes:BsasA and Manes:BsasB, belonging to the β- -substituted alanine synthase (Bsas) family of enzymes, were isolated. Previous work suggest that MANes;BsasA has an important role in cysteine biosynthesis because it shows high cysteine synthase activity, which is a key enzyme important for the cyanide detoxification pathway. Our project focusses on the functional characterization of MANes;BsasA gene in mutants of the model plant *Arabidopsis thaliana*. *A. thaliana* has nine genes of the Bsas family of enzymes, each with different cysteine synthase and β-cyanoalanine synthase activity. Mutants lacking each of these proteins will be used for the characterization of the novel cassava genes. MANes;BsasA gene has been introduced into pKYLX vector behing 2X35S promoter and is being utilized for agrobacterium mediated transformation of Arabidopsis floral buds. Our objective is to measure cysteine synthase activity of the transgenic mutants, the non-transgenics mutants and wild type plants to better understand the functionality of MANes;BsasA gene.

USING MITOCHONDRIAL CONTROL REGIONS TO DETERMINE POPULATION STRUCTURE IN THE SHINY COWBIRD *MOLOTHRUS BONARIENSIS* IN SOUTHWESTERN PUERTO RICO

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The shiny cowbird, *Molothrus bonariensis*, is an obligate interspecific brood parasite, meaning it always lays its eggs in the nests of other bird species. This behavior can cause severe damages to host populations by reducing the survival of the hosts' nestlings. The critically endangered yellow-shouldered blackbird (*Agelaius xanthomus*) is one of the hosts most severely affected by this parasite. Recent observations (T. Nakamura, unpublished data) suggest there are two distinct populations of cowbirds, that look and sound different, and most importantly, parasitize different hosts. Population models used for management decisions currently assume that there is only one population. The models' predictions, and thus conservation actions, would be dramatically different if there are in fact two populations. We evaluated this hypothesis by examining the DNA of shiny cowbird samples that were collected from two areas in southwest Puerto Rico (Lajas and Cabo Rojo). Two DNA fragments located in the mitochondrial control region (1,290 bp and 690 bp) where amplified and sequenced, and the sequencing results were evaluated for genetic distance between the populations. Our results will provide valuable information about shiny cowbirds in Puerto Rico, informing yellow-shouldered blackbird conservation efforts.

SPORE VIABILITY AFTER EXPOSURE TO DESICCATION OF TWO TROPICAL MOSSES (*NECKEROPSIS DISTICHA* AND *FISSIDENS ANGUSTIFOLIUS*) WITH OCCURRENCE IN CONTRASTING SUBSTRATES

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Spore viability is a qualitative trait that indicates how long a spore can survive under stress without losing its ability to germinate. The viability of moss spores contained in propagule banks can be affected by disturbances. One common disturbance is reduced availability of water during dry season. Studies in temperate regions have shown that the lack of water, which submits moss spores to desiccation, can hinder their viability. However, few studies have examined the effect of desiccation in tropical mosses. To address this information gap, we submitted spores of *Neckeropsis disticha* and *Fissidens angustifolius*, two tropical moss species that occur on contrasting substrates, to desiccation treatments in order to evaluate their viability. The desiccation treatments consisted of exposure of the naked spores to 12-hours-long day/night photoperiods at a flux of 2,200 lumens. After culture in ½ MS media, viability was assessed by regression analysis of the number of germinated spores. Results show a decreasing linear relationship between time of exposure to the treatment and viability. Spores of *N. disticha* appear more desiccation-tolerant than those of *F. angustifolius*, which implies that they are more resistant to disturbances than the latter. This suggests a trade-off between viability and bank type. Spores of *N. disticha* are likely found in the temporal bark propagule bank, which is subject to more frequent disturbances than the permanent propagule soil bank where *F. angustifolius* spores are present.

DOES MORPHOLOGY PLAY A PART IN THE ART OF SYMBIOSIS? PHYLOGENY AND MORPHOLOGICAL EVOLUTION OF GALEOMMITODEAN CLAMS OF SOUTH MADAGASCAR

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High levels of morphological disparity within closely related species is one of the most intriguing observations in evolutionary biology. Finding patterns of morphological evolution is an ample topic that remains to be studied furthermore. Consider the superfamily of marine bivalves or clams known as Galeomatodidea, it is comprised of clams that display a large array of morphological diversity as well as two life styles, commensals/softbottom or free-living/hardbottom.With this knowledge one can speculate that the evolution of the size and shell shape of commensals may be constrained by their special habitats (hosts).Hence in this study we hypothesized that the morphological evolution of these clams may be influenced by their habitat choice. We tested the hypothesis that the free living/ hard-bottom dwelling clams would exhibit higher variance in shell morphology and larger shell size compared to the commensal/softbottom dwellers. We used a preserved collection of fifty six (7 commensals, 40 free living and 9 of unknown lifestyle) galeomatoidean clams from south Madagascar and reconstructed their molecular phylogeny using one nuclear marker. We performed landmark-based morphometric analyses to display the variety of shell shapes and sizes. Comparative analyses revealed that the free-living clades have higher shell shape variances and larger shell sizes in general. Our results indicate that lifestyle/habitat choice may play an important role in the size and shape evolution of galeommatoidean clams.

GENERATION AND SCREENING OF METAGENOMIC LIBRARIES FROM GUANAJIBO MANGROVE SOIL SAMPLE USING A DIRECT METHOD OF DNA EXTRACTION

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The mangrove soil is a rough environment to the microorganisms due to high salinity, sulfide content, and bacterial inhibitory components such as tannins. Most of the mangrove microbial studies involve the use of traditional cultivable methods which represent only 1% of the total microbial flora there. In order to access the uncultivable flora (99%) of the mangrove as an ecosystem, and search for activities with application in biotechnology and biomedical research, an environmental DNA library needs to be generated. The main objective of this research is to generate a large insert metagenomic library from mangrove soil samples using a direct method and perform screening for the presence of activities such as cellulases, DNases and antibiotic resistance. A metagenomic library of approximately 3,100 clones was generated using a soil sample obtained in the mangrove of Guanajibo in Mayaguez. The freezing and thawing direct extraction method was used to obtain the DNA in order to generate this metagenomic library. The DNA was electro-eluted, end-repaired, and the ligation procedures were done to generate concatemers followed by phage packing and infection of EPI300 T1R Escherichia coli strain. The presence of clones with inserts was confirmed by restriction analysis. We are in the process of screening the metagenomic library generated for the production of cellulases and DNases using carboxymethylcellulose media, and DNA Toluidine Agar respectively. Also, Luria Bertani Agar supplemented with tetracycline (5 and 10ug/mL) was used to select for tetracycline resistant clones.

MICROFUNGI FROM RED MANGROVE EPIBIONT SPONGES AT LA PARGUERA CAYS, LAJAS, PUERTO RICO

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Red mangrove's rhizosphere provides one of the most species-rich habitats in the marine environment. This unique habitat is of great value to a variety of organisms because it serves as a reproduction site and nursery for some, while others spent their entire life there, including fish, crustaceans, echinoderms, mollusks and sponges. Marine sponges depend on a constant flow of water through their bodies to obtain food, oxygen and to remove wastes. Marine sponges form complex symbiotic associations with bacteria, and their symbiotic products have great pharmacological potential. Because of this important contribution their relationships have been extensively studied. However, the relationship between marine sponges with fungi has received little attention in the past and knowledge in this area is very limited. To understand the possible relationships between marine sponges and fungi, information about the fungal diversity associated with these organisms is a priority. The main purpose of this work was to document the diversity of fungal associates associated with two marine sponges from the red mangrove rhizosphere at La Parguera cays in Lajas, Puerto Rico. Samples consisting of sponges and surrounding water were collected and placed in sterile bags avoiding contact with air and then transported in ice to the laboratory for immediate processing. For culturing purposes two different approaches were taken: sponges were hand triturated for dilutions (10-1 to 10-4) and spread over solidified media. Alternatively, pieces of approximately 1 cm2 were inoculated directly over selected culture media. Plates were incubated at 25°C for a maximum period of 60 days to allow growth of slow-growing colonies. Direct plating method resulted in a higher number of isolates, including mycelial fungi and yeasts, while the dilution plate method only yielded mycelial fungi. A total of 96 fungal morphotypes were isolated from both sponge species representing at least 8 identified genera.

THE MARINE ENVIRONMENT SELECTS FOR RIFAMPICIN RESISTANT ENTEROCOCCI

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The enterococci are a diverse group of bacteria abundant in the gastrointestinal tract of animals, from invertebrates to humans. Their presence at a population density higher of 104CFU/100ml in recreational marine waters suggests fecal pollution. Although they are not classified as true pathogens, their capacity to carry virulence factors makes them a dangerous opportunistic pathogen which is among the leading etiological agents in nosocomial infections worldwide. Previous studies in our laboratory have shown that Rifampicin resistance is more prevalent among environmental isolates (beach, 66%) than in clinical samples (56%), septic tanks (40%), and human feces (24%). In this study we attempt to proof that the resistance to Rifampicin confers the environmental isolates with a selective advantage in the marine environment and that this phenotype arises from susceptible strains by selective mutations. Susceptible isolates were exposed to seawater conditions by preparing growth media (BHI) with sterile seawater. A single colony was inoculated in the medium and incubated at 32°C for one week. Daily samples were drawn, serially diluted, and enumerated on plates with and without the antibiotic (5ug/ml). Our preliminary data suggest that the resistance phenotype developed from the susceptible strains in as little as 24hrs when exposed to seawater. Within this culture condition, the resistant phenotype became a significant portion of the total population at 48hrs; in the absence of seawater this was not observed. Taken together our results suggest that exposure to the marine environment promotes the Rifampicin resistance within the population of Enterococci increasing health risk to humans.

ASSOCIATION BETWEEN ALLELE FREQUENCIES OF THE DRD4 GENE AND ADHD IN PUERTO RICO

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Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder that affects a great number of children, and its symptoms tend to persist throughout the lifespan of affected individuals. It is a complex disorder influenced by genetic as well as environmental factors. There have been a number of studies that link ADHD to certain genes, among these the human dopamine receptor D4 gene (DRD4). It encodes for a protein known as dopamine receptor D4 and is primarily found in areas of the brain that control cognition and attention. Most of the diversity of this gene is the result of the length variation in a 48 base-pair tandem repeat (VNTR) in exon 3. Variant alleles ranging from 2 (2R) to 11 (11R) repeats have been found, but the most common variants are the 2R, 4R, and 7R, representing 90% of all gene copies in most populations. The allele frequencies of DRD4 48 bp VNTR vary across populations and the 7R allele is found at a higher frequency in groups that have migrated farther away from Africa and have multi-ethnic ancestries. Puerto Rico is an admixed population with elements of European, African and Native American ancestry, with high levels of genetic diversity with many alleles that can be correlated with risk of a disease or a disorder. In this study, we estimated allele frequencies of the repeat region of DRD4 and the frequencies of several other informative variable alleles found in comparisons between individuals from several regions across Puerto Rico, and discussed potential implication of their distribution on the incidence of ADHD on the island.

INVESTIGATION OF THE UTILITY OF THE SIMPORT-MATRIX CHAPERONES FOR AVIAN INFLUENZA VIRUS SAMPLE STORAGE AND TRANSPORTATION

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Due to the transmission of avian influenza virus (AIV) H5N1 from birds to humans, an increase in surveillance activities occurred. Surveillance studies are important for discovering potential reservoirs or sources of new strains of influenza virus that could be a threat to human and animal health. Success of detection by current surveillance methods depends on sample handling and maintenance of samples at low temperature is critical. Broadly used sampling strategies for AIV in wild birds consists of collecting oropharyngeal, tracheal, and/or cloacal swabs and placing them in viral transport media (VTM) while maintaining the samples on ice in the field and during shipping. The objective of this study was to evaluate a sample collection system in which maintenance of the cold-chain is not critical to the preservation of sample integrity in the field during sample collection and transport. We hypothesized that the Simport Matrix-Chaperone (SMC) system is useful and effective for the storage and transportation of AIV samples. Stability of a low pathogenic H5N3 AIV was tested using three different matrices: MicroCrystals, MicroSpheres, and Elastomer. Our studies show that samples reached complete dehydration after 24 hours of storage at room temperature. Following three days of room temperature storage, the samples were retrieved from the different matrices and tested for viability of the virus by virus isolation and presence of AIV matrix gene by real-time RT-PCR. The AIV matrix gene was detected in all three matrices tested and was isolated from the MicroSpheres and MicroCrystals. This study showed that the SMC system could provide an advantageous and simple system for collection and transport of samples by reducing the need for maintenance of the cold chain.

CELLULOLYTIC ENZYME ACTIVITY AND DEGRADATION OF HYDROCARBONS AND ALGINATE: INSIGHTS FROM BACTERIAL COMMUNITIES ASSOCIATED WITH THE FIDDLER CRAB *UCA RAPAX*

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Mangroves are one of the most important ecosystems in the tropics because they protect coastal areas from erosion. The fauna in the mangroves is diverse, among the invertebrates we can find the fiddler crab, *Uca rapax*. These marine invertebrates are detritivores feeding on detritus or suspended organic material in the sediment of mangroves. Because fiddler crabs do not have the required enzymes for the degradation of some organic materials we assume they have a gut microflora responsible of secreting enzymes that may assist in degradation of organic materials. We tested two isolate groups: (1) isolates only found in the hindgut of *U. rapax* (*Pseudomonas, Paenibacillus, Paracoccus, Achromobacter, Klebsiella, Geobacillus, Echinicola, Micrococcus, Brachybacterium, Vibrio* and *Bosea*), and (2) isolates of common genera that were found in mangrove sediment from the crab habitat (*Bacillus, Microbacterium*, and *Streptomyces*). We investigated cellulolytic enzyme activity through the reducing sugar method; and hydrocarbon (toluene, xylene, and commercial diesel) and alginate (major component of seaweed) degrading capacity using a basal defined medium containing 1% of each compound as the only source of carbon. Positive results for hydrocarbons and alginate were determined by direct observation of bacterial growth. Most tested strains were capable of degrading diesel (83%), toluene (91%) and xylene (87%), and 89% of them also degraded alginate. These results show the importance of bacteria in nutrient recycling in mangrove ecosystems. Future assays will be conducted to test other compounds.

