¡BIENVENIDOS AL SEXTO 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 Sexto 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 artísticas de nuestros estudiantes y colegas.

El comité organizador agradece profundamente el apoyo del Departamento de Biología-Recinto Universitario de Mayagüez 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.

Comité Organizador

Dimaris Acosta

Sandra L. Maldonado Ramírez

Carlos Muñoz

Vivian Navas

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Jeanine Vélez Gavilán

Itinerario del Sexto Simposio de Investigación Subgraduada

30 de abril de 2016

8:00 am	Registro
8:45 am	Bienvenida: Auditorio de Biología (B-392)
8:45–9:45 am	Primera Sesión Plenaria Fishing and Ecotourism: Opportunities for Puerto Rico Dr. Wes Neal, Associate Extension Professor, Mississippi State University Auditorio de Biología (B-392)
9:45 am	Sesión I
	Genética no Humana (B-180)
	Medicina (B-181)
	Ecología I (B-182)
	Microbiología I (B-280C)
12:00 pm	Almuerzo (Lobby)
12:45–1:45 pm	Segunda Sesión Plenaria Microbiologists as Instruments of Global Health: stories from the field Commander Dr. Guillermo Pimentel, Microbiologist, United States Navy Auditorio de Biología (B-392)
1:45 pm	Sesión II
	Genética Humana (B-181)
	Ecología Microbiana (B-182)
	Ecología II (B-280C)
3:45 pm	Sesión III
	Bioquimica (B-180)
	Microbiología II (B-181)
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

Glycosyl Hydrolase Expression at Different Carbon Sources in the Haloarcheon *Halogeometricum borinquense*

Salazar Morales, Aldo Ivan,[1, 2] Mara F. Cuebas Irizarry,[1] and Rafael Montalvo Rodriguez[1]

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Abstract

Since the discovery of Archaea over 30 years ago, little is known about regulation of carbohydrate utilization by gene expression. For example, carbon catabolite repression, which is controlled by glycosyl hydrolases expression (GH), has barely been studied. The objective of this study is to understand the regulation of glycosyl hydrolases expression in the presence of different carbon sources at high salinity. To achieve this goal, the halophilic archaeon *Halogeometricum borinquense* was selected, as it has a shorter generation time than other halophilic archaea. In addition, *H. borinquense* has the ability to use different carbon sources, which include: glucose, maltose, mannose, fructose, xylose, trehalose, cellobiose, raffinose, and glycerol. Cells of *H. borinquense* were grown in minimal media with maltose, sucrose, and glucose as the only carbon source; and also in rich medium until late logarithmic phase. Enzyme assays were performed to test glycosyl hydrolase expression in all four media. Results revealed low or no levels of glycosyl hydrolase activity in rich medium and minimal medium with glucose as carbon source, compared to considerably higher activity in sucrose and maltose. The specific activities of the α-glucosidase, β-glycosidase, and β-galactosidase were calculated for maltose, sucrose, and rich media. Enzyme activities in sucrose, maltose, and rich media showed a 13-,15-,5-fold increase for α-glucosidase, and a 136-,272-,16-fold increase for β-glycosidase in comparison to glucose activity levels. These studies provide evidence of a putative CCR system in halophilic archaea. Preliminary analyses suggests that GH regulation depends on carbon source.

Mutación de Pérdida de Función en CCBE1 Como Posible Causa de Enfermedades en los Puertorriqueños

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Resumen

Las mutaciones de pérdida de función son variantes que suelen abolir la función de genes humanos que codifican para proteínas. Estas mutaciones recesivas, suelen estar asociadas al desarrollo de enfermedades en el ser humano. Se pretendía identificar polimorfismos, asociados a mutaciones de pérdida de función, que tengan un impacto sobre la salud de los puertorriqueños. A través de análisis del Proyecto 1000 Genoma, se identificaron mutaciones de pérdida de función que tenían una prevalencia en Puerto Rico mayor que en el resto del mundo; se buscaba que tales mutaciones tuvieran un efecto sobre todas las isoformas del gen, que el gen tuviera un tamaño de transcripción significativo y una expresión a nivel fetal en los humanos. Entre estas mutaciones se encontró una en la posición 57,147,471 del cromosoma 18, correspondiente al gen CCBE1 ("Collagen and Calcium binding EFG domains 1"). CCBE1 provee instrucciones para hacer una proteína que se encuentra en la matriz extracelular y está asociada a la formación del sistema linfático. Se realizó un genotipado usando Real Time PCR con muestras de ADN recolectadas de diversas áreas geográficas de Puerto Rico en busca de individuos que presentaran tal mutación. Se espera que la mayoría de los individuos muestreados que presenten la mutación sean de naturaleza heterocigota, ya que la manifestación homocigota de la mutación se encuentra relacionada al desarrollo de una enfermedad autosómica recesiva letal denominada Síndrome de Hennekam. Aquellos individuos que sean heterocigotos para esta mutación son potenciales portadores de esta enfermedad y podrían padecer problemas tiroideos.

Halorubrum tropicale sp. nov., a Novel Halophilic Archaeon Isolated from the Solar Salterns in Cabo Rojo

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Abstract

A novel strain (V5) belonging to the halophilic archaea was isolated from the Cabo Rojo solar salterns in Puerto Rico, for which the name *Halorubrum tropicale* is proposed. Strain V5 is a pleomorphic cocci. The organism was isolated from an artificial seawater agar medium with pyruvate as sole carbon source at a pH of 7.2. Optimal growth factors were obtained for salinity (20% w/v), temperature (45ºC), and pH (7.5). Analysis using the 16S rRNA gene revealed that the V5 strain belongs to the genus *Halorubrum*. Phylogenetic analyses using the *rpoB*, *ppsA*, and *atpB* genes by multilocus sequence analysis indicate that strain V5 indeed represents a new species. Analysis of the 16S rRNA gene revealed that strain V5 was most closely related to *H. terrestre* and *H. distributum* with 99% similarity. As for the rpoB and atpB genes, the most related organism was *H. xinjiangense* (97%). The ppsA gene showed a 95% similarity with *Halorubrum arcis*. The average nucleotide identity was determined by comparing all available *Halorubrum* genomes with strain V5, concluding that all species of *Halorubrum* were between 81%-88% similar, the most similar species being *H. litoruem* with 88.36%. The genome consists of 3.57 MB in length with a G + C content of 67.6%. It includes 4 rRNAs, 52 tRNAs, and 3246 protein-coding sequences. A series of characterization tests were conducted, including NaCl concentration, temperature, pH, carbon sources, hydrolysis, gas production, and antibiotic resistance.

Solirubrum puertoriconensis: Characterization and Genome Sequence of a Novel UV-Resistant Bacteria

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Abstract

Ultraviolet (UV) radiation resistance in bacteria is mediated by a DNA enzymatic repair system 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 for isolating UV resistant bacteria in Puerto Rico, a dry soil sample was aseptically collected from La Parguera, in the town of Lajas. One gram of soil was suspended in sterile distilled water, where it was irradiated with UV-light (254nm) in a microbiological hood. *Escherichia coli* was used as negative control. The suspension was serially diluted and plated in R2A medium and incubated in the dark for several days to avoid photoreparation. A Gram-negative, rod-shaped, red-pigmented bacterium, *Solirubrum puertoriconensis* (strain MC1), was successfully isolated for further characterization. Strain MC1 grows at room temperature. Sequence analysis using the 16S rRNA gene showed 96.8% similarity to *Hymenobacter deserti* and values lower than 91% to other *Hymenobacter* species. This result suggests that MC1 might represent a novel genus within the Bacteroidetes. Polyphasic taxonomy is currently being performed to provide full characterization of this novel organism.

Cultivable Fungi from Fresh Casts of *Yuisia olgae* (Annelida: Oligochaeta: Achantodrilidae)

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Abstract

The ecology of the recently discovered earthworm genus *Yuisia* is unknown, and the work presented here is the first attempt to describe the mycobiota of one of its species, *Y. olgae*, from fresh casts. This is not only the first attempt to understand *Yuisia*'s ecology, but also the first attempt to describe the mycobiota associated with tropical earthworm's casts. For this research two specimens of *Y. olgae* were placed on individual transparent sealed plastic bags with sterilized moist paper towel. After 24 hours, the earthworm defecated the majority of their intestinal contents on the moist paper towel and 1g of these feces were collected using sterile instruments and mixed with 10 mL of a sterile solution of 10% KOH. A serial dilution was performed and two sets of samples were cultivated: one inoculating 1ml from each original and diluted solution and another one, with inoculating 0.1 mL. All inoculations were homogeneously distributed on commercial Potato Dextrose Agar. A total of 15 species and morphotypes of filamentous fungi were isolated, 11 representing species of *Cylindrocarpon*, *Fusarium*, *Penincillium*, *Aspergillus*, and *Mucor*. The other four isolates lacked reproductive structures. *Fusarium* was the most abundant isolate, followed by *Cylindrocarpon*. This contrasts with prvious reports, as *Fusarium* and others plant pathogens abundance seems to decline in earthworm casts. This correspond to the first report of the genus *Cylindrocarpon* isolated from earthworm casts.

Characterization of the Microchromosome 27 of the Puerto Rican Parrot (*Amazona vittata*)

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Abstract

The endangered Puerto Rican parrot (*Amazona vittata*) began to be investigated in genomic terms after having their genome recently sequenced. To extend the knowledge regarding the type and amount of protein-coding genes, sequences rearrangements and translocations, we are focusing on the Puerto Rican Parrot microchromosome 27 from the latest genome assembly (http://genomes.uprm.edu/parrot). Different annotation resources and data-bases were used such as NCBI's BLAST Nucleotide Sequence, UCSC Genome Browser, Repeat Masker, GIRI-Sensor, AUGUSTUS, FGENESH, and GENSCAN. The chicken (*Gallus gallus*) sequence files were used as the principal templates for the annotation, but in order to acquire better results it was necessary to use other bird genomes such as that of the budgerigar (*Melopsittacus undulatus*), Kea (*Nestor notabilis*), zebra finch (*Taeniopygia guttata*), turkey (*Meleagris gallopavo*), saker falcon (*Falco cherrug*), and the peregrine falcon (*Falco peregrinus*). A total of 169 genes were found after analyzing the 21 scaffolds belonging to microchromosome 27. Two gene clusters were found in two scaffolds; the first one with genes of the HOXB family that are involved in developmental process and the second one with feather β-keratins. The total length of all scaffolds adds to 4.9 Mb and the homologous chromosome in chicken has a length of 5.3 Mb. The microchromosome 27 is in synteny with all the bird species genomes analyzed; but two scaffolds show unique gene inversions for *A. vittata*. Results obtained from gene predictors suggest the presence of more genes, especially β-keratins.

