

¡BIENVENIDOS AL QUINTO SIMPOSIO DE INVESTIGACIÓN SUBGRADUADA EN BIOLOGÍA!

Nuevamente 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. 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 Quinto 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. Este año, continua la colaboración entre biología y arte con exposiciones artístico-científico de nuestros estudiantes y colegas.

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 Institutes), y su Directora, la Dra. Nanette Diffoot Carlo, la Asociación de Profesores del Recinto Universitario de Mayagüez (APRUM), y de la Oficina del Decano, Facultad de Artes y Ciencias del Recinto Universitario de Mayagüez, y el Decano, el Dr. Manuel Valdés Pizzini. Deseamos agradecer el apoyo y colaboración de los todos los mentores de investigación de los estudiantes presentadores y de los colegas que fungen como jueces en la evaluación de los trabajos presentados. Además, queremos agradecer profundamente a nuestros estudiantes graduados y a las asociaciones estudiantiles de nuestro Departamento por su ayuda durante la celebración del simposio. Nos gustaría reconocer la colaboración del Sr. Ramiro Vidal por la preparación de la página electrónica para someter los resúmenes y las inscripciones y al Sr. Martín Rosas por hacer disponible la infraestructura para el proceso de recibir las presentaciones. Además, nuestro agradecimiento al Dr. Carlos Muñoz por la preparación de la portada para el libro de resúmenes y a nuestras secretarias en el Departamento de Biología por su apoyo y colaboración: Brenda Soto, Mitzy Zavala, Alicia Collazo, Magda Bermúdez y Mary Jiménez.

Comité Organizador

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Itinerario del Quinto Simposio de Investigación Subgraduada

2 de mayo de 2015

8:00 am	Registro
8:45 am	Bienvenida: Auditorio de Biología (B-392)
9:00 am	Primera Sesión Plenaria Dr. David A. Jenkins, Research Entomologist, United States Department of Agriculture, Agricultural Research Service, Tropical Agriculture Research Station Auditorio de Biología (B-392)
9:45 am	Sesión I
	Génética I (B-180)
	Medicina (B-181)
	Ecología I (B-182)
	Microbiología I (B-280C)
	Microbiología II (B-282)
12:00 pm	Almuerzo (Lobby)
1:00 pm	Segunda Sesión Plenaria Dr. Gregory J. Quirk, Principal Investigator, Department of Psychiatrics, Laboratory of Fear Learning, University of Puerto Rico, Medical Sciences Campus Auditorio de Biología (B-392)
1:45 pm	Sesión II
	Génética II (B-180)
	Bioquímica (B-181)
	Ecología II (B-182)
	Microbiología III (B-280C)
3:45 pm	Merienda (Lobby)
	Abren las exhibiciones de arte (B-019A)
4:15 pm	Premiación y Clausura Auditorio de Biología (B-392)
5:00 pm	Foto del Grupo de Participantes, escaleras detrás del edificio de Biología

Presentaciones
Científicas

Scientific
Presentations

Detection of Parvoviruses PARV4 and B19V in Several Human Tissues by PCR

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Abstract

Human Parvovirus 4 (PARV4), a member of the Parvoviridae family, was recently discovered in humans. It has not yet been linked to any particular disease, but its prevalence in immunocompromised patients suggests its involvement in diseases. In previous studies, diverse solid tissue sample analyses have shown higher viral prevalence in myocardium, lung, and liver tissues. In order to determine the presence of PARV4 along with Parvovirus B19V (B19), a member of the same family that infects humans, tissue samples from the myocardium, lung, and liver were collected. DNA extraction and a PCR of all samples are being performed using specific primers for PARV4 genotypes 1 and 2 and all three B19 genotypes. Furthermore, samples believed to be positive for viral presence will be sequenced. Data suggest that both PARV4 and B19 are present in the examined tissues. Risk factors that may be associated with certain diagnoses and viral presence will be evaluated. In accordance with prior publications, a finding of higher prevalence of PARV4 in the liver, followed by myocardium and lung tissues is expected, with B19 prevailing over PARV4. This is the first study assessed to detect both viruses in tissues of the Puerto Rican population. Study results may help in relating cardiac, hepatic, and pulmonary diseases to PARV4 infection.

Dihydroartemisinin Induces Ferritinophagy Through the Lysosomal Pathway

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Abstract

Ferritin is a protein complex capable of storing iron in a non-toxic but bioavailable form. Thus, its expression protects cells from iron-induced oxidative damage. However, when cells are under iron-depleted conditions, the stored iron can be released from ferritin through the process of ferritinophagy. The upstream molecular mechanisms that regulate ferritinophagy are still unclear. Moreover, ferritinophagy could occur via lysosomal or proteasomal pathways. In this study, we aimed to compare the effects of two small molecules, Ferristatin II (FS II) and Dihydroartemisinin (DHA), on ferritinophagy using the leukemia cell line K562. The cells were incubated with μM of DHA or FS II for 24 hours, cell lysates were collected, and protein was analyzed using western blots. Although both small molecules degraded the iron importer transferrin receptor 1 (TfR-I), only DHA induced ferritin degradation, suggesting these small molecules control iron metabolism by independent pathways. We also examined the mechanism through which ferritin is degraded. Co-incubation with leupeptin, an inhibitor of lysosomal proteases, blocked the effects of DHA on ferritin degradation indicating the involvement of the lysosome. In conclusion, this study provides evidence that the small molecule DHA induces ferritinophagy through the lysosomal pathway. Pharmacological induction of ferritinophagy could have therapeutic potential in various diseases such as cancer and anemia.

Landscape Effects on Bird and Arthropod Diversity in Soybean Agroecosystems

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Abstract

Biodiversity studies in agricultural lands have shown that landscape-scale patterns of land cover influence the diversity and abundance of birds and arthropods that are important ecosystem service providers. Most studies to date, however, focus on the amounts of natural land cover and do not consider the land-cover heterogeneity of crop areas. We studied the roles of configurational heterogeneity and the amount of natural land cover in determining the diversity and abundance of arthropod and bird natural enemies in soybean agroecosystems of SW Ohio. We hypothesized that configurational heterogeneity would affect species diversity and abundance of natural enemies through differences in the area of and distance between similar land-cover types. We also tested the alternative hypothesis that the amount of forest cover increases natural-enemy diversity. To test the effect of configurational heterogeneity, we pre-selected 3km radius landscapes with overall high crop cover (~60–80%) and sorted them by mean patch area of crop types. We also determined the amount of forest cover in each landscape. Birds were sampled through three 60 min surveys in three soybean fields within each landscape. Arthropods were sampled using sweep samples near the edge (10m) and interior (100m) of soybean fields. Our results suggest that both birds and predatory arthropods responded positively to greater landscape configuration but predatory arthropods were more sensitive to variation in the amount of natural cover compared to birds.

Diversity of Hydrogenotrophic Partnerships in Successive Syntrophic Enrichments

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Abstract

Methane is a gas fuel biologically produced by a special group of prokaryotes. This highly energetic biogas is the end product of metabolism of a diverse group of methanogens. Among the methanogens, the hydrogenotrophs are the required partners for bacteria responsible to degrade volatile fatty acids (VFA) in natural environments where carbon dioxide is the dominant electron accepting process. However, the exact mechanisms to develop these inter-domain microbial interactions, described as a mutualistic symbiosis are unknown. Our goal is to determine if a preference for a specific hydrogenotrophic partner exists in natural ecosystems. Sediments from different subtropical environments were enriched with successively smaller volatile fatty acids with a starvation period between each individual substrate addition. Syntrophic couples were purified by serial dilution with each successional substrate; benzoate, butyrate, propionate, and acetate respectively. Metabolism was confirmed by end product chemical analysis using a Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC) for substrate consumption. Preliminary microscopic analysis of our different serially diluted enrichments suggests that the substrates dictate the morphological diversity of the enriched microorganisms along with the origin of the sediments. Interestingly, some morphologies were observed consistently across all environments and substrates, suggesting that these substrates are degraded by different specialists that depend on ubiquitous hydrogenotrophs. More time is required to confirm these findings using molecular techniques.

Telomere Length and Telomerase Activity Affect the Lifespan of *Amazona vittata*

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Abstract

A highly conserved repetitive DNA sequences found at the ends of eukaryotic chromosomes, known as the telomeres, are responsible for limiting the division of cells leading to senescence. As an organism ages, cells divide and with each division telomeres experience shortening or loss of telomeric DNA. It has been suggested that this mechanism was developed to target and terminate spread of the rapidly dividing cancer cells. Normally, telomeres protect chromosome ends from deterioration leading to instability and inability to replicate. Essential to telomeres and their stability is the enzyme telomerase: both in germline cells and cancer cells, shortening of telomeres is apparently balanced by their elongation, achieved by the synthesis of additional repeats in telomeric sequences by telomerase. Recent studies suggest that telomerase activity and the length of telomeres in organisms cells can be useful indicators of an organism's age and potential lifespan. Environmental factors can contribute to shortening of telomeres, and thus shortening of the life time. This is true not only about humans, but other long living organisms, specifically parrots known to have very long lifespans. It has been suggested that individuals released from the captive breeding program lose telomeres under the environmental stress. There has been no experimental approach developed so far to evaluate this hypothesis for the captive breeding program of the puerto rican parrots (*Amazona vittata*). Using molecular methods, a qPCR telomere assay has been developed earlier that permits the quantification of this enzyme's activity and the length of the telomeres in avian species cells. We adopted this method as a useful tool to estimate and the assess the potential life spans of individuals in this endangered species, as new populations from the captive breeding program are introduced to the wild.

Origin and Diversity of Plant Specie in Markets in Western Puerto Rico

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Abstract

Modern technology allows humans to modify landscapes and supply resources at great scales. By 2050, the world's population is estimated to increase by two billion and food demand will continue to increase exponentially. This calls for more farming and food transport services. In this study we compare small and large food markets in the western area of Puerto Rico in terms of their fresh fruit and vegetable diversity and the percentage of imported versus locally grown species. The results confirmed our hypothesis: larger food markets had a larger amount of plant species, as well as a higher percentage of imported plant products. A deep study of the impacts of importation on the quality and nutrient content of our food may be a topic of interest for future investigations. Plans to support and develop small markets with locally grown species are also a priority.

Lepidoptera Use of Native and Non-Native Plants in Puerto Rico

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Abstract

In Puerto Rico, Lepidoptera have received renewed attention in recent years, however, this is no complete work describing larvae-plant interactions. Food source for butterfly larvae is valuable information but this aspect of butterfly biology has been poorly studied. The main objective of this study was to gather all the known (published and non-published) information about the Lepidoptera species (and subspecies) reported in Puerto Rico, and to categorize these species as native or endemic. Plants reported as food-sources for caterpillars were classified into native and non-native genera. Data were merged into a matrix for statistical analysis to compare the use of native or non-native plants among groups of endemic and native butterflies. According to chi-square test, the proportions of native and non-native plant hosts used by native and non-native caterpillars did not differ. Therefore, endemic and native lepidopterans used non-native plants in the same proportion as food for their caterpillars, which suggests that non-native plants could be beneficial to endemic butterflies. A comparison on plants between native and endemic Lepidoptera showed that a 25% of the Lepidoptera shared the same plant genera and 26% shared the same plant family as food sources, suggesting that native and endemic Lepidoptera use similar plants. Further work should be done at the species level to better elucidate complex Lepidoptera-host plant interactions.