FREQUENCY OF IL33 POLYMORPHISMS IN THE PUERTO RICANS WITH ASTHMA

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Asthma is one of the most recognized human disorders that disproportionately affect the Puerto Rican population. This chronic inflammatory disorder of the airways affected more than 15% of Puerto Ricans from 2000 to 2007, reaching an alarming 20% in 2003, and has become a substantial problem for health and treatment costs. Interleukin-33 (IL33), a gene that has been previously linked to asthma disorder, codes for a cytokine with allergic inflammatory properties. We test for a correlation between variations on the IL33 gene and atopic asthma predisposition. This study is to inquire if the asthma samples have different variants in the IL33 gene affecting the expression of the protein as compared to the reference samples chosen at a southwestern region of Puerto Rico using the Local Genome Diversity Studies Panel (LGDS), a cohort collected by the undergraduate students at the UPRM. We genotyped 96 asthma samples and 182 matched controls from the LGDS study using IL33 assay on the Real-time PCR (RT-PCR). After our initial evaluation of IL33 variation, further projects can be developed to have a clearer view on the possible implications of the analyzed gene in Puerto Ricans. Future work will assess the allelic frequencies of different asthma related SNPs to establish a possible pattern for a more effective identification and to develop diagnostic strategies for asthma and other disorders.

COMPARING LEAF TRAIT VARIATION BETWEEN NATIVE AND NON-NATIVE DRY FOREST TREES

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Assessing the range and significance of functional trait variation both within and among species is a major focus in community ecology. This is particularly important to restoration ecology, because restoration is often focused on increasing native species diversity as a way to increase ecosystem function. Thus, it is important to understand how species diversity relates to functional trait diversity. We compared leaf trait variation among native and non-native trees within actively restored (native dominated) and degraded (non-native dominated) patches of dry forest in the Laguna Cartagena National Wildlife Refuge. We hypothesized that actively restored patches would show higher variation in SLA than degraded patches. Within 6 plots (2 in each patch type), we collected 10 leaves from each of the 3 most common tree species in each plot. Leaves were scanned and their area measured using Image J software. After drying in the oven, leaves were weighed, and SLA (specific leaf area) calculated to provide a measure of functional trait variation. Our results supported our hypothesis as both the average SLA and coefficient of variation tended to be highest in patches where active restoration techniques had been applied. Higher SLA values and higher variation may indicate dominance by species with higher photosynthetic rates and rapid growth. In contrast, lower SLA values in degraded plots may indicate increased stress tolerance by the dominant species in those patches. Information on leaf trait variation may help us predict how forest patches will respond to environmental changes.

THE EFFECT OF VIRULENCE FACTORS AND SUGAR AVAILABILITY ON ENTEROCOCCAL BIOFILM FORMATION

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Enterococci are a common inhabitant of the human body. They can become pathogenic with a change in its environment. This is partly because of the virulence genes developed, or attained, in order to avoid both host defense and antibiotics damage. Bacteremia, urinary tract infection, and infective endocarditis, are three known infections enterococci produce in humans. In the latter two, enterococci take advantage of the high density population and form a 3D structure irreversibly attached to nearby tissue to colonize and survive. This structure, known as biofilm, is a byproduct of genetic and environmental factors. This research takes into account the presence of virulence factors (asa1, gelE, and cylA) and quantitatively measures the growth of the biofilm produced by the bacteria in different growth conditions (sugar-gradient). This is in order to observe the pathogenesis of the isolate, and whether it is affected by the presence of virulence factors. A biofilm assay was developed and optimized to measure the biofilm formation by optical density. So far, the clinical isolates under study have shown an indirectly proportional relation between the biofilm formation and the number of virulence factors present in the cell. Also, the biofilm production drops at 0.2-0.3% of the dextrose-gradient. This data implies that the level of pathogenesis is inversely affected by the number of VF but is also affected by the availability of sugar present. Moreover, isolates with either asa1 or gelE genotype produce almost twice as much biofilm as other isolates (asa1/gelE; asa1/gelE/cylA).

EVALUATING CHROMOSOMAL REARRANGEMENTS IN THE ENDANGERED PUERTO RICAN PARROT

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Last year the Puerto Rican Parrot genome was sequenced to become the first community-funded genome project with the aim of contributing to the U.S. Fish and Wildlife Service – led conservation and recovery program. Currently, The Puerto Rican Parrot Genome project focus in on the annotation: describing and comparing the parrot genome to other avian genomes (like the chicken genome) in silico using bioinformatics tools. In this study, chromosomal translocations between parrot and chicken genomes were identified by aligning 25 kb-long fragments from the start and end of each of the 3,097 scaffolds from the parrot genome to the reference chicken genome by the BLAT program. Of the largest 435 scaffolds representing 908 Mb of the 1.6 Gb parrot genome, 35 (8.0%) showed at least one translocation, and two scaffolds showed two or more. By mapping scaffold segments to the chicken reference chromosomes we have found extensive chromosomal rearrangements between the chicken and the parrot evolutionary lineages, including mostly inversions and possible duplications after the divergence of these species. With our ongoing research, we expect to be able to describe the chromosome rearrangements that have occurred after the divergence of Psittacidae and Phasianidae, and contribute to the general understanding of the avian genome evolution.

CELLULOSE-DEGRADING ABILITY OF ACTINOBACTERIA ASSOCIATED WITH THE GUT OF THE TERMITE *NASUTITERMES COSTALIS*

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Actinobacteria are known for their ability to produce secondary metabolites that can inhibit growth of other microorganisms. Also, they are capable of degrading complex structural polysaccharides such as chitin and cellulose. The association between Actinobacteria and insects has been extensively studied in some cases. The Actinobacteria function in protecting the host from pathogenic microorganisms and providing access to nutrients that the host cannot degrade. In this study we want to determine the cellulose-degradation ability of Actinobacteria isolated from the gut and the nest of the termite *Nasutitermes costalis*, which is a common, xylophagous termite in Puerto Rico. Actinobacteria were isolated and identified from the termite gut and nest in a previous study. We screened all Actinobacteria isolates for cellulose degradation using a qualitative plate assay method. We detected 42 isolates capable of degrading cellulose from the gut of the termite, and 46 isolates able to degrade cellulose from the nest which were further analyzed. We used a quantitative method to characterize the degradation process. Cell growth was determined through optical density to standardize inoculum. Isolates were cultivated in cellulose agar for 72 hours at 25°C. Iodine staining was used to observe the hydrolysis halo and recorded. The qualitative test revealed that 90.5% of the bacteria in the termite gut tested were positive for cellulose degradation, and 90.2% of the bacteria in the nest were also positive. We are further characterizing the positive isolates through quantitative analysis of cellulose degradation.

GENETIC DETERMINATION AND PREDICTION OF SIZE IN ADMIXED DOGS FROM PUERTO RICO

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Domestic dogs (*Canis familiaris*) show a large variety in size, more than any other species ever studied. They range from breeds less than one foot tall, like Chihuahuas and Yorkshire Terriers, to larger breeds such as the Great Dane and Fila Brasileiro. Recently, two genetic variants have been identified to be associated with the dog size: one single nucleotide polymorphism (SNP), and a short interspersed nuclear element (SINE), in the IGF1 gene of dogs. All these studies have been done on pure breeds only which give a limited perspective on the use of the genetic test in dogs of mixed breeds, which in the case of Puerto Rico, represent a large proportion of the canine population (best known as satos). It is unclear, if the size can be determined for satos. Owners have to wait until they grow to the full size. In this study, we intended to test the genetic variants of the IGF1 gene in purebred and mixed breed dogs performing PCR. Results from the SINE are easily assessed by evaluation size variants with a gel electrophoresis, while the allele in the other locus is determined by sequencing. The overall goal of our experiment is to validate the existing correlation between genetic markers and dog size in order to develop a standard test for the assessment of dog size in mixed breeds from Puerto Rico. This will help the future owners to have an idea of how big or small the dog will grow to be. We would like to offer this test to dog shelters and veterinarians across the island.

AFRICAN HIPPOPOTAMUS TO AID THE CAPTIVE BREEDING STRATEGY AT THE PUERTO RICO ZOO JUAN A. RIVERO

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When closely related animals of the same species mate, this can results in an increased chance of homozygous genotypes, some of which could allow expression of lethal or otherwise harmful recessive alleles. This, in turn leads to inbreeding depression, causing health and physic defects: recessive genetic diseases, fertility reduction in females, sperm viability in males and a weak immune system. Inbreeding depression has been a problem in zoo populations which many breeding programs trying to avoid. Genetic testing provides a tool of determining relatedness of individuals prior to breeding. However, most of exotic animals do not have standard genetic markers for relatedness and paternity testing, and often tests are designed when the need for such testing arise. Our laboratory is working to develop a test that would allow the determination of relatedness between the African hippopotamus (Hippopotamus *amphibius*) at the Puerto Rico Zoo Juan A. Rivero. The main purpose my research work this semester is to develop and implement the use of restriction enzyme haplotypes in the control region of the mitochondrial DNA, as molecular marker for this species. First, we develop primers that would amplify a variable region of mtDNA and treated it with a number of restriction enzymes. If a polymorphic site was found that showed different alleles, then the animals must have descended from different mothers and the breeding can proceed. In the future, we will continue to apply the molecular tests to the relatedness analyses of many different species under captivity at the Puerto Rico Zoo.

FROZEN ZOO: ORGANIZATION OF DNA COLLECTION FOR CONSERVATION GENETICS AND COMPARATIVE GENOMICS PROGRAM AT THE BIOLOGY DEPARTMENT, UPRM

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Conservation of DNA from different species can help us preserve genetic material of a variety of animals, some of which are in danger of extinction. The first frozen zoo was founded in 1972 at San Diego Zoo's Institute for Conservation Research as a repository for skin-cell samples from rare and endangered species. We have started a similar project at the University of Puerto Rico at Mayagüez in collaboration with Puerto Rico Zoo Juan A. Rivero. Blood, feathers and hair of animals are collected during routine veterinarian procedures. Different protocols are applied for DNA extraction of the different tissue samples. Tubes containing genetic material of different species are sorted with respect to class, order, family, genus, and species. Subsequently, stored at very low temperatures (-85°C) for optimal long-term preservation. Our objective is to create a scientific and educational collection of animal DNA from a wide variety of species available either from the Puerto Rico Zoo, or other collaborative sources for the future studies in comparative genomics. In addition, these samples will preserve genetic variation of rare and endangered species that may contribute for their conservation. In order to make our collection available for other researchers, we are designing a web page containing information about specimens where visitors could search for samples and the associated information about the animal species using our browser. This initiative is a first step in the collaborative effort to develop a research direction at Puerto Rico Zoo Juan A. Rivero.

ISOLATION OF PIGMENTED CULTIVABLE ANTIBIOTIC RESISTANT BACTERIA FROM CAVE SOILS

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Antibiotic resistance may become the main cause of human demise. Pigments in bacteria have been related to higher antibiotic resistance strains (*Streptococcus*), while in the other hand, red pigmented *Serratia marcenses* have been found more susceptible to antibiotics than non-pigmented subjects. In order to understand resistance, researchers utilize cultivable approaches (CA) and culture independent technologies such as metagenomics. This research focus on the isolation of cultivable gentamicin (gen) and kanamycin (kan) resistant microbes from cave soil in Puerto Rico, and determine its relation with pigmentation. The cave soil samples were serial diluted and spread on Luria Bertani agar, supplemented with gen or kan at 50ug/ml. The plates were incubated at 25°C and 37°C for up to 7 days. The isolates were classified based on the macroscopic morphology and pigmentation. For gen, 4 isolates were capable of growing at 37°C and 8 at 25°C, all pigmented. In contrast, in kan, 13 isolates were obtained at 25°C and two at 37°C. The samples of Kan were gram tested, and 2 non-pigmented at 37°C. The results showed seven cocci, four bacilli. In addition to the microscopic characteristics, tests are being conducted to assess the maximum resistance concentration (MRC). Samples were grown in LB broth with concentrations of kan from 50ug/mL to 200ug/mL. The majority of the candidates grew at the concentration tested. Further increase in the antibiotic concentration is underway. These findings support the concept of searching for cultivable antibiotic resistant bioprospects in low impact environments, considering morphological pigments as a potential indicator.