Caracterización de Secuencias Homólogas al CHR7 de *Gallus gallus* en Cromosoma AVI6 de *Amazona vittata*

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Resumen

La cotorra puertorriqueña (*Amazona vittata*) es un ave endémica de Puerto Rico que se encuentra en peligro de extinción. Su genoma fue secuenciado con el propósito de conocer más sobre su genética y así poder ayudar en los proyectos de conservación. Como parte de estos estudios genómicos se realizó una hibridación fluorescente in situ entre los cromosomas de la gallina y la cotorra puertorriqueña; y se encontró que el cromosoma 6 en *A. vittata* (AVI6) hibridó con el 6 y 7 de *Gallus gallus*. Esta translocación de cromosomas es única hasta el momento del orden de los Psittasiformes, ya que en el 2013 se logró identificar además dicho arreglo en el genoma del guacamayo rojo (*Ara macao*). Partiendo de estos resultados, se lograron identificar 66 andamios que parean con estos dos cromosomas en *G. gallus*. Esta investigación está enfocada en caracterizar las secuencias del AVI6 que presentan homología con el cromosoma 7 de la gallina. Utilizando programas y bases de datos como NCBI, UCSC Genome Browser, Repeat Masker, Gene Panther y diferentes predictores de genes se busca poder caracterizar las secuencias, analizar las translocaciones previamente identificadas y describir y anotar genes que codifiquen para proteínas. Actualmente se ha trabajado con los 17 andamios más grandes y la longitud de éstos suma a 11.7 Mb. Hasta el momento un 26.9% de los genes identificados cumplen un papel en la regulación biológica y la localización de productos. Se estima que el tamaño del cromosoma AVI6 debe estar cercano a las 72 Mb.

Análisis del Contenido Estomacal de la Rana Invasora (*Osteopilus septentrionalis*) en Sembradío de Agüacates en Isabela, Puerto Rico

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Resumen

Las especies invasoras son uno de los grandes problemas ecológicos que existen en todas las partes del mundo. La introducción de la Rana Cubana (*Osteopilus septentrionalis* - Hylidae) puede ser un factor a considerar en la disminución de especies nativas y endémicas en el Caribe y en EEUU. Dado su éxito marcado en diferentes hábitats a los que ha sido introducida, esta especie ha sido utilizada para examinar su impacto en comunidades nativas. Este trabajo analiza la dieta de esta rana en un área cercana al punto de introducción de esta en PR (Agüadilla). Las ranas fueron capturadas en la Estación Experimental Agrícola de Isabela, en siembra experimental de árboles de aguacates (*Persea americana*) y colocadas en bolsas plásticas para su transporte al laboratorio. Eutanasia de los especímenes se efectuó por congelación (protocolo aceptado). Se efectuó disección de especímenes bajo estereoscopio con el propósito de remover tracto digestivo (estómago y tracto intestinal completo). Se analizó el contenido de éstos tractos de 13 individuos (6 hembras y 7 machos). Se encontraron mayormente invertebrados (insectos, ácaros, moluscos) y un vertebrado (cráneo de *Eleutherodactylus antillensis*). Se propone llevar a cabo este estudio en áreas naturales cuya diversidad de presas es mayor en donde se ha establecido esta especie. Estrategias para el control de esta especie en Puerto Rico no se han implementado, y muy poco se sabe sobre el impacto de esta especie sobre especies nativas de anfibios, reptiles y otros.

Distribution of a Wilson's Disease Causing Loss of Function Mutation in Gene ATP7B in Southwest Puerto Rico

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Abstract

The ATP7B gene encodes for an ATPase with a fundamental role in the cellular transport of copper within hepatocytes. Ninety percent of Wilson's Disease (WD) cases are caused by a loss-of-function mutation in this gene, leading to a decreased excretion of copper into bile. Copper then accumulates in the liver and eventually flows through the bloodstream and is accumulated in other organs, such as the kidneys and the brain with fatal consequences. A mutation altering the acceptor splicing site of intron #11(rs367956522) has been detected in a family afflicted by WD in southwest Puerto Rico. We have found it in its homozygous form in two cases, and in its heterozygous form in sixteen cases within four families out of 219 samples assayed in southwest Puerto Rico. We have further found that homozygous and heterozygous individuals are conversely homozygous or heterozygous for a second mutation located within the pyrimidine tract of intron #16 of the gene (rs140708492). Three samples heterozygous for only rs140708492, a rare variant specific to Europe, were found. Therefore we deduce that the SNPs are linked and that the mutation generating rs367956522 occurred in a chromosome that already had rs140708492. People heterozygous for any of the mutations are being given free access to medical tests that will allow us to study the phenotypic effects of a single copy of the mutation. An expanded survey of rs367956522 in Puerto Rico will allow us to determine the viability of recommending newborn tests for this SNP.

Polysaccharide-based Polyelectrolyte Multilayers: Physicochemical Characterization and In Vitro Studies

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Abstract

Implants over the years have caused many unpleasant reactions and infections. This occurs because the body rejects them after a period of time since they are not biocompatible. One alternative to improve their biocompatibility is to generate a bioactive surface that helps in cell processes such as cell adhesion and therefore improves biocompatibility. Our work was composed of developing three and six bilayers of Chitosan (Chi) and Heparin (Hep) over a silicon substrate using the layer-by-layer (LBL) method, simulating the surface of the tissue's extracellular matrix to enhance the biocompatibility and cellular adhesion of the substrate. NIH/3T3 cells were used for in vitro studies. Infrared Variable Angle Spectroscopic Ellipsometry (IR-VASE) allowed us to study the thickness and chemistry of the CHI/HEP bilayers, while cellular adhesion and morphology was observed via scanning electron microscopy (SEM). The IR-VASE analysis reported the distinctive peaks of polysaccharides, enabling us to see their chemistry and presence in the substrate. SEM demonstrated that three bilayers were not able to completely cover the silicon surface while six bilayers were able to cover completely the silicon substrate and generate a homogenous surface. Our in vitro studies reported that six bilayers generate an attractive surface for cells to adhere and survive in comparison to the three bilayers. This demonstrates that the development of these polymeric bilayers can become a promising way to increase the biocompatibility of biomaterials' surfaces.

Listado del Género *Auricularia* en el Área Oeste de Puerto Rico

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Resumen

El género *Auricularia* se caracteriza por agrupar hongos comestibles y descomponedores de madera, asociados generalmente a áreas húmedas y de poco sol. Se han reportado especies de este género con propiedades antibacteriales, antioxidantes y antiparasíticas. A pesar de la importancia de estos hongos, al presente no se han realizado estudios sistemáticos para conocer la diversidad de este género en Puerto Rico. Se colectaron muestras aleatoriamente en zonas boscosas del área oeste de Puerto Rico para documentar la diversidad del género en esta región. Las muestras fueron removidas del sustrato utilizando una cuchilla y transportadas en fundas de papel para ser identificadas a nivel de especie utilizando técnicas morfológicas tradicionales como cortes y tinciones. Entre las especies identificadas se incluye *A. fuscosuccinea*, *A. delicata* y *A. auricula-judae*. Estas identificaciones fueron realizadas utilizando una hoja de datos que facilitó la caracterización del hábitat y de caracteres morfológicos.

Enfermedades Asociadas al Polimorfismo rs41341748 en el Gen MSR1 y su Frecuencia en Puerto Rico

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Resumen

Dado los avances en genómica y bioinformática, hoy día se pueden estudiar con mayor facilidad las anomalías presentes en el genoma humano. En este contexto, esta investigación se centra en el estudio de enfermedades mendelianas causadas por mutaciones de pérdida de función. Utilizando los datos de la primera y tercera fase del Proyecto "1000 Genomas" se identificó el polimorfismo rs41341748 presente en el gen MSR1 el cual ha sido asociado a la incidencia de enfermedades como Esófago de Barret y Adenocarcinoma Esofágico. Este polimorfismo de un solo nucleótido (SNP) cambia una citosina por una timina en un codón para el aminoácido arginina formando así un codón de terminación prematuro. Dicha mutación afecta todas las isoformas del gen. Actualmente se está utilizando un ensayo específico para el SNP por "Real Time PCR" para determinar la frecuencia y distribución genográfica del polimorfismo en Puerto Rico genotipando 396 muestras recolectadas por toda la isla. Como control positivo se está utilizando al individuo HG01075, que es uno de siete individuos heterocigotos para la mutación encontrados por el Proyecto del 1000 Genomas de entre un total de 104 puertorriqueños. Los resultados preliminares no han sido conclusivos. La alta frecuencia del polimorfismo en Puerto Rico nos permite predecir una mayor incidencia de algunas de las enfermedades asociadas a la mutación.

Analysis of Loss-of-Function Mutations Identified by the 1000 Genomes Project in Puerto Rico

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Abstract

Loss-of-function mutations are mutations in the DNA that cause a protein to malfunction. The purpose of this experiment is to study different loss-of-function mutations that have been identified by the 1000 Genomes Project in Puerto Rico. Each chromosome was screened and thoroughly analyzed using bioinformatics tools to create a reduced list of the 12 most detrimental and interesting mutations. Here, chromosomes 4, 5, and 14 were assigned. The analysis included observing the frequency of the mutation in Puerto Rico, and whether it caused a frame shift, premature stop codon, or damaged splice site. It also included revising if the mutation affected all isoforms of the protein, how high the P(rec) of the mutated gene was, its GERP, transcript size, and whether it appeared in databases such as OMIM and Cosmic. In case of the creation of premature stop codons, it was examined whether is caused non-sense mediated decay. After the list with the 12 mutations was created, the mutation most likely to abolish gene function completely and affect a function without redundant genes was selected. A mutation in the SEMA 3A gene was selected, which is found on chromosome 7. It is a member of the semaphorin family and can function as a chemorepulsive or chemoattractive agent. Mutations in it have been related to different disorders such as hypogonadotropic hypogonadism, head and neck squamous cell carcinoma, endometriosis, and more. Currently, samples from the Puerto Rican population are being tested in the lab to determine if they contain this mutation.

Biodiversity Responses of Different Testate Amoebae Communities to Tropical Climate Change Extremes

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Abstract

Testate amoebae have been proven to be successful climate change proxies in temperate environments because of their sensitivity to water table depth and substrate moisture. However, little is known about their bioindicator capacities in tropical landscapes, where the effects of climate change are particularly unpredictable. Insight into the climate sensitivity of tropical testacean communities may provide scientists with new ways to gauge the effects of climate change in tropical ecosystems. Given the moisture-driven changes in composition, species richness and abundance of testaceans in temperate freshwater ecosystems, we hypothesize that altered levels of precipitation will change the abundance, richness, and community composition of testaceans in freshwater tropical ecosystems. In this study, we tested this hypothesis by experimentally controlling the amount of precipitation from baseline levels in tank bromeliads (Bromeliaceae) from three tropical countries and identifying the testacean communities therein. Our results show that testacean response to changes in precipitation varies with country; some changed in composition, richness, and abundance, others only in composition, and some did not respond to changes in precipitation. These results highlight the possibility that there is no single biological response to climate change. We conclude that further research should elucidate the mechanisms behind these differences and identify these location-specific factors for a more precise use of testaceans as indicators of climate change in tropical countries. Due to the threat that climate change poses in the tropics, it is imperative that scientists find new, varied and trustworthy ways to measure precisely how climate change is altering our environment.

