

Primer Simposio Subgraduado de Biología 2011

biología organísmica• evolución • genética • ecosistemas
• celular y molecular • bioquímica • otros



sábado, 7 de mayo de 2011 Departamento de Biología Recinto Universitario de Mayagüez







Eukaryotes

ryarchaeota enarchaeota-Eocyte:

¡Bienvenidos al Primer Simposio de Investigación Subgraduada en Biología!

El Departamento de Biología del Recinto Universitario de Mayagüez celebra y reconoce la diversidad y la labor investigativa de sus estudiantes subgraduados. Actualmente, nuestro departamento cuenta con un promedio de 1,400 estudiantes, de los cuales un por ciento en aumento está encaminado hacia el diseño y ejecución de proyectos científicos. Entendemos que el método científico es completado una vez se divulgan los resultados dentro del contexto de la comunidad cívica y científica a la cual pertenecen.

El Primer Simposio de Investigación Subgraduada tiene como propósito crear el espacio para consolidar el conocimiento adquirido durante la experimentación en el laboratorio y para practicar las destrezas de comunicación oral científica.

El comité organizador agradece el apoyo de: Departamento de Biología-Recinto Universitario de Mayagüez, Role Model-H.H.M.I. (subvención de Howard Hughes Medical Institute) & Puerto Rico Science, Technology and Research Trust.

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- o Lucy B. Williams

Itinerario Simposio de Investigación Subgraduada

7 de mayo de 2011

| 8:00 | Registro |
|-------|--|
| 8:30 | Bienvenida (B-392) |
| 9:00 | Sesión la |
| | B-180 Ecología I 4/8 B-181 Fisiología 4/7 B-182 Biotecnología 4/8 |
| 10:00 | Merienda |
| 10:15 | Sesión Ib |
| | B-180 Ecología I 4/8 B-181 Fisiología 3/7 B-182 Biotecnología 5/8 |
| 11:30 | Almuerzo |
| 12:30 | Charlas de Desarrollo Profesional (B-392) |
| 12:30 | Dra. Nanette Diffoot Carlo |
| 12:45 | Dr. Luis Ríos Hernández |
| 1:00 | Dr. Matías Cafaro |
| 1:15 | Dr. David Logue |
| 1:30 | Sesión IIa |
| | B-180 Ecología II 4/8 B-181 Evolución 4/8 B-182 Genética 4/8 B-184 Medicina 4/9 |
| 2:30 | Merienda |
| 2:45 | Sesión IIb |
| | B-180 Ecología II 4/8 B-181 Evolución 4/8 B-182 Genética 4/8 B-184 Medicina 5/9 |
| 4:00 | Panel de Discusión (B-392) |
| 5:00 | Premiación y Clausura (B-392) |

| NAME | TITLE | ADVISOR | ORDER | START TIME | ROOM |
|---------------|--|--------------|-------|---------------|-------|
| Gonzalez | Effects of Thermal Oxidation of Gamma-Titanium Aluminide at 500°c and 700°c on Human Osteoblast Cell Adhesion | N. Diffoot | 1 | 9:00 | B-182 |
| Mendez | Growth And Biocompatibility Studies Of Osteoblast Cells (Hfob1.19) Cultered On Titanium Alloys With Hydroxyapatite | N. Diffoot | 2 | 9:15 | B-182 |
| Chaparro | A Microbial Food Sensor To Support A Self-Sustaining Environment In Space | P. Ortiz | 3 | 9:30 | B-182 |
| Santana | Search, Isolation And Characterization Of Radiation Resistant Bioprospects From Extreme Environments In Puerto Rico | C. Ríos | 4 | 9:45 | B-182 |
| Ledesma | Isolation Of Toxin Component Interacting Partners Using T7 Phage Display | C. Ríos | 5 | 10:15 | B-182 |
| Surrillo | Development Of Anaerobic Microcosms Capable Of Biotransforming Alginate Into The Alternative Renewable Energy Source Methane. | L. Ríos | 6 | 10:30 | B-182 |
| Marquez | Biomass Of Dioscorea Bulbifera And Mucuna Pruriens As Raw Material For The Anaerobic Biotransformation Into A Renewable Energy Source | L. Ríos | 7 | 10:45 | B-182 |
| Rodriguez, B. | Microbial Fuel Cells As Enrichment Devices For Electricigenic Microbes | C. Rodríguez | 8 | 11:00 | B-182 |
| De Jesus | Photosynthetic Purple Non-Sulfur Bacteria From The Tropical Hypersaline Microbial Mats At Cabo Rojo Salterns | C. Ríos | 1 | 9:00 | B-180 |
| Millan | Detection Of Microbial Bioprospects From The Phytotelmata Of The Pitcher Plant Nepenthes Sp | C. Ríos | 2 | 9:15 | B-180 |
| Del Valle | Impacted Mangrove Habitats As Stable Reservoirs Of Antibiotic Resistance Genes | C. Rodríguez | 3 | 9:30 | B-180 |
| Narvaez | Nutrient Analysis And Algal Characterization At Cartagena Lagoon, Lajas, Puerto Rico | C. Santos | 4 | 9:45 | B-180 |
| Morales | Abundancia De La Almeja Asiatica, Corbicula Fluminea (Müller), En Diferentes Sedimentos Del Embalse Guajataca | C. Santos | 5 | 10:15 | B-180 |
| Ramirez | Caracterizacion De La Dieta Del Diablito Rojo, Amphilophus Labiatus (Actinopterygii), En El Embalase Guajataca | C. Santos | 6 | 10:30 | B-180 |
| Alvarez | Relación Entre Densidad Y Biomasa De Amebas Testadas A Través De Diferentes Niveles Tróficos | D. Acosta | 7 | 10:45 | B-180 |