GENETIC DIVERSITY IN MYOCARDIAL DISEASE SUSCEPTIBILITY LOCI ACROSS PUERTO RICO

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The Local Genome Diversity Studies (LGDS) is a project that aims to collect DNA from the samples of saliva, from all the 78 municipalities of Puerto Rico, with the purpose of using them for the identification of the admixture present in the modern population of Puerto Ricans. The information that this admixture can provide us can be very useful for the identification of genetic disorders and diseases. Several different undergraduate projects are based on this collection. For instance, my own project during this semester is based on the identification of the LTA4H gene, whose rs2247570 and rs2660898 alleles of African ancestry could encode for heart attack problems in the Puerto Rican population. In order to show this association, I helped organizing collection of samples from five different municipalities (Loíza, Fajardo, San Lorenzo, Naguabo and Humacao) located on the eastern part of the island, and carrying the most of the African admixture based on the earlier studies. For each sample, we extracted the DNA and prepared them for the concentrations suitable for the nReal Time PCR.

FUNCTIONAL ROLE OF THE SINGLE NUCLEOTIDE POLYMORPHISM LOCATED AT -224 A>G & -599 C>T IN NEUROPEPTIDE RECEPTOR Y2 (NPY2R) GENE IN PREDISPOSITION TO HYPERTENSION

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Hypertension (HTN) or high blood pressure is a disease that affects one in three adults worldwide. Genetic, environmental and lifestyle factors influenced the risk of having HTN. Previous work in human population have identified genetic variants in the promoter region of the Neuropeptide Y receptor 2 (NPY2R) associated with hypertension. We hypothesized that single nucleotide polymorphism (SNP) in the promoter region of the NPY2R at -224 A>G and -599 C>T will affect the expression levels of this gene. To examine this hypothesis the DNA of 16 individuals were amplified to produce two fragments of 1025bp (R3) and 349bp (R4) of the regions of interest in NPY2R. These fragments were inserted into the luciferase reporter system vectors (pGL4.10 and pGL4.23 respectively) and transfected in the HEK 293 cells. Expression levels of the luciferase reporter gene were measured and compared for the different constructs. Our data provided strong evidence that both alleles (-224 A>G & -599 C>T) can regulated the level of expression of the promoter in NPY2R. However, the insert containing both SNPs (-599 & -224) appears to exert a greater expression in gene regulation. This may be due to the combination of both SNPs and the influence of the promoter region belonging to NPY2R.

CONFRONTATIONS BETWEEN ACTINOBACTERIA ISOLATED FROM *CYPHOMYRMEX MINUTUS* AND MICROFUNGI WITH PATHOGENIC POTENTIAL

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In the fungus-growing ant symbiosis, the ants cultivate a fungus (Basidiomycota) and protect their cultivar from specific parasites (Ascomycota) using the antibiotic production capacity of Actinobacteria. Three different genera of fungus-growing ants are known from Puerto Rico. Of these, *Cyphomyrmex minutus* is the only species that cultivates its fungus in yeast form. The cultivar pathogen Escovopsis has not been described for any yeast agriculture ant species. The main objective of this research is to study potential antibiotic producing Actinobacteria isolated form *C. minutus* against other fungus-growing ant's cultivar pathogens. We selected six of the most frequent actinobacteria previously isolated and identified from *C. minutus* for bioassays and confrontations. Six microfungi known for their pathogenic potential against the cultivar of others fungus-growing ants were used in the bioassays. Actinobacteria were inoculated in yeast malt extract agar (YMEA) on the center of the plate and incubated at 25°C for 21 days. The microfungi was inoculated at 21mm away from the actinobacteria and incubated for 30 days. At this point the zone of inhibition (ZOI) was measured. The interaction between the actinobacteria and the fungi was reported. *Streptomyces* 8-1 and *Nocardia* sp. look like good candidates for producing a zone of inhibition against other microfungi. We will be exploring their antibiosis potential and as future work testing the production of secondary metabolites.

ANALYSIS OF PHYTOPLANKTON IN LAKE PATILLAS

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This research is based on the analysis of phytoplankton in Lake Patillas, a man-made reservoir located in the municipality of the same name to the southeast of Puerto Rico. A sample was collected with two attached Bongo nets in January 2013, and preserved in formaldehyde. In our preliminary analysis, we identified and quantified the phytoplankton in the reservoir. Then, we compared our results with also preliminary analyses done in 2012. However, we observed that, per year, the lake's predominant phytoplankton species changed depending upon some factors in the reservoir. An ochrophyte, *Dinobryum* sp., predominated in our phytoplankton preliminaries of 2013, followed by the green alga *Pediastrum simplex*. The results in the preliminaries of 2012 were the opposite: *Pediastrum simplex* was the predominant phytoplankton followed by *Dinobyium* sp.

ISOLATION OF TOXIN COMPONENT INTERACTING PARTNERS USING T7 PHAGE DISPLAY TECHNIQUE WITH HUMAN CDNA LIBRARIES

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Since 9/11 attacks, biological warfare research has taken an important role; specifically understanding the toxins mode of action with biomolecular partners. Proteomics is a method that elucidates biochemical and regulatory pathways in which proteins functions. The identification of interacting partners of known function with those, whose role is uncharacterized, makes possible to infer the biological activity, allowing the development of therapeutics in biomedical research. Phage Display (PD) is a proteomics method to study protein-protein interactions. This "in vitro" technique allow the generation of pools of combinatorial nucleotides displayed as peptides on a virus (T7) surface. The main focus of this project is to use T7 PD to isolate interacting partners of lethal factor, part of the protein secreted by Bacillus anthracis. The display of human cDNA fragments has successfully been used to identify putative interacting candidates from purified wild type and mutant lethal factor as targets. Premade T7 PD from Human Stomach and Colon cDNA phages clones interacting with putative toxin were isolated after several rounds of affinity selection (biopanning). The cDNA was amplified by PCR, and the amplicons sequenced and analyzed in silico. Preliminary data, for the colon cDNA, suggest consensus regions with different known proteins associated with cellular differentiation, among others. In contrast, the stomach cDNA interacting partners did not show similarity to any known protein indicating novel interacting domains. Ongoing studies are made to obtain more candidates for analysis. Also specificity test will be done to confirm the ability of the peptides to discriminate between targets.

DIVERSITY OF BENTHIC OSTRACODS FROM CARIBBEAN MESOPHOTIC REEFS

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Mesophotic coral reefs (MCEs), reefs at depths of 50 m to 150 m, are receiving renewed interest from marine scientists and managers because they are linked physically and biologically to their shallow water counterparts, have the potential to be refugia for shallow coral reefs, and can be a source of larvae that could contribute to the resiliency of shallow water reefs. The lack of knowledge in regard to population dynamics between mesophotic corals and their shallower counterparts impairs our broader understanding of the ecology, evolution, biodiversity and connectivity of coral reef communities, essential to conservation efforts such as the delineation of marine reserves. In an effort to increase our knowledge of the biodiversity, abundance and distribution, benthic ostracods, one of the most successful microcrustaceans of marine ecosystems were selected in this study. Several sediment samples from MCEs of Puerto Rico and US Virgin Islands were collected at different depths (52-102m) using technical diving. Ostracods were either hand sorted directly from the sediment or after a Ludox AM-30 colloidal silica resuspension and centrifugation step, used for mass-extraction of meiofauna and macrofauna. The highest densities of ostracods were found in the deepest samples (≥ 67m) and these were the most abundant and diverse assemblages. The total community of ostracods collected belongs to the orders Myodocopida and Podocopida. Podocopid ostracods showed the highest number of individuals and species. Using a morphological and molecular barcoding approach, we provide the first report of the biodiversity of ostracods in the MCEs of NE Caribbean.

IDENTIFICATION OF SIMPLE SEQUENCE REPEATS IN THE GENOME OF THE PUERTO RICAN PARROT (*AMAZONA VITTATA*)

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In this study we used BLAT comparative alignment in UCSC's Bioinformatics database to annotate scaffolds from the Puerto Rican Parrot (*Amazona vittata*) genome assembly using information from the reference chicken (*Gallus gallus*) genome. Of the 2,660 scaffolds covering 235.3 Mb of the 1.6 Gb genome, less than 5% found no homologous sequence. Most scaffolds aligned with the Z chromosome (approximately 35%). Using the approximate start and end points, scaffolds were reorganized into proper sequential order according to chromosome and position. Using the default parameters in the RepeatMasker Web Server of the Institute for Systems Biology, the organized scaffolds were scanned against the reference chicken genome again to find potential Simple Sequence Repeats (SSRs), also known as microsatellites. We identified SSRs interspersed every 4 Mb to create a genetic map and study recombination in the parrot pedigrees. SSRs of three or more base units were ideal, because their lower mutation rate guarantees a reliable mode of inheritance. On the other hand, SSRs of 15 or more repeats are more informative, because they demonstrate higher mutation rates. Of the hundreds of SSRs identified, most belonged to the 2-base unit type, and none were without an adenine or thymine nucleotide. Few repeats were classified 3-base unit type, and even fewer exceeded 15 repeats. We will continue this study with the eventual goal of creating reliable markers for recombination-based genetic maps and population studies in *A. vittata*.

DISTRIBUTION OF ANTIBIOTIC RESISTANCE PATTERNS AND VANCOMYCIN RESISTANCE GENES IN ENTEROCOCCUS SPP. ISOLATED FROM CLINICAL, HUMAN FECAL, ANIMAL FECAL, RIVER AND BEACH SAMPLES

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A significant rise in nosocomial infections caused by Vancomycin-resistant Enterococci was reported in the United States since 1989. The genes that confer resistance are vanA which presents high resistance to vancomycin, vanB confers moderate resistance, and vanC confers low resistance and have been found as an intrinsic characteristic in motile Enterococci. Interestingly, the enterococci are the gold standard fecal indicator of pollution in recreational waters and potentially a reservoir of these organisms. In this work we determine the distribution of vancomycin resistance genes in the enterococci population found in different habitats including river, beach, clinical, animal, and human fecal samples. Out of 517 isolates, only 155 were selected using their intermediary or resistant phenotype base on the Kirby-Bauer antibiotic resistance method using vancomycin. These were further characterized by a Multiplex PCR assay where we found a broad frequency of genes throughout the sources: the beach was the most diverse source with vanA (4.5%), vanB (5.7%), vanC1 (1%), and vanC2/3 (44%); in clinical (32% vanA, and 7% vanB); in river vanB (20%); in animal fecal vanC2/3(14%) and vanA (2%); and in healthy human fecal samples we did not detected any genes. Interestingly, some isolates presented a negative genotype suggesting the existence of a new van genotype or a new sequence variant dominant in most habitats. This data suggest that these genes are not limited to clinical isolates, the beach isolates contain a higher diversity of genes than any other source supporting that these isolates do not originate from humans.

ASTROCYTES EXPRESSING NEF PROMOTE APOPTOSIS AND SMAD SIGNALING IN NEURONS

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HIV is known to infect cells in the brain causing significant neurological impairments. Damage ranges from mild to moderate HIV Associated Neurocognitive Disorders (HAND) or severe HIV Associated Dementia (HAD). Previous work in our lab found learning impairment in rats infused with astrocytes expressing the HIV protein negative factor (Nef). Histological studies performed on the brain tissues of those rats showed a loss of neurons near the site where Nef was present as well as evidence for TGFβ; signaling. We set up in vitro experiments to test for relationships between astrocytic Nef and TGFβ; signaling as well as neuron loss. We propose this loss is due to an apoptotic response of the neurons to the astrocytes expressing Nef. We grew neuronal cells and exposed them to astrocytes expressing Nef or green fluorescence protein (GFP) as a control. Annexin V staining was used to measure apoptosis and a reporter assay measured SMAD activity by TGFβ; signaling. Results suggest that Nef causes neuronal apoptosis when expressed by astrocytes. The neurons also show an increase in SMAD reporter activity at 48 hours after exposure to astrocytes expressing Nef. These studies suggest that the loss of neurons in rats may have been due to apoptosis cause by the astrocytic expression of Nef. These studies are relevant to understanding the high prevalence of HAND among patients receiving effective antiretroviral treatment.

GRAMMAR FOR TROUPIALS: SYNTAX IN THE COMPLEX SONG OF VENEZUELAN TROUPIALS (*ICTERUS ICTERUS*)

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Humans and songbirds are among the small set of species that learn their vocal signals. Researchers have used songbirds to study various aspects of vocal learning, including syntax: the orderly temporal arrangements of acoustic units. Venezuelan Troupials (*Icterus icterus*) sing both simple repetitive songs and complex songs with two or more syllable types, but their song structure has never been investigated before. This report describes the structure of their complex songs, with special attention to paid to syntax. Free living Troupials were recorded during their breeding season in Cabo Rojo, Puerto Rico. We made spectrograms of over a hundred complex songs, identified syllable types, and examines the syllable order. Preliminary findings indicate that birds use different syllables in the beginning, middle, and end of songs. We suggest that Troupial songs are characterized by phonological syntax: Only some combinations of elements are allowed but elements have no information value when emitted singly.

SYNERGISTIC PATHWAY BETWEEN CHEMICAL AND MECHANICAL INDUCED INFLAMMATION IN HUMAN EPITHELIAL CELLS

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Acute lung injury (ALI) is a major cause of respiratory failure and mortality in critically ill patients. These patients suffer from severe hypoxia and must be placed on a mechanical ventilator in order to survive. However, mechanical ventilation is known to further exacerbate the initial injury and it may lead to ventilator induced lung injury (VILI). The main focus of this work is to show that the inflammation of ALI is a result of both a pathogenic infection and the effect of the mechanical forces exerted on the lung tissue. To explore the synergistic relation between mechanical and chemical inflammation, an adenocarcinomic human alveolar basal epithelial cell line from the lung (A549) and polarized human small airway epithelial cells (HSAECs) were grown to confluence on transwell culture inserts and treated with different concentrations (5 μg/ml, 10 μg/ml and 50 μg/ml) of Lipopolysaccharide (LPS) to simulate a Gram-negative bacterial infection. After treatment, the cells were exposed to cyclic pressure (± 10 cm H2O at 0.2 Hz) for 8 hours. Samples without LPS treatment and/or pressure were used as controls. After treating the cells, an ELISA allowed the measurement of different cytokines secretions, such as IL-6, IL-8 and IL-1β, resultant from the inflammation caused by the given conditions. A clear response in cytokine secretion is seen after the pressure treatment suggesting a relation between mechanical stimuli and inflammatory response.