Deciphering the Regulation of Natural Killer Cell Development by lncRNA23

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Abstract

Novel Long noncoding RNA 23 (lncRNA23) transcript and its gene locus was identified through RNA sequencing by probing various subsets of CD4+ T immune cells (unpublished data from Williams Lab). Examining lncRNA23 expression in various other immune cell populations identified a conspicuous expression of lncRNA23 in natural killer (NK) cells. In-vivo knockout studies in mice using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 a genome-editing technology, indicates that the lncRNA23 gene locus is essential for NK cell development. CRISPR-Cas9 mediated deletion of the lncRNA23 gene locus decreases the expression of transcription factors important in NK cell development. Thus, based on these primary observations we sought to elucidate the mechanisms by which lncRNA23 regulates gene expression using overexpression and knockdown strategies. Initially, the 2kb lncRNA23 cDNA was cloned into a mammalian lentivirus expression vector. Lentiviruses containing lncRNA23 plasmid were produced, and transduced into mouse kidney fibroblast (MK4) cell line to determine the efficiency of transduction and lncRNA23 expression in these cells. Unexpectedly, the results obtained in MK4 cells so far indicate that overexpression of lncRNA23 down regulates factors important for NK cell development. We speculate that this inverse regulation might be due to a squelching mechanism; nevertheless an immediate future investigation on this hypothesis is warranted. In short, this research aims to understand the novel mechanisms of lncRNA23 in regulating gene expression, and its role in immune cell identity, signaling and differentiation.

Distribución de una Mutación de Pérdida de Función en el Gen VPS13A en la Población Puertorrigueña

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Resumen

Las mutaciones de pérdida de función son variantes genéticas que interrumpen la función de genes, al truncar o eliminar el polipéptido para el que codifican, en algunos casos resultando en susceptibilidad a enfermedades complejas, como lo es la enfermedad neurodegenerativa coreoacantocitosis. Diversas variantes genéticas han sido identificadas como responsables del padecimiento de esta enfermedad, entre las que se encuentra la mutación sin sentido c.3889C>T en el gen VPS13A. Esta mutación fue reportada en el proyecto de los 1000 Genomas en un individuo de la población puertorriqueña, por lo que se decidió validar y estudiar la frecuencia de esta variante en Puerto Rico. Se utilizaron navegadores genéticos como "UCSC Genome Browser" y "Ensembl" para determinar el efecto de la mutación en las isoformas del gen. Se utilizó "ViiA7 Real-Time PCR System" con el propósito de validar la mutación en el individuo identificado como HG00554 en el proyecto de los 1000 Genomas, así como para genotipar 396 muestras recolectadas alrededor de la Isla. Se encontró que el SNP afecta a todas las isoformas presentadas en los "browsers," resultando en la eliminación de aproximadamente 2000 residuos de la proteína codificada por VPS13A. Resultados preliminares demuestran que el individuo HG00554 es heterocigoto para el alelo que contiene el SNP y el resto de los individuos estudiados son homocigotos para el alelo sin la mutación. Estos datos demuestran la tendencia de mutaciones de pérdida de función a estar presentes en baja frecuencia en una población.

Characterization of Chromosome 25 in *Amazona vittata*

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Abstract

Amazona vittata, known commonly as the Puerto Rican parrot, is one of the endemic birds of Puerto Rico, and the only one that belongs to the genus *Amazona*. This bird is also the only surviving native parrot species in a United States territory. The purpose of this project is to characterize, annotate the protein coding genes, and make a map of *A. vittata*'s chromosome 25 to determine if the chromosome maintains synteny with *Gallus gallus*'s chromosome 25. Fourteen scaffolds corresponding to chromosome 25 were analyzed using NCBI's BLAST, DNA Stats, Repeat Masker, GENE-Panther, GENSCAN, AUGUSTUS, FGENESH, and UCSC Genome Browser. The species that were used as reference for these analyses were *G. gallus*, *Melopsittacus undulatus*, *Nestor notabilis*, *Falco peregrinus*, *F. cherug*, *Meleagris gallopavo,* and *Nipponia nippon*. The total length of chromosome 25 in *G. gallus* is 2.91Mb, while the chromosome in *A. vittata* is 2.68Mb, according to the sum of the 14 scaffolds identified. After analysis, 137 genes were identified as part of the chromosome. The predominant molecular function of these genes is binding, followed by catalytic activity. Two clusters of beta-keratin genes of claws, feathers and scales were found on two different scaffolds. Some of the genes detected could not be confirmed if they belong to chromosome 25, since they were unplaced in reference species. In conclusion, *A. vittata*'s chromosome 25 contains keratin gene clusters and does not maintain synteny with *G. gallus* as predicted.

Estudio Aerobiológico de Hongos Cultivables en el Edificio de Biología en UPR-Mayagüez

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Resumen

La aerobiología es el estudio de los microorganismos y partículas biológicas suspendidas en el aire y que son transportadas por este medio, como por ejemplo las esporas y el polen. En aerobiología se aplican conceptos de regulación de factores ambientales y monitoreo con el propósito de mantener un control en los niveles aceptables de partículas biológicas con relación a la calidad del aire. El objetivo principal de la investigación consistió en colectar muestras de hongos cultivables suspendidas en el aire dentro del Edificio de Biología en UPR-Mayagüez para determinar si el mismo cuenta con una buena calidad de aire. Entre los meses de enero a marzo de 2016 se llevaron a cabo dos muestreos para recolectar muestras ambientales utilizando el Andersen Cascade Impactator con platos Petri conteniendo Corn Meal Agar. Las colonias recuperadas se contaron y se aislaron para identificarlas a nivel de género. Hasta el momento se han identificado aislados pertenecientes a los géneros *Cladosporium*, *Curvularia*, *Fusarium* y *Stachybotrys*, un hongo altamente toxigénico. Se espera poder completar la identificación de todos los aislados para establecer una comparación con los datos obtenidos en semestres anteriores.

Lignolytic Enzyme Activity by *Purpureocillium lilacinum*

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Abstract

Purpureocillium lilacinum (formerly known as *Paecilomyces lilacinus*), is an entomopathogenic fungi that may be used as a biocontrol agent. Although this fungi is known to be collected from soil, it also may cause infections in immunodeficient patients. Based on previous studies in our laboratory, *Purpureocillium lilacinum* is capable of using naphthalene as its only carbon source. The purpose of this experiment was to determine if naphthalene can induce lignolytic enzymes activity in *P. lilacinum*. The fungi was inoculated in a liquid basal medium with 10% naphthalene and 10% glucose concentration for 20 days at 28°C, with 110 rpm of agitation. To measure the presence or absence of naphthalene, we measured the change in absorbance in the samples at 320 nm. As a result in glucose, there is no enzymatic activity whereas on naphthalene there is. In conclusion, *P. lilanicium* is a fungi that may be used as a possible agent in the area of bioremediation.

Aspergillus caelatus as a Potential Degrader of Congo Red Dye

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Abstract

To the best of our knowledge, the ecological role or behavior of *Aspergillus caelatus*, a species in *Aspergillus* section *Flavi*, hasn't been studied. The majority of the studies of *A. caelatus* are descriptive about its reproduction and morphology. Over the past few years, a great deal of efforts has been put to find fungi with the ability to degrade xenobiotics. The purpose of this study was to observe the capacity of *A. caelatus* to use a xenobiotic like Congo Red and to determine if its capable of inducing ligninolytic enzymes. The fungi were put to growth in a basal medium containing 0.01 mg/ml of Congo red for a period of 20 days at 28° C with an agitation of 110 rpm. The ability of fungi to degrade Congo Red was measured directly by determination the residual Congo Red by spectrophotometry at 490 nm. By day 20, *A. caelatus* had a biomass of 44.98 mg and the concentration of Congo Red in the medium was of 0.0056 mg/ml with a maximum decolorization percentage of 97.52%. The data obtained in the present study advanced our knowledge of the capability of *A. caelatus* to degrade in it could be used in the future as a possible bioremediation.

Characterization of Sex Chromosomes Z and W of the Puerto Rican Parrot (*Amazona vittata*)

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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 chromosomes Z and W 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, 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. Chromosome W did not show any signal. We started the annotation by identifying 937 scaffolds of the parrot genome matching to the chicken (*Gallus gallus*) chromosome Z and 31 for chromosome W. 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 (*Fidecula albicolis*), saker falcon (*Falco cherrug*), and peregrine falcon (*Falco peregrinus*). We analyzed the larger 66 scaffolds of the Z chromosome, found in them 211 genes and 1.3% of them are involved in reproduction. The total length of these scaffolds adds to 23.3 Mb, which represents approximately 28.4% to 29.1% of the Z chromosome. Only two scaffolds from the 31 corresponding to chromosome W were validated and we found in them two genes.

Assessing a Loss-of-Function Mutation in the WDPCP Gene in Puerto Rico

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Abstract

The 1000 Genomes Project has identified loss-of function-mutations (lof) in different populations. This type of mutation causes a protein to lose its function. A number of lof mutations were found in the Puerto Rican population and this research focuses on the WDPCP gene from chromosome 2. This gene is involved in ciliogenesis and regulation of cell migration and cell polarity. The phenotypes associated with mutations in this gene include Bardet-Biedl Syndrome, polysyndactyly, and heart defects. Current efforts are being made to identify the presence and distribution of the WDPCP mutations in Puerto Rico. If the mutation is present in the sampled population and it is affected by factors such as inbreeding, it will appear predominantly in samples obtained from towns in the center of the island. The mutation was validated in the lab using genotyping techniques. Samples from random towns of Puerto Rico were assessed to determine if the mutation is present. The presence of this mutation in samples from across the island has not been confirmed. Future applications include the study of patterns of geographic distribution of the mutation and possible phenotypic effects of the heterozygous condition.