Assessing Cassava Leaf Proteins for Animal Feedstock

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Abstract

Cassava is one of the crops with increasing popularity among scientists because it has a high yield and contains a high amount of starch in the roots, while being a versatile crop, resisting harsh environments. This opens up a great range of possible uses for this crop, including, but not limited to cultivating it on countries where environmental stress, hunger or nutrient deficiency is an issue. However, the roots are currently the main component being used in the industry, which considers the leaves as a mere byproduct. The use of leaves as a protein source for animal feedstock could eventually replace human based diet components, such as corn, soybeans, and wheat, among others. This research aims to achieve an optimization of the extraction and processing of the leaves, in order to efficiently obtain a higher protein yield. We observed the differences in protein yield by comparing the age of the plants, position of the collected leaves and the various conditions during storage and handling, such as temperature and storage duration. The preliminary data collected showed that there was no difference in the leaves' protein content when our collection and storage variables were changed. This can positively suggest that the protein yield remains relatively constant, which can translate to more efficient production methods at a lower final cost. Throughout this research we will continue adding variables that can potentially lead to the maximization of the protein yield in Cassava's leaves, in order to help pave the way for further research.

Screening for Antimicrobial Agents Production in Metagenomics Libraries Generated from Guajataca Water Reservoir in Puerto Rico

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Abstract

Antimicrobials have revolutionized human health care, but in recent years, their effectiveness has been diminishing. Antimicrobial resistance (AMR) in health care facilities is a global public health concern. Over 70% of bacterial pathogens found in US hospitals are resistant to at least one antibiotic and more than 14,000 patients die annually from resistant nosocomial infections. AMR also increases the health costs for the government and the patients. Being aware of the enormous repercussions of this problem, it is imperative to do more research in order to reduce the impact of this issue. Knowing the scarce discovery of novel antibiotics by culture-dependent methods is necessary to implement culture-independent methods such as Metagenomics as an alternative solution for the increasing antimicrobial resistance issue. This work focuses in searching for novel antimicrobial agents from aquatic metagenomic libraries (AML) generated from Guajataca water reservoir (GWR) in Puerto Rico. Once determined the appropriate number of clones per plate to perform the detection analysis. A screening was performed to 1% of the 2.5M Guajataca AML using a double-layer agar assay with *Bacillus subtilis* as a target. While no positive clones were found using this strategy, modifications in the methods of screening have been implemented including clone induction and new microbial targets. This project will serve to open research in the discovery of novel antimicrobial agents by applying emerging disciplines, resulting in an alternative to AMR issue and the social implications that it has.

Optimization of rHbI His-Tag and its Mutant Containing HisE7 Crystals

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Abstract

Hemoglobin I (HbI) from *Lucina pectinata* is a model hemeprotein used to understand H₂S interactions with hemeproteins. Recombinant protein hemoglobin I (rHbI) with Gln in the E7 position and a Histidine tag (His-Tag) was prepared in a 5L bioreactor. Kinetically, studies showed similar behavior between HbI and rHbI, therefore, the latter is suitable as the native protein analog. A mutant with Histidine in the position E7 with a His-Tag was obtained to analyze the difference between an H₂S protein carrier and an O₂ one. Structurally, SAX-WAX data has been obtained, which support that there is no structural differences between these hemeproteins. To ensure this and further explore the internal structural orientation of the amino acids and the effect of His-Tag in the rHbI structure, crystal diffraction patterns are needed. Crystallization of rHbI His-tag and the mutant were conducted by the hanging drop vapor diffusion and counter diffusion techniques at 4° C and 25° C. Crystal formation was previously observed in buffers containing Lithium Sulfate. Buffer #49 from the Hampton Research Screening Kit 1, variations from this one, and Triana™ Crystallization Kit with pH variations were used as precipitant agents in the screening. The protein sample was prepared at different concentrations with or without cyanide as a ligand. The crystals have a reddish-brown color and a circular or rectangular shape with a size range from 9.5 μm to 70.0 μm, respectively. The seeding technique and further optimization of conditions are being used to enhance crystal quality for X-ray diffraction.

Molecular Identification of Fungi Associated With Birds in Boquerón

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Abstract

This investigation studies the different types of fungi associated with migratory birds in Puerto Rico. The two bird species that were sampled include the local moorhen *Gallinula chloropus* and the migratory blue-wing teal *Anas discors*. The common moorhen lives in Puerto Rico wetlands and is one of the species that can be hunted in limited quantities during the hunting season (November-January). On the other hand, the blue-wing teal migrates to Puerto Rico in October to winter. Samples from the cloaca with a sterile cotton swab and from feathers were collected from dead birds hunted in the “Refugio de Vida Silvestre de Boquerón” in two hunting seasons, 2013-2014 and 2014-2015. Samples were cultured on SDA or PDA media containing antibiotics. Isolated fungi were transferred to fresh media and subcultured for DNA extraction using the CTAB protocol. The 18S rDNA gene was amplified by PCR using the fungal specific primers NS1 and NS4. We processed 167 fungal isolates including yeast and filamentous fungi. Several samples amplified in the first attempt, but many needed increase in MgCl₂ concentration and high-specificity additives. So far we have obtained 72 amplification products ranging between 575–500 bp in size, which is expected for this gene. Sequencing of samples is currently underway, but morphological identification has shown the presence of the genera, *Aspergillus*, *Cladosporium*, *Cylindrocladium*, *Fusarium*, *Microsporum*, *Mucor*, *Paecilomyces*, *Penicillium*, and *Pestalotia*.

Synthetic Biology Approach to Modify and Express Bacterial Micro-Compartments Into Heterologous Systems

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Abstract

Cyanobacteria are photosynthetic microorganisms that play an important role in the planet's primary production. Recently, they have been proposed as renewable production “microfactories” because of their amenability to genetic engineering, their fully sequenced genomes and because they do not compete for arable land. As opposed to plants, cyanobacteria fix carbon dioxide in the carboxysome, a protein based organelle that encapsulates the enzymes RuBisCO and carbonic anhydrase. The carboxysome shell concentrates carbon dioxide inside this compartment and keeps out the competing substrate oxygen, therefore increasing the efficiency of the carboxylase activity of RuBisCO. Our projects seek a better understanding of the structure and organization of carboxysomes. First, variants of the carboxysomal shell protein CcmO were expressed in *E. coli* and purified by FPLC to further crystallize them to determine its structure. Second, different sets of carboxysomal proteins were expressed in the heterologous host *E. coli* to determine the minimum protein units required for a pseudo-carboxysome formation. Successful assembly was analyzed by transmission electron microscopy and FPLC/Western Blot. We envision that this research could be a step into the incorporation of carboxysomes into diverse applications. In the industrial sector they could be used to encapsulate toxic or valuable compounds during fermentations; they could be used as well for protein scaffolds, biofuel production, etc. In agriculture they may represent a new tool to achieve food security by decreasing photorespiration of plants, which may result in higher yields.

Correlates of Cryptic Diversity in Parasitic Worms

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Abstract

Cryptic species present a major problem in biodiversity studies. Parasites are particularly challenging because they are small, hidden in hosts, and possess few stable morphological characters that differ reliably between species. Thus, molecular studies are required for cryptic species discrimination. In 2011, Robert Poulin (University of Otago) published a meta-analysis of encounter of cryptic species in 33 studies of 40 parasitic helminth taxa. He observed that the number of cryptic parasite species detected is correlated with both number of parasites in which DNA is sequenced and, less strongly, with the number of host species sampled. We screened 31 out of the 33 studies and accounted for another variable that might be expected to affect encounter of cryptic parasite species, namely the spatial scale of each study. Our recompilation and re-analysis produced similar but not identical results. The main discrepancy was that the best predictor of the number cryptic parasite species encountered is the number of host species examined. This suggests cryptic parasite diversity is often host specific.

Evaluating Relative Contribution of Genetic and Environmental Factors to Bacterial Profiles in Parrot Species

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Abstract

The genetic differences among species, as well as their ecological and geographical separation all contribute to the complexity of microbial and viral meta-communities. Recently, next-generation sequencing has allowed us to bypass many challenges in culturing representative isolates in order to identify taxonomic groups. The first is called community profiling, which includes a group of methods that are based on the direct amplification and analyses of a conservative DNA marker. An example of this is to use the small subunit ribosomal RNA gene to generate profile(s) of microbial communities. Studies have already employed this approach to describe the diet of a variety of animals. Quantitative gene concentration analysis can reveal both species-specific and habitat-specific metagenome fingerprints that reflect specific genetic determinants, as well as physical and biological characteristics of the sampled environments. However, it is not clear how much of the metagenome is determined by the environment, and how much by the genetics of the species. In our experiment, we are trying to standardize the environment to see if and how much of the difference between the species is determined by the genetic differences. Fecal samples are collected from 22 parrots (five species) kept at a private farm and fed exactly with the same diet. These samples are used to obtain genomic DNA, and profiled using the Ion Torrent PGM at the Caribbean Genome Center, where bacterial diversity can be assessed by 16S rRNA amplicon sequences and analyzing sequencing reads on the phylum, class, order, family, and genus levels.

Will Changes in Precipitation Affect the Stability of Protist Communities?

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Abstract

There is recent interest in understanding the effect of climatic change, particularly precipitation, on the diversity and composition of tropical trophic webs. One model suggested that some tropical forests would dry whereas others will over saturate. We don't have enough data to predict the effects of precipitation on the dispersion of microbial-mediated diseases, on nutrient cycles but also on local biodiversity. In this project we examined the diversity of testate amoebae as a model to predict whether there will be changes in the community structure of microbial trophic webs. Given that testate amoebae are sensitive to humidity, we expect to find significant differences in species richness, abundance and species composition among precipitation treatments. A total of 33 bromeliads were assembled as mesocosms to avoid water entrance. Rainfall was collected to simulate precipitation, and subsequently added in a controlled manner to emulate dryness vs water saturation. No significant differences in species richness and abundance were found between the pre sample (control) and the post sample (precipitation simulated). Similarly, no significant differences were found in those parameters among the dry, moderate and wet treatments. Based on an ANOSIM analysis, there were no significant differences in species composition. However, controlling for the cosmopolitan species, the dry mesocosms were dominated by different species in comparison to the wet treatments. If this trend is consistent, we should be able to depict the trend as more data is included in the matrix.

Dragonflies in Areas Covered and Devoid of the Floating Fern *Salvinia* spp.

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Abstract

Mangroves conform productive ecosystems that provide opportunities to develop a diverse community of aquatic macroinvertebrates. These macroinvertebrates are widely used as bioindicators of water quality. The Boquerón Natural Wildlife Reserve at Cabo Rojo (NRWL) is the largest mangrove forest in western Puerto Rico. This mangrove forest has been invaded by floating ferns (*Salvinia* spp.). Since the dragonflies (*Odonata*) were among the most abundant macroinvertebrates present in the mangrove, we measured the possible effects of *Salvinia* spp. on these insects. *Odonata* from the mangroves of Puerto Rico have never been described to species level, and their distribution and abundance have not been studied. *Odonata* are very important as bioindicators because they reflect changes in the ecosystem. Nymphs process organic matter present in the water and promote the distribution of nutrients to other organisms. Nymphs are aquatic predators; they need a considerable amount of nutrients to complete the metamorphosis from aquatic larvae to aerial adult, which takes one to three years. The adult stage is also a predator insect, and it deposits its eggs in leaves near aquatic environments. There were six selected sampling zones, three with *Salvinia* spp. and three without *Salvinia* spp. This study showed there are differences in insect communities between areas with the floating ferns and those devoid of them. Also, mangroves were used as foraging areas by odonates in the family Coenagrionidae and as nursery areas by members of the family Protoneuridae.