| Otero | Description Of The Prokaryotic Community In The Bivalve Lucina Pectinata In Marines Wetlands Of Cabo Rojo, Puerto Rico | J. López | 8 | 11:00 | B-180 |
|-----------|---|-------------|---|-------|-------|
| Flores, | Distribution Of Hawkmoths (Sphingidae) In Puerto Rico | N. Franz | 1 | 1:30 | B-180 |
| Acosta | Sondeo De Actividad Acústica En Una Comunidad De Anfibios En La Reserva Natural "El Tallonal" – Arecibo, Pr. | F. Bird | 2 | 1:45 | B-180 |
| Gonzalez | Performance Levels Of Sexual Signals In Adelaide's Warbler, Dendroica Adelaide | D. Logue | 3 | 2:00 | B-180 |
| Schraft | Song Sharing In Two Neighborhoods Of Adelaide's Warblers (Dendroica Adelaidae) | D. Logue | 4 | 2:15 | B-180 |
| Sierra | The Evolution Of Vocal Duetting In Perching Birds | D. Logue | 5 | 2:45 | B-180 |
| Tarazona | Abundancia Y Riqueza De Moluscos Asociados A Bosques De Spathodea Campanulata En Zona Aluvial Y Karstico | I. Sastre | 6 | 3:00 | B-180 |
| Segui | Effects Of Fire On Leaf Phenology Of Bucida Buceras, A Native Dry Forest Tree In Puerto Rico | J. Thaxton | 7 | 3:15 | B-180 |
| Ramos | Assessment Of Parasitism In Pterois Volitans From Coastal Waters In Puerto Rico | L. Williams | 8 | 3:30 | B-180 |
| Labiosa | Mantenimiento Y Digitalización De La Colección Herpetológica Del Recinto Universitario De Mayagüez. | F. Bird | 1 | 1:30 | B-181 |
| Matos | Actinobacteria Associated To The Yeast Agriculture Ant Cyphomyrmex Minutus | M. Cafaro | 2 | 1:45 | B-181 |
| Antonetti | Actinobacteria Associated To Paratrechina Sp. Ant In Guanica Dry Forest. | M. Cafaro | 3 | 2:00 | B-181 |
| Castro | Microfungi Associated With The Fungus-Growing Ant, Cyphomyrmex Minutus | M. Cafaro | 4 | 2:15 | B-181 |
| Ruiz | Comparative Genomic Studies Of Indels In Humans And Two Asian Primates: Pongo Abelii And Macaca Mulatta | T. Oleksyk | 5 | 2:45 | B-181 |
| Vázquez | Class Size And Infestation Relationship Between Enterobryus Halophilus (Opistokonta: Ichthyosporea) And Molecrab Emerita Portoricensis (Crustacea: Anomura) | M. Cafaro | 6 | 3:00 | B-181 |