Validating the Presence and Geographic Distribution of rs145497708 in Puerto Rico

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Abstract

The polymorphic DRD5 gene encodes for the production of the D5 dopamine receptor in neurons and liver tissue. Mutations in this gene have been associated with susceptibility to ADHD, a childhood-onset neurological disorder characterized by hyperactivity, inattention, and impulsivity; and blepharospasm, an adult-onset focal dystonia that causes continued and involuntary closing of the eyelids. rs145497708, identified in Phase III of the 1000 Genomes Project, is a rare, loss-of-function mutation in this gene that produces a premature stop-codon which removes the end of the intramembranous region of the D5 receptor. It is possible that this mutation may also be associated to neurological disorders. Because no human may be used as a true control, data provided by the 1000 Genomes Project must be validated to ensure the absence of false positives or false negatives. For this reason, we are seeking to validate the presence and geographic distribution of this mutation in PR by acquiring genotypic information of 396 Puerto Rican individuals from municipalities throughout the island using custom-designed primers for PCR amplification and HinfI or DdeI digestion. As positive control, the Puerto Rican individual identified as HG11082 in the 1000 Genomes Project, heterozygous for the mutation, is also being tested. If rs145497708 is a true mutation, then it is expected to be found in other members of the Puerto Rican population. Tests for the presence or absence of this mutation for other individuals throughout the island are still being awaited.

Chracterization of the Chromosome 11 in *Amazona vittata*

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Abstract

The Puerto Rican parrot (*Amazona vittata*) is endemic species of Puerto Rico, and since 1967 it was listed as an endangered species. For this reason different agencies are working with recovery programs and the establishment of different populations. This project is also focused on the conservation of the species but in a genomic context, and we are focusing on the characterization of *A. vittata* chromosome 11. We started the description of the protein coding genes, rearrangements and conserved regions for this chromosome by identifying 412 scaffolds of the parrot genome matching to the chicken (Gallus gallus) chromosome 11. Some annotation resources and tools used for this work were UCSC Genome Browser, Blast from NCBI, DNA Stats, Repeat Masker, Gene Panther, and three different gene predictors (Augustus, Fgenesh, and Genscan). To be more accurate we also compared the Puerto Rican parrot scaffolds with the budgerigar (*Melopsittacus undulatus*), zebra finch (*Taeniopygia guttata*), turkey (*Meleagris gallopavo*), collared flycatcher (Fidecula *albicolis*), saker falcon (*Falco cherrug*), and peregrine falcon (*Falco peregrinus*). We analyzed the larger 211 scaffolds and their lengths add to 18.1 Mb, which represents approximately 85% of the total length of the chromosome in the chicken. A total of 120 genes have been identified and 68% of them are involved in metabolism, cellular process and biological regulation. We proposed a high grade of conservation for the chromosome because most of the scaffolds are in synteny with all analyzed species and only small gene inversions were identified.

Análysis Genotípico del Gen RAD548 Para la Mutación de Perdida de Función RS145900595 en la Población Puertorriqueña

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Resumen

Durante los últimos años la comunidad científica se ha enfocado en el estudio de mutaciones de pérdida de función debido a su gran importancia clínica. Por esta razón y utilizando los datos del Proyecto 1000 Genomas, se realizó un cribado para identificar mutaciones de pérdida de función que expresaran fenotipo con trasfondo clínico y tuvieran representación en la población puertorriqueña. A partir de los datos analizados en las bases de datos NCBI, Genome Browser, OMIM y Ensembl se seleccionó el polimorfismo de un solo nucleótido (SNP) rs145900595 como la mutación a ser estudiada. Este SNP está localizado en la posición 95,479,761 del cromosoma 8 y se encuentra solamente una vez en el mundo, específicamente en Puerto Rico. La mutación afecta parcialmente las isoformas del gen RAD54B al sustituir una citosina por una timina en un codón para el aminoácido arginina, lo cual provoca la generación de un codón de terminación prematuro. Debido a esto la proteína pierde todos sus dominios ya que se acorta a dos amino ácidos de longitud. RAD54B codifica para una proteína involucrada en la reparación y recombinación del ADN y está asociado a enfermedades clínicas como cáncer de colon, linfoma de Hodgkin, adenocarcinoma en los pulmones y Síndrome de Werner. Utilizando la técnica de reacción de polimerasa en cadena en tiempo real se espera genotipar 396 individuos puertorriqueños para ver la frecuencia de este SNP en Puerto Rico. Hasta el momento se han analizado 66 individuos y ninguno de estos posee la mutación.

Movement and Macrohabitat Use of the Invasive Snake (Boidae: *Boa constrictor*) in Puerto Rico

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Abstract

Alien species constitute one of the top threats to biodiversity around the world. Humans have contributed to the displacement and introduction of these species to areas where they have not lived before. Snakes are among the most common invasive species, especially *Boa constrictor*, which has recently been established in continental areas (South Florida) and in islands (Aruba, Cozumel, and Puerto Rico). Native to the Neotropics, *B. constrictor* is a diet and habitat generalist with one of the biggest distributions. In Puerto Rico, *B. constrictor* lacks predators, has high amount of food sources and are parthenogenetic, causing population increase. Information regarding macrohabitat and movement patterns for each snake was gathered to better understand its biology and implement rigorous management laws for this invasive species. Data collection was done by surgically implanting transmitters in ten snakes to evaluate microhabitat use as well as measuring total movement and distance per move of the snakes from July through September 2014 and October through December 2015. Partial data collected included assessing encounter of snakes in open versus closed canopy (59% of the time), as they used a habitat with a 50% or more open canopy. Mean total distance was 141.19 m and mean distance per move was 135.41 m (six out of 10 individuals). Even though the relocations were more frequent on forests than forest edges, snakes found at forest edges preferred being closer to roads, presumably because roads offer good food sources and thermoregulation habitat.

The Response of Testate Amoebae to Climate Change: Can it be Predicted Across Latitudes Through a Single Model

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Abstract

Besides the current increase in temperature of 2°C, climate change will include a new precipitation schedule across some biogeographical latitudes. Some ecosystems like tropical forests could dry out while others may be saturated through rainfall. The response of plants and animals to these changes in precipitation is just starting to be understood. One group that could provide rapid information about this response to precipitation changes is the protists. In this study, we hypothesized that given that testate amoebae (TA) are bio-indicator species, they should respond in the same manner to three precipitation treatments across latitudes (Costa Rica, Colombia, and French Guiana). With all other environmental variables controlled, three precipitation treatments (dry, moderate, and wet) were applied to tank bromeliads for three months. Each treatment considered two hydrological variables (amount of water and number of days with water). Testate amoebae were fixed with Lugol's fixative, identified, and enumerated. We found that changes in the TA community structure differed among latitudes (countries), and that the hydrological parameter induced different responses. For instance, in Colombia the abundance responded to the number of days with water (k) whereas in Costa Rica, the abundance responded to amount of water (mu). Furthermore, we found that the saturated treatments were not conducive to more diversity. The lack of a single response of TA suggests that perhaps elucidating the effects of climate change on biodiversity might be more complex than expected.

Mycorrhizal Effects and Infection Percent in *Capsicum chinense* Grown in Different Soil Types

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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. Chemical fertilizers are commonly used to help grow these plants, overlooking biological fertilizers. Symbiotic relationships between mycorrhizal fungi and the roots of vascular plants can serve as biological crop enhancers, but their effects have not been compared to those of chemical fertilizers. The purpose of this research was to determine the effects caused by mycorrhizae on *C. chinense*, and to determine the mycorrhizae percentage associated with this crop after being grown in different soil treatments. The "ají dulce" plants were grown under six treatments using commercial Promix® mixes. In order to calculate the percentage of infection, the roots were stained with Trypan blue 0.4% and viewed under the microscope. Significant differences in plant characteristics were obtained when the plants in the different treatments were compared using ANOVA, obtaining a p-value.

Detection and Identification of Human Lungs Proteins that Binds *Bacillus anthracis* Lethal Factor Using t7

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Abstract

Anthrax is a serious infectious disease caused by gram-positive, rod-shaped bacteria known as *Bacillus anthracis*. After a bio-terrorist attack on 2001, *B. anthracis* has become a serious target to biomedical research. *B. anthracis* toxin action is due to a tripartite protein components: protective antigen (PA), lethal factor (LF) and edema factor (EF). While the virulence of LF causes death in the cell by the inhibition of the MAPK pathway, the interaction with other protein families has been proposed. For its virulence factor, understanding of anthrax lethal toxin and its interaction with new ligands is an important question that needs to be answer. This project is focused on identification of LF specific interaction proteins using T7 Phage Display. Human Lung cDNA libraries expressed on the surface of a T7 Phage were exposed to wild type and mutant LF as targets. After three biopannings, 1 x 103 plaques with putative displayed interaction peptides were found suggesting the presence of putative interaction peptides. A total of thirty plaques were isolated, fifteen from each type of LF. The in silico analysis of the putative interacting peptides with LFWT suggest the presence of Golgi associated protein, involved in sulfate transfer to proteoglycans. On the other hand, putative interacting peptides with LFMT include domains involved in nuclear transduction signals, transcription factors and regulators. This combinatorial technique have demonstrated to be useful in detecting potential interacting partners that could help understand the molecular pathogenesis of LF as well as the development of biosensors.

Assessing Papaya Virus Presence in Puerto Rico

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Abstract

Papaya is one of the fruits with increasing popularity among scientists as well as the general public because it acts as a good source for vitamins and carbohydrates. Papaya's properties make it an ideal crop to cultivate on countries where nutrient deficiency is an issue. However, diseases seriously hinder this crop's development. Recent efforts in the research of papaya have been on the study of viruses such as Papaya Ringspot Virus (PRSV). This virus has been reported among papaya in Puerto Rico; therefore, our research aims to assess if other worldwide reported papaya viruses are present in Puerto Rico, along with PRSV. The following seven viruses were reported by the scientific community as being present in papaya: Papaya Leaf Curl Virus (PLCV), Papaya Mosaic Virus (PapMV), Alfalfa Mosaic Virus (AMV), Papaya Leaf Distortion Mosaic Virus (PLDMV), and Zucchini Yellow Mosaic Virus (ZYMV). The primer sequence given in the literature for the coat protein gene of each virus was used along with ten cDNA samples of PRSV positive papaya, to prepare a PCR for the different viruses. The preliminary results of each virus amplification showed little to none band discrimination or presence in a 1% agarose gel. This can positively suggest the absence of these seven papaya viruses in Puerto Rico. The future aim of this project is to order a synthetic coat protein gene of each virus, in order to have definitive proof of virus absence in our papaya samples.