Isolation of UV-C Radiation Resistant Bioprospects from Puerto Rico Soils

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Abstract

Exposure to Ultraviolet (UV) radiation has increased throughout the last decade due to atmospheric complications such as ozone depletion. UV radiation causes many health complications, for it is harmful and even lethal in large doses, although this problem can be fought using bioremediation and protection strategies. UV radiation resistant microorganisms can be used as models to understand UV-resistance and develop methods to help confront this situation. The objective of this research is to find cultivatable bacteria capable of tolerating ultraviolet radiation and determine their degree of resistance. Radiation resistant bioprospects were isolated from different environments of Puerto Rico by irradiating soil samples from the sampling sites with ultraviolet radiation. To determine the extent of resistance of the bacteria they were exposed to UV radiation from 15 to 120 seconds and grown at 37°C for 24 hours, covered in aluminum foil to avoid DNA repair by photo-reactivation. The survival percentages per dose of radiation were calculated and plotted. After processing 6 different soil samples from Cerro Punta, 12 potential candidates were isolated. Also, three gram-positive bacilli previously isolates from the Cabo Rojo Salterns had a higher survival percentage than the control *Escherichia coli*, their survival rates were 10%, 1.83% and 0.86%. A total of 9 isolates from Cerro Punta have been tested and 2 of these isolates have shown resistance. The bacteria found are potential candidates to find the gene(s) or physiological strategies responsible for their resistance and to use as biological models in the fight against UV radiation induced damage.

Characterization of the Z-Chromosome of the Puerto Rican Parrot

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Abstract

Puerto Rican parrot (*Amazona vittata*) genome data became publicly available thanks to the community effort of the people of Puerto Rico. To advance the description of rearrangements, conserved regions, protein-coding genes and other important protein and gene features, we are focusing on sex chromosome Z from the latest genome assembly (<http://genomes.uprm.edu/parrot>). Some annotation resources and tools used for this work are: Stand-alone Blast, Blast from NCBI, UCSC Genome Browser, Gene Model Checker, Ensembl, MEGA 6, and Repeat Masker. Fluorescence in situ hybridization of chicken's chromosomes against Puerto Rican Parrot shows that chromosome Z from both species hybridize completely and do not present any translocation with other chromosomes. We started the annotation by identifying 937 scaffolds of the parrot genome matching to the chicken (*Gallus gallus*) chromosome Z. Chicken and budgerigar (*Melopsittacus undulatus*) sequence files were used as major templates for this work, but occasionally it was necessary to use other genomes such as that of the zebra finch (*Taeniopygia guttata*), turkey (*Meleagris gallopavo*), collared flycatcher (*Ficedula albicollis*), saker falcon (*Falco cherrug*) and peregrine falcon (*Falco peregrinus*). We analyzed the larger 66 scaffolds and found in them 211 genes, most of them with a molecular function for binding or catalytic activity. The total length of these scaffolds adds to 23.3 Mb, which represents approximately 28.4% to 29.1% of Z chromosome. Most of the analyzed scaffolds are in synteny with other bird species, but Scaffold 605 shows a unique inversion for *A. vittata*, and corresponds to the beginning of the chromosome.

Genomic Dissection of Anthracnose Resistance Locus Present in SC112-14

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Abstract

Sorghum (Sorghum bicolor (L.) Moench) is the fifth most important grain crop in the world, however, its productivity is been constrained by fungus diseases. Anthracnose (*Colletotricum sublineolum*) is one of the most destructive diseases, which affect all aerial tissues of the plant. The most effective strategy for its control is the incorporation of resistance genes. Recently, the anthracnose resistance response present in the sorghum line SC112-14 was delimited to a 1.0 Mb genomic region at the distal region of chromosome five (locus Cs). Nevertheless, Cs locus is too large to identify candidate genes (>100 genes) for the observe anthracnose resistance response. Therefore, the objective of this study is to reduce the length of the Cs locus to identify candidate genes for further functional genomics studies. Thirteen genes evenly distributed within the Cs locus were strategically selected for the development of molecular markers. Firstly, a total of 38 pairs of primers were designed to amplify the introns of selected genes. Once PCR reactions were adjusted for the primers, fourteen pairs of primers representing five genes showed correctly amplification. Subsequently, these primers were used to amplify susceptible (PI 609251) and resistant (SC112-14) lines used for the development of a mapping population. These amplicons were sequenced by BigDye terminator chemistry for the identification of single nucleotide polymorphism (SNPs). The further SNPs genotyping of recombinants from this mapping population will allow us to reduce the length of the Cs locus.

Phylogenetic Analysis of the Epiphytic Community from Coffee Leaves

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Abstract

Coffee is one of the most important worldwide crops. Agricultural practices in coffee plantations have been attributed with an array of benefits and ecological impacts. In this study we aim to describe the microbiome of coffee at the phyllosphere level, and examine if sun-grown and shade-grown coffee growing practices affect its composition. To understand the impact of agricultural practices in the microbial composition of the phyllosphere community we sequenced the 16S rRNA gene from coffee leaves. DNA was extracted from sun-grown and shade-grown plots within the same coffee plantation in Adjuntas, Puerto Rico, using phenol:chloroform extraction. We obtained amplicon sequencing from five samples using the Illumina Mi-SEQ platform and analyzed using MG-RAST and Parallel-META. In addition, Scanning Electron Microscopy (SEM) was performed to confirm the presence of bacteria on the abaxial and adaxial surfaces of the leaves. SEM images suggest adaxial surfaces might be more abundant than abaxial surfaces. Bacilli-like structures were observed inside and with association to leaves' stomas. Microbial communities present in the coffee phyllosphere were dominated by *Pseudomonas*, *Acinetobacter*, *Butiauxella*, *Escherichia*, and *Citrobacter*, with some difference in its relative abundance. Over 500 phylotypes were detected thus demonstrating the complexity of these communities. However, the microbial communities in sun-grown and shade-grown leaves were undistinguishable. The high degree of diversity in the phyllosphere community strongly argues of its role in plant health and perhaps coffee bean quality.

Tissue 3D Printing as a First Phase to Organ Graft Fabrication

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Abstract

In an effort to create complex structures such as organs, a 3D printer will be configured to print cells mixed homogeneously in agarose. The printer can read digital 3D models, which can be obtained from MRIs and other imaging methods, and convert them to coordinates to deposit material. The proposed design for material extrusion includes an Archimedes Screw Pump controlled via Arduino to coordinate extrusion relative to the print surface displacements. A mixture of SeaKem 1.0% Agarose has been chosen as in situ scaffold material. This agarose concentration has a gelling temperature at approximately 37° C, which is a safe temperature to mix the cells before printing. Furthermore, the agarose will completely solidify at 33° C. Thus, the agarose and cell mixture will be fed to the printer at the gelling temperature of agarose which will then be progressively lowered to 33° C; when the printer tip comes into contact with the print surface ensuring appropriate stiffness for maintaining the structure being printed. Multiple parts will be printed in a petri dish and then transferred to an incubator which will maintain it in ideal growth conditions. A time frame will be established for each part and will be monitored to observe the manner cells in which cells are proliferating and their survival rate. A honeycomb infill pattern was chosen to maximize surface area and porosity of the gel in order to provide uniform growth factors to all the cells and help increase survival rates of the cells.

Snake Trap Methods: Efficiency of Funnel Traps for Puerto Rican boas (*C. inornatus*)

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Abstract

The Puerto Rican Boa (*C. inornatus*) is an endemic snake species from Puerto Rico, listed as endangered since the 1970s, and of which few individuals are known. Currently, a study to understand population size, activity patterns, and habitat use of this snake is being conducted at Fort Buchanan Army Base, which is located in the urban sprawl of the San Juan, Puerto Rico metropolitan area. In order to understand population size, activity patterns, and habitat use of this snake in a fragmented habitat, boas have been caught opportunistically by hand throughout field season. Looking for options that could improve the chance of captures, it came to our attention that in the past, funnel traps have proven to be an effective method in the capture of large reptiles. No previous studies about the effectiveness of funnel traps to capture *C. inornatus* specie have been documented. During two weeks, ten double-ended funnel traps with snake lure where placed randomly in two forested sites where snakes have been reported. The traps were checked twice a day during four days, collecting abiotic data every time. During the second week, traps were changed to another eleven randomly chosen sites in a known boa habitat. If anything was found inside the traps it was documented, and the animal immediately released. The data were analyzed to determine the effectiveness, if any, of funnel traps as a capturing method for Puerto Rican Boas. In general, no snakes were caught, although one boa was found five feet away from one trap, and various *Borikenophis* sp., *Rhinella marina*, and anoles where found in other traps. In conclusion, funnel traps do not seem to be effective to capture the Puerto Rican boa. Factors such as trap location, the species being studied, and the presence of bait or lure may affect the results. For future studies, live bait may be used on the pit traps to lure the snakes in.

Lactase SNP Diversity in the Human Population of Puerto Rico

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Abstract

The lactase gene, located in human chromosome 2, encodes for an enzyme that hydrolyses the glycosidic bond of carbohydrates such as phlorizin and lactose. Previous analyses have demonstrated that the gene has a single nucleotide polymorphism (SNP) named rs2322659 and is located at position 4927 of the mRNA. It is an adenine to guanine substitution, resulting in an amino acid change from asparagine, a neutral amino acid, to serine, a polar amino acid that may affect the individual's ability to digest lactose. We are studying the allelic geographic distribution of this SNP in the population of Puerto Rico. For the development of the assay, saliva samples were collected randomly from people within 20 municipalities in Puerto Rico. The municipalities were also chosen in a randomly way, but representing the northern, southern, western, eastern, and central regions of the island. The DNA from the saliva samples was extracted using proteinase K digestion followed by ethanol precipitation. After quantifying the DNA concentration of each sample, they were diluted to a concentration of 10ng/μL. Genotype differences were analyzed in a ViiA7 Real-Time PCR System, using the Taqman Assay. Because Europeans are the only population where the G allele represents the majority, we expect to find the highest frequencies of this SNP in the regions with the highest European ancestry.

Degradation of Aromatic Compounds by *Purpureocillium lilacinum*

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Abstract

Purpureocillium lilacinum is a saprophytic filamentous fungus found in soils, decaying vegetation, insects, nematodes, animals, humans, and indoor air. It has potential uses as a biocontrol agent, as it has been extensively studied against insects and root-knot nematodes. It is also a clinically important species, as it can cause infection in both, immunocompromised and immunocompetent patients. However, its potential as a bioremediation agent has not been studied. The objective of this study was to determine its potential ability as a bioremediation fungus. *Purpureocillium lilacinum* was isolated in 5% NaCl PDA from red mangrove wood. Congo Red and naphthalene degradation assays were conducted to determine its degradation potential. Samples were incubated in the shaker at 30° C, 140 rpm, for 10–20 days. The absorbance reading was done every two days at 320nm for naphthalene and at 490nm for Congo Red. Lignolytic enzyme assays were conducted for all samples. After 10 days, *P. lilacinum* reached 98.95% Congo Red and 46.70% naphthalene degradation. Our data show that *P. lilacinum* has a high potential as a bioremediation fungus, since both compounds were degraded.