| Toro | Identification Of Fungi Associated With Nasutitermes Termite Nests | M. Cafaro | 7 | 3:15 | B-181 |
|--------------------|---|---------------------------|---|------|-------|
| Ferrer | Experimental Validation Of Insertions And Deletions In Pairwise Comparisons Of Homologous Coding Sequences Between Humans And Two African Primates. | T. Oleksyk | 8 | 3:30 | B-181 |
| Cruz- Hernández | Functional-Based Metagenomics: Unraveling Urease Activity In Metagenomic Libraries Generated From Tropical Rain Forest Soils In Puerto Rico | C. Ríos | 1 | 1:30 | B-182 |
| Pabon | Searching For A Genetic Tool: Isolation Of Rhodobacter Sphaeroides Bacteriophages From Water Samples In Puerto Rico | C. Rios | 2 | 1:45 | B-182 |
| Cruz | Surveillance Of Tetracycline Resistance In Metagenomic Libraries Generated From Tropical Rain Forest Soils In Puerto Rico | C. Ríos | 3 | 2:00 | B-182 |
| Rodríguez | Probing For Integron–Encoded Antibiotic Resistance Among Fecal Indicator Bacteria. | C. Rodríguez | 4 | 2:15 | B-182 |
| Perez | Proyecto Adopte Un Gen | J.Martinez | 5 | 2:45 | B-182 |
| Muñiz | Distribution Of Integrons Among Different Environments In Puerto Rico | R. Montalvo | 6 | 3:00 | B-182 |
| Almodovar | Host-Defense Peptide Mimetic Foldamers | P. Ortiz | 7 | 3:15 | B-182 |
| Ríos | Assessment Of Genetic Diversity In Sweetpotato Ipomoea Batatas L. Germplasm Collection In Puerto Rico – Preliminary Study | D. Siritunga | 8 | 3:30 | B-182 |
| Latorre | Monitoring The Efficacy Of Antimicrobial Photodynamic Therapy In A Murine Model Of Cutaneous Leishmaniasis Using L. Major Expressing Gfp | Akilov (U Pittsburgh) | 1 | 1:30 | B-184 |
| Cabrera | Identifying Rare Variants In The Chrnd And Chrng Genes | Ehringher (CU Boulder) | 2 | 1:45 | B-184 |
| Adorno | Estrogen In Breast Cancer | Kreeger | 4 | 2:15 | B-184 |
| Irizarry | Investigating Potential Drug Leads From The Marine Red Macroalgae Peyssonnelia Sp. | Kubanek and Myers | 5 | 2:45 | B-184 |
| Vélez | Mir-17-3p Regulates Metabolism Of The Prostate Cancer Cell | MARC Program | 6 | 3:00 | B-184 |

| Rodríguez, C | Magnetic Nanoparticles For Cancer Therapy | O. Perales | 7 | 3:15 | B-184 |
|--------------|--|---------------------|---|-------|-------|
| Munoz, | Biodegradable Nanocarriers For Photodynamic Therapy | J. Ramirez- Vick | 8 | 3:30 | B-184 |
| Ares | Role Of Imp2 And Hmga2 In Adipocyte Differentiation And Type 2 Diabetes | Wrighting | 9 | 3:45 | B-184 |
| Martínez | Study Of H2s Binding Mechanism, Stability And Release Of Hbi With Different Ligands Using Flash Photolysis Technique | MARC Program | 1 | 9:00 | B-181 |
| Pasische | Effect Of Ph Media On Bryophyte Growth: A Study For Protocol Optimization | I. Sastre | 2 | 9:15 | B-181 |
| Mojica | Biomechanical Properties Of Bovine Intervertebral Discs Under Combined Compressive And Torsional Loadings | P. Sundaram | 3 | 9:30 | B-181 |
| Martines | The Effect Of Stlt-1 On Bacteria-Activated Neutrophils | A.Washington | 4 | 9:45 | B-181 |
| Ortiz | Tlt-1 Mediates Platelet-Neutrophil Interaction | A.Washington | 5 | 10:15 | B-181 |
| Santiago | Determinacion De La Presencia De Tlt-1 En El Cerebro De Raton | A.Washington | 6 | 10:30 | B-181 |
| Torres | Effect Of Stlt-1 On R848-Activated Neutrophils | A.Washington | 7 | 10:45 | B-181 |

SONDEO DE ACTIVIDAD ACÚSTICA EN UNA COMUNIDAD DE ANFIBIOS EN LA RESERVA NATURAL "EL TALLONAL" – ARECIBO, PR.

Acosta, I., S. Velázquez, L. Laboy, K. Pantoja, X. Labiosa

Departamento de Biología, UPR- Recinto de Mayagüez

Abstract

La utilización de técnicas de sondeos acústicos para documentar especies de anfibios representa una herramienta de mucho valor para el estudio a largo plazo de comunidades. ΕI establecimiento de estaciones acústicas automatizadas, programadas para grabar a intervalos determinados de tiempo (1 minuto/30 minutos/12 horas-noche) de forma repetitiva (diariamente) permite establecer la presencia de especies en el área seleccionada sin la presencia de personal de muestreo. Las grabaciones de actividad acústica sirven como récord de presencia/ausencia de especies de interés (endémicas, invasoras, y en peligro de extinción) pero solo a posteriori de Esta actividad acústica, junto con información de precipitación y temperatura la actividad. permiten analizar el patrón temporal de utilización de hábitat por las diferentes especies. En la Reserva Natural de El Tallonal (Arecibo, PR), se ha establecido una estación acústica con el propósito de rastrear la actividad reproductiva de una especie de anfibio endémico, en peligro de extinción (sapo concho del norte de PR – **Peltophryne lemur**) que se está re-introduciendo a su hábitat natural. La competencia ecológica con una especie invasora (*Rhinella [Bufo] marina*), tanto por las charcas de reproducción como por el nicho acústico, son retos que los biólogos de la conservación enfrentan en esta reserva natural. Los datos obtenidos de las estaciones acústicas revelan que la especie invasora domina el nicho acústico tanto en cantidad como en intensidad. A tales efectos, las estaciones acústicas no registran de forma efectiva las vocalizaciones de la especie de interés (*Peltophryne*) por la interferencia acústica de la especie invasora. No obstante, la información obtenida de estas estaciones nos permiten establecer patrones de actividad reproductiva en diferentes especies de anfibios, tanto de hábitos acuáticos (Peltophryne lemur, Rhinella marina, Leptodactylus albilabris y Lithobates catesbeianus) como de hábitos terrestres y arbóreos (Eleutherodactylus coqui, E. antillensis, E. cochranae, *E.richmondi*). Se espera en un futuro cercano, el que las vocalizaciones recogidas por la