Variante de Perdida de Función en el Gen KRT83 y sus Efectos en la Población Puertorriqueña

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Resumen

Estudios de secuenciación genómica indican que los seres humanos presentan una cantidad de variantes genéticas que causan pérdida de función en los genes codificantes de proteínas mucho más alta que lo esperado. Hicimos un análisis de mutaciones de pérdida de función vaticinadas por el Proyecto 1000 Genomas para Puerto Rico a base de la secuencia genómica de 104 puertorriqueños, buscando aquellos que tenían una mayor probabilidad de causar fenotipos negativos sobre el individuo. De esta manera identificamos una mutación en el gen KRT83 que sustituye una base por otra creando así un codón de terminación prematuro. El gen KRT83 codifica para una queratina tipo II, la cual se heterodimeriza con una queratina del tipo I para formar filamentos de cabello. Se han descrito mutaciones no sinónimas en estos genes que causan alopecia y queratosis pilaris de forma autosómica dominante. Nos interesa saber si esta mutación hallada en Puerto Rico se expresa de forma dominante o recesiva. Esto es importante porque se halló en forma heterocigota en 3 de los 104 puertorriqueños secuenciados, dándole una frecuencia de 1.5%. Este experimento se llevó a cabo por medio de la cuantificación, dilución, genotipación y amplificación de PCR con muestras de diferentes pueblos de Puerto Rico. Para hacer una mejor descripción de su distribución geográfica en el país.

Utility of the UPRM Invertebrate Collection (INVCOL) Database

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Abstract

The UPRM Invertebrate Collection (INVCOL) is one of the few well curated collections of invertebrates in the Caribbean. It comprises many type and expertly identified specimens making it a important resource. Here I describe how we digitize the collection making it publicly available to the scientific community.

Accessing Antibiotic Resistance in Pristine vs Anthropogenic Impacted Environments Using Metagenomics

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Abstract

The outbreak of infectious diseases with Antibiotic Resistance (AR) has threat the first line of the medical defense in the world. During years, classic microbiology tried to resolve this problem by producing new antibiotics but its continuous overuse only increased the frequency of AR pathogenic infections. In other hand, functional metagenomic analysis have reveal that world's ecosystems have a diverse collection of AR genes. The purpose of our research was to generate metagenomic libraries (ML) from two different types of conditions: from a pristine environment and a human anthropogenic impacted ecosystem (HAIE). Then, explore for AR genes against ampicillin, kanamycin and tetracycline. Two ML from compost, a vegetable waste and a human biosolid, were generated (representing HAIE) and two ML from soils of Isla de Mona, PR were also generated (representing 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, kanamycin and tetracycline were determined. The AR clones from the libraries were isolated by selection on culture media supplemented with 1X-10X MIC of each antibiotic separately. 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 HAIE are potential reservoirs of β-lactams AR in contrast to the pristine environment. Further analysis will confirm AR against the other two antibiotics.

Effect of CaS Nanostructures on the Survival and Growth Rate of *E. coli*, *P. aeroginosa*, and *B. cereous* In Vitro

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Abstract

CaS nanostructures patented by this group have recently been used to inhibit the proliferation of human adenocarcinoma cancer cells with no detectable effect on the proliferation of normal human fibroblasts in vitro. The effect was attributed to the difference in the acidic and basic pH used to promote cell culture growth among the two types of cells. In the acidic media employed in the cancer cell culture the CaS nanostructures break into free calcium ions and sulfides, both of which are known to cause apoptosis. No such effect is expected in the normal fibroblasts that are fed with a neutral of slightly basic media. We have extended the work to explore the affect of CaS nanostructures in the survival and proliferation rate of *E. coli*, *P. aeruginosa*, and *B. cereous* in vitro. These bacteria are fed with media that has a neutral or slightly basic pH. We used Kirby Bauer method in a Mueller Hinton medium to grow these bacteria. Nanostructures with an average size of 1 and 10 nm were employed in the study. We found that the nanostructures had no effect on the growth and survival rate of these bacteria as compared with controls, consistent with our earlier hypothesis. Ongoing work focuses on pursuing the effect of CaS nanostructures on the survival rate of bacteria that are fed with acidic media as part of the cell culture procedure, some species of study may be *T. prosperus* and *T. acidophilus*.

Diversity and Molecular Phylogeny of the Opiliones (Harvestman) of Puerto Rico

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Abstract

Taxonomic and phylogenetic relationships among the harvestmen (Arachnida: Opiliones) species of Puerto Rico are poorly known, and systematic work has been limited largely to species descriptions. There are 12 species formally reported for Puerto Rico in five families (Agoristenidae, Baintanidae, Cosmetidae, Gonylepidae, and Phalangodidae). Representatives from the Invertebrate collection of the UPRM-Biology and samples from different forests were analyzed and specimens photographed for specific taxonomic features to classify them taxonomically. We are obtaining genomic DNA to compare among representatives of endemic genera. Actually, we identified useful morphological differences not reported before for many of species. In the genus *Yunquenus*, we studied specimens that do not meet the description of the sole, type species. *Yunquenus*, *Metacynortoides*, and other cosmetids in the Island seem to conform species complexes. Phylogenetic analysis is being carrying out to prove our hypothesis.

Loss of Function Mutations: ABCC2 Gene

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Abstract

Loss of function (LOF) mutations are a common genomic variant event that causes phenotypes related to genetic diseases. Sequencing, identification, and characterization of these LOF variants may provide an insight for future novel applications related to treatment approaches and disease characterization. The project focuses on determining heterozygous individuals in Puerto rican population samples for a novel LOF mutation that introduces a Single Nucleotide Polymorphism (G>A) in a splice consensus sequence. This mutation was identified through the 1000 Genome Project database and was selected for having particular in vivo functions, was identified in a Puerto rican individual, and it possessed the potential to express a non-functional phenotype. The affected gene codifies for an ATP binding cassette transporter: ABCC2 involved with biliar and drug export located in the apical membrane of hepatocytes. Mutations in the ABCC2 gene have been found to develop Dubbin-Johnson Syndrome phenotype which is related to high bilirubin concentration in blood. Current and future analytical methods consist in DNA sample quantification and genotyping Taqman assays using ViiA 7 Real Time PCR to identify presence of the ABCC2 polymorphism in the population samples.

Using Multiplex-PCR to Detect Virulence Factor Genes From *Vibrio parahaemolyticus* Isolates from the Oyster *Crassostrea rhizophorae* and the clam *Phacoides pectinatus*

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Abstract

Infections with pathogenic strains of *V. parahaemolyticus* is the leading cause of food-borne acute gastroenteritis worldwide. In Puerto Rico, there are no regulatory agencies that control the handling and quality conditions for selling raw shellfish such as oysters and clams. Detection of pathogenic strains of this gram negative bacterium can allow us to potentially predict and compare outbreaks in PR with other countries and to raise awareness to local population about food-born infections. Our goal is to determine the presence of pathogenicity associated genes in *V. parahaemolyticus* isolates from the oyster *Crassostrea rhizophorae* and the clam *Phacoides pectinatus* from the southwest coast of PR. DNA extractions were performed from isolated V. parahaemolyticus. The extracted DNA was processed following the standardized multiplex-PCR protocol recommended by the FDA. Multiplex PCR was performed for the amplification of tdh and trh genes associated with pathogenic activity, and tlh a specie specific marker. A total 3 samples for each oyster and clam were obtained. All resulted positive to *V. parahaemolyticus* based on the presence of a tlh gene amplicon and none of them resulted to be pathogenic due to the absence of a tdh and trh amplicons by modified electrophoresis in a 3% agarose gel. The isolates analyzed in this study lack pathogenicity markers and might not represent a risk to the population in terms of *V. parahaemolyticus*' associated infections. Future monitoring is necessary using this molecular techniques to detect the pathogenic levels of any *V. parahaemolyticus* isolated from the coasts of Puerto Rico.

Purple Non Sulfur Bacteria as Bioprospects in the Production of Antibacterial Substances

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Abstract

In 2010, the Center for Disease Control and Prevention (CDC) reported that at least 2 million people in the United States have been infected with antibiotic resistant bacteria, of which 23,000 died by these infections. For this reason, efforts have been focused in the search of new scientific strategies to address this problem. The Purple non-sulfur Bacteria (PNSB) are a ubiquitous, physiological versatile and diverse group that due to their bioremediation capabilities and biotechnological applications make them potential bioprospects for antimicrobial agent's production. We aim to study the capability of 52 PNSB isolated from water reservoirs in Puerto Rico to produce antimicrobial substances against several microbial targets. The PNSB were cultured in LB Miller and Sistrom agar under aerobic, anaerobic and phototrophic conditions. After this, 5µL of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were transferred to 6mL of top agar and poured over media with PNSB. After 24 hours, the PNSB showing inhibition zones were scored and analyzed. A total of 6 PNSB were capable of producing inhibition in LB against *B. subtilis* only in aerobic conditions. In contrast, 19 PNSB produced inhibition against *S. aureus*; 15 against *B. subtilis*; 2 against *E. coli*; and 1 against *K. pneumoniae* under aerobic conditions. In silico analysis using 16S rDNA showed that these bacteria belong to *Rhodopseudomonas* and *Rhodobacter* genres. These results suggest that the PNSB in Puerto Rico could be used as potential bioprospects in the fight against antimicrobial resistance.

Antimicrobial Potential of Fungal Endophytes from the Black Mangrove *Avicennia germinans*

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Abstract

The search for new antimicrobial 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. One hundred and four fungal endophytes were isolated from which 20 genera were screened for antimicrobial secondary metabolite production. From these, we selected the fungal strains that showed the highest antimicrobial potential: *Aspergillus flavus* and *Aspergillus clavatus*. Three bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and the yeast *Candida albicans*, were used to perform bioassays. Growth curves were prepared to determine the different growth phases of yeast and bacteria. Fungal extracts (10 ul) were added at the beginning of the lag phase, during the exponential growth, and at the stationary phase. Our results showed that *A. flavus* extract strongly inhibited the growth of *C. albicans* while *A. clavatus* inhibited the growth of *E. coli*. There was no apparent inhibition in the growth of *P. aeruginosa*. These results could lead to future discoveries of new antimicrobial or antifungal compounds.

Identificación de la Diversidad de Helechos del Bosque Estatal de Río Abajo, Puerto Rico

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Resumen

En Puerto Rico hay aproximadamente 29 familias, 100 géneros y 400 especies de helechos. Desde el comienzo de estudios botánicos en Puerto Rico, cada esfuerzo dedicado al conocimiento de los helechos ha resultado en un aumento significativo de las especies reconocidas, con el último tratamiento comprensivo siendo el de George Proctor en 1989. De los pocos helechos endémicos a Puerto Rico, más de la mitad fueron descritos recientemente, sugiriendo que podría haber más especies endémicas. Nuestro estudio intenta establecer una base de datos moleculares de secuencias de ADN que puedan servir para identificar y documentar las especies de helechos presentes en la isla. Colectamos más de 60 muestras de herbario de helechos en el Bosque Estatal de Río Abajo. De estos seleccionamos 48 para extraer y amplificar ADN utilizando PCR. Intentamos la amplificación de varios diferentes marcadores de ADN que han sido propuestos como regiones de Código de Barras de ADN para helechos utilizando cebadores publicados. De todos los marcadores que intentamos solo tuvimos éxito con la región cloroplástica trnL-F, de la cual obtuvimos aproximadamente 1,000 bases de 27 muestras. Hay un patrón taxonómico en las muestras que no amplificaron, sugiriendo que cebadores adicionales serán necesarios. Las 27 muestras exitosamente secuenciadas representan 22 especies, de las cuales solo hay datos en GenBank de cinco. Se puede concluir que se necesita más investigación de los helechos de Puerto Rico y esta investigación ha servido como un proyecto piloto para conocer y aportar información de las especies de helechos le la región.