Does Size of the Freshwater Snail *Tarebia granifera* (Thiaridae) Relate to Periphyton Composition on Bed-rocks and on Snail Shells in Two Streams of Western Puerto Rico?

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Abstract

Does size of the freshwater snail *Tarebia granifera* (Thiaridae) relates to periphyton composition on bed-rocks and on snail shells in two streams of western Puerto Rico? *Tarebia (Thiara) granifera* is an invasive parthenogenetic snail found in freshwater bodies throughout tropical and subtropical areas, including Puerto Rico and other islands of the Caribbean. The ecological impacts of this invader remain largely unknown, but the species is known to very competitive against other freshwater snails and are believed to affect the periphyton composition on natural substrates. Two streams, Quebrada de Oro and Quebrada Cojolla, were selected as study sites due to their variability in body sizes and population densities of *T. granifera*. Four stations were established in each stream from down-stream to up-stream (0 m, 50 m, 100 m, and 150 m) to relate snail and periphyton composition of natural substrates (mainly rocks). Ten snails were removed from each station; these were measured, their shells scrapped and the periphytonic algae present on their shells were assessed. The study also aimed to correlate limnological parameters such as turbidity, dissolved oxygen and temperature with snail-size and the abundance and biodiversity of periphyton on bed-rock. Diatoms (*Ochrophyta*) dominated in both streams, with species of *Gomphonema* dominating in Quebrada de Oro and species of *Cocconeis* and *Denticula* dominating in Quebrada Cojolla. The same pattern of dominance was observed for samples scrapped from rocks and those obtained from snail-shells, regardless of their size. The relationships between snail-size and limnological parameters are under study.

Isolation of a Novel UV-Resistant Bacteria From Dry Soil in La Parguera, Puerto Rico

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Abstract

Ultraviolet (UV) radiation resistance in bacteria is mediated by an enzymatic repair of DNA through various pathways. Tolerance to UV radiation has been related to the solar UV level present in its natural habitat, which makes the system more efficient in repairing DNA. In an attempt of searching for UV resistant bacteria in Puerto Rico, a dry soil sample was aseptically collected from La Parguera, PR. One gram of soil was suspended in sterile distilled water (5 mL), where 100 µL was irradiated with UV-light (254 nm) in a microbiological hood, using *E. coli* as negative control. The suspension was serially diluted and placed in the heterotrophic media R2A for several days, covered with aluminum paper to avoid photoreparation. A Gram-negative, rod-shaped with red pigment bacteria was successfully isolated for further characterization. This strain was named "MC1." Phylogenetic analysis based on 16S rRNA gene sequences indicates 96.8% sequence similarity to *Hymenobacter deserti* based on EZ-taxon database. This result suggests that "MC1" belongs to a novel genus in the Bacteroidetes phylum. The isolated strain grows at room temperature. Polyphasic taxonomy is currently being performed to provide full characterization of this novel organism.

Monitoring Antibiosis in Natural Water Bodies in Puerto Rico Using Metagenomics

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Abstract

Nowadays, it is commonly seen that treatment for microbial infection is not as effective as a few decades ago. Actual antimicrobials no longer have effect in controlling microbial reproduction and growth, thus we need to develop new methods to counteract this resistance by finding new antimicrobial agents, or modifying the existing ones. Metagenomics is one of the most promising methods since it provides access to the entire genetic content of a particular environment by non-cultivable methods. In this study, we explored antimicrobial activity in two different natural aquatic environments. Specifically by screening metagenomic libraries (AMLs) generated from Playa Sucia beach (PS) and the Río Grande de Añasco river (RGA). First, a cultivable “crowded plate” assay was performed to the water samples, where potentially inhibition zones for PS were detected. Then, whole PS (1,500 clones approximately) and 1% of RGA (870 clones approximately) AMLs were screened using the double-layer agar assay. Bacterial targets essayed for antimicrobial activity in the libraries were *Klebsiella pneumoniae* and *Bacillus subtilis*. While antimicrobial activity was detected at cultivable level, no antimicrobial production was found in the libraries for the method and targets studied. There is work in progress to complete the screening of AMLs, and to test the activity using cells lysis. This project represents an important effort to learn and search for novel antimicrobials in natural water bodies that could reduce the increasing antimicrobial resistance problem. Also, in a long term, will sheds light in the understanding of antimicrobial agent’s genes, their distribution and prevalence.

Abiotic Factors Affect H₂O₂ Production of Sea Anemone’s Endosymbionts

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Abstract

Reactive oxygen species, such as H₂O₂, produced by cnidaria’s symbiotic algae under irradiance and temperature stress, result in symbiosis breakdown. The intertidal sea anemone *Anthopleura elegantissima*, hosts two very different symbionts: *Symbiodinium muscatinei* (zooxanthellae) and *Elliptochloris marina* (zoochlorellae). To test for differences in H₂O₂ production by the two symbionts, intact *A. elegantissima* tentacles containing either zooxanthellae or zoochlorellae were exposed to irradiances of 0, 100, or 900 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at 10° C and 20° C. Although similar studies have been done with symbionts extracted from the host, it is known that extraction from the tentacles stresses the cells, potentially increasing H₂O₂ production. In our study, temperature had no significant effect on H₂O₂ production in either symbiont, but both zooxanthellate and zoochlorellate anemone increased H₂O₂ production significantly in the highest light treatment. The effect, however, was more dramatic in anemones hosting zooxanthellae, suggesting that anemones hosting zooxanthellae have a greater H₂O₂ burden despite the superior ability of zooxanthellae to photoacclimate to high temperature and irradiance.

Expression of Focal Adhesion in Response to Inducing Cellular Orientation Through Mechanical Transduction

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Abstract

In Tissue Engineering, the synthesis of scaffolds is a method by which a biologically derived or synthesized material is implanted into an artificial structure capable of supporting three-dimensional tissue formation. The scaffold will later on be implanted into a human subject in order to replace damaged or diseased tissue and regrow newly, healthy tissue. To achieve this goal of tissue reconstruction, scaffolds must meet an important set of requirements such as: high porosity, biodegradability, mechanical properties and biocompatibility. We are specifically interested in this last requirement, biocompatibility, since it plays an important role in numerous biomedical applications such as bone and joint replacement techniques as well as dental implants and improving prosthetics for amputees. In recent literature, there are no defined methods to directly quantify the adhesion of a biomaterial to a living tissue. Our main objective is to study focal adhesion mechanics in different cell lines on PDMS (Polydimethylsiloxane) of varying stiffness subjected to static and cyclic loading. Our plan is to analyze and quantify the images by immunofluorescence imaging to identify if there are similar or unique patterns in the development of focal adhesions between different cell lines due to a particular loading frequency or substrate's stiffness. Our future interest in this project is to develop the skills and knowledge necessary to move onto live cell imaging that will give us substantially more data in real time of the cell's behavior during mechanical transduction.

Genetic Differentiation Between Eurafrikan and Caribbean *Octopus vulgaris*

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Abstract

The *Octopus vulgaris* species (Lamarck 1798) has a widespread distribution, inhabiting warm waters of the Caribbean Sea as well as the Mediterranean, South Indian Ocean, and Japan. The main objective of this study is to estimate the connectivity patterns between eastern Caribbean and Eurafrikan populations of *O. vulgaris*. To achieve this, specimens were collected off the coast of Greece, Spain, and Caribbean Islands, and evaluated by sequence analysis. DNA was extracted using a DNeasy kit and tissue protocol, and samples were revised before sequencing using PCR. In this part of the project, comparisons were based off the amplified 16s gene. Data analysis is underway and further research must still be conducted.

Mycorrhizal effects on ‘Ají dulce’ (*Capsicum chinense*)

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Abstract

Capsicum chinense is widely cultivated in the Caribbean. Most types are pungent, but in Puerto Rico, non-pungent types are preferred and referred to as “ají dulce,” or sweet chili pepper. This is one of the main crops cultivated on the island and is part of the local cuisine. It is known for being a good source of vitamins A, C, E, and for its antioxidant properties. Currently, in commercial agriculture, the use of chemical fertilizers dominates the local market, while biological ones are overlooked. Symbiotic relationships between mycorrhizal fungi and the roots of vascular plants can serve as biological crop enhancers. The purpose of this research was to determine, characterize and identify the mycorrhizae associated with locally grown *C. chinense* in the western area of Puerto Rico. We collected “ají dulce” roots and surrounding soil from plants growing in experimental plots at UPRM. After processing and staining the samples, fungi were morphologically identified using taxonomic keys. Among the genera identified so far we found *Glomus* and *Acaulospora*. We also grew “ají dulce” under six treatments using commercial general purpose Promix® BX, Promix® Mycorrhizae (*Glomus intraradices*), Promix® with 25% soil from UPRM, greenhouse Promix® with fertilizer, Promix® Mycorrhizae (*Glomus intraradices*) with fertilizer and Promix® with 25% soil from the UPRM greenhouse with fertilizer. Significant differences were obtained when compared using T-test, obtaining a p-value of < 0.0001 between Promix® Mycorrhizae and Promix® BX treatments, where the first one resulted in greater stem length, fruit, and leaf number.

Subestimación de la Población de Enterococos Totales en Aguas Recreacionales

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Resumen

Los enterococos son bacterias gram positivas, fermentadoras, parte de la flora intestinal de animales de sangre caliente y el estándar como indicadores de contaminación fecal en aguas recreacionales. La cuantificación adecuada de estos organismos es esencial para denominar si un cuerpo de agua contiene contaminación fecal y representa un riesgo para la salud. En este estudio, utilizamos tres cultivos puros de los enterococos más abundantes en aguas recreacionales de Puerto Rico; estos son *Enterococcus casseliflavus*, *Enterococcus faecalis*, y *Enterococcus faecium*. El crecimiento de 24 hrs de los cultivos puros en agar BEA se utilizó para hacer un estándar con una densidad óptica de 0.5 a 600 nm en una solución amortiguadora de fosfato y cloruro de sodio. La densidad del cultivo se midió haciendo diluciones en serie hasta la extinción y su conteo en agar BHI y simultáneamente en tubos con el medio Enterolert (Idexx). Al cabo de 24 hrs se determinó la población de cada cultivo en los dos medios y su población se comparó. Nuestros resultados preliminares indican que la cuantificación de *E. faecalis* y *E. faecium* fue idéntica en ambos medios de cultivo. Sin embargo, Enterolert subestimo la población de *E. casseliflavus* por tres órdenes de magnitud cuando se comparó con BHI. Esto sugiere que la enumeración de enterococos utilizando este medio comercial subestima la población total de enterococos en ambientes dominados por *E. casseliflavus*. Esto adquiere importancia cuando otros estudios en nuestro laboratorio han demostrado que *E. casseliflavus* es el enterococo dominante en las aguas de Puerto Rico.