estación acústica puedan ser transmitidas en tiempo real a un servidor computadorizado que pueda identificar las mismas por medio de un algoritmo.

ESTROGEN IN BREAST CANCER

Adorno, V., Kreeger, P. and Dawn Tian

Department of Biomedical Engineering, University of Wisconsin, Madison

Abstract

Breast cancer is second most common cause of cancer in women. Estrogen induces proliferation of breast cancer cells. Several experiments were performed to measure estrogen induced proliferation in MCF7 cells. Other chemicals, known as Estrogen Disrupting Chemicals (EDC's), were also tested to determine their effect on MCF7 proliferation. Cell Titer Glo and Crystal Violet assay were used as proliferation assays, however, results are variable and no significant estrogen response was obtained. Future experiments include using a new MCF-7 strain and testing other proliferation assays.

HOST-DEFENSE PEPTIDE MIMETIC FOLDAMERS

Almodóvar Cruz, J. M. 2

Co-authors: Francisco J. Chaparro Carrasquillo1,3, Carlos J. Martes Martínez2, Juan C. Nieves-Christie2, Patricia Ortiz1

1. Department of Chemical Engineering, University of Puerto Rico at Mayaguez

2. Industrial Biotechnology Department, University of Puerto Rico at Mayaguez

3. Department of Chemistry, University of Puerto Rico at Mayaguez

Abstract

β-peptides are short nanorods made from artificial amino acids designed to mimic natural antimicrobial peptides. Key structural features of these β -peptide have been identified as the antimicrobial activities. However, the mechanisms of action of these peptides are not completely understood. In order to gain more insight about this mechanism of action this research proposes the utilization of a Saccharomyces cerevisiae strain (SPY760) exposed to a random gene mutagenesis by means of a mini-Tn3::LEU2 transposon. This strain along with a nonmutagenized Saccharomyces cerevisiae strain (SKY760) were exposed to several Minimal Inhibitory Concentration (MIC) assays utilizing (AVK)3 β -peptides with the goal of obtaining resistant mutants, which were observed at a concentration of 32µg/mL. Genomic DNA from these resistant mutants was successfully extracted and digested with restriction enzymes. Vectorette PCR revealed different amplification products, therefore pointing out different genes that may be involved in β -peptide resistance. Several genes whose function range from unknown to mediation of oxidative stress have been identified using bioinformatics tools. Next steps involve performing genomic knock outs of identified genes verifying their involvement in β -peptide resistance, characterize genes of unknown function and perform MIC assays with other β -peptides to assess potential mechanistic differences as a function of different structures.

ACTINOBACTERIA ASSOCIATED TO PARATRECHINA SP. ANT IN GUANICA DRY FOREST

Antonetti Negrón, K. and Hernández Reyes, R.

Department of Biology, University of Puerto Rico, Mayagüez Campus.

Abstract

Actinobacteria are gram-positive bacteria that produce secondary metabolites, which are economically important to humans due to clinical and industrial applications. In nature, some Actinobacteria establish symbiotic associations with plants and insects. The most studied association with insects has been in the fungus-growing ant symbiosis. These bacteria produce antibiotics that help protect their crop from other pathogenic microorganisms. Other ants, like Paratrechina sp., which is not a fungus-growing ant, also have bacteria associated with them, but no ecological role has been assigned. The goal of our study is to identify Actinobacteria associated with this species of ant using culture-independent methods. We collected Paratrechina sp. ants in the forest of Guanica for direct DNA extraction and amplification of the 16S rDNA gene in this project. Metagenomic libraries of the 16S rDNA gene were prepared in Escherichia coli JM109 competent cells. We performed colony PCR to confirm the presence of insert in our clones before RFLP analysis. We observed seven different restriction patterns with Hinfl. Those samples that did not show variation with Hinfl were cut with Haell restriction enzyme and eight extra different patterns were found in our samples. Clones that showed different restriction patterns were sequenced; our preliminary results indicate that Streptomyces species were the most abundant taxa associated with Paratrechina sp. ants.