Screening and Detection of Antimicrobial Agents Production Bioprospects, in Metagenomic Libraries from Aquatic

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Abstract

Antimicrobials have revolutionized human health care, but in recent years, their effectiveness has been diminishing. Being aware of the scarcity of novel antimicrobials discovered by culture-dependent methods is necessary to implement culture-independent methods such as Metagenomics. For this, an antimicrobial agent production functional analysis methods were performed to four high molecular weight aquatic bodies metagenomic libraries (AML) generated from samples of Guajataca water reservoir (GWR- G1 and G2), Rio Grande de Añasco (RGA), Playuela Beach (PB). The functional screening was done using two assays: (1) overlay inhibition test and (2) Kirby Bauer assay (KBA) using two supernatants: one after centrifuging the un-lysed AML culture (S1) and the other one after lysing the AML culture (S2). In the first test, 500 clones of the respective AML were spread on Petri plates with Luria Bertani (LB) and after 24 hrs of incubation, an overlay of *Bacillus subtilis* as target was done on top of the AML clones. After spreading *B. subtilis* on LB plates, 5mm individual disks impregnated with S1 and S2 were placed on top and incubated for 24 hrs. While no antibiosis halos were detected for S1, halos with variable inhibition zones were found in the S2 tested. For PB 35.7% of the disk demonstrated inhibition halos, for G1 14.3%, for G2 42.9 % and 36% for RGA. These results demonstrated the reliability of Metagenomics in the discovery of antimicrobial products in these environments, representing an opportunity and a new alternative in solving the antibiotic resistant issue.

DNA Barcoding of the Solanaceae Family of Plants in Puerto Rico

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Abstract

The Solanaceae family is one of the major groups of angiosperms, with more than two thousand species and approximately a hundred genera. This family contains species with worldwide agricultural and economical importance like potatoes (*Solanum tuberosum*), eggplants (*Solanum melongena*), tomatoes (*Solanum lycopersicum*) and peppers (*Capsicum* sp.). In Puerto Rico we have six endemic species of Solanaceae: *Brunfelsia desinfolia*, *Brunfelsia lacteal*, *Brunfelsia portoricensis*, *Goetzea elegans*, *Solanum ensifolium* and *Solanum woodburyi*. The habitat of these plants have been reduced by land loss, which is the main reason for these species to be threatened and endangered in the wild. The research project's objective is to study the genetic diversity of the Solanaceae in Puerto Rico using DNA barcoding. We will compare the genetic diversity between species by amplifying different chloroplast regions with the primers trnH-psbA, psbI-psbK and MatK KIM. With this technique we can study genetic variability among species to see if the geographic site contributes towards its variation. A phylogenetic tree will be made with our collected DNA sequences and the NCBI database sequences to study the evolutionary pattern of this family in Puerto Rico. The overall objective is to collect approximately 50 different species of Solanaceae including the six endangered species in different geographic sites of Puerto Rico. In this presentation we will present the DNA barcoding of 15 of those species. Understanding the phylogenetic distribution and the genetic diversity of the Solanaceae in Puerto Rico can be useful to design a conservation plan for those threatened and endangered species.

Screening *Camelina* Mutated Lines by TILLING for Biofuel Oil Quality Improvement

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Abstract

The emergence of biofuel production has been one triggered by the growing concerns of climate change, carbon dioxide emissions, and the limited supply of fossil fuels. Biofuels, including biodiesel generated from oilseed crops, provide an alternative and renewable energy source to supplement fossil fuel use. *Camelina sativa* is an emerging oilseed crop due to its low input requirements (e.g., water and fertilizer) and rapid generation time. However, a major limitation of camelina oil for biofuels is its low oxidative stability arising from a high content of the polyunsaturated fatty acids (PUFAs) linoleic acid (18:2) and alpha-linolenic acid (18:3). Our goal is to generate camelina oil enriched in the more oxidatively stable oleic acid (18:1) and low in PUFAs, making it ideal for biofuel production. Wild type camelina seeds were treated with ethyl methanesulfonate to produce point mutations in genes responsible for its content of PUFAs 18:2 and 18:3. This process is called TILLING (Targeting Local Lesions IN Genomes), a reverse genetics, non-transgenic strategy. After being taken to the homozygous stage, the 825 lines were screened by gas chromatography to assay the fatty acid composition of the seed oil. Three lines presented the most significant distinctions in their fatty acid composition compared to wild type camelina with decreased PUFAs and increased oleic acid, and these lines were further analyzed. The genes coding for the desaturases responsible for the synthesis of the PUFAs linoleic and alpha-linolenic acid (FAD2 and FAD3 respectively) were PCR amplified and sequenced to detect the point mutations. In addition, 425 of these lines were screened by high performance liquid chromatography in search of increased vitamin E content. Two of these lines were notable because of the high amount of vitamin E or presence of α-tocopherol. The presence of α-tocopherol is significant because of its high antioxidative ability. These germplasm will ultimately be crossed into elite camelina lines for use as a biodiesel feedstock.

Micro-Habitat Selection of *Sphaerodactylus nicholsi* Egg-Bearing Females at the Cabo Rojo Salt Flats

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Abstract

Species in the genus *Sphaerodactylus* (Gekkota: Sphaerodactylidae) include some of the smallest amniotes in the world. Their small size poses some problems with heat conservation and there has been no documentation of any physiological adaptation to deal with heat loss. The reproductive cycle of some sphaerodactylids in the Caribbean has been described as year-round with seasonal spikes of egg-bearing females in the summer months and hatchlings in the winter months. Lack of a known heat conservation mechanism coupled with their small size poses questions about how egg-bearing sphaerodactylids deal with environmental parameters. We studied *S. nicholsi* micro-habitat selection in the Cabo Rojo Salt Flats and examined the presence of egg-bearing females with environmental temperature, relative humidity, substrate, over story cover, SVL and TL relative to the overall *S. nicholsi* population. Although our preliminary results from January 2016-April 2016 are yet to be compared with the warm season (June-September), we expect differences in environmental temperature and substrate between egg-bearing females and non-egg bearing individuals. This would suggest differences in microhabitat selection by egg-bearing females. Relationships with over story cover, SVL and TL are yet to be determined.

Do *Rhynchonycteris naso* Have a Voice Signature in their Echolocation Calls?

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Abstract

Bats, as any species, communicate through multiple mediums with both, conspecifics and heterospecifics. They use echolocation and social calls to transmit information, to maintain their social behavior or just to obtain more details about their surroundings. There are numerous studies suggesting that bats have individual signatures in their social calls but only few studies investigated if echolocation calls possess individual signatures. *Rhynchonycteris naso* live in social groups that are stable over many years and would profit from recognizing their group mates based on their echolocation calls. To test for individual signatures in the echolocation calls of *R. naso*, we captured individuals in three different colonies and released them inside a flight cage to record their echolocation calls. We obtained call parameters using two different methods (Avisoft SASLab Pro and MATLAB voicebox), the latter of which is a new tool that extracts acoustic parameters based on voice cues. Our data suggest the presence of a strong individual signature in echolocation calls of *R. naso*. Avissoft SASLab Pro (64.5% classification success) performed better than the MATLAB voicebox (50.9% classification success). Moreover, by combining the acoustic parameters obtained with the above mentioned techniques, we were able to increase classification success to 70.1%. Therefore, our results provide first evidence that *R. naso* may be able to discriminate individuals based on their echolocation calls and that voice cues might be useful in the study of bat social communication.

Identification of Putative Mercury Reductase Genes in Purple Non-sulfur Bacteria (PNSB) from Aquatic Environments

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Abstract

A mayor concern has arisen after the Environmental Protection Agency revealed in 2014 that 79.7% of rivers and streams used as drinking supply in Puerto Rico were impaired with metals such as mercury. This metal is bio-accumulative and its consumption leads to kidney and liver damage. Several cases of human intoxication have been reported after mercury intake. For example, the Agency for Toxic Substances and Disease Registry recorded the poisoning of 290 civilians in Puerto Rico after eating fish grown in mercury contaminated waters. Evaporation, filtration and other techniques have been employed to extract these metals from aquatic environments. Unfortunately, byproducts like toxic waste, low effectivity and high costs limit their use. Bioprospectors such as PNSB has been studied for its possible use in mercury removal from aquatic environments. The benthic hypersaline microbial mats, water reservoirs, bromelia's and heliconia's phytotelmata PNSB collection of the Laboratory of Microbial Biotechnology and Bioprospecting was studied for the presence of genes related to mercury resistance. During this study, 16% of the water reservoir's isolates presented a positive amplification for mercuric reductase genes (1,238 bp). Other amplifications from 31% of bromeliads, 16% of Microbial Mats and 33% of heliconia's isolates will be proved by in silico analysis to validate their genetic identity. Same analysis in 16SrDNA suggested that PNSB isolates belong to Rhodopseudomonas, Rhodospirillaceae and Rhodobacter family. The results suggest an unknown biotechnological potential of these isolates from ubiquotous environments by the presence of mercury reductase genes and further bioremediation applications in metal resistance.

Distribución Geográfica en Puerto Rico de una Mutación de Pérdida de Función del Gen NOD2

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Resumen

NOD2 es un miembro de la familia de receptores para dominios de oligomerización para la unión a nucleótidos (NLRs) localizados en el citosol. Sus ligandos son moléculas de la pared celular de bacterias, y su actividad es parte esencial de las respuestas inmunes innatas y adaptativas, incluyendo la apoptosis. La oligomerización de NOD2 redunda en el reclutamiento y la activación de caspasas, y tiene además una relación directa con el factor de transcripción NF-κB cuya función es coordinar respuestas inflamatorias. Diversos polimorfismos en NOD2 están asociados a la enfermedad de Crohn. El propósito de la investigación es determinar la frecuencia y distribución geográfica en Puerto Rico de la mutación de NOD2 rs2066847. La mutación consiste en la inserción de una citosina, lo que implica un desplazamiento del marco de lectura y la pérdida de función del gen. La mutación se ha encontrado en 30 individuos en el mundo, 6 de ellos entre 104 individuos en Puerto Rico. Por lo tanto, esperamos encontrar unos 24 individuos heterocigotos para la mutación entre las 396 muestras que se probarán en 28 municipios de Puerto Rico. La prueba se hará utilizando un iniciador con una disparidad a su secuencia complementaria que producirá un sitio de restricción ApaI solamente en presencia de la mutación. El estudio servirá para identificar la región en Puerto Rico con la mayor incidencia de la mutación para entonces iniciar un estudio sobre sus posibles efectos fenotípicos.