Spatio-Temporality of the Earthworm Community in an Ephemeral Wetland

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Abstract

An ephemeral wetland is body of water that contains liquid for some part of the year. We assume that an increase of evapotranspiration, resulting in a loss of water, affects the distribution and composition of the communities that inhabit the soil inside and outside the system. The effect on earthworm communities created by the seasonal dry-down of ephemeral wetlands is unclear. We chose to study the spatio-temporality of the earthworm communities in and around Lake Arnold, an ephemeral wetland located at Blandy Experimental Farm, Virginia, U. S. A. We created four transects of 30 meters at four different points around the lake. The four transects had one plot every five meters, but we were only able to work in the area located outside the lake. As the lake dried up and exposed more ground, we proceeded to work in it, following the same procedure. Every two weeks, the hand sorting method was used for extracting the earthworms, using an area of 35 x 35 cm, and in a depth of approximately 45 cm. The extracted adult earthworms were classified into their respective genera. We have documented that some soil variables can be affected by this increase of evapotranspiration, such as temperature, moisture, and pH. Richness, abundance, and diversity were affected by some soil variables. Earthworm density steadily changed by plot distance to the wetland shore.

Quantifying Calorie Units Generated by Different Feedstocks

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Abstract

The world population growth has increased the energetic demand exponentially. The bioenergy topic has been trending for several years now, however there is no global consensus on how sustainable are they. This AD is mostly done using microbes. It has been determined that pretreating the feedstocks can improve methane yield when anaerobically decomposed. Unfortunately, the thermal energy (calories) each biomass has as stored energy is still unknown. Without this data, the amount of energy that is transformed into methane by AD is unknown. Thus, the efficiency of each feedstock becomes impossible to state. Our objective is to determine the thermal energy some types of lignocellulosic biomass release when ignited using an Oxygen Bomb Calorimeter.

Land-Use Impacts on Bees and Hunting Wasps

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Abstract

Bees and wasps play a significant role in pollination and in hunting of other organisms in natural ecosystems and agricultural ecosystems. We sampled bees and wasps from a commercial farm with different types of crops and land uses to observe their distribution and investigate the preferences of local species. We found that honey bees and other bees were distributed around the farm regardless of land use patterns. One kleptoparasitic wasp, *Nysson* sp., was restricted to certain areas of the farm. This preliminary study helps understand how agricultural land use patterns impact pollinators and predators.

Plant Diversity and Conditions in the Urban Environment of the University of Puerto Rico-Mayagüez Campus

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Abstract

Plant diversity, and the benefits it provides to humans and other organisms, has been exponentially degraded by urbanization. In the University of Puerto Rico-Mayagüez Campus, plant diversity creates a better living environment for students and faculty members by providing a natural scenery that contrasts the usual concrete surroundings of the city of Mayagüez's urban environment. Our objective was to survey plant diversity and conditions on an urban university campus, and compare our results to a previous campus-wide census conducted in 2004. Using previous census data and maps, we located and identified trees, and assessed their condition. Most trees were adults that were present in the previous census, some trees died and others were removed for the construction of parking spaces. Also, a great quantity of the trees surveyed showed termite damage. Although our inventory is ongoing, these data suggest that more care will be needed to maintain plant diversity on this campus.

Role of A2 Neurons in Fear and Aversion

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Abstract

Fear triggers a combination of responses mediated primarily by the amygdala. Photostimulation of A2 noradrenergic neurons in the nucleus of the solitary tract (NTS) has been shown to induce aversion (an affective component of fear) in mice, indicating that the A2 neurons play a role in fear. We hypothesized that these neurons project to neurons in the parabrachial nucleus (PBN), which, in turn, project to the amygdala, thus mediating aversion. The proposed neural circuit was investigated by optogenetically stimulating the A2 neurons while simultaneously photoinhibiting projections from the PBN to the amygdala. Aversive behavior was measured using operant conditioning and compared between mice expressing YFP/ChR2 in A2 neurons and ARCH/GFP in glutamatergic PBN neurons. Mice were monitored in basal conditions, during concurrent stimulation/inhibition with blue and yellow light, and during stimulation with blue light only. Results support that A2 neurons indeed play a role in eliciting aversive behavior in mice and that inhibition of projections from the PBN to the amygdala reduces the aversion prompted by stimulating A2 neurons. Neuronal activation was also measured during predator-related fear response by quantifying c-fos expression in the NTS after exposure to TMT, a component in fox urine. However, our results for this experiment are inconclusive and subject to further study. Identifying A2 NE neurons' role in fear not only contributes to our understanding of how this emotion and fear-related disorders such as phobias or PTSDs are mediated by the brain, but it also provides a subject for future study of possible genetic targets.

Culture-Dependent Diversity of the Cabo Rojo Solar Salterns

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Abstract

The use of the 16S rRNA gene sequences to establish phylogenetic relationships has long been the universal method in taxonomy. However, the fact that the gene is highly conserved, sequence results cannot be used to differentiate closely related species. Furthermore, intraspecific heterogeneity of the 16S rRNA genes has further hindered the capability to use it as a phylogenetic marker. The recently developed method of Multilocus Sequence Analysis (MLSA) has been proposed as an alternative to differentiate closely related organisms at the species level. The method consists of using the sequence data of 3–7 concatenated housekeeping genes to form phylogenetic relationships. The purpose of this study is to analyze the archaeal diversity from the solar salterns in Cabo Rojo isolated using different culture media conditions and utilizing MLSA as our main tool for strain characterization. We compared the isolation efficiency of media with different solidifying agents (agar and agarose), water (artificial sea water and water from the solar salterns), and carbon sources (pyruvate and glycerol). Samples were collected from the solar salterns and grown in the aforementioned culture media variables. After obtaining pure strains, the next step is to utilize MLSA by amplifying the genes *rpoB*, *atpB*, and *ppsA* and construct phylogenetic trees with the sequence data obtained. Preliminary data showed that more growth was obtained in media containing agarose and water from the solar salterns; however we observed more diversity in media containing agarose and artificial seawater. Recently, new data has shown the possibility of new species of Haloarchaea.

Regulation of Glycosyl Hydrolase Expression in *Halogeometricum borinquense*

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Abstract

The regulation of gene expression in the Archaea domain has aroused a lot of interest due to their property for thriving in extreme environments. However, few reports describe the essential pathways they accomplish under adverse conditions. Specifically, for halophilic archaea, little is known about genes involved and regulated in carbohydrate metabolism. The purpose of this study is to understand the function of glycosyl hydrolases in carbohydrate utilization at high salinity. The haloarchaeon *Halogeometricum borinquense* was selected as model since it displays shorter generation times when compared to other haloarchaea. Also, *H. borinquense* uses glucose, mannose, fructose, xylose, maltose, trehalose, cellobiose, raffinose, and glycerol as sole carbon sources. Cells of *H. borinquense* grown in rich media, until mid-logarithmic phase, were irradiated with ultraviolet light (UV) 254 nm to induce genomic mutations. Irradiated cultures were transferred to rich media and cells with the capacity to grow after treatment were selected. Putative mutants were tested in minimal media containing two sugars as its only carbon source: 0.4% (w/v) sucrose and 0.6% (w/v) maltose. Enzyme assays focused on alpha-glucosidase, beta-glucosidase and beta-galactosidase were performed on these mutants. Physiological and enzymatic studies revealed drastic changes in the growth of several mutants versus wild-type suggesting that high doses of UV-light affected the production or function of glycosyl hydrolases. Findings might lead to future studies about the expression of genes and proteins involved in catabolite repression in extremely halophilic archaea.

Estrogen Metabolizing Bioprospects in Guánica Dry Forest Soil

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Abstract

Over recent years the contamination in bodies of water with estrogenic steroid hormones has caused worldwide problems such as the feminization of fish. In processed water, the Environmental Protection Agency (EPA) has reported minimal concentrations of 0.4 and 0.9 ng/L of 17 β -estradiol (E2) and 17 α -Ethinylestradiol (EE2) respectively. In order to address this problem we propose cultivable approach to search for bioprospects capable of metabolizing estrogen. The screening of a soil sample was performed using M9 minimal salt medium EE2 and E2 as the sole carbon sources. The candidates isolated were characterized macroscopically and microscopically, in order to determine the identity of the microbe responsible for the activity. Monitoring of one soil sample yielded a total of 21 cultivable bioprospects that grew on M9 media enriched with both estrogens. Macroscopically the majority of these bioprospects showed white or beige pigmented colonies, most of which are opaque, translucent and transparent. The size and morphology of the colonies also varied including circular, irregular and rhizoids; with an average size of 0.5 mm. Microscopically the bacteria were bacilli of varying sizes with Gram's staining resulting in 19 gram positive and 2 gram negative bacteria. The 16S rDNA sequencing analysis showed that most of them belong to but are not limited to the genus *Bacillus*. The data obtained demonstrate the presence of hormone-metabolizing bioprospects in Puerto Rico. This research can lead to the development of biosensors and novel bioremediation procedures for water treatment in plants keeping the hormones from reaching bodies of water or human consumption.

Isolation of Toxin Component Interacting Partners Using T7 Phage Display from Human cDNA Libraries

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Abstract

Biological warfare has caused deaths worldwide proving its efficiency by causing terror. Since the use of *Bacillus anthracis* spores in 2001, research driven to understand the biological mechanism of this bacterium Lethal factor (LF) toxin component has increased. The cleavage performed by LF to the mitogen activated protein kinase kinases results in a cascade of reactions that promote cell apoptosis. Moreover, the damage caused by LF to recently identified substrates suggests novel interactions with other unknown proteins. This research focuses on the isolation of LF-interacting partners by T7 Phage Display (PD), a technique used to identify protein-protein interactions. Using LF as target, rounds of biopannings were performed with human premade cDNA libraries from stomach expressed on the surface of a bacteriophage (T7). LF-interacting partners isolated were amplified, sequenced and analyzed in silico. Later on, a specificity test was performed to detect interaction between individual T7PD candidates and LF. The data suggest interaction of LF with 10 proteins from the lipase and peptidase families, among others. In addition, a member from the peptidase family showed interaction to casein and LF, but higher affinity to the last. The minimum concentration of LF for interaction with PA1-46 candidate was perceived to 1 µg/mL. These results will increase the understanding of the molecular pathogenesis of anthrax disease in order to generate new therapeutic agents.

Characterization of Dominant Alleles of the SLD2 DNA Replication Protein

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Abstract

Sld2 is an essential protein required for *S. cerevisiae* DNA replication initiation. During S-phase, cyclin-dependent kinases (CDK), phosphorylates Sld2 driving incorporation of the phosphorylated protein into a multi-protein complex that is required to activate the replicative DNA helicase. Despite their importance, it is unclear how these interactions trigger the initiation of DNA unwinding and new DNA synthesis. Using a library of Sld2 mutants, generated by transposon-based mutagenesis, we screened for dominant-negative mutants in *S. cerevisiae* that interfere with the function of wild-type Sld2 upon overexpression. We identified three mutants, and selected one (Sld2_135) for further characterization by fluorescence activated cell sorting (FACS). The Sld2_135 mutation inserts five amino acids between a known and a potential S-CDK phosphorylation site. Consistent with a DNA replication defect, FACS studies indicate that this mutant arrests cells in G1 phase. We plan to use in vitro replication assays to investigate how this mutant protein alters the events of helicase activation. Our studies will provide a clearer view of how phosphorylation of Sld2 contributes to the protein-protein interactions that stimulate DNA replication initiation.