SONG TYPE MATCHING IN A SPECIES WITH A SPLIT REPERTOIRE

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"Song-type matching" occurs when a bird replies to the song of a rival by singing the same song-type. Male Adelaide's warblers (*Setophaga adelaidae*) exhibit song-type matching. Like other New World Warblers (Parulidae), Adelaide's warblers have repertoires that are split into two classes: B-type songs are given before dawn during the breeding season, and A-type songs are given at all other times. We are using this system to conduct the first study of song-type matching across singing modes. We recorded nine free-living males during both A-type and B-type singing, compared rates of song-type matching between the two singing modes, and tested the hypothesis that individual males are consistent in their tendency to match song types during A-type and B-type singing. We found that birds both matched more, and were matched more during B-type singing. Data on male tendencies to match are forthcoming.

SOCIAL BEHAVIOR AND FACIAL SHAPE IN THE EUSOCIAL PAPER WASP *MISCHOCYTTARUS MEXICANUS CUBICULA* (HYMENOPTERA, VESPIDAE)

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The division of labor and the morphological characteristics that improve differentiation in the workers force in social species is one of the angular stones in the understanding of the evolution of social behavior. Six wild nests of the eusocial paper wasp *Mischocyttarus mexicanus cubicula* were located in Susua forest P. R., each of the wasps that were found in the nest were single paint-marked with the intention of recording their individual behavior. Video recordings of wasp's behaviors were conducted for two days for each nest. Ethograms were developed using focal sampling method. At the end of the two-days of recordings, the whole nest was collected and each wasp's face was photographed under a stereoscope in order to perform geometric mophometrics analysis for the purpose of recognizing morphological differences with a mathematical algorithm. There are five different behaviors that are clearly observed by the frequency of the task performed; these are nursing, foraging, nest building, guarding and dominance. The wasp's face morphometrics and the behavioral observations, suggests a relationship between tasks performed and facial features.

SCANNING ELECTRON MICROSCOPY ANALYSIS OF THE MICROFLORA ASSOCIATED WITH THE HINDGUT OF THE FIDDLER CRAB *UCA RAPAX*

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Bacteria have important ecological roles in marine ecosystems. A significant amount of nutrients are available in these environments, but they are not completely accessible to the marine fauna. A diversity of bacteria can be found, especially associated with marine invertebrates, which facilitate nutrient access and mobilization, essential in nutrient recycling. It is our main concern to characterize the microflora associated with the hindgut of the 'fiddler crab' *Uca rapax* and its colonization in the mangrove ecosystem of Bosque Estatal de Boquerón, Cabo Rojo, PR. Hindgut associated bacteria facilitate nutrient uptake providing enzymes for detritus degradation and absorption. Given the abundance of bacteria present in the gut, morphological and physiological plasticity is expected to occur as an adaptive response to nutrient limitation. Our objective is to observe, through scanning electron microscopy (SEM), the diversity of bacteria associated with the hindgut of *Uca rapax*, to characterize the microscopic population and to determine whether the population is resident or transient. Hindguts dissected from the live crabs were fixed in glutaraldehyde (4% solution) and dehydrated in ethanol (10-100% series solution) prior to critical point drying. Hindguts were dissected at different times during processing. Samples were coated with a gold layer for further characterization of the bacterial diversity and analyzed through SEM. Previous culture dependent studies demonstrated a dominance of Firmicutes in *Uca rapax* hindgut, while the microscopic analysis showed rods as well as cocci-shaped bacteria; the latter being the predominant form.

EFECTO DE LA COMBINACIÓN DE FEROMONAS DE NITIDÚLIDOS Y COATRAYENTES SOBRE ESCARABAJOS POLINIZADORES DE ATEMOYA (ANNONA SQUAMOSA LINN Y ANNONA CHERIMOLA MILLER: ANNONACEAE)

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El árbol de atemoya, un híbrido entre *Annona squamosa* Linn y *Annona cherimola* Miller (Annonaceae), posee un gran potencial para ser cultivado en las zonas tropicales y subtropicales gracias a un fruto de alta calidad. La evolución de este género ha desarrollado un método de reproducción por cantarofilia, polinización por escarabajos, en el cual los nitidúlidos juegan un papel importante. Sin embargo, el obstáculo de un bajo rendimiento y baja calidad de frutas han impedido el mercadeo a gran escala de la fruta, el cual es producto de visitas poco frecuentes de polinizadores. En nuestro estudio se monitorio la atracción de 10 tratamientos, los cuales consistían de combinaciones de feromonas disponibles comercialmente y co-atrayantes, utilizando trampas universal de alevillas en un cultivo de atemoyas en Puerto Rico. Los resultados mostraron que todos los tratamientos significativamente atraen a las especies de escarabajos recolectadas. La atracción de los tratamientos fue dependiente tanto de las especies como en los periodos en los que se llevaron a cabo los muestreos, pero los especímenes con mayor potencial de explotación para la polinización lo fueron *Brachypeplus* sp. y *Carpophilus dimidiatus* (Coleoptera: Nitidulidae) los cuales obtuvieron los mayores promedios en los muestreos realizados. Algunos de los tratamientos aplicados (i.e. dátil e higo + masa de pan y feromona de nitidulides + dátil y higo + pan) demuestran que podrían ser utilizados en huertos comerciales de atemoyas, sin embargo es imperativo llevar a cabo estudios que determinen el efecto preciso de cada tratamiento sobre escarabajo polinizador.

ANALIZANDO POLIMORFISMOS INFORMATIVOS DEL GEN APOE, SU FRECUENCIA EN LA REGIÓN DE ADJUNTAS, P.R. Y SU RELEVANCIA EN LA ENFERMEDAD DE ALZHEIMER

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El Alzheimer es una enfermedad neurodegenerativa que afecta la memoria, la forma de pensar y el carácter o la manera de comportarse de una persona. Esta enfermedad es la forma más común de demencia, representando un 60% a 80% de los casos de demencia, y hoy día se estima que alrededor de 5.4 millones de estadounidenses poseen Alzheimer. Según estudios realizados, el riesgo de desarrollar Alzheimer en los hispanos es 1.5 veces mayor que en los norteamericanos, y aunque todavía no se conoce qué causa esta enfermedad sí se han encontrado factores ambientales y genéticos que contribuyen a su desarrollo. Actualmente se conocen varios genes asociados a Alzheimer pero entre ellos una variante del gen APOE, el alelo APOE-4, es considerada como el mayor factor genético que contribuye al desarrollo de esta enfermedad. El gen APOE codifica para una proteína conocida como apolipoproteina E(ApoE). Esta proteína es sintetizada en mayor cantidad en el hígado por las células parénquimas, seguido por el sistema nervioso central, donde es sintetizada y secretada por los astrocitos. ApoE es crítico para el transporte de lípidos a través del plasma y para la homeostasis de colesterol en el sistema nervioso central. En este proyecto, se analizaron los haplotipos de 55 individuos puertorriqueños, gracias a la información publicada por el proyecto 1000 Genomes, para identificar polimorfismos informativos sobre las familias de haplotipos más comunes en Puerto Rico y que tuvieran alguna relevancia médica. La frecuencia de estos polimorfismos en el pueblo de Adjuntas será presentada.

PURIFICATION OF A NEW LYSINE TAG FUSED SULFIDE REACTIVE HEMOGLOBIN FROM *LUCINA PECTINATA*

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Hemoglobin I (HbI) from the clam *Lucina pectinata* is capable of binding hydrogen sulfide (H2S) with very high affinity through its heme group. Therefore, this protein is an ideal biological component that possesses great potential to be developed into a highly selective H2S biosensor. The incorporation of anchoring tags to proteins, such as the HbI, is a very convenient procedure for optimization of purification purposes. That is why the HbI was fused with lysine residues at the C-terminus, using a cloning strategy. The purified fused HbI can then be utilized for biomedical and sensor engineering applications. This study presents the optimization of the purification of Lys-tagged recombined HbI (rHbI). Initially, the purification strategy consisted of the separation of the cell debris of *E. coli* (that produced our protein of interest) from the rHbI and other present intracellular proteins via centrifugation. This was followed by different chromatographic techniques such as: size exclusion chromatography (SEC), cationic exchange chromatography (CEC) and anionic exchange chromatography (AEC). The total protein concentration of each aliquot was determined through the Bradford protein assay. Then, the aliquots were analyzed by SDS-PAGE and Ultraviolet-visible spectroscopy to observe molecular weight of the proteins and characteristic bands of HbI, respectively. The analyzed SDS-PAGE gels and spectrum showed that the purification processes of the rHbI require further optimization. That is why additional optimization of these techniques is currently being effectuated in order to purify the rHbI.

GENERATION AND SCREENING OF METAGENOMIC LIBRARY FROM MANGROVE SOIL USING DIRECT METHOD OF DNA EXTRACTION

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Mangroves soil has a high content of sulfide, organic matter, trace of metals, and variable acid and salt content. The combination of these factors makes mangrove soil, an unusual microenvironment to unravel and understand its cultivable and uncultivable microflora. Metagenomic is an emerging discipline that uses culture independent methods, to access the uncultivable. It consists in microbial community DNA isolation and cloning into a special vector known as fosmid, which will be introduced in a cultivable strain to perform diversity and functional studies. The main purpose of the research is the generation of a metagenomic library (Ml) from mangrove soil using a direct extraction method, and performs screening for the presence of chitinase, protease and amylase production. The mangrove soil was subjected four times to freeze (-80°C) and thaw (60°C) for 45min. The high molecular weight DNA was sizing in an agarose gel, and electroeluted overnight. The DNA was end repaired, ligated with pCCIFOS1 and in vitro packaged into Lambda particles. The *Escherichia coli* Epi300 was used as a host, and a total of 3,000 clones ML was generated. Several MG clones were randomly chosen, and the DNA extraction and restriction analysis confirmed the presence of an insert. A monitoring for the proposed activities was performed using Luria Bertani Agar supplemented with starch, skim milk, and minimal media containing chitin as sole carbon source. The culture media for the selections and screening have been inoculated, and we are in the process of determining the presence of any positive clones.

REGULATION OF WNT PATHWAY BY PRB IN OSTEOBLASTS

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pRb is a tumor suppressor that plays an important role in the co-activation of the osteoblast-specific transcription factor Runx2. The Wnt pathway also plays an important role in osteoblastic differentiation. Signal activation of Wnt hinders β;-catenin degradation, which promotes its re-localization to the nucleus, where it co-activates the transcription of Wnt target genes. Therefore, β;-catenin nuclear localization is considered a predominant indicator of Wnt activation. In contrast, the inhibition of Wnt leads to the recruitment of a protein complex responsible for targeting β;-catenin for degradation. Since the deregulation of both, pRb and Wnt, is concomitantly observed in osteosarcomas, we were interested in determining if these pathways are functionally related. The aim of this study is to determine whether pRb is capable of affecting Wnt signaling by regulating β;-catenin localization. We hypothesized that a functional pRb is necessary for β;-catenin nuclear localization. Immunocytology for β;-catenin in cultured pRb-expressing and pRb-deficient MC3T3 cell lines was conducted in order to assess β;-catenin localization. We also performed β;-catenin immunohistochemical (IHC) analysis on normal bone and osteosarcoma tissues. Our qualitative immunocytology shows an increase in nuclear β;-catenin localization in the presence of a functional pRb. These correlations demonstrate that pRb function promotes β;-catenin's nuclear localization. Interestingly, in osteosarcoma tissues we observed a correlation in which pRb's localization determines β;-catenin's localization. Importantly, we observed that cells with cytoplasmic pRb in osteosarcoma tissues also had cytoplasmic β;-catenin localization. We conclude that pRb promotes β;-catenin nuclear re-localization, showing that pRb plays an important role in Wnt activity.

ISOLATION AND CHARACTERIZATION OF AMMONIA OXIDIZING MICROORGANISMS IN MARINE, SALINE, AND HYPERSALINE ENVIRONMENTS

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Nitrogen is one of the essential elements for life. It is an important part of cells biomass being present in proteins, DNA and other molecules. The nitrogen cycle is composed by four main reactions: fixation, nitrification, denitrification and annamox (anaerobic nitrification). During nitrification, ammonia is converted to nitrite, and to nitrate by an additional reaction. This reaction is the most important of the Nitrogen cycle because most living organisms can only assimilate nitrogen as nitrate. The objective of this project is to study the populations that oxidize ammonia in marine (3.5% - 5.0% NaCl), moderate halophilic (10.0% - 15.0% NaCl) and extreme halophilic (20.0% - 25.0% NaCl) environments from Cabo Rojo, Puerto Rico. Several strains showing a very slow growth rate were isolated from these three sampling sites using a medium that has ammonia as the electron source and sodium bicarbonate as the solely carbon source. A total of 48 isolates are being characterized using morphological and physiological properties. In silico analysis of 16S rRNA gene sequences have shown that the isolated strains belong to the bacteria and archaea domains. Preliminary 16S rRNA gene sequence analysis on strains MC2A, MC2B and MC4 showed that they are closely related to the genera *Halovenus*(95.7%), *Halomicrobium*(95.1%) and *Halovenus*(93.6%). These isolates are possible autotrophic ammonia oxidizers belonging to the extremely halophilic archaea. Further studies like physiological tests, identification of specific molecular markers such as amoAand accgenes is currently on their way. The study of these isolates can provide useful information on the possibility of ammonia oxidation in high salinity environments.