ROLE OF IMP2 AND HMGA2 IN ADIPOCYTE DIFFERENTIATION AND TYPE 2 DIABETES

Ares, S.I., Wrighting, D.M., and Altshuler, D.

Department of Medical and Population Genetics, Broad Institute Summer Research Program in Genomics

Abstract

Peroxisome proliferator-activated receptor gamma (PPARy), high mobility group AT-hook 2 (HMGA2), and insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2) are genes that have been associated with Type 2 Diabetes risk. Properly differentiated adipose tissue plays an important role in glucose regulation; in fact, PPARy is known to be a major regulator of adipocyte differentiation and function. Recent studies have shown that HMGA2 affects adipocyte formation and accumulation in mice, and acts as a transcriptional regulator of IGF2BP2. In order to study the function of these genes in adipocytes, we investigated methods of inducing overexpression and gene knockdown in human pre-adipocytes. We showed that nucleofection of human pre-adipocytes resulted in ~70% of cells expressing GFP, while transfection using Lipofectamine LTX resulted in <1% expression. For gene knockdown, siRNA's were used to silence expression of PPARy, HMGA2, and IGF2BP2 in pre-adipocytes. Using a fluorescent control, we showed that siRNA delivery was successful and had moderate efficiency. In the future, we hope to assess the potential role of HMGA2 and IGF2BP2 using these optimized protocols.

IDENTIFYING RARE VARIANTS IN THE CHRND AND CHRNG GENES

Cabrera Montalvo, C. J.1, Kamens, H.2, Ehringer, M.2

 University of Puerto Rico at Mayagüez, Department of Biology
 University of Colorado, Boulder, Department of Integrative Physiology, Institute of Behavioral Genetics

Abstract

Even with all the known health and social problems they bring, smoking and drinking alcohol are still some of the most preventable causes of death. Common action sites both substances are the nicotinic acetylcholine receptors (nAChR). Variants in the genes encoding the subunits that form these receptors, known as Single Nucleotide Polymorphisms (SNPs), have been associated with behaviors like number of drinks or of cigarettes, and age of onset of dependence. This project optimizes a PCR protocol to deep-sequence the genes of the delta and gamma subunits of the nAChR, CHRND and CHRNG, respectively. Upon their sequencing, we hope to discover new significant SNPs that might offer us a more complete understanding of the molecular mechanisms behind alcohol and nicotine dependence.

MICROFUNGI ASSOCIATED WITH THE FUNGUS-GROWING ANT, CYPHOMYRMEX MINUTUS

Castro, K., M. Medina-Rivera and L. Fernández Lebrón

Biology Department, UPR- Mayagüez Campus, Mayagüez, Puerto Rico 00680

Abstract

In the fungus-growing ant symbiosis, the ants cultivar a fungus (Basidiomycota) and protect their culture from specific parasites (Ascomycota) using the antibiotic production capacity of Actinobacteria. Three different genera of fungus-growing ants are known from Puerto Rico. Of these, Cyphomyrmex minutus is the only species that cultivates its fungus in yeast form. Although the interaction in the attine ant symbiosis has been extensively studied, the yeast-cultivating ants and their microbial associates in the Caribbean have not been described. The main objectives of this project are isolate, classify and characterize fungi associated to Cyphomyrmex minutus nests, using culture dependent techniques, microscopy and molecular techniques. We have isolated the ant cultuvar (yeast) and all associated microfungi (mycelial fungi) including potential pathogens. Samples were taken from seventeen nests in the Cambalache Tropical Forest, Puerto Rico during the rainy season. Microfungi were classified and characterized using morphology and molecular methods. Isolations were obtained from the inoculation of the yeast cultivar on Potato Dextrose Agar with antibiotics. Morphology of these isolates was investigated with light microscopy with phase contrast and scanning electron microscopy. To further characterize the microfungi we isolated and amplified the ITS 1 and 2 regions of ribosomal DNA. From the seventeen nests we isolated fourteen different genera of microfungi associated to the ant yeast cultivar. These results represent an important contribution to understanding the interaction between yeast-growing ants, their symbionts and their nest.

A MICROBIAL FOOD SENSOR TO SUPPORT A SELF-SUSTAINING

ENVIRONMENT IN SPACE

Chaparro-Carrasquillo, F.J.1-2

Coauthors: Pinzón, C.A.2, Rinaldi, C.2, Acevedo, A.2 and Ortiz, P.2

University of Puerto Rico at Mayagüez, Department of Biology-Department of Chemistry