Characterization of the Genetic Diversity of Papaya in Puerto Rico

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Abstract

Papaya (*Carica papaya* L.) is a highly cultivated fruit crop in tropical and subtropical areas in the world. It is well known for having many beneficial properties such as being a great source of antioxidant nutrients, fiber, and vitamin B among others. Even though papaya is not one of the most important fruit crops in the Puerto Rican economy or diet, it is culturally important, and many citizens consume it regularly. In Puerto Rico, papaya diversity has not been studied at a molecular level. This project aims to assess the genetic diversity of papaya in Puerto Rico using Simple Sequence Repeat (SSR) markers. In this study 20 SSR markers are being analyzed with 15 samples from different regions of the island. We extracted the DNA from our samples, used Polymerase Chain Reaction (PCR) to amplify the 20 SSR markers, and ran the resulting amplicons in polyacrylamide gel electrophoresis to obtain the allelic distribution for further statistical analysis. Genetic estimators will be achieved using GenAEx 6.5 and Power Marker 3.25 software programs. Knowing the genetic diversity of papaya could be very helpful for future agricultural projects such as in crop improvement and will expand our knowledge of papaya in Puerto Rico.

The Role of Protein Translocation in *Campylobacter jejuni* N-linked Glycosylation

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Abstract

Asparagine (N)-linked glycosylation is a protein modification that is important for various cell processes, including cell-cell interactions, cell-pathogen interactions, and protein folding and stability. In eukaryotes, N-linked glycosylation occurs primarily co-translocationally, as substrate proteins are translocated through the general secretory (Sec) pathway from the cytosol to the endoplasmic reticulum lumen. In contrast, little is known about this process in bacteria, specifically if N-linked glycosylation occurs after substrate proteins have been successfully translocated into the periplasm or while they are being translocated. Previous reports have demonstrated that the bacterial oligosaccharyltransferase (OTase) is capable of glycosylating fully folded proteins, in flexible and exposed acceptor sites, posttranslocationally. However, these findings are not comprehensive, as only several bacterial glycoproteins have been examined in this context. *Campylobacter jejuni*, a food-borne pathogen and one of the most common causes of human gastroenteritis worldwide, encodes an N-linked glycosylation system that has been well studied. N-linked glycosylation in *C. jejuni* is essential for proper host colonization and infection. JlpA, a surface-exposed adhesin in *C. jejuni*, has two N-linked glycosylation sites, one of them located in a non-structured area of the fully folded protein, and the other in a structured alpha helix. To determine whether protein glycosylation in bacteria is dependent on translocation, we tested the efficiency of glycosylation of the two sites in JlpA when the protein is translocated into the periplasm by the Sec (native pathway of JlpA) or TAT (twin-arginine translocation, non-native pathway of JlpA) machinery in *Escherichia coli*.

Genotoxic Effects of Ferrocene Derivatives by Cytokinesis-Block Micronucleus Assay

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Abstract

Ferrocene and ferricenium are commonly used to synthesize drugs for chemotherapeutic treatment. For many years, researchers have driven their attention to functionalization of ferrocene or ferricenium complexes to increase their toxicity and genotoxic damage in tumorigenic breast cancer cells (MCF7). It is well known that metallic compounds based on ferrocene or ferricenium caused direct damage to DNA. Ferrocene complexes produce reactive oxygen species (ROS) that lead in chromosome breaking. In this study the genotoxic damage of new ferrocene derivatives were assessed by Cytokinesis-block micronucleus assay (CBMN). A measure of DNA damage was calculated based on micronuclei formation. Micronuclei are extranuclear chromosome fragments expressed on divided cells and they measure the amount of DNA damage. For CBMN assay, the compounds previously synthesized, 1,1'-4-(1H-pyrrol-1-yl)phenyl ferrocenedicarboxylate, ("Fc-(CO₂-Ph-4-Py)₂"), 1,4-(1H-pyrrol-1-yl)phenyl, 1'-carboxyl ferrocenecarboxylate ("Fc-(CO₂-Ph-4-Py)CO₂H") and 4-(1H-pyrrol-1-yl)phenyl ferroceneacetylolate ("Fc-CH₂CO₂-Ph-4-Py") were exposed to the cells for 72 h. The cells were then treated with cytochalasin-β, a filament inhibitor of the cytokinesis in cells, to obtain binucleated cells which could express the micronuclei forming activity. The studies revealed that the presence of these substances induce micronucleus formation in binucleated cells and that Fc-(CO₂-Ph-4-Py)₂ exhibits a higher micronucleus formation compared with the other complexes studied.

Asellaria jatibonicua in the terrestrial isopod *Litthorophiloscia culebrae*

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Abstract

The trichomycetes are microorganisms associated with the gut of arthropods. They are an ecological group composed of protists (Ichthyosporidia) and fungi (Kickxellomycotina) that converged in a similar life style evolutionary solution with key adaptations to their gut environment. Isopods in particular harbor fungal members belonging to the Asellariales. One essential aspect of the ecology of the Asellariales is to understand the environmental conditions associated to the isopods. For instance, the different environmental fluctuations of temperature, water precipitation and relative humidity affect isopod lifestyle and thus, the life cycle of these fungi. In this study, isopods were collected in the University of Puerto Rico - Mayagüez and dissected in the laboratory the same day. We determined an average prevalence of 26% over 18 months and observed seasonality behavior of *Asellaria jatibonicua* associated with the terrestrial isopod *Litthorophiloscia culebrae* Moore. We have found *L. culebrae* in different forests of Puerto Rico, including Río Abajo, Guajataca, and Toro Negro and in an urban locality in Mayagüez. Data show a low prevalence of the fungus in the less humid months (with the lowest prevalence of 7% in August 2012 and 8% in March 2013) increasing towards the more humid months (with the highest prevalence of 44% in September 2012 and 55% in December 2012). In addition, during this study we found another trichomycete in the same host, *Parataeniella* sp. (Ichthyosporidia: Eccrinales), which represents a first record for Puerto Rico and a new species for science.

Fungal Bioprospects Present in *Nepenthes* sp. Trap Fluids

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Abstract

Carnivorous plants (CP) have been around for thousands of years, they have developed unique structures and traits in order to attract their prey and fulfill their nutritional requirements. *Nepenthes* sp. is a CP with a unique trap, referred to as a pitcher, with a fluid at the bottom in which the plant secretes digestive enzymes. The trap/fluid serves as a microenvironment (phytothelmata), which could result as a source of microbial bioprospects capable of degrading complex molecules with biotechnology applications. The focus of this research is the isolation and characterization of fungi present in the trap fluids of the CP *Nepenthes* sp. Combined fluids of CP phytothelmata were dispersed and cultivated on Petri plates with a non-defined enriched medium at pH 4 and pH 6. The inoculated plates were incubated at 25° C until microbial growth was observed. The fungi present were isolated and purified in Potato Dextrose Agar (PDA), and further analyzed macro and microscopically. A total of 19 different fungi were isolated from the pitcher, most of which were mycelial. Most of the isolated showed pigmentation ranging from red and pink to green and brown. Microscopically, 11 of the isolates showed cynocitic hyphae. The presence of asexual reproductive structures like phialydes, and budding cells were detected and identified as well. Based on the mycological analysis, some of the isolated fungi belong to the genera *Aspergillus* and *Fusarium*. Ongoing research is in progress to characterize the fungi genetically and perform functional analysis to the discovery of new complex molecules degrading enzymes.

Qualitative and Quantitative Analysis of Cell Proliferation Restriction Due To Metal Trace Elements Released from Ti Alloys

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Abstract

Metals such as titanium and its alloys are often used for bone replacement in orthopedic biomedicine due to their excellent biocompatibility. Ti-6Al-4V is a titanium alloy mostly used as implant material for bone reconstruction. This alloy is considered to possibly release vanadium (V) ions that are known to cause cytotoxic effects. The V-free gamma-TiAl alloy has shown to be an excellent biocompatible alloy with high corrosion resistant properties. hFOB cells were cultured on both Ti alloys thermally oxidized at temperatures of 121° C, 500° C, 700° C, and 800° C. A MTT Assay Technique was used in order to compare the viability of hFOB cells at a qualitative level, while Atomic Absorbance Spectroscopy using Graphite Furnace (GF-AAS) was used to evaluate Vanadium traces from Ti-6Al-4V. The presence of V trace elements was observed in media from cell cultures on Ti-6Al-4V treated at temperatures from 500° C, 700° C, and 800° C. Data obtained suggests a direct relationship with V presence and increase in degree of cell death of hFOB cells cultured in relation with Ti-6Al-4V alloys. Further studies of V-free alloys are highly recommended in order to obtain the best biocompatible material for orthopedic implants.

Diversity of Fungi Transported by Migratory Birds in Boqueron, Cabo Rojo

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Abstract

Migratory birds are important vectors for different forms of life including fungi and other microorganisms. Migratory birds are vulnerable to climate change because of limited wintering areas. The objective of this investigation is to establish the fungal diversity transported by migratory birds to Puerto Rico. During the hunting seasons 2013–2014 and 2014–2015 at Refugio de Boqueron, Cabo Rojo we sampled birds killed by hunters. Species sampled belonged mostly to *Anas discors* (blue wing teal), but others were represented: *A. americana*, *A. clypeata*, *A. platyrhynchos*, *Aythya collaris*, *Ay. affinis*, and *Gallinula chloropus*. Samples were taken from the cloacae with a sterile cotton swab, which was then placed in Sabouraud media with antibiotics until processed in the laboratory. Fungal isolates were identified by morphology under light microscopy. In the first sampling we collected samples from 344 birds obtaining 101 fungal isolates. In the second sampling we collected a total of 150 samples. Comparing data from both years, we determined minor changes in fungal diversity. Common environmental fungi were identified in both seasons: *Cladosporium*, *Mucor*, *Aspergillus*, and *Penicillium*. During the first hunting season we also detected *Fusarium*, *Pestalotia*, and *Paecilomyces*, which were not isolated in the second season. In the latter we isolated *Rhizopus*, not found previously. Based on these results and comparing to other genera found in European migratory birds, isolates from *Rhizopus*, *Mucor*, *Penicillium* sp.3, and *Aspergillus* sp.4 seem to be local representatives. We have partially identified our samples through morphology and further characterization will be done using molecular techniques.

Spatial Summation Properties of Neuron Subtypes in Mouse Visual Cortex

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Abstract

In the primary visual cortex (V1) of mammals, the activity of many neurons is attenuated when they are stimulated outside of their receptive field center. This phenomenon, known as surround suppression, is thought to be critical for integrating contextual information in visual processing. It has long been hypothesized that inhibition underlies surround suppression; however, the role of different inhibitory neuron subtypes remains unclear. In this study, we compare the response of excitatory pyramidal cells (PC), inhibitory somatostatin (SOM) and parvalbumin (PV) cells to grating stimulus of different sizes to gain insight of the mechanisms that underlies surround suppression. We used two-photon calcium imaging to measure size tuning of these cell types of awake, head-fixed mice. We fit the response in a difference of Gaussians model and used the size of maximal response and the degree of suppression to compare between neuron subtypes. As expected, excitatory neurons preferred smaller stimulus sizes and were strongly suppressed by large stimuli. In contrast, PV inhibitory neurons showed a preference for medium sizes, but were also suppressed, similar to PCs. In contrast, SOM neurons showed lower suppression in compared to the other neuron subtypes and they responded maximally to medium and large sizes. This preference for large sizes is consistent with the hypothesis that feedback inhibition from SOM neurons is responsible for suppressing PC and PV neurons in layer 2/3 of mouse V1. Future studies should aim to describe the connectivity between these three neuronal subtypes to better understand the mechanisms responsible for surround suppression.