EXTRACTOS NATURALES CONTRA CANDIDA SP

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Candida albicans es un hongo levaduriforme que forma parte de la flora normal del cuerpo. Cambios fisiológicos en el individuo pueden causar crecimiento excesivo del patógeno oportunista *Candida albicans* produciendo candidiasis. Candidiasis digestiva se considera una micosis sistémica porque afecta el sistema digestivo provocando la rápida proliferación de la levadura, daños a las paredes de los intestinos y la colonización de otros microorganismos. Esta investigación tenía como objetivo probar el efecto anti fúngico de extractos naturales en *Candida albicans*. Los extractos naturales utilizados fueron canela, Pau D' Arco y aceite de orégano. Se realizaron diluciones de cada uno de los extractos naturales y se utilizaron discos de sensitividad impregnados con los extractos a diferentes concentraciones: 100%, 75%, 50% & 25%. Se cultivó la levadura en medios de Sabureaud Dextrose Agar (SDA) en tres pH diferentes; pH 5.2 (control), pH 4.5 & pH 3.9, esto para simular el pH del sistema digestivo. Todos los platos fueron incubados por ±72 horas a una temperatura de 37ºC. Se observó que el extracto de canela no tiene efecto antifungal al no mostrar halos de inhibición en los discos de inhibición. En cambio los extractos de Pau D' Arco y aceite de orégano si presentaron halos de inhibición en las diferentes concentraciones y medios de SDA utilizados, por lo que si exhiben efectos anti fúngicos contra la levadura *Candida albicans*. Se proyecta realizar futuras variaciones en los pH de los medios, tiempo y temperatura de incubación y utilizar extractos naturales que contengan compuestos similares a los utilizados, como el aceite de tomillo.

THE RELATIONSHIP BETWEEN ABUNDANCE AND SIZE IN BENTHIC FORAMINIFERA: A TEST FOR THE ALLOMETRIC THEORY

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The variability in foraminiferal body size has been used as an indicator of environmental disturbance; however, there is additional information that can be derived from body size and abundances. For instance, the latter relationship can also be used to link community structure and resource availability, to estimate rates of energy transfer and to predict resource availability. The allometric theory suggests that as body size decreases there is a concomitant increase in abundance and vice versa. In this work we will quantify the size of different foraminifera and its relative abundance in two sites in southwestern Puerto Rico to determine whether it fits the allometric model. If foraminiferal density scales with body size within the trophic levels as predicted in a Lindeman trophic web, then the rate of change should be -3/4. We collected 10 random samples and extracted all foraminifera. The cells were measured using known geometrical shapes and will be regressed with relative abundance. Based on the work with multi trophic level of testate amoebae, we expect to see a fit model for foraminifera across levels.

ANOTACIÓN DEL CROMOSOMA 28 DE LA COTORRA PUERTORRIQUEÑA

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Como parte del Proyecto del Genoma de la Cotorra Puertorriqueña, se llevó a cabo la anotación del cromosoma 28 de la cotorra que es homólogo al cromosoma 28 de la gallina (*Gallus gallus*), siendo el genoma de referencia en aves. Por medio del alineamiento con Stand-Alone BLASTde mRNAs de genes en el cromosoma 28 de la gallina, al genoma de la cotorra se identificó un total de cinco andamios con secuencias del cromosoma 28. Entre todos, los cinco andamios cubren una longitud de 2.5 Mb de los 4.7 Mb del cromosoma. Estos tienen una densidad de 45 genes por Mb, mayor que la obtenida al presente de los cromosomas 8 y 11. Los genes fueron anotados utilizando tBLASTx para el alineamiento de cada exón. Típicamente, el primer exón de cada gen sufre de una divergencia muy alta que impide su anotación utilizando al genoma de la gallina como referencia. A pesar de ser un cromosoma corto, se observan inversiones frecuentes y translocaciones intracromosómicas en relación ante el genoma de referencia. La identificación de elementos repetitivos usando Repeat Masker reveló que, contrario al caso en humanos donde el número de SINES duplica el número de LINES, en la cotorra el número de LINES presenta un orden de mayor magnitud que el de SINES. Esto se debe mayormente a una escasez de SINES, dado que el porciento del cromosoma con elementos repetitivos es bajo (3.3%). Esta investigación continuará realizándose utilizando los cromosomas de referencia de la gallina y la misma tendrá la finalidad de establecer la sintenía y los re-arreglos cromosómicos que poseen ambos genomas.

CHEMICAL ANALYSIS OF NEST MATERIAL FROM TWO TERMITE SPECIES IN PUERTO RICO

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Two species of termites in the genus *Nasutitermes* (*N. costalis* and *N. acajutlae*) are common in Puerto Rico. They build their nests on trees out of wood, soil, fecal secretions and saliva. Termite nests not only provide shelter but also food and energy storage for the termite colony. External appearances of nests from both of these species seem to be the same. We determined macro and micronutrients nest composition, pH, NH4+, NO3-, percentages of organic carbon and organic matter from three nest samples of *N. acajutlae* from Guánica and six nest samples of *N. costalis* (three from Joyuda and three from Miradero) through chemical analyses using an Optical Emission Spectrometer. Our results showed that samples from *N. acajutlae* nests have a higher NH4+, NO3-, P and Mn content with relative values of 206.6 ppm, 15.3 ppm, 83.86 ug/g, 4.816 ug/g, respectively. On the contrary, the K content is higher in *N. costalis* nests with values higher than 3647.6 ug/g. In general, organic matter and carbon content showed no significant differences between nests of both species. A curious observation was that high Zn content was determined in *N. costalis* nests, but *N. acajutlae* nests show complete absence of this micronutrient. It is probable that the differences found in nests from both species of termites are due to species-specific differences given that *N. costalis* nests were sampled from different ecosystems but still showed similar composition with each other.

A COMBINED THERAPY OF HYPERTHERMIA AND CURCUMIN ENCAPSULATED IN NANOCAPSULES FOR AUGMENTED CANCER CELL DESTRUCTION

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Curcumin has been shown to have excellent antioxidant, anticancer, and pro-apoptotic properties. However, its poor solubility in water and short half-life in circulation are two main hurdles for its wide application as a therapeutic agent. To overcome this problem, curcumin was physically encapsulated in nanocapsules synthesized using two biocompatible polymers: Pluronic F127 and chitosan. The curcumin-loaded nanocapsules (nano curcumin, N-Cur) had an average diameter of 22.0 ± 0.7 nm at 37°C. Cancer cells were treated with various drug concentrations of free curcumin (F-Cur) and N-Cur and mild hyperthermia (HT) was applied at different time points. It was found that the N-Cur had a much better anticancer effect than F-Cur. Moreover, the combination of N-Cur and HT after one hour of drug treatment can decrease the IC 50 of prostate cancer (PC-3) cell destruction by more than 14 times (1.26±0.57 vs. 18.75±11.32 μg/ml) and 11 times (1.26±0.57 vs. 11.61±8.71 μg/ml) compared to F-Cur with HT and N-Cur without HT, respectively. Preliminary studies suggest enhanced apoptosis is the mechanism of the improved cell killing when N-Cur is combined with HT. In addition, cell cycle analysis shows that cells 1-3 hr after the curcumin treatment in the late G1 and early S phase are the most vulnerable to the combined treatment. This study shows the great potential to develop a novel combined cancer treatment modality of hyperthermia and nanoparticle-mediated delivery of curcumin or other therapeutic agents for effective cancer cell destruction.

GENOTYPING OF THE HUMAN IGFBP-2 LOCUS IN THE RENAL CARCINOMA CELL LINE A498

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In humans, variation in expression of the insulin-like growth factor binding protein 2 (IGFBP-2) has been associated to various types of cancer. Its presence has been identified as an insufficient marker of cancer predisposition, while high concentration of this protein has been linked to the presence of a tumor. Our research objective was to genotype a renal carcinoma cell line (A-498) for reported single nucleotide polymorphisms (n=8 SNPs; GenBank Accesion: AY398667) located at intron-2 of IGFBP-2 and to identify potential new mutations. This region was chosen because of evidence generated in our lab suggesting homology between bovines and humans (two regions: 138 and 98 bp) offering the option of an animal model, since IGFBP-2 intron-2 in bovines is highly polymorphic (n=12 SNPs; GenBank Accession: BV680048). Also, DNA was isolated from human saliva of one subject for nucleotide sequence trace comparison. Primers to amplify the selected region in humans were designed using the sequence available at the NCBI database (GenBank Accesion: AY398667) and the IDTDNA primer design tool. The PCR amplified fragment (1,300 bp) was sequenced in forward and reverse (Macrogen, Seoul, Korea) and analyzed using the sequence-editing tool BioEdit. A novel thymine (T) insertion/deletion was identified in a 9-10 mononucleotide (T) short tandem (microsatellite) repeat. Both A-498 and the human subject were heterozygotes for the INS/DEL, while the reported sequence from NCBI is homozygous for the insertion. Homozygous individuals for both the insertion and the deletion allele need to be identified to further confirm this discovery. Moreover, we need to elucidate the potential effects of a reading frame shift in the non-coding DNA/RNA (immature mRNA), since the 8 reported human IGFBP-2 Intron-2 SNPs are located downstream of the T INS/DEL, and its ultimate effect on cell proliferation and prospective relation to neoplastic processes in the kidney or other anatomical structure.

GENE ANNOTATION AND CHARACTERIZATION OF REARRANGEMENTS IN THE EVOLUTION OF THE PUERTO RICAN PARROT (*AMAZONA VITTATA*)

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The Puerto Rican parrot (*Amazona vittata*) was listed as an endangered species in 1967 and since this date conservation projects were developed. Last year, the *A. vittata* genome was the first vertebrate genome sequenced a community-funded project, making the genome data publicly available for research. Our study is part of the next step in the parrot genome project: the annotation of the chromosomes, description of the protein-coding genes and other important protein and gene features. We focused on chromosomes 8 and 11, and compared them to the reference genome of chicken (*Gallus gallus*) and the zebra finch (*Taeniopygia guttata*). These species are used for different reasons: the *G. gallus* genome is the most thoroughly studied among birds, while *T. guttata* is the most closely related species to the Puerto Rican parrot. However, information from other avian species could be used as well, for instance the turkey genome (*M. gallopavo*) also shows the high evolutionary conservation for some genes in the parrot genome. We have thoroughly annotated 3.9 Mb of the chromosome 8 and 0.7 Mb of the chromosome 11, and found 46 and 12 genes, in these regions respectively. On chromosome eight, 27 genes have been completely annotated, whereas in the other 19, some regions showed divergence that has not allowed full annotations. On chromosome 11, only one gene was too divergent for annotation. Some interesting features have been identified by the comparison, for instance an exon fusion, four frame shift introns, and one premature stop codon.

INITIAL GERMINATION AND SEEDLING GROWTH OF *MORINGA OLEIFERA*: VARIATION IN RESPONSES TO SCARIFICATION, LIGHT INTENSITY AND SUBSTRATE TYPE

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Moringa oleifera Lam. has been widely hailed as a "miracle tree" in dry tropical areas due to its multiple uses. Optimal conditions for cultivation should be understood prior to the establishment of commercial plantings in Puerto Rico. A first step is learning about initial seed germination and seedling growth. We used germination tests to assess viability of locally collected unhulled seeds stored for different periods of time (1, 2, 3 months). We obtained 100% germination in Petri plates for seeds stored 1 month, but after 2 months storage germination was reduced to ~50% and to 15% for seeds stored 3 months. In a second experiment, we tested germination of fresh seeds in 3 substrates (commercial potting soil, paper towels and field soil from Lajas Agricultural Experimental Station). A total of 350 seeds were sown in replicate trays or Petri plates and monitored daily over 10 days. Germination percentage was highest in commercial potting soil and lowest on paper towels. In a third experiment in the greenhouse, survival and growth of seedlings transplanted from the second germination experiment were monitored in two different light levels: ambient light and deep shade. Initial results indicated that scarification reduced germination, while shade decreased survival and growth. Furthermore, seedlings from seeds germinated in the Lajas soils type were taller and had more leaflets than those germinated in the commercial soil type. These results suggest that this species can be germinated readily from seed but seed handling and processing affect initial germination and performance of seedlings.

CAN THE MOSS' PROTONEMA BE DISPERSED BY HIDROCHORY?