Abstract

In order to build sustainable and long-term human presence in space, attention should be placed on assessing the quality of nutritional sources. This research focuses on the development of a low-cost, biocompatible, and biodegradable microbial sensor for the determination of microbial concentration in liquids, based on the response of gel-encapsulated magnetic nanoparticles. To achieve this, the biosensor was prepared and tested as follow. A solution of 1g chitosan (low or medium molecular weight) and 100mL of 3% acetic acid was mixed with a second solution containing 20g of 3% acetic acid and a variable amount of glutaraldehyde. Here, chitosan acts as the biopolymer; acetic acid is the solvent, and glutaraldehyde is used as the cross-linker. The final solution was sonificated for two minutes, and patentized nanoparticles of ferrite were added. This solution is poured into a petri plate, dried for 12h at 50°C and lyophilizated. A biological agent, Escherichia coli (E.coli), was used to test the toxicity of the gel film. While growing, these microorganisms were in contact with the film. Depending on the synthesis properties and time of interaction, the toxicity of the biosensor can be measured by analyzing the inhibition halo and concentration of microorganisms in the liquid media. When compared to low molecular weight chitosan at higher concentration of glutaraldehyde, swelling studies showed that films with medium molecular weight of chitosan absorbed greater quantities of water. Qualitative toxicity results showed that at higher glutaraldehyde concentrations the inhibition of *E.coli* increase. Analysis of various physicochemical properties of chitosan-based gels, including swelling, leaching of nanoparticles and the interaction of biological agents with the gel film, would help in the improvement of this device for microbial concentration detection in liquids. The next step is to start nanoparticles leaching assessment experiments to know whether the nanoparticles are maintained within the film.

FUNCTIONAL BASED-METAGENOMICS: UNRAVELING UREASE ACTIVITY IN METAGENOMIC LIBRARIES GENERATED FROM TROPICAL RAIN FOREST SOILS IN PUERTO RICO

Cruz-Hernández J. C.¹ and C. Ríos-Velázquez²

University of Puerto Rico at Mayagüez, ¹Industrial Biotechnology Program and ²Biology Department

Abstract

Currently, most of the enzymes with industrial and biomedical applications were isolated from microorganisms by traditional/culture dependent methods. Metagenomics has allowed unraveling the presence of novel microbial groups and new enzymatic activities with application in Biotechnology by using culture independent approaches. Urea is the main metabolite of protein degradation, which can be used as fertilizer and can become in some Industries a waste product. The identification of new enzymes that show a high rate of catalysis than those documented in Puerto Ricans soils, will provide an alternative way to biodegrade industrial wastes. Last year, metagenomic libraries from a tropical rain forest and a dry forest in Puerto Rico were screened for urease activity (UA). Several fosmids pools showed UA, a few individual clones were isolated, but no strategies to determine the gene(s) responsible for the activities were performed. The main focus of this research is to confirm the individual clones for UA phenotype, and determine the gene responsible for such activity. The metagenomic urease positive clones were confirmed by extraction and re-electroporation of the fosmids in an isogenic strain. Transposon mutagenesis was performed to interrupt the expression of gene responsible for urease phenotype. The mutagenized gene in the fosmid showing urease loss of function, was amplified by PCR using Tn specific primers, and sequenced for in silico analysis. A total of 37 putative urease positive and re-confirmed clones were isolated. Restriction analysis of the fosmids indicates that 22 of them are fosmids with different cloned fragment. The in silico analysis suggest the presence of sequences similar to several enzyme activities such as deacetylaces, phosphates and alpha/beta hydrolases. Primer walking of the cloned fragments is being performed to have a better understanding of the inactivated region and relate it with the UA found.

SURVEILLANCE OF TETRACYCLINE RESISTANCE IN METAGENOMIC LIBRARIES GENERATED FROM TROPICAL RAIN FOREST SOILS IN PUERTO RICO

Cruz-García¹, J; P. Ondina¹, R. Santiago², M. Ortega³ and C. Ríos-Velázquez⁴ ¹Industrial Biotechnology Program, University of Puerto Rico, Mayagüez, PR ²Department of Bacteriology, University of Wisconsin, Madison, WI ³Department of Chemical Biology, University of Illinois at Urbana-Champagne ⁴Biology Department, University of Puerto Rico, Mayagüez, PR

To date, most of the knowledge regarding resistance mechanisms in bacteria has been derived studying cultivable microorganisms. The generation of metagenomic libraries has become a powerful culture independent technique that has facilitated the unraveling of novel activities, including antibiotic resistance. Two years ago, we began functional genomic analyses in tropical rainforest soils in Puerto Rico by generating a metagenomic library from these areas. We also began monitoring for antibiotic resistance by selection of the library on solid media containing ampicillin, gentamicin, and tetracycline. The presence of a cloned fragment was confirmed by restriction analysis. Though an appreciable number of clones were found, we observed less diversity for clones resistant to tetracycline when comparing restriction patterns to clones resistant to other antibiotics tested. This research focuses on the study of these clones resistant to tetracycline. In order to identify the gene responsible for the tetracycline resistance phenotype, in vitro transposon mutagenesis was used. The mutagenized fosmids were then electroporated into an isogenic host. Sequencing candidates were isolated by detecting loss of antibiotic resistance phenotype using replica plating. Clones were sequenced outwards of the transposon using Tm specific primers. The in silico analysis suggests that the sequences are related or similar to aminoglycoside and carbohydrate modifiers/inhibitors of microbial origin.