An Ovine Model to Study Osseointegration of Gamma Titanium Aluminide

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Abstract

Titanium-based metallic materials are commonly used as implants in dental and orthopedic applications. The most popular alloy is Ti6Al4V which has been found to be toxic due to released vanadium. In addition the alloy possesses low osteoconductivity requiring the use of luting agents (a cement that adheres implant to bone), which in some cases breaks off causing loosening of the implant. The purpose of this research is to test a new type of vanadium free titanium alloy whose osteoconductivity diminishes the use of luting agents. Gamma titanium aluminide appears to demonstrate enhanced osteoconductivity with a surface treatment known as Plasma Electrolytic Oxidation (PEO). The treatment protects the implanted surface and deposits calcium and phosphorus, known to induce bone formation, on the implant surface. This study uses an in vivo ovine model to test the enhanced osseointegration of gamma titanium aluminide alloy. A total of nine 14mm long cortical screws were designed and later implanted in the iliac arch of two sheep, three Ti6Al4V, three gamma TiAl (without treatment) and three gamma TiAl (with PEO treatment). The first sheep was sacrificed after a three month implantation period. The torque needed to remove each screw was taken as a measure of osseointegration. The torque for PEO-treated gamma TiAl screws was at least 50 Ncm greater than that for the other six screws. The second sheep will be sacrificed six months after the implantation. Histology of the tissue adjacent to the implanted screws will be performed to further understand the extent of osseointegration.

Frequency Distributions of CYP2C9 Gene Variants in Puerto Rico

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Abstract

CYP2C9 is a cytochrome P450 enzyme coding gene located in chromosome 10. The enzyme is involved in the NADPH-dependent electron pathway and in the oxidation of many compounds such as steroids, cholesterol, and fatty acids. It contributes to the metabolism of drugs used for a variety of treatments, such as epilepsy, blood clotting and diabetes. Variations in the sequence of the gene alter enzyme effectiveness. A survey of the variants of this gene in the Puerto Rican sample set of the 1000 Genome Project showed two polymorphisms predicted by Polyphen to be probably (rs1799853) or possibly (rs1057910) damaging. Both polymorphisms are non-synonymous and have been associated to the metabolism of warfarin and nonsteroidal anti-inflammatory drugs such as ibuprofen. Whereas rs1057910 is at a frequency of 4%, rs1799853 is at a 17% frequency, which is highest for all world populations sampled by the 1000 Genome Project. Because the 1000 Genome Project sample set for Puerto Rico is skewed toward a higher representation of the western region, we are assaying genotype frequencies for both polymorphisms in different geographic regions to find out if their frequency distributions are homogeneous across the island.

A Transition at an Intronic Acceptor Site in the ATP7B Gene May Cause Wilson's Disease in Southwestern Puerto Rico

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Abstract

Wilson's Disease is an autosomal recessive genetic disorder caused by a deficiency in copper metabolism, which henceforth accumulates in body tissues such as the liver or brain. Neuropsychiatric and hepatic symptoms are observed in subjects suffering from the disease, but symptoms may be misdiagnosed as psychiatric or hepatic problems with subsequent mistreatment and aggravation of the disease. Because Wilson's Disease often remains asymptomatic until extensive damage has been caused, and diet-based treatment may be effective for avoiding further damage, it is important to establish straightforward genotyping assays for rare disease-causing variants that may be frequent in contained geographic areas. Wilson's Disease has been associated to mutations located on the ATP7B gene, which codes for a copper transporter to the bile and to the major copper-carrying protein in blood, ceruloplasmin. In most cases (90%) subjects are either homozygous or heterozygous for a loss-of-function mutation in this gene. Through TaqMan genotyping assays, we have detected a transition at the acceptor site of an intron in the ATP7B gene in southwestern Puerto Rico that may abolish its splicing, thus producing an mRNA with a premature stop codon. This transition is apparently linked to another rare variant located within the pyrimidine tract of another intron, as subjects carrying the first variant always carry the second one. To determine if any of the variants is interfering with copper metabolism, subjects with any of the variants will be asked to visit collaborating specialists in internal medicine and ophthalmology, who will assay copper metabolism function.

Microbial Diversity of Hypersaline Lagoons in Cabo Rojo Using Metagenomics

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Abstract

Hypersaline lagoons are considered extreme environments due to their high salt content, which can be inhabitable for many organisms. The solar salterns in Cabo Rojo are supplied by two hypersaline lagoons known as Candelaria and Fraternidad. They have been the subject of microbial diversity studies by traditional culture-dependent methods mostly for phylogenetic studies. However culture-dependent methods are limited and biased by media restrictions. Metagenomics is an alternative that can provide a more direct insight into the diversity present in these environments. The use of this methodology can also provide a more comprehensive analysis than the traditional culture-dependent methods. The main purpose of this research project is to study of the prokaryotic diversity at Candelaria lagoon using a metagenomic approach. Water samples were collected from the Candelaria lagoon and filtered. DNA extraction was performed on these membranes, further purified and subsequently sequenced for analysis. A preliminary metagenome is presented with particular emphasis on the 16SrRNA gene sequence to determine Operational Taxonomic Units (OTU's) frequency, abundance and diversity. There was a high diversity in bacterial phyla, which composed 25 of 27 groups. However there was more individual diversity in Archaeal groups particularly in Euryarcheota. Comparisons between both Candelaria and Fraternidad lagoons are still to be conducted as well as full metagenome analysis.

Characterization of the Hrp1 Prion in Wild Yeast

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Abstract

Prions are self-propagating, transmissible proteins that have the capacity to switch between the native and prion conformations. Prion domain (PrD) prediction algorithms show that the most abundant functional group of proteins that harbors a PrD is the group of RNA-binding proteins (RBPs). Hrp1 and Pub1 are two RBPs that harbor both a PrD and an RNA Recognition Motifs binding domain. A screen was conducted examining the SDS-resistant properties of Hrp1 and Pub1 in 280 wild yeast strains; 48 strains exhibited SDS-resistant aggregates as detected by a Dot-blot assay. Some of these were revalidated by SDD-AGE. In order to better understand the structural properties of these proteins in the different yeast strains, we tagged the endogenous proteins with GFP. Hrp1 protein was endogenously tagged with GFP in the W303 lab strain and in a wild yeast strain that exhibited an SDS-resistant aggregate form of Hrp1 (YJM326). The tagging of Hrp1 was validated by western blot analysis. As previously shown, Hrp1 localizes to the nucleus in the W303 strain. Surprisingly, in the YJM326 strain almost all the Hrp1 observed was in cytoplasmic aggregates under normal conditions. This is striking given that, for Hrp1 in the lab strains, induction of cytoplasmic aggregation is toxic to the cell. Pub1 was also tagged in the lab strains and exhibits cytoplasmic localization with distinct puncta in some cells. We are currently characterizing the cellular localization of Hrp1 and Pub1 in several other wild strains and examining the effect of manipulation of the chaperone machinery on the structural attributes of these PrD-containing RBPs.

Functional Analysis of Bioprospects from *Caracolus marginella* Gut

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Abstract

A microbiome is a collective of microorganisms that lives within another organism. Thanks to the advances achieved by disciplines such as genomics, culture dependent strategies to unravel microbial flora (metagenomics), and the lessons and technology developed by the Human Genome Projects; in 2009, the human microbiome project (HMP) was established. As the HMP, many microbiomes initiatives has been initiated including in mammals such the Giant Panda to understand diet behavior, and in invertebrates such as oysters and marine snails, analyzing their capability to adapt to different food resources in the environment they colonize. *Caracolus marginella* is a pulmonate mollusk native from some Caribbean Islands and Central America, where the gut microbiome has not been studied. This research seeks to analyze the *C. marginella* gut microbiome using culture-dependent and independent approaches. Specimens of *C. marginella* were collected and after dissecting the gut, samples were used to isolate cultivable microflora and to generate a metagenomic library. Isolated microorganisms (culture-dependent) and metagenomic libraries microorganisms (culture-independent) were tested for cellulose and amylose degradation. A total of 32 cultivable bioprospects were isolated, 50% of them were positive to cellulose degradation and 7% for amylose degradation. The *C. marginella* 1% metagenomic library clones tested directly and by cell lysis showed no specific degradation of the carbon sources tested. There is ongoing research to identify the cultivable isolates genetically, as well as analyze and compared the genes responsible for the activity assayed. Also, functional analyses to determine xenobiotics degradation capability are in progress.

Antibiotic Resistance Genes in Microbial Communities Using Metagenomics

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Abstract

Antibiotic Resistance (AR) infections has placed the world in a new age against potential microbial pathogens: the Post-Antibiotic Era. While classic microbiology studied those microorganisms using culture-dependent approaches, functional metagenomic analysis shows that the AR genes present in microbial pathogens at hospitals was first originated in microbial communities of the world's environments and that more of the 99% of them cannot be cultivable. The focus of our research was to generate metagenomic libraries (ML) from two different types of conditions: from a pristine environment and an anthropogenic impacted ecosystem (HAIE). Then, explore for AR genes against ampicillin. Two ML from compost, a vegetable waste and a human biosolid, were generated (representing the HAIE) and two ML from soils of Isla de Mona, PR were also generated (representing the pristine environment). The high molecular weight (40kbp) DNA of each one was end repaired, electro eluted, and ligated into the fosmid vector pCCFOS1, then, transduced to *Escherichia coli* Epi300-T1R using T1 bacteriophage. The Minimal Inhibitory Concentration (MIC) of ampicillin (15µg/mL) was determined. The AR clones from the libraries were isolated by selection on culture media supplemented with 1X-10X MIC ampicillin. While many clones in the ML of composts showed AR to 1X-10X MIC of ampicillin, no AR to ampicillin was detected in the clones from the pristine environment. These results represents that anthropogenic impacted environments are potential reservoirs of β -lactams AR in contrast to the pristine environment. Now, work is in process to detect the AR gene(s) responsible for the activity.

Testing the Neutral Mutation-Drift Hypothesis on the 1,000 Genomes Data Set

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Abstract

It has been proposed that much of the variation observed at the nucleotide level should be neutral or nearly neutral with respect to natural selection. As neutral variations are less likely to influence the phenotypic function of an organism as a whole, they would be less likely to be associated to genetic disease. Thus, in line with the goals of the 1,000 Genomes project, it would be of interest to know what proportion of the variation found in the whole genome can be considered to be neutral, as well as the precise mapping and identification of such regions, thus lending priority to those loci that are undergoing some evolutionary process. Initially focusing on the sample of Puerto Rican origin, we ran an array of tests and other data summarizing procedures such as site frequency spectra (SFS) on the data set. In order to determine whether alleles at a locus were selectively neutral or were undergoing an evolutionary process, we used Tajima's test to calculate a test statistic over a sliding window for each chromosome separately. In the case of chromosomes 21 and 22, we observed an excess of singleton sites in the SFS compared to what we would expect under neutrality, suggesting the possibility of there being regions under negative selection. Tajima's test, however, indicated the presence of regions under balancing selection. In the future, historical information as well as population structure must be taken into account, other than the obvious need sequencing of additional samples.