Identification of *Rhodobacter sphaeroides* Genes Involved in Phage Infection

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Abstract

Bacteriophages can be utilized as an efficient way to introduce genetic material into host cells. This process, known as transduction, is used as a bio-prospecting tool in order to perform genetic and biochemical analysis. Transduction is also very useful in Biotechnology, where it is commonly used in order to mass produce desired molecules. Our laboratory has isolated and characterized several *Rhodobacter sphaeroides* specific bacteriophages able of performing lysis, but to date, no transduction capability has been detected. The goal of this research is to identify genetic targets in *R. sphaeroides* that are involved in the susceptibility of infection by the phages and the integration of the phage into the *R. sphaeroides* genome. Several *R. sphaeroides* 2.4.1 and 630 Tn mutants were generated and its susceptibility for infection by seven different bacteriophages were determined by an overlay plaque assay. A total of 23 *R. sphaeroides* 2.4.1 and 290 R. *sphaeroides* 630 Tn mutants have been generated. There is a notable difference between the mutation success rate of the mutants of *R. sphaeroides* 2.4.1 and those of *R. sphaeroides* 630. This seems to be caused by a slight difference in replication time that causes the different species reach log phase at different times. Viral plaque assays will be performed on the isolated mutants in order to screen for a change in infection rate by the previously isolated phages. Once we obtain a positive result in the viral plaque assay, we will sequence the mutant and identify the genes that are responsible for the integration and susceptibility of infection.

Identificación de Clones Termo-tolerantes Presentes en Bibliotecas Metagenómicas de Diversos Ambientes

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Resumen

Nos encontramos en una época interglaciar donde el planeta está calentándose poco a poco. Desde el comienzo de la era industrial, las concentraciones de gases de efecto invernadero, en especial CO2 han aumentado gravemente y con éstas, las temperaturas ambientales promedio. Nos interesa conocer si los microorganismos que viven en ambientes donde no existen temperaturas extremas, están preparados para tolerar cambios rápidos de aumento en temperatura y de esta manera poder sobrevivir. Usando metagenómica funcional, se seleccionaron clones de bibliotecas metagenómicas de Cueva Ventana (CV, Arecibo PR), tapetes microbianos (TP, Salinas de Cabo Rojo PR) y Centralia (CE, Pennsylvania USA) capaces de tolerar temperaturas mayores de 50oC. A los fósmidos de estos clones se le realizó mutagénesis por transposón para detectar la identidad de los genes responsables de este fenotipo por su inactivación. Se lograron aislar 20 clones de TM y 10 de CE capaces de crecer a 50oC. Los análisis de restricción mostraron que mientras los clones de TM tienen el mismo patrón genético, los de CE son más diversos con 5 patrones. Los fósmidos en los clones que fueron reinsertados en células isogénicas mostraron la adquisición del fenotipo. Finalmente, los estudios bioinformáticos de los fragmentos en los fósmidos no mostraron similitud con genes conocidos en bases de datos. Por el momento, los datos sugieren que la diversidad de clones en cada ambiente evaluado con respecto a la variabilidad de la temperatura presente, puede ser un factor que afecte la presencia de clones capaces de tolerar altas temperaturas.

Molecular and Physiological Characterization of Fungi Present in *Nepenthes* sp. Trap Fluids

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Abstract

Carnivorous plants (CP) evolved thousands of years ago in response to environments with low nutritional content and created a unique microenvironment described as acidic, and high Nitrogen concentration. *Nepenthes* sp. is a CP that developed a unique trap in order to fulfill its nutritional requirements. The *Nepenthes* sp. trap includes a pitcher like extension in which the digestive enzymes are secreted. This microenvironment (phythothelmata), may lead to the presence of a microbial agents capable of degrading complex molecules, with biotechnological potential. The focus of this research is the isolation and characterization of fungi present in the trap fluids of the CP *Nepenthes* sp. Last year the isolates were presented and further analyzed macro and microscopically determining identity based on morphology. This time, the molecular and physiological characterization of some of the isolates is presented. Out of the 9 different fungi isolates, 5 showed coenocytic hyphae with diverse types of macroconidia. Molecularly, after DNA extraction using bead beating, the ITS region of the mycelial fungal isolates was amplified, sequenced and analyzed. The in silico analysis revealed that most of the isolates belong to the *Fusarium* genus, specifically *F. oxysporum* and *F. beomiforme*. The remaining four isolates were identified as yeast for which physiological identification using degradation of carbohydrates was performed. These tests suggests that all the isolated yeast are different, and that these yeast are not *Candida krusei*, *C. albicans* nor *C. tropicalis*. Ongoing research is in progress to characterize the remaining isolates and perform functional analysis.

Assessing Diversity and Divergence Among *Solenodon paradoxus* of Dominican Republic

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Abstract

Solenodon paradoxus is a species of insectivorous mammals, which inhabits the Hispaniola. This species was thought to be almost extinct until 1907, and it is important because it diverged in the Cretaceous period nearly 76 million years ago and has not evolved ever since. Currently, it is considered an endangered species because of population decrease. We collected blood and tissue samples from 4 Hispaniolan solenodons coming from the northern side of the Dominican Republic. We design 13 primer pairs to amplify the whole mitochondrial genome. We successfully amplify 12 out of the 13 segments, and using Sanger sequencing we were able to obtain the mitogenome of the 4 individuals. Their mitogenome was compared with previously published data from other 5 individuals of the southern side of the Island. Aligning control regions sequences from the additional individuals suggested that solenodons from the northern (n=4) and southern (n=5) Dominican Republic grouped separately in a network. We also calculated FST, and results showed divergence among the species. This preliminary pattern is consistent with a previous morphological analysis that suggested dividing *S. paradoxus* into northern (*S. p. paradoxus*) and a southern (*S. p. woodi*) subspecies, which would also suggest separate conservation management of northern and southern Hispaniolan solenodons.

New Genus and New Species of Earthworm (Oligochaeta, Acanthodrilidae, Benhamiinae) from Puerto Rico

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Abstract

Puerto Rico is known for having a rich diversity of fauna and flora, and its earthworm fauna (Oligochaeta) is the best studied in the Greater Antilles. The subfamily Benhamiinae (Acanthodrilidae) is widely distributed through the tropical regions of America and Africa, with *Dichogaster* being the most distributed genus, with native species, and *Eutrigaster* distributed mainly on Neotropical areas. After a series of small surveys, the genus *Yuisia* n. gen. is proposed to accommodate *Eutrigaster*-like species, and *Yuisia jenkinsi* n. sp., *Y. olgae* n. sp., and *Y. aebiana* n. sp. are described from Puerto Rico. The specimens were found under the cork of a trunk in decomposition and its decomposed fibers at the Quebrada de Oro area, Mayagüez, and at an agricultural area at Minillas Valle, San Germán. The earthworms were collected by hand and preserved initially in ethanol 95% and transferred into formalin 10%. A phylogeny analysis was made using maximum parsimony, with morphological characters, to compare the new species to *Eutrigaster* and *Dichogaster*. The morphological phylogeny supports the separation of the new species into a new genus. The new species can be easily distinguished by dissection; *Yuisia* species differ from the *Eutrigaster* species by the location of the first dorsal pore, spermathecal structure and number of penial setae associated to the first prostates. With these new records, it became obvious that the terrestrial oligochaete taxonomy of Puerto Rico and its smaller islands is still unfinished.

Identificación Molecular y Filogenia de las Polillas (Lepidoptera) de Puerto Rico

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Resumen

En Puerto Rico hay aproximadamente 1,000 especies de Lepidoptera, las mariposas y polillas. Un poco mas de 100 de estas son mariposas y las demás son polillas. Hasta la fecha no hay un solo recurso autoritativo para identificar polillas en la región, pero si hay muchas imágenes disponibles en línea. Para este proyecto colectamos polillas, las fotografiamos, y les extraímos ADN. No se preservaron especimenes, sino que las imágenes sirven como testigos de las colecciones. Después de extraer ADN amplificamos la región Cytochrome c oxidase subunit I (COX1). De 58 muestras tuvimos éxito con 38 especimenes. Usamos estas secuencias en búsquedas BLAST en GenBank para identificar a especie las muestras. Los resultados de las búsquedas de BLAST y análisis filogenéticos revelan que estos 38 especimenes representan 30 diferentes especies de polillas, una especie de Neuroptera (Crisopas o Lacewings) y una especie de Hemiptera (True Bugs). Nuestro muestreo fue oportunista, ósea que no tratamos de evitar colectar la misma especie múltiples veces. El que solo re-colectamos tres especies múltiples veces (una dos veces, una tres veces y una cuatro veces) sugiere que hay una gran diversidad de polillas en Puerto Rico. Las búsquedas de BLAST identificaron a 10 especies con un nivel de identidad de 99% o mas. Las demás solo se pudieron identificar a género u orden, porque no están aún en GenBank. El uso de esta metodología hace posible documentar la diversidad de polillas en Puerto Rico aún no siendo expertos en su identificación.

A Worldwide Phylogenetic Analysis of *Phyllanthus* (Phyllanthaceae)

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Abstract

Phyllanthus (Phyllanthaceae) is one of the 20 largest genera of Angiosperms, with size estimates ranging from 700 to 1,200 species. One reason for this range is that some taxonomists recognize *Breynia* (~ 60 species), *Glochidion* (~ 300 species), and *Sauropus* (~ 40 species) as distinct genera, while others include them within *Phyllanthus*. Phylogenetic studies on the group to date have primarily been regional, with their results being influenced by the skewness of their sampling. We set out to perform as broad a phylogenetic analysis as possible of *Phyllanthus*. We downloaded from GenBank and combined DNA sequences of six different markers (nuclear: PHYC and ITS; chloroplast: matK, psbA-trnH, trnL-F, rbcL) for approximately 235 species, most of which have only been sequenced for 1–3 markers. We supplemented this with sequences of approximately 30 species for which we sequenced 4–6 markers. Our results strongly support the inclusion of *Breynia*, *Glochidion*, and *Sauropus* within *Phyllanthus*, which will require numerous taxonomic changes. We recover the small herb P. maderaspatensis sister to the rest of *Phyllanthus*, suggesting an Old World origin for the group. We also infer the ancestral habit of *Phyllanthus* as being herbaceous, with multiple independent origins of large, woody taxa such as the 20 m tall *P. skutchii* of Central America. Our analyses are an example of how to use and combine existing, published data to address questions beyond those under consideration by previous workers, and will serve as the basis for research proposals on this large and challenging genus.