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Bryophyte use sexual and/or asexual modules, and various mechanisms (water, wind, and animal) for dispersal. Among these, asexual structures (gametophyte stem and leaf fragments, bulbils, gemmae, and protonema) and their dispersal has not been studied thoroughly. In addition, there is no information regarding the dispersal of the protonema by any mechanism. Therefore, we are interested in knowing if the protonema disperses and how the dispersal and survival of these structures are affected by water (ombrochory-raindrop splash). To develop protonemata, we surface sterilized spores of *Taxyphyllum taxirameum* and *Callicostella belangeriana*, which were cultured on ½ MS under laboratory conditions. Protonemata of each species (n= 100; according to treatment) were subjected to raindrop simulation dispersal that consisted of three sequential dyed raindrops dropped at 1 and 2 meters from a burette. The dispersal distance was measured (cm) from the source. Distance was analyzed statistically (Kruskal-Wallis test & linear regression) to see if the species, height, or relationship with protonema size were affected by ombrochory. Protonemata dispersed for each species. Maximum dispersal was 45 cm and 80 cm for *C. belangeriana* and for *T. taxirameum*, 52 cm and 71 cm (1 & 2 m, respectively). Drops from 1m dispersed the protonema less than drops from 2 m; in contrast, species traits did not affect protonema dispersal distance. There was no linear correlation between protonema size and dispersal distance. Ombrochory aids protonemata in short distance dispersal. Height of drop dispersal enables the protonemata to travel further distances, which, helps colonize new substrates.

THE USE OF INSECTS AS INDICATORS OF RESTORATION SUCCESS IN TROPICAL DRY FOREST

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A frequent goal of restoration activities is to increase diversity and structural complexity of native plant communities. Because arthropod diversity is strongly determined by the structural diversity of the plant community, they may serve as efficient indicators of restoration success. We compared insect diversity among native and non-native trees within actively restored (native dominated) and degraded (non-native dominated) patches of dry forest in the Laguna Cartagena National Wildlife Refuge to determine: 1) if there is a difference in insect species diversity, and 2) if this difference is related to restoration history. This was achieved through quantification and identification (to taxonomic order level) of insects from Malaise traps samples from six plots (three in each patch type). Insect orders present in both actively restored and degraded patches included: Diptera (true flies), Hemiptera, Lepidoptera, Collembola, Hymenoptera, Coleoptera, Psocodea, Blattodea, and Orthoptera, with Diptera having the most individuals. Orders Strepsiptera and Neuroptera were found only in the non-native tree plots and represented by just one individual. While there was no difference in average richness between patch types, average richness per individual tended to be higher in non-native tree plots. Average abundance tended to be higher in native tree plots. This trend was probably due to the overwhelming amount of Dipterans, which could have overcome the short distances between plots due to flying capability. Our data could prove useful in assessing restoration success by understanding how insect taxa respond to restoration activities that increase cover and diversity of woody plants.

COMPARISON AND DOMINANCE OF ENTEROCOCCI STRAINS SHARING THE SAME VIRULENT GENOTYPE FROM DIFFERENT AQUATIC ENVIRONMENTAL ISOLATES USING A FINGER-PRINTING PCR TECHNIQUE

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Enterococci have a dual role in the human body. They are commensal microorganisms and part of our gastrointestinal tract typically associated with health benefits. On the other hand, as an opportunistic pathogen, they are responsible for causing urinary tract infections, bacteremia, and endocarditis. These infections are exacerbated by the presence of virulence factors that help the bacterium to adhere to surfaces and create biofilms, namely aggregation substance (asa1) and gelatinase (gelE). Interestingly, our previous research demonstrated that these two virulence factors are also present in the Enterococci inhabiting our waterways. In this research we used environmental isolates from around the world to determine the prevalence of virulent factors using a multiplex PCR. The positive isolates, with the same virulence genotype, were further characterized using a finger printing technique to identify strains of the same species. Our preliminary data supports that the virulence genotype asa1/gelE dominates among *E. faecalis* isolates from around the world. Furthermore, the finger printing technique demonstrates that each site contains a diverse population; in PR at least five different strains coexist. Interestingly some strains are distributed around the world, for instance, strain-1 is present in PR, Hawaii, California, and Australia; while other two strains are only present in PR and Hawaii. In conclusion, our data suggest that Enterococci with the virulence genotype of asa1/gelE are selected by the marine environment worldwide. Furthermore, this genotype is limited to a narrow set of strains that are distributed and selected by similar environments around the world; the environment selects.

TAXONOMIC STRUCTURE OF THE MEIOFAUNA FROM CARIBBEAN MESOPHOTIC REEFS

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Biodiversity surveys with emphasis on meiofauna associated with mesophotic coral ecosystems (MCEs) has been studied as an extension of a long-term research program (Deep Cres) of the Department of Marine Sciences, University of Puerto Rico at Mayagüez. Mesophotic coral reefs in the Caribbean, one of the most diverse regions in the world, are among the least documented. This study is a research effort aimed to establish a characterization of the meiofauna taxonomic structure that could help to evaluate the biodiversity of these MCEs, and the connectivity between them and their shallow water counterparts. Several mesophotic reefs of Puerto Rico and adjacent regions were sampled at different depths (3 to more 100 m) by SCUBA diving during several cruises. Organisms were hand sorted from sediments or after Ludox AM-30 colloidal silica resuspension and centrifugation step. The total community of organisms were counted and separated into orders. Copepods were the most abundant taxa at all stations, representing 77% of all organisms collected. Acari followed in relative abundance with a 8.7%. Amphipods ranked third in relative abundance (5.61%), and the other orders have relatives abundances lower than 5%. El Seco station at Vieques Island represents the place with the highest abundance and the highest diversity of all stations sampled. Significant differences were found among depths and taxonomic groups, the highest densities of organisms were found at the greatest depths sampled and were far more abundant and diverse that shallow and intermediate water depths.

POPULATION DIVERSITY OF *ENTEROCOCCUS* SPP. IN POULTRY FECAL SAMPLES

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Enterococci are normal flora of the gastrointestinal tract of humans and animals, they are also commonly used as indicators of fecal contamination of recreational waters. *Enterococcus* is also found inhabiting soils, water, and plants. Since enterococci seem to be ubiquitous in natural habitats is difficult to interpret the significance of exceedance of enterococci in a recreational water sample in tropical habitats. In this research, describe the diversity of enterococci commoly found in poultry fecal matter to examinate it possible contribution as a contamination source. The fecal samples were collected in a homestead from individual specimens, composted, homogenized and then serially diluted and planted on mE agar. Once incubated, isolated colonies were picked at random and characterized biochemically and specieted using a PCR-RFLP method using the atpA gene. Phenotypic and genotypic charasterists were determined using pigmentation, motility, resistance to antibiotic, virulence factors, and strain identification by BOX-PCR. The population of enterococci was dominated by *E. casseliflavus*, *E. faecalis*, *E. faecium*, and to a minor extent *E. gallinarum*. Resistance to tetracycline dominated among the population (70%), followed by Rifampicin (24%) and very low resistance to piperacillin and vancomycin. The genotype of virulence was dominated by gelE and asa1/gelE with few avirulent isolates. Interestingly, the population of *E. casseliflavus* was limited to one strain, while the *E. faecalis* was more diverse containing at least 5 different strains. This work describes the population of enterococci within poultry feces as part of a comprehensive survey of animal feces as possible source of recreational water contamination.

CHARACTERIZATION AND QUANTIFICATION OF PHYTOPLANKTON IN GUAJATACA RESERVOIR, PUERTO RICO

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Guajataca is reservoir located mostly on karstic ground, between the municipalities of Isabela and Quebradillas, Puerto Rico. The main use of this reservoir is water supply for agriculture and urban areas. Duplicate samples were collected on February 1, 2013 using bongo nets to analyze the phytoplankton of this mesotrophic reservoir. A total of 16 taxa were observed. Both replicates had the same algae as most abundant: *Pediastrum simplex* and pennate diatoms. However, one of the bongos (A) had a relative abundance of 43% *Pediastrum simplex*, while the other (B) had 64%. Pennate diatoms had an average relative abundance of 38% on bongo A and 14% on bongo B. Preliminary results suggest an assemblage typical of a dry season, with abundance of organic matter.

DETERMINATION OF ESTROGEN DEGRADING BIOPROSPECTS USING CULTIVABLE AND CULTURE INDEPENDENT APPROACHES

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Estrogens have been attracting a lot of attention in the scientific communities due to their natural inability to biodegrade and continued exposure in high quantities to the environment. The excessive use of estrogen related compounds like, but not limited to, birth control pills, natural supplements, prescriptions for menopausal women, and other derivatives to further increase production of foods has raised concerns on a public health level. A publication by the U.S. Geological Survey (2001), reported the presence of reproductive hormones (40%) and estrogenic alkylphenols (70%) in surface waters. To come in contact with significant concentrations of estrogenic compounds has been linked to diseases such as breast and testicular cancer, endometriosis, Alzheimer, lower sperm counts, birth defects, and feminization of male fish worldwide. The purpose of this research is to find novel bioprospects using cultivable and culture independent approaches, capable of fully degrading estrogenic compounds. Screenings of composts and forest metagenomic libraries, and soil samples from diverse places were serially diluted and spreaded on minimum media (M9) supplemented with 17β-estradiol and 17α-ethynilestradiol as the only carbon source. Growth in the media will suggest that the bioprospect can use the estrogen, as the sole carbon source, thus further analysis will be performed to identify the gene(s) responsible for this activity. To date, possible candidates have been found in the Golden Creek behind the Biology building, but neither of the metagenomic libraries tested have shown any ability to grow under the described conditions. Other screenings of different metagenomic libraries, soils and water samples are in progress.

SCREENING FOR AMYLOID-β DEGRADATION ACTIVITY ON METAGENOMIC LIBRARIES FROM PUERTO RICO SOILS

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Diseases such as Parkinson and Alzheimer (AD) are related to the presence of protein aggregates (plaques) within the brain cells. These plaques are composed of the amyloid β; (Aβ40-42). An enzyme system that degrades the plaques will be needed in order to reduce the development of the disease. Metagenomics allows accessing, by culture independent approaches, activities of uncultivable microbes which represent 99% of the total microbes in soil. In our laboratory, novel enzymes activities like involved in antibiotic resistance, have been found in metagenomic libraries generated from forest soils. This fact leads us to propose if culture independent approaches allow the access of the majority of the microbial community in the environment, then, activities related by β40 degradation could be present. Four soil metagenomic libraries from two forests in Puerto Rico, dry and rainy forests, were used to screen the ability of using Aβ40 as the sole carbon source. The libraries were inoculated in Luria Bertani for 3 hrs, and after an OD600 of 0.2, the cells were washed and inoculated on minimal media (M-9) supplemented with Aβ40. After the incubation at 37°C, 132 clones from one rainy forest ML, and both MLs from the dry forest (215 clones total) showed growth in Aβ40. Some of the potential clones from the ML grew in 7 days, and showed differences in morphology (bigger colonies) from the control, which took 14 days to grow. The results suggest the presence of metabolic activity using amyloidβ, an important step to initiate studies in medical bioremediation.

SEROPREVALENCE OF PARVOVIRUS B19 IN PUERTO RICO

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Parvovirus B19 (B19) is a single-stranded DNA virus of the Parvoviridae family, known to cause erythema infectiosum and hydrops fetalis, and suspected of causing diverse idiopathic conditions. Due to B19's small size and resistant viral particle, it is of concern for transfusion products, as it may cause donor-recipient infections. Seroprevalence studies provide information useful in gauging the impact of a pathogen in a population, and how its prevalence varies in different regions and with time. In a first attempt to study the seroprevalence of B19 in the Puerto Rican population, blood samples were gathered from blood banks and verified for immunoglobulin G specific for B19 by an indirect ELISA. Preliminary data resulted in a seroprevalence of 59%, coinciding with a current study that resulted in a seroprevalence of 59% for a high risk population (elementary school teachers) in the island. The data suggests that there is no significant difference between the seroprevalence of the general populations and teachers in Puerto Rico. This contrasts with the tendency that has been described for this virus, where the high risk population has a higher seroprevalence than the general population. The value is similar to that in European countries, but lower than in the United States. We suggest that this may be due to significant differences between the populations under study. We recommend nucleic acid testing of transfusion products in the island due to the high seroprevalence and the ability of B19 DNA to infect.

ISOLATING AND IDENTIFYING CULTIVABLE ANTIMICROBIAL PRODUCING AGENTS BIOPROSPECTS FROM HYPERSALINE MICROBIAL MATS AND FORESTS' SOILS IN PUERTO RICO

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Today, the increase of antibiotic resistant microorganisms and the increase and prevalence of infections over other diseases is estimated between 45% and 87%. To counteract this issue, approaches such as understanding the antibiotic resistance phenomena, or searching for novel antimicrobial agents have been developed. The main focus of this research is to find cultivable bioprospects from Microbial Mats (MM) and forests soil (FS) in Puerto Rico, capable of producing new antimicrobial agents. The microbial targets included *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus epidermidis*. Serial dilutions using samples from MM and FS were performed using 0.85% NaCl. The diluted samples were spread on Nutrient Agar and Tryptic Soy Agar. The samples were incubated at 25ºC and 37ºC, for 24 hrs and colonies showing an inhibition halos were isolated as putative antimicrobial agents producing bioprospects (PAAPB). The isolates were tested for inhibition activity against the targets, by Kirby Bauer method. A total of 37 PAAPB were isolated on this study. The preliminary testing of the candidates confirmed that two bioprospects inhibited *K. pneumoniae*, two *S. epidermidis*, two *P. aeruginosa* and five *E. coli*. The bacterial DNA was extracted effectively and its 16S rDNA amplified by PCR. In silico analysis was done to determine the identity of the isolates. A 350 genomic library clones of one of the isolates was generated to identify the gene(s) responsible for the inhibition. After identifying the gene(s) responsible for the inhibition by Tn mutagenesis, combinatorial chemistry approaches will be performed to increase the inhibition potential.