The distribution of the invasive *Harrisia* Cactus Mealybug on Puerto Rico

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Abstract

Cacti are a key component of Puerto Rican dry forests ecosystems. Currently, four native columnar cacti, including two endemic species, are threatened by the invasion of *Hypogeococcus pungens*, the *Harrisia* cactus mealybug (HCM). Little research has been published concerning the consequences of HCM in the island's cacti, and its distribution and intensity of infection are still not well understood. We conducted a survey of eleven plots in different parts of the island, where cacti were identified to species and severity of infection was determined using an index from 0–5. Zero are healthy, uninfected cacti; one, light infection with < 10 galls > 2 cm in diameter; two, light infection with 10–20 galls, > 6 cm in diameter; three, moderate infection, 20–40 galls, > 10 cm diameter; four, heavy infection, < 30 galls, < 10 cm diameter; and five, very heavy infection, < 30 galls, < 10cm diameter, and signs of necropsy and dead branches. Survival, seedling recruitment, and infection evolution was determined by revisiting the plots. HCM was located in all plots, but intensity of infection differed greatly between plots. Plots within the same area differed greatly in terms of severity, indicating a limited dispersion of the HCM. Mortality rate differs by species, with *Melocactus intortus* suffering higher mortality, and appearing to be more susceptible to HCM relative to other cacti species. Also, the occurrence of invasive plants (a vine and a grass), as well as anthropogenic fire disturbances, could have an impact on the survival of the cacti species. To our knowledge, this is the first study on the distribution of HCM on the island of Puerto Rico.

Analyzing the Genetic Diversity of *Phyllostachys* Through DNA Barcoding

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Abstract

Phyllostachys is a genus of temperate bamboo, a group of perennial grasses within the grass family Poaceae, that includes 76 species and an estimated 140 varieties and cultivars. This genus has been considered the most economically important group of the monopodial bamboos, since for centuries it has been the principal source of paper pulp, timber and handicraft materials in China and in Japan. Currently, phylogenetic analysis within woody bamboos presents difficulties since these rarely bear flowers, which are the traditional basis of identification; thus, taxonomists must rely on vegetative characters and other means for this end. DNA barcoding has demonstrated to be a promising tool for taxonomic research that allows for rapid and accurate identification of plant species through the use of a short, standardized DNA sequence of a well-known gene. In addition, it has largely contributed to environmental genomic studies to analyze diversity for conservation of biodiversity. This project aims to analyze the genetic diversity of 18 species within the genus through single-locus DNA barcodes, while verifying the species resolution efficiency of this tool at a genus level. Current results suggest that single-locus DNA barcodes do not have adequate sequence variation for species discrimination within the *Phyllostachys* genus.

DNA Barcoding of Tropical and Temperate Bamboo Species in Puerto Rico

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Abstract

Bamboo species compose the fourth largest group of the grass family, Poaceae, of temperate and tropical forests worldwide. Despite its usefulness as both a building material and a food source, which make it inherently important both economically and culturally, a high degree of uncertainty remains at the taxonomic level of the Bambusoideae subfamily. DNA barcoding, a technique in which a universally accepted short DNA sequence is employed for the identification of species, can be used to resolve the evolutionary relationships of temperate and tropical species, which have been difficult to determine traditionally, relying solely on morphological data. Although coding regions are typically used in reconstructing deep-level phylogenies, noncoding regions render a greater number of substitutions that are neither biased, nor reflect the functional evolution of the gene. This project aims to resolve the phylogenetic relationship between bamboo species using two barcodes: the noncoding, chloroplast intergenic spacers trnH-psbA and psbK-psbI. Preliminary results show 12 new psbI-psbK sequences, three new trnH-psbA sequences, and 5 species with different barcoding sequences from the ones available in databases of the Bambusa species. For temperate species, 11 and seven sequences were determined using trnH-psbA and psbK-psbI, respectively, including an unidentified species.

vfl2 and Wild Type Centrin-Sfi1p21 Complex Crystallization Screenings

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Abstract

Human centrin is a 20 kDa calcium sensor protein, belonging to the EF-hand superfamily, which has both structural and regulatory roles. Sfi1 is a 1242 amino acid protein comprised of 23 tandem centrin binding sites (CBS). These centrin binding sites are not conserved allowing for a systematic study of the interaction. Structural information is available for the yeast homologue of this complex known as Cdc31-Sfi1p (PDB 2DOQ) associated with the duplication of the half bridge, yet the effect of a naturally occurring mutation with well-defined phenotype known as vfl2 has not been investigated to date. In *Homo sapiens* the centrin (E105K) variant corresponding to the vfl2 phenotype has been link to no centriole duplication. Our goal is to investigate the structural consequence of the centrin mutation (E105K) and its effect on the key salt-bridge interaction required for complex formation and to determine the possibility of an alternate salt-bridge interaction within the centrin (E105K)-Sfi1p21 complex. Crystal screens were performed in collaboration with the HTS Lab and Visual data examined for Hs centrin 1 WT-, Hs centrin 1(E105K)- and Hs centrin 2 (E105K)-Sfi1p21 complexes. Optical identification of possible complex crystals was achieved with the result of said visual data which was composed of images taken with novel Second Order Non-linear Imaging of Chiral Crystals (SONICC), UV-T, and light microscopy. Suspected centrin-Sfi1p21 complex crystals were found in similar crystallization conditions. Crystals that were positive in all three viewing methods were considered to have a high possibility of been the crystallized centrin-Sfi121 complex.

Full Annotation of the CaMKI, lgs, and bip2 Genes of *Drosophila elegans*

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Abstract

Bioinformatics tools have made possible the documentation of the starting sites and coding exons of genes, which are useful for a deeper understanding of a species. This part of the investigation studies the genes CaMKI, lgs, and bip2 with all their isoforms, including the untranslated regions. These genes are located on chromosome 4 of *D. elegans*. This was done using various bioinformatics programs such as BLAST, GEP UCSC Genome Browser, Flybase, Gene Record Finder and Gene Model Checker. The gene sequences of *D. elegans* were compared to those of *D. melanogaster*, the model species. When sequences were to divergent, further comparisons were made with *D. rhopaloa*, a more closely related species. The methods used in the investigation were able to confirm that the genes exist in *D. elegans* and can be annotated manually almost to completion. Future applications include the extrapolation of the techniques to other organisms.

Cataloging *Garcinia* Species Through DNA Barcoding

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Abstract

DNA Barcoding allows easy identification of species and the study of biological diversity in organisms. This makes cataloging of genetic diversity possible and accessible to everyone. Plant species can be identified at genetic level even using small or damaged specimens. In addition to this, it is important for the conservation of species diversity to avoid the impact of global climate change in genetic diversity. Another important aspect of DNA Barcoding is to determine occurrences of fraud and the use of protected species. This project is focused on using DNA Barcoding in the *Garcinia* collection of the Tropical Agriculture Research Station (TARS) in Mayagüez, Puerto Rico. *Garcinia* family consists of species such as achacharu, purple mangosteen, giant leaf madrono, palo de cruz, mameyito, monkey fruit, cherry mangosteen, and lemon drop mangosteen, among others. Most species have a commercial value as an edible fruit but some have recently gained prominence as nutritional supplements. DNA barcoding of this family will be achieved through the amplification of locusses commonly used in plant DNA barcoding such as trnH-psbA and psbI-psbK. The preliminary results from our project shows unique samples where no molecular data is present in GenBank database. The expected final results are to have a catalog of genetic diversity, confirm the identity of species in the TARS *Garcinia* Collection, and provide new information to the current database.

Western Blot Detection of the Tyrosine-Protein Kinase Src in Breast Cancer Cell Lines Non-infected and Infected with the LuIII Parvovirus

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Abstract

Src tyrosine kinase is a governing protein that is related to cancer progression. It has been found that Src is over expressed or aberrantly activated in colon and breast cancers. Parvovirus LuIII infects cancerous cells. To confirm that Src tyrosine kinase expression is elevated during cancer progression, a Western Blot protocol was performed using the Non-phospho-Src (Tyr 527) primary antibody to obtain densitometric data and compare the results for three different breast cell lines: non carcinogenic (MCF-12ATM), intermediate carcinogenic (MCF-7TM), and stage III carcinogenic (MDA-MB-231TM). The same procedure was done for the same three breast cell lines, infected with the LuIII virus to see how Src responds in its presence. The cell lines were cultured (and some infected with LuIII), followed by protein lysis and quantification by the Lowry assay. Preliminary data show that the expression of Src was more elevated in the intermediate carcinogenic cell line, for both, the non-infected and infected cell lines. However, Src had more expression in the non-infected cell lines in comparison with the infected cells in all the three cases. The data suggests that the infection with the LuIII virus reduced the Src tyrosine kinase activity. Further, the LuIII virus showed infectivity in mammary cancerous cells in addition to the lung cells. Finally, a different banding pattern for Src was observed in the non-infected versus infected cells suggesting an effect of parvovirus LuIII on the expression of Src.

Endophytic Fungi From the Black Mangrove *Avicennia germinans* in Puerto Rico

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Abstract

The search for new antimicrobial and antifungal compounds has been of great interest in recent years. These compounds are naturally produced by microorganisms, including endophytic fungi isolated from mangroves. This study focuses on endophytic fungi isolated from leaves of the black mangrove (*Avicennia germinans*) from Bahia Salinas, Cabo Rojo, Puerto Rico. We isolated 42 fungal endophytes, from which five identified strains were tested for antimicrobial and antifungal secondary metabolite production. We selected fungal strains from *Penicillium*, *Aspergillus flavus*, *Purpureocillium*, *Engyodontium*, and *Bionectria* for the analysis. Four bacterial strains: *Escherichia coli*, *Pseudomonas*, *Serratia*, and *Staphylococcus*; and two yeast strains: *Candida albicans* and *C. tropicalis* were used to perform bioassays. Growth curves were prepared to determine the log phase of yeast and bacteria. Fungal extracts (10 ul) were added after exponential growth started (2–4.5 hours) to a culture well of 100 ul. Our results showed that *Aspergillus flavus* extract strongly inhibited the growth of *C. albicans* and *E. coli*, and but increase the growth in *Staphylococcus*. There was no apparent inhibition in the growth of *C. tropicalis* or the bacterial strains. Although, *Serratia* was slightly inhibited by *Aspergillus flavus*, *Purpureocillium*, and *Bionectria*. These results could open a way for future discoveries of new antimicrobial o antifungal compounds.

Degradation Capacity of Crude Glycerol by Microorganisms Associated with Fiddler Crab (*Uca rapax*) Intestine

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Abstract

As an alternative to the use of fossil fuels, industries have relied on the production of biodiesel. Glycerol, 1,2,3-propanetriol, is a byproduct from biodiesel synthesis. Due to the large residue amounts and low cost of glycerol the idea of using microorganisms to degrade the glycerol and convert it to a renewable fuel source is warranted. For this work, 57 microorganisms previously isolated from the fiddler crab (*Uca rapax*) intestine were used to determine their capacity to degrade crude glycerol. The microorganisms were inoculated in three different synthetic media: negative control without a carbon source, positive control with dextrose and for the experimental, with 5% glycerol. They were incubated at 28° C for 4–7 days. Eight microorganisms were capable of growing using crude glycerol as a primary carbon source; but only four of them were able to alter the medium color meaning that they produce secondary metabolites. The strains that produced secondary metabolites were identified as *Streptomyces* sp., *Bacillus* sp., and *Enterobacter* sp. Also, these strains showed faster growth in comparison to the others.

Gracias por su
participación y apoyo

Thank you for your
participation and support