Invertebrados Asociados a la Hojarasca de Bosque Nativo y Exótico en Susúa, Puerto Rico

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Resumen

Los invertebrados en el Bosque Estatal de Susúa, Sabana Grande, Puerto Rico, han sido poco estudiados. Este estudio pretende comparar la biodiversidad de invertebrados presentes en diferentes tipos de hojarasca, y observar los órdenes que predominan mediante comparación. Investigamos los invertebrados encontrados en la hojarasca de Caoba Dominicana (*Swietenia mahagoni*) y hojarasca de árboles no introducidos. Se colectaron 240 muestras mediante dos métodos: (1) la recolecta de hojarasca, la cual fue luego procesada mediante trampas Berlese y (2) trampas de caída. Los organismos fueron observados utilizando un estereoscopio. Se identificaron 28 órdenes de invertebrados: los órdenes Acari, y Collembola fueron los más abundantes, superando el 57% del total de individuos en hojarasca y 53% en trampas de caída, respectivamente. El orden Ostrácoda es inesperado por ser principalmente acuático y no haber un cuerpo de agua adyacente al área muestreada. Un total de 39465 individuos fueron observados en las muestras; según el análisis de varianza (ANOVA) no se encontró diferencia significativa entre los organismos totales encontrados en la hojarasca de caoba dominicana versus la hojarasca de árboles no introducido (p-valor: 0.3113). Sin embargo, al realizar el análisis excluyendo los ácaros se encuentra diferencia significativa con un p-valor de 0.0313.

Detection of Fungal Capabilities to Metabolize 2,4,6-Trinitrotoluene Using Cyclic Voltammetry

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Abstract

2,4,6-Trinitrotoluene (TNT) is a man-made explosive used in military shells, bombs, and grenades, in industrial uses, and in underwater blasting. Biodegradation is regarded as an option to clean up many environmental pollutants. However, there is a need for TNT biodegradation experiments using fungi where the metabolic activity can be easily detected. Cyclic voltammetry (CV) is an electrochemical technique that applies an alternating potential, oxidizes and reduces molecules, producing a detectable current signal. The main focus of this research is the use of CV as an effective technique to detect the TNT degradation capabilities of an Aspergillus sp. isolated in Puerto Rico. Spores suspensions were spreaded agar, and after seven days of incubation, disks were placed in tubes with aqueous media with 68 ppm TNT for 58 days. After applying CV for TNT, three reduction peaks were observed with decreasing potential and a prominent oxidation peak in the return sweep. In the first five days of incubation, the TNT concentration decreased almost 10ppm on average as per Peak 1 and Peak 4. After 58 days of incubation, the TNT concentration had decreased almost 20 ppm on average for the mentioned peaks. For Peak 2 and 3 we did not found a drastic decrease in concentration, only a reduction of 8ppm on average after 58 days of incubation. Further studies to determine the biodegradation product are underway.

Targeting Protein-Protein Interaction Between MLL-Fusion Proteins and DOT1L

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Abstract

Mixed lineage leukemia (MLL) is an aggressive, therapeutic resistant leukemia. MLL rearrangements are associated with a poor prognosis, where the MLL gene undergoes a chromosomal translocation and fuses with one of more than 60 fusion partners. MLL fusion proteins gain oncogenic potential to up-regulate the expression of MLL target genes through the recruitment of the histone methyltransferase DOT1L (disruptor telomeric silencing 1-like). This work focuses on studying the protein-protein interaction (PPI) between DOT1L and two common MLL-fusion proteins MLL-AF9/ENL as a potential therapeutic target. A high throughput screening using a single dose fluorescent polarization (FP) assay was performed as a primary screening method. Out of 120,000 compounds, several hit compounds were identified and further tested in counter screening methods like Surface Plasmon Resonance (SPR). We were able to identify several compounds as promising hits that showed dose-dependent inhibition in FP and SPR. These results strongly suggest that these compounds will be good candidates for further biophysical and biological characterization. Upon further validation, these compounds will be developed using medicinal chemistry methods and leading toward the development of novel and potent inhibitors of the MLL-AF9/DOT1L PPI.

Isolation of Bacterial Prospects With Protease Activity

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Abstract

Proteases are enzymes that degrade or hydrolyze proteins, they cleave the peptide bonds that make up the backbone of polypeptides. This gives the microorganism that produces the protease access to the individual amino acids. Due to their diverse functions, proteases are wildly used in the food, textile, and detergent industries for the production and processing of these materials. The focus of this research is the isolation of microorganisms capable of producing proteases from various coastal areas (CA) in Puerto Rico. Water and sand samples where collected from one CA in Cabo Rojo, three in Ponce and two in Mayaguez. The samples where serially diluted and spread on Skim Milk Agar. After being incubated at 32oC, the colonies that showed a characteristic degradation zone were purified and the activity reconfirmed. The isolated bioprospects were classified as strong or weak on a degradation standpoint. All the CA sampled have shown colonies positive to protease production, being the Ponce CA the area with the highest number of protease bioprospects (19). From the twenty-five total different colonies positive for protease activity, eighteen were classified as strong and seven weak. Work is in progress for the further isolation and identification of bioprospects microbiological and genetic identities. This way the comparison of different types of microorganism and enzymes that degrade protein throughout the CA of Puerto Rico is possible.

Evaluating the Effects of Degree of Crosslinking and Addition of RGD Peptide Over Cellular Adhesion

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Abstract

Bioactive coatings are known to increase biocompatibility and cellular adhesion of implantable biomaterials. Applying the layer-by-layer technique, biomaterial surfaces have been successfully coated, thus improving the biocompatibility, compared to uncoated biomaterials. The objective of this work is to evaluate the effect of crosslinking in polymeric multilayer combinations and the addition of a peptide to the multilayers. In this study, multilayers of chitosan and heparin with top layers of heparin-maleamide and heparin-RGD peptide were evaluated. After preparing six bilayers, the multilayers were crosslinked using EDC/NHS chemistry at varying concentration. RGD peptide functionalization was carried out using maleimide chemistry in combination with EDC/NHS chemistry. 1H-NMR was used to confirm effective conjugation of heparin with maleamide and heparin/maleimide with RGD. NIH-3T3 cells were used to assess biocompatibility and cell-adhesion. Fluorescence microscopy was used to assess cell adhesion. Our results indicate that non-crosslinked surfaces do not represent an attractive surface for cells. Multilayers crosslinked with 30 mg/mL of EDC showed improved cellular adhesion, having a preference in adhesion on HEP-terminated multilayers. Multilayers crosslinked with 70 mg/mL of EDC, showed increased cellular adhesion, independent of the combination of polymer/polysaccharide composing the multilayers. These results demonstrate that polymeric layers composed of HEP and CHI, with high degree of crosslinking provides a more apt surface for cellular adhesion. Studies utilizing NIH-3T3 cells show an increased in cellular adhesion in wells containing the heparin-RGD peptide combination. Our work has demonstrated that chitosan and heparin crosslinked multilayers functionalized with RGD peptide have higher cell adhesion, therefore increasing applications in the field of tissue engineering.

Microbial Community Associated With Cilantro Rhizosphere

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Abstract

The aim of this study is to determine the microbial community associated with cilantro (*Coriandrum sativum*) rhizosphere to create baseline data for future research including responses to activated sludge treatments as fertilizer. We want to understand the effect of bacteria in *C. sativum* after fertilization with sludge from the treatment plant wastewater in Mayagüez (AAA). The first stage is the characterization of the microbial community associated with the rhizosphere of the plant. Cilantro is grown in sterile soil in a hydroponic solution for 4 weeks; then the roots were cut and placed in phosphate buffer. After mixing, 500µl of the solution was plated with yeast extract malt agar media (YMEA). Bacteria were transferred to fresh media until pure colonies were obtained. Currently, 37 bacterial strains have been isolated from *C. sativum* rhizosphere. In addition, fungal colonies were recovered. Preliminary morphological identification indicates the genus *Trichoderma* is abundant in the rhizosphere of the plant. To identify bacterial strains traditional morphological and molecular techniques will be implemented. Current research will determine if the compost extract has positive effects on plant development and growth by measuring stem length, number of leaves and other morphological characteristics.

Haloarcula rubripromontorii, sp. nov., a Novel Halophilic Archaeon from the Cabo Rojo Solar Salterns

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Abstract

During a microbial diversity study, strain SL3 was isolated on growth medium containing glycerol as the sole carbon source and natural saltern pond water from the Solar Salterns at Cabo Rojo, Puerto Rico. This coral-red pigmented archaeon was subjected to biochemical, morphological, genotypic, and phylogenetic taxonomical studies. The cells are pleomorphic cocci form and stain Gram positive. Acid production was observed from galactose, glycerol and glucose. Growth concentrations for NaCl are from 15% to 30% (optimum 20%) and 0% to 10% (optimum 2%) w/v for Mg+2. Meanwhile growth was observed at pH ranging from 6.5 to 9 (optimum 7.5) and at temperatures ranging from 30°C to 50°C (optimum 38°C). Strain SL3 has multiple sequences encoding 16S rRNAs which can possess up to 5% divergence. The entire genome was sequenced and presented a GC content of 61.9%. Phylogenetic analysis revealed that one of the copies of the 16S gene is most closely to *Haloarcula salaria* HST01-2R, while the other copy is most closely related to *Haloarcula hispanica* (99% similarity each). As for the rpoB gene, phylogenetic analysis revealed that the most closely related organism was *H. hispanica* (95% similarity). However, the average nucleotide identity between strain SL3 and all other available *Haloarcula* genomes revealed that all species of *Haloarcula* were 88% to 89% similar, with the proposed cut-off for species boundary being at 95% to 96%. *H. vallismortis* was the most similar with 89% similarity.

Changes in Sediment Porewater Chemistry and Wild Rice Growth as a Result of Reduced Hyporheic Flow

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Abstract

Wild rice (*Zizania palustris* and *Z. aquatica*) is economically and culturally important to Native Americans and there is increasing interest in restoration efforts, but the factors that influence its distribution are not well known. Recent work suggests that sulfide, a byproduct of bacterial decomposition in anaerobic sediments containing sulfate, can be toxic to plants. We investigated whether the reduction of hyporheic (sub-surface) flow would negatively impact wild rice growth by (1) decreasing nutrient replenishment in the sediments and/or (2) allowing the buildup of natural stressors, such as ammonia and sulfide. To test these hypotheses, we manipulated hyporheic flow at several locations in a stand of wild rice in Michigan and sampled the changes in porewater chemistry and wild rice growth over 5.5 weeks. To evaluate rice growth, we measured root biomass and length before and after the manipulation, and measured plant heights every two weeks during the experiment. Our results suggest that while there was no difference in sediment porewater chemistry between control and manipulated plots, reducing hyporheic flow may negatively affect the growth of wild rice.

Gracias por su participación y apoyo

Thank you for your participation and support