TESTING FOR RHYTHMIC METER IN VENEZUELAN TROUPIAL (*ICTERUS ICTERUS*) SONGS

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Rhythmic meter describes the pattern of stressed and unstressed beats in music and poetry. In music, stressed beats occur at the beginning of each measure and at small integer fractions of the measure. No non-human animals have been shown to use rhythmic meter in their acoustic signals. If birds do exhibit rhythmic meter, we would expect to find stressed (high amplitude) beats at points corresponding to the beginning of each song syllable, and at small integer fractions (e.g., x/3, x/4, or x/5, where x is a small integer) within syllables. Observations in the field suggested that Venezuelan troupial (*Icterus icterus*) songs exhibit rhythmic meter. We used a sample of over 500 songs to test this hypothesis. We measured the time at the beginning and end of each syllable, and at the beginning of each note within the syllables. The duration from the beginning of the syllable to the beginning of each note within the syllable was divided by the syllable duration. These values were compared to the distribution of values expected given random timing. Our preliminary results indicate that troupial songs exhibit rhythmic meter. Specifically, troupials appear to divide their syllables into evenly sized thirds, fourths, and fifths. By demonstrating that an animal can use rhythmic meter, this study opens the door to an improved understand of music cognition, and the functional significance of rhythm in acoustic signals.

A COST-EFFECTIVE METHOD FOR IDENTIFICATION OF COMMON SNAKE SPECIES IN PUERTO RICO

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Rapid and effective way for identification of animal tissues is crucial for forensics and construction of genetic libraries, and can be used in education, for medical purposes, and to help the conservation of endangered species. Our laboratory has been collaborating with the Puerto Rico Zoo in identification of reptilian remains, specifically, DNA of native and exotic snake species found in Puerto Rico. Since DNA sequencing can be expensive, and requires special equipment, we proposed a cost-effective alternative - species assignment with use of restriction enzymes. Six species of snakes are commonly found on the island: *Python reticulates* (Reticulated Python), *Python regius* (Ball Python), *Python sebae* (Rock Python), *Python molurus* (Burmese Python), *Boa constrictor* (Boa constrictor), and *Pantherophis guttatus* (Corn Snake). We extracted DNA, quantified and PCR amplified the mitochondrial 16S rRNA gene, then performed restriction enzyme digestion and electrophoresis on all the six species. We selecting two restriction enzymes that proved the most informative differentiation between the snakes: Nla III, and Ase1 I. The results in the electrophoresis gels show different enzymatic cut patterns for each individual species and provide an easy way for identification of all six snake species in Puerto Rico. Our method can be adopted by the forensic laboratories, as well as veterinary or medical clinics, used for surveys of natural populations of snakes, or educational purposes.

ISOLATION AND DIVERSITY OF ACTINOBACTERIAL MORPHOTYPES FROM RIO CUPEYES

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Recently, scientists have focused their studies in the discovery of novel Actinobacteria due to their capacity for secondary metabolites production. Actinobacteria are mainly terrestrial but they are found also in aquatic ecosystems. Therefore, we can consider Puerto Rico as a promising source for the discovery of Actinobacteria due to the diverse environments present in the island. This research focused in the isolation of Actinobacteria from Rio Cupeyes, a stream with relatively low human impact. Sediment and water samples were collected at three different sites and at different seasons to determine if Actinobacterial diversity changes across time in a set segment of the river. Actinobacteria were initially isolated using chitin agar supplemented with antifungals as selective medium. Morphological features were observed using yeast-malt extract agar (YMEA). A total of 49 Actinobacterial morphotypes were obtained from Rio Cupeyes. The highest quantity of morphotypes was recovered in the second sampling, during the summer season. Preliminary results suggest that there is a relatively high diversity of morphotypes and that these are particular for each sampled season. The significant difference in the isolated morphotypes between seasons might be related to the temperature of the water and to the runoff in the vicinity of the river. Future work will be conducted to determine the identity of the isolates via genetic analyses and whether they are producers of secondary metabolites.

SEARCHING FOR FUNGAL BIOPROSPECTS CAPABLE OF METABOLIZING 2, 4, 6 TRINITROTOLUENE

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Fungi are characterized for their cell wall made of chitin, tubular structures (hyphae) and saprophytic capabilities. Its morphological diversity and physiological versatility are also distinguishing traits. Fungi have been used for environmental applications in bioremediation, being capable of degrading complex molecules and hydrocarbons. In this research, fungal bioprospects where tested for 2,4,6-trinitrotoluene degradation, also known as TNT. The fungal candidates were inoculated on potato dextrose broth (PDB) supplemented with TNT (2ppm), except the negative control. The plates were incubated for two weeks, collecting samples every three days. Each sample was analyzed using High Performance Liquid Chromatography (HPLC) with a mobile phase of 70% water and 30% methanol. The peak on the chromatogram corresponding to TNT appeared 7.2 minutes approximately after injection for each sample. According to these results, three candidates were capable of degrading TNT at different rates. Although there was a reduction of TNT, the media did not present degradation which suggests the assimilation of TNT. According to morphological characterization, two bioprospects may belong to the *Aspergillus* and *Geotrichum* genus. Molecular characterization involved genomic DNA extraction, and amplification of 18S rDNA and the internal transcribed spacer (ITS) regions by PCR. The amplicons were further sequenced and in silico evaluated. The analyzed 18S rDNA suggest that the isolates may belong to *Aspergillus versicolor*, *Aspegillus* sp. and *Tricosporum* sp. genus. However, ITS analysis suggests that the bioprospects belong to the *Aspergillus siplowii* and *Aspergillus nomius* species. Experiments are in progress to understand the bioprospects usage of TNT, and further molecular characterization.

GES-21, A NEW EXTENDED SPECTRUM BETA-LACTAMASE DETECTED ACROSS WASTEWATER-POLLUTED COASTAL ECOSYSTEMS FROM PUERTO RICO

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GES (Guiana extended spectrum) determinants are an emerging group of bacterial enzymes with an extended substrate range that facilitates the degradation of a wide variety of beta-lactam antibiotics, including those used as last resort. Genes encoding GES enzymes are known to reside within a genetic system designated as mobile resistance integron. Resistance integrons (classes 1-3) are laterally transferred among clinical isolates of gram negative bacteria and are implicated in the acquisition as well as in the dispersal of multiple antibiotic resistance mechanisms. However, these elements are also being recently detected outside the hospital environment, particularly in settings impacted by fecal pollution. Herein we describe the occurrence of a novel genotype of a GES-like beta-lactamase (GES-21) which is encoded by a class 1 integron and was exclusively detected in metagenomic DNA retrieved from various coastal environments highly impacted by point and non point sources of fecal pollution. The detection of GES-21 under such circumstances demonstrates that human influence can turn local coastal ecosystems into stable reservoirs of bacteria highly adapted to gain and spread genes that compromise the effectiveness of medically important antibiotics. Functional analyses of GES-21 through molecular dynamics simulations and gene expression are currently in progress.

SONG ORDER IN ADELAIDE'S WARBLER

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All human languages are characterized by a property called "lexical syntax." Lexical syntax describes a set of acceptable orderings of elements that also provide information when given individually. It appears to be rare in non-human animals. If we consider a given bird song as an independent and complete unit of information, then birds that consistently sing their song types in an ordered manner could be said to exhibit lexical syntax. We tested for this type of lexical syntax in male Adelaide's warblers (*Setophaga adelaidae*). Preliminary analysis of morning singing from three individuals indicated non-random song-type delivery. Syntactical rules differed between pre-dawn and post-dawn singing. Specifically, we found strong associations between pairs of song types before dawn, but repetition of the same song type and then slow transitions to another song type after dawn. We developed a variety of graphical representations to visualize our data. Results suggest that the information the warbler transmits may not only depend on the song choice but also on choice of song order. Future analyses will integrate contextual information, with the goal of understanding the function of the birds' syntactical rules.

LOCAL GENOME DIVERSITIES STUDIES: GENETIC DIVERSITY AND FREQUENCY OF CCR5 Δ32 DELETION IN THE MUNICIPALITY OF BARCELONETA

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The genetic variation present in Puerto Rico makes the island a great scenario for admixture studies. Puerto Rico has an admixed population composed of three major ancestries: African, European, and Native American, and high genetic variation across the 78 municipalities of the island have been documented. Our goal is to demonstrate genetic variation in one of the disease candidate loci present in the municipality of Barceloneta. This town was founded on July 1, 1881 and was one of the last municipalities founded by the Spanish government in Puerto Rico. It was named after Barcelona, the capital of Catalonia, Spain. The name may be an indication that at the time of its foundation, there were many Catalonians living in the area. The population has increased from 7,835 citizens at the time the town was incorporated to 25,013 citizens today, of which 77.9% are self-classified as European-American and 6.9% as the Afro-Americans, according to the 2010 census. For this study, we collected 96 saliva samples from people of the town and extracted their DNA. We then amplified, and analyzed the frequency of the deletion of 32 base pairs in the CCR5 gene using electrophoresis. This characteristic mutation, which is more common in people of European ancestry, confers a degree of protection against HIV. We expect to find at least 6% of the population with the polymorphism.

SPEEDBALL ALTERS THE VPR-MEDIATED APOPTOSIS IN NEURONS

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HIV infection leads to Associated Neurocognitive Disorders (HAND) in nearly 50% of patients. HIV infects microglia and astrocytes in the brain. Infected astrocytes normally do not support viral replication but do express viral proteins. The HIV-1 viral protein R (Vpr) is an important factor in viral replication and contributes to HAND by eliciting apoptosis in neurons. HIV+ drug abusers show higher neurotoxicity than their non-abusing counterparts. Speedball is a commonly abused drug mixture of cocaine and heroin. We hypothesize that speedball enhances the Vpr-mediated neuronal apoptosis. We co-cultured SH-SY5Y neurons with Vpr-transfected SVGA astrocytes in the presence of different concentrations of speedball. We then measured markers of cell apoptosis, cell viability and toxicity using a fluorescent assay. Vpr caused apoptosis and cytotoxicity in neurons. Speedball effects were dependent on dose with higher doses reducing Vpr toxicity. Viability measurements were unchanged under the conditions we tested. These data suggest that the combination of Vpr and high dose speedball induced a protective response in neurons. Supported by NIH: DA026722, MD007579 and GM096955.

SONG MODULATION IN RESPONSE TO A SIMULATED TERRITORY INTRUSION IN ADELAIDE'S WARBLERS (*SETOPHAGA ADELAIDAE*)

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Bird song is a multifunctional signal that plays an important role in territory defense. Although early research indicated that adult song was "crystallized", recent studies suggest that songbirds dynamically optimize song characteristics according to their behavioral context. This kind of adaptive plasticity may be especially important during territorial conflicts, when signals are predicted to communicate the singer's aggressive motivation and fighting ability. Alteration of song sound frequency parameters during simulated contests has previously been demonstrated in canyon wrens and *Montezuma oropendolas*. Our study will determine the presence and extent of song modulation in response to a simulated territory intrusion in male Adelaide's Warblers (*Setophaga adelaidae*). We will compare songs that were recorded before and during a simulated territory intrusion, measuring song length, song high and low frequency, frequency with maximum power frequency, as well as trill rate. Our findings will shed light on the facultative vocal adjustment hypothesis.

COMPARING THE DIVERSITY PRESENT IN VENTANA CAVE SOIL METAGENOMIC LIBRARY AND THE SOIL SOURCE USING DENATURING GRADIENT GEL ELECTROPHORESIS

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Metagenomics is a tool to evaluate genomes in an environment, unraveling novel activities and microbial diversity using culture independent approaches. A Metagenomic Library (MgL) from Ventana Cave soil in Puerto Rico has been studied using functional analysis, but how representative is the diversity found in the MgL when compared to the diversity in the cave is still needed. The goal of this research is to compare the microbial diversity present in the MgL with the diversity present in its respective soil source using Denaturing Gradient Gel Electrophoresis (DGGE). Fosmid DNA from induced MgL was extracted, and a direct DNA extraction to the soil used to generate the MgL was made. Universal 16S-rDNA-PCR using domain-specific primer was performed. Amplicons were run using a 6.5% polyacrylamide gel (37.5:1) with a denaturing gradient of 40%-60%. Band numbers where quantified and patterns were compared. Also, the bands were excised, amplified, and sequenced. The soil source band pattern was a subset of the fosmid bands from the MgL which suggests PCR bias. Sequence data analysis showed that the majority of the MgL-16S-rDNA band pattern included uncultivable bacteria. Some cultivable bacteria present included *Pseudomonas*, *Vibrio*, and *Luteimonas*. In the soil-16S-rDNA band pattern, the diversity assessed consisted of mostly uncultivable bacteria, nonetheless the presence of *Actinomyces*, *Microbacterium*, and Promicromonosporaceae was detected. Even though it has been said that metagenomic assesses the totality of the microbial diversity in a given environment, DGGE demonstrated that the MgL partially represents the diversity present in the soil source.