PHOTOSYNTHETIC PURPLE NON-SULFUR BACTERIA FROM THE TROPICAL HYPERSALINE MICROBIAL MATS AT CABO ROJO SALTERNS

De Jesús-Cruz, M. Soto-Feliciano, K., and Ríos-Velázquez, C.

Department of Biology University of Puerto Rico- Mayagüez Campus

Abstract

Microbial mats are stratified communities that develop at the interfaces between the water and solid substrates in extreme environments. These contain organosedimentary-laminated structures, influenced by light, dissolved oxygen and sulfide. When fully developed; microbial mats have three distinctive layers. The top layer (green pigmented), having organo-heterotrophic cyanobacteria and other aerobic microbes. The middle layer (pink pigmented), characterized by anoxygenic phototrophic microorganisms; and a black layer at the bottom containing sulfate-reducing bacteria. In order to study the diversity, physiology and the geochemical processes in the mats, a Microbial Observatory (MO) was developed at the Tropical Hypersaline Microbial mats at the Cabo Rojo Saltern in Puerto Rico. As part of the MO and due to their versatile physiology and potential application in Biotechnology, our laboratory focuses on isolating and characterizing of cultivable purple non-sulfur bacteria (PNSB) present in benthic and ephemeral mats at the Cabo Rojo Salterns during rainy and dry seasons. Microbial mats were dissected and the pink layer was cultivated in solid and liquid media. After being incubated anaerobically in the presence of light, a characteristic pigmentation was observed. The colonies with PNSB pigmentation were isolated and characterized microscopically, biochemically and molecularly. A total of 10 and 13 isolates of the rainy and dry seasons were isolated respectively. Microscopic analysis revealed gram-negative rods of variable sizes. The cultured PNBS exhibited a variety of salinity tolerance, and a diverse metabolism of carbon compounds as energy sources. Biochemical and molecular analysis revealed the presence of the bacteriochlorophyl (800 and 850nm) and carotenoids peaks (520 nm). Amplicons from all the isolates were obtained using specific primers for pufM and 16S rDNA. In silico analysis suggests the presence of Rhodospirillaceae bacterium, Rhodospirillum salexigens and various unidentified species in both mats. Altogether, these results suggest microbial mat-specific as well as seasonspecific purple non-sulfur bacteria.

IMPACTED MANGROVE HABITATS AS STABLE RESERVOIRS OF ANTIBIOTIC RESISTANCE GENES

Del Valle, L., Álvarez, O. & Rodríguez, C.

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Abstract

Mangrove ecosystem serve as a nursery environment for marine species that are important for commercial and recreational fishing industries. In the last decade the spread of antibiotic resistance (ABR) outside the clinical scene has been linked to the degradation of aquatic environments due to wastewater contamination. This has raised concerns about the dissemination of resistant strains by exposure to recreational aquatic activities of through the food chain. To evaluate the impact of this problem in Puerto Rico we analyzed the potential of mangrove habitats to serve as reservoirs of ABR genes. To this end we used a PCR-based technique to detect antibiotic resistance mechanism encoded by a genetic system known as integrons, which is laterally transmissible among pathogens and harmless bacteria. Samples from the water column, sediments and rhizosphere from impacted (La Parguera, Lajas) and relatively undisturbed (Ensenada Dakity, Culebra) mangrove environments were analyzed. Results from mangrove habitats with minimal impact (Ensenada Dakity, Culebra) suggest that natural factors may influence the prevalence of integron-encoded, resistance genes as these were undetected during the dry season but were found during the rainy season. Nevertheless, ABR gene were detected only in samples from sediments and rhizosphere material at this site, indicating that mangrove habitats may serve as transient reservoirs of ABR genes when the impact is moderate. In contrast, sampling of the disturbed mangrove site (La Parguera, Lajas) indicates that a sustained impact of wastewater pollution may turn the mangrove ecosystem into a stable reservoir of resistance determinants. Our results indicate that wastewater pollution of mangrove ecosystem may constitute a risk to human health due to the introduction of bacterial populations harboring potentially transmissible antibiotic resistance genes.

LOCALIZATION OF TLT-1 IN MOUSE BRAIN

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Abstract

The brain is prone to suffer several types of damage usually caused by inflammation, stroke, traumatic brain injury and brain ischemia, among others. Triggering receptor expressed on myeloid cells like transcript-1 (TLT-1) is a transmembrane receptor, specific to platelets and megakaryocyte α -granules. TLT-1 is involved in inflammation, modulates the activation of neutrophils and possibly other leukocytes, and facilitates platelet aggregation at sites of vascular injury. Our recent data demonstrate a more aggressive nature to wild type mice compared to null mice. These differences prompted us to look for a unifying hypothesis as to the cause of the behavioral discrepancies. Closer evaluation of TremI1-/- mice demonstrates that these mice have lower heart rates compared to wild type mice. We hypothesized that TLT-1 may be found in the brain. To address the question we dissected the brain into its various sections and probed the tissue by western blot analysis. Our results show that TLT-1 is found in various sections in the brain including the cerebellum and medulla where expression was the highest. Expression of TLT-1 in the medulla is consistent with null mice having a lower heart rate than wild type and suggests a role for TLT-1 modulating motor function. Future expectations are to identify what cells express TLT-1 in the brain and to clone the cDNA which appears to run at a lower molecular weight that platelet TLT-1.

EXPERIMENTAL VALIDATION OF INSERTIONS AND DELETIONS IN PAIRWISE COMPARISONS OF HOMOLOGOUS CODING SEQUENCES BETWEEN HUMANS AND TWO AFRICAN PRIMATES.

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Abstract

Comparisons between primate genomes are important to further understand evolution of our own genome and its structure. Nucleotide insertions and deletions, known has indels, are important genetic and evolutionary markers that differentiate between us and our closest relatives, chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*). In our current research, a database of 61 indels discovered by reference genome alignments between the three species was validated by PCR amplification and electrophoresis in two humans, two chimps and two gorilla samples. Primers were designed to amplify only the indels observed in coding regions, because they have the largest potential impact on the protein product.

From 61genes containing candidate indels, 57 have been tested in the laboratory. Of these, 30 have been validated. One indel was a false positive of the reference genome alignment. We found two indels showing polymorphism between humans and chimpanzees. These will be good candidates to survey in multiple human populations. The remaining 27 indels are still in process of validation in the laboratory.

Indels sum up a large fraction of differences between human and their close relatives, the African apes. These differences may be an important part of what defines us as a human species. The information collected in our project can be further used in surveys of different human populations, and as candidate loci for discovery of human disease. In the future, we will continue this project by genotyping the Human Diversity Panel (a database containing samples from 52 human populations) and in cohorts of kidney and HIV patients in collaboration with the National Cancer Institute.

DISTRIBUTION OF HAWK MOTH'S (SPHINGIDAE) IN PUERTO RICO

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Abstract

The Sphingidae are a family of Lepidoptera commonly known as hawkmoths. They are most diverse in the tropics, including Puerto Rico. The goal of this project is to catalogue the diversity of hawkmoth species of Puerto Rico based on the holdings of the UPRM Insect Collection (<u>http://invcol.uprm.edu/</u>). At present the collection holds 33 species and approximately 380 specimens of Sphingidae, each with data on the collecting records which span across many decades. These data were used to identify the location and thus ultimately document the known distribution of each species in Puerto Rico. However, the collection has a sampling bias; most of the specimens are from Mayagüez, leading to the conclusion that this area is the most diverse for hawkmoths in Puerto Rico. This outcome could be tested by adding records from other collections, such as the Insect Museum in Río Piedras.

EFFECTS OF THERMAL OXIDATION OF GAMMA-TITANIUM ALUMINIDE AT 500°C AND 700°C ON HUMAN OSTEOBLAST CELL ADHESION

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Abstract

Prosthetic technology is in search for cost efficient materials that comply with the specifications of biocompatibility, corrosion resistance and optimal mechanical qualities. Thermal oxidation seems to be a promising method to generate highly corrosion resistant and biocompatible surfaces for implant applications. In this experiment, titanium alloys: gamma-TiAI and Ti-6AI-4V, where thermally oxidized at 500°C and 700°C in air to generate an oxide layer. Human Fetal Osteoblast cells (hFOB1.19I) were used to examine cell adhesion and osseointegration on thermally oxidized surfaces by the presence of focal adhesion points. hFOB1.19 cells were grown for 24 hours on titanium surfaces. An inmunofluorescence labeling assay was performed to determine the expression of vinculin, a protein present at focal points. Preliminary results show that hFOB1.19 cells were able to attach on gamma-TiAI disks thermally oxidized at 500°C and 700°C.

PERFORMANCE LEVELS OF SEXUAL SIGNALS IN ADELAIDE'S WARBLER,

DENDROICA ADELAIDAE

González-Quintero, G., Logue, D., & Pereira, D.

Abstract

In Adelaide's warblers (*Dendroica adelaidae*), males sing multiple song types, though it is not clear how individual males choose which signal to use in situations of competition and female attraction. We are working to test Logue and Forstmeier's model of communication, which states that focal males in the vicinity of another male will have an advantage if they can match a song when it surpasses the quality of the original. If they cannot match the quality, it is advantageous to not match the signal in case of eavesdropping