ISOLATION OF PURPLE NON-SULFUR BACTERIA FROM THE PHYTOTELMATA OF ZINGIBERALES

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Purple non sulfur bacteria (PNSB) are members of the alpha and beta Proteobacteria. They are versatile physiologically, which makes them candidates for bioremediation. The PNSB are ubiquitous, previously found in our laboratory in phytotelmata of bromeliads. Phytotelmata are small aquatic environments held by leaves or flowers of plants. This accumulation of water provides a suitable habitat for microbes and various aquatic organisms. The flowers of Zingiberales like Heliconias has phytotelmata that could serve as microecosystem for PNSB. The purpose of this investigation is to detect and isolate PNSB from the phytotelmata of plants from the order Zingiberales on different locations in Puerto Rico. A total of 14 samples were collected from the phytotelmata of Zingiberales located in Mayaguez and Las Marias. The samples were spread on Sistrom's minimal media and incubated anaerobically with light for a week. A total of 30 PNSB were isolated from all the phytotelmata tested, 15 from each town. Genomic DNA was successfully extracted from all isolates, and 25 16SrDNA amplicons were obtained by PCR. The in silico analysis showed that eighteen isolates were most closely related to *Rhodopseudomonas* sp., four to *Rhodobacter sphaeroides* and three to *Rubrivivax gelatinosus*. The fifteen isolates from Mayagüez have also been tested for their capability to grow in minimal media with biodiesel, and diesel, as the sole carbon source. Seven of the fifteen isolates grew with diesel and biodiesel. Future work aims to further confirm these results and determine the biotechnological potential for bioremediation of the isolates.

THE OPERANT CONDITIONING BIRD CAGE

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The Operant Conditioning Bird Cage (OCBC) is a research tool for Behavioral Ecologists studying mating signal preferences in female birds. Using this system, researchers can conduct operant assays to examine the Darwinian process of female choice. Our system allows researchers to assign different stimuli to each of three special perches inside the bird cage via a computer application specifically designed for this system. When a bird hops on a perch, a sound stimulus (e.g., a male bird song) is reproduced through speakers inside the cage. The OCBC automatically tallies the time of each choice for the duration of the session. The OCBC's flexibility will allow researchers to address questions about female choice that would not be tenable with alternative testing apparatuses. Among the intended applications are a test of the hypothesis that signal alignability affects female preference, and an assay that allows females to design synthetic songs.

IS EVERYTHING REALLY EVERYWHERE? ABUNDANCE AND SPECIES RICHNESS OF BENTHIC FORAMINIFERA IN TWO SOUTHWESTERN COASTAL ZONES OF PUERTO RICO

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Benthic foraminifera have been used in previous studies as bioindicator tools for paleoecology, ocean acidification and biogeographical patterns. However, there is few data regarding the current distribution of foraminifera. With the new climatic change we need to know the current distribution of foraminifera to then predict the new upcoming environmental change. We will estimate foraminifera species richness and abundance in zones that have different anthropogenic disturbances with two objectives: determine if everything is everywhere across latitudes and across similar and contrasting disturbance regimes. If everything is everywhere (the Beijerick-Becking axiom), then there should be similar diversities between Parguera and Buye samples under similar and contrasting disturbance regimes. Moreover, our species lists should be similar to that published elsewhere. Twelve random samples were collected from two sites in southwestern Puerto Rico, Buye and La Pargera. Within the site, two areas were selected that had contrasting disturbance regimes (high and low). Based on preliminary data, species composition, diversity and abundance seem higher on sites with high disturbance, although most of the species are shared among sites. This should be explained by a possible resemblance, in the structure and composition, of the sand. Upon completion, our species list will be compared with that published at different locations around the world like New Zealand, Saudi Arabia, Hawaii and other locations.

ASSESSING THE GENETIC DIVERSITY OF NATURALIZED CACAO (*THEOBROMA CACAO* L.) THROUGH AN ISLAND-WIDE SURVEY IN PUERTO RICO

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Cacao (*Theobroma cacao L.*) originated in the tropical rainforests of South America, was mainly harvested in Central America and later followed its arrival to the Caribbean islands. Cacao is one of the most important crops for many countries worldwide. Many studies have reported genetic diversity of cacao, but little is known about the existing types of cacao in Puerto Rico. Therefore, it is imperative to retrieve and analyze genetic information of *T. cacao* for the preservation, conservation and understanding of existing types available in Puerto Rico. With this purpose, cacao pods and leaves were obtained through an island-wide survey. Furthermore, morphological characterizations and DNA extractions from the leaves were performed using a commercial kit. DNA was assessed for quantity and quality and arrayed on PCR plates to be analyzed using a Single Nucleotide Polymorphism (SNP) genotyping technique. By assessing the genetic diversity of naturalized cacao in Puerto Rico, a much better understanding of the history and sources of introduced cacao is garnered; unique germplasm identified in the study will be acquired, propagated and included in the official germplasm collection at the USDA-ARS TARS site. As an implication, this research could promote the relevance of *T. cacao* as a key component of local agriculture, and further incorporation of cacao varieties to the studies concerning sustainable development and public health.

IMPLEMENTATION OF 1-D ANALYSIS SOFTWARE QUANTITY ONE® TO IMPROVE ACCURACY OF BOX-PCR DNA FINGERPRINTING FOR THE IDENTIFICATION OF ACTINOBACTERIA ASSOCIATED WITH *NASUTITERMES* NESTS

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Nasutitermes is a genus of higher termites within the eusocial insects. Their condition makes them vulnerable to pathogenic attacks because their nests are constructed among common waste buildup and because they are genetically more similar between themselves than to other populations. It has been shown that other social insects (e.g. ants, bees) have developed symbiotic relationships with Actinobacteria to protect themselves from such attacks. To help describe this type of relationship, BOX-PCR fingerprinting and electrophoresis techniques were implemented in the identification of Actinobacteria strains present in the nests of *Nasutitermes* termites. Phylogenetic trees were manually constructed by computing the transitive closure between each strain based on dissimilarity matrices. Due to the time and effort this process required, a 1-D analysis using the Quantity One® software by BIO-RAD was implemented in order to develop neighbor-joining phylogenetic trees of 100 different Actinobacteria strains. This method provided more accurate analyses of the electrophoresis gels due to lane-based functions used to determine molecular weights, isoelectric points, other values, and a wider tolerance for distortions in the shape of lanes and bands. These results were then verified by constructing corresponding phylogenetic trees using data from DNA sequences of the 16S rRNA gene of each isolated Actinobacteria strain tested. After comparing the phylogenetic trees developed with each method, we observed that the phylogenetic relationships established were congruent. Our observations indicate that the implementation of the Quantity One® 1-D analysis software provides a faster, more dependable method of establishing phylogenetic relationships with great precision.

THE PROTIST TRICHOMYCETE *ENTEROBRYUS* ASSOCIATED WITH *ANADENOBOLUS MONILICORNIS* IN GUANICA DRY FOREST

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Symbiosis is the association between two non-related organisms. The common yellow-banded millipede, *Anadenobolus monilicornis* (Diplopoda: Spirobolida: Rhinocricidae) and the protist *Enterobryus* sp. (Ichthyosporea: Eccrinales), a species of hair-like microorganism that inhabits its gut, forms a commensalistic relationship. *Enterobryus* was once part of a fungal class (trichomycetes), but now it is classified as a protist. Other members of *Enterobryus* have also been reported associated with other non-carnivorous arthropod hosts including beetles, crabs and millipedes. We collected millipede specimens in Playa Jaboncillo within Guanica Dry Forest to study the prevalence of *Enterobryus*. We characterized morphologically the *Enterobryus* species that inhabits *A. monilicornis* through measurements of key structures and statistical analysis. Traditionally, *Enterobryus* species are difficult to identify due to high intraspecific variation. Thus, statistical analysis of character measurements is included in an attempt to investigate character stability. Millipedes were dissected; gut linings with attached *Enterobryus* were removed. Carefully, the hindgut was cut into smaller pieces and placed on a slide, they were hydrated with ddH2O, then the slide was preserved utilizing lactophenol cottonblue, the mounted slide was then observed under a light microscope. Based on the observed characters we hypothesized that this is a new species of *Enterobryus*. Morphometric data of thalli, sporangiospores and holdfasts presented a normal distribution of parameters except for the basal disk width of the holdfast, which showed extreme variation. This character, although used to describe *Enterobryus* species is not reliable in the new species when using the mean or range values in taxon descriptions.

COMPARISON OF LEAF AND ROOT TRAITS OF NATIVE AND NON-NATIVE GRASSES IN TROPICAL DRY FOREST

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Invasive non-native plants are often capable of outcompeting and displacing natives. Both leaf and root traits may play a role in the outcome of these interactions. We compared leaf (SLA = specific leaf area) and root (SRL = specific root length) traits among seven species of native and non-native grasses in the Guánica dry forest in Puerto Rico. We focused on dry forest because this ecosystem type, particularly on islands, tends to be susceptible to invasion. We hypothesized that invasive non-native grasses would show traits associated with faster growth (high SLA and SRL) while native grasses would show traits associated with greater drought tolerance (low SLA and SRL). We collected three individuals (clumps) of each species. From each of those we took a sample of 10 leaves and 10 roots. The leaves and roots were hydrated for 48 hours and then scanned using ImageJ to find the area of the leaves and the lengths of the roots. To measure the weight we dried them at 48˚C for 48 hours. We found differences in SLA and SRL among species, but differences were not consistent between natives and non-natives. The highest values of SLA occurred in *Bouteloua repens* (native) and *Botriochloa pertusa* (non-native) which are common on rock outcrops. SRL was highest for *Melinis repens* a non-native that is rapidly invading some areas of the forest. Variation in leaf and root traits may contribute to different ecological strategies of grass species in the forest.

ANTIFUNGAL PRODUCTION IN ACTINOBACTERIA ASSOCIATED WITH THE EXOSKELETON OF THE TERMITE *NASUTITERMES COSTALIS*

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The functionality of microorganisms associated with multicellular eukaryotes has been studied extensively. Currently, we know that Actinobacteria exert antimicrobial functions in insects such as ants, bees and wasps. We also know that termites have Actinobacteria in their gut and exoskeleton, although their exact role is still under debate. In the intestine, it is believed that Actinobacteria function as degraders of cellulose, termite's primary food source. In the exoskeleton, we suspect a defense function against pathogens as reported in other social insects. Pathogenicity of the fungi *Metarhizium anisopliae, Beauveria bassiana, Fusarium oxysporum*, *Trichoderma harzianum* and *Aspergillus* sp. has been reported in many arthropods, including termites. In this research we want to study the antifungal capacity of Actinobacteria associated with *Nasutitermes costalis*, which is a common arboreal, xylophagous termite in Puerto Rico. We have previously isolated and identified Actinobacteria from the exoskeleton of *N. costalis*. Most of our isolates belong to the genus *Streptomyces*, well-known antibiotic producers. We selected 15 isolates based on their phylogenetic position from our previous analyses. We evaluated their antifungal capacity against entomopathogenic fungi through bioassays. Our preliminary results show total inhibition of 47.4%, 40%, 92.3%, 80% and 90.9% with *M. anisopliae, B. Bassiana, F. oxysporum, T. harzianum* and *Aspergillus* sp., respectively. Most of the strains produced total or partial growth inhibition of the pathogenic fungus 12 days after incubation. Also, antifungal efficiency could be specific to the fungus since the Actinobacteria W1SI96[1] had the more capacity of inhibition against *M. anisopliae* and the least in *B. bassiana*.

GENETIC DETERMINANTS OF HAIR AND EYE COLOR IN PUERTO RICANS

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Genetic markers have been used to predict phenotypic characteristics like eye color, but is difficult to predict since many loci are involved, and different alleles come from different continents. We set out to determine variation and frequency in loci known to influence hair and eye pigmentation in Puerto Rico. Using custom TaqMan® essay panel, we genotyped six single nucleotide polymorphisms (SNPs): four from the OCA2 gene and two from the MC1R gene that are all previously associated with eye and hair color. In the preliminary study using 40 samples from the municipality of Santa Isabel, we found higher genetic variation in OCA2 than in MC1R. In the current study, we created a diversity panel using DNA from 192 saliva samples collected with the help of the Local Genome Diversity Studies (LGDS) in Puerto Rico, specifically focusing on the East side of the island where a high concentration of African ancestry has been found. Also, we used a set of samples that included samples from Europe, Africa, Asia and South America, and analyzed them for the same genetic variants. Despite the common misconceptions of Puerto Ricans having all the same eye and hair color, genetic tests reveal the existence of variation in the genes. We will genotype samples from municipalities across the island to obtain a clear picture of genetic variation on these pigmentation genes that have been associated with a higher risk of melanoma and obesity. If this test is successful, our findings can be applied in forensic investigations.

DIVERSITY OF THE PAPAYA RINGSPOT VIRUS IN PUERTO RICO

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Carica papaya (papaya) is an important crop in many diverse localities such as Hawaii, India, Taiwan, Florida, Puerto Rico etc. It is an economically significant crop and a source of nutrients such as vitamin C, antioxidants and papain, a digestive enzyme used commercially and industrially. But its production is being affected and limited by the presence of the Papaya ringspot virus (Prsv). The virus belongs to the family of potyviruses and it has two infecting types: Prsv-W that affects all cucurbits but not papaya, and Prsv-P that affects papaya only. The latter one is found mostly in tropical and subtropical regions where the crop is usually grown. Prsv's virion particle is filamentous and consists of a monopartite single-stranded positive RNA. It is transmitted in a non-persistent manner by aphids. Thus, within a few seconds the virus can enter the aphid, which will then infect another papaya plant. The virus affects papaya systemically causing symptoms such as chlorosis, leaf distortion, stunted growth, fruits with bumps and 'ringspots', among others. In order to assess the diversity of Prsv in Puerto Rico, we are analyzing the coat protein gene sequence of the virus genome, which is required for viral transmission. This sequence analysis will allow us to measure its variability between isolates of the island and compare to others from around the world. Consequently, the proper identification of the gene sequence of the coat protein can lead to a potential target in the production of transgenic Prsv-resistant Puerto Rican papaya in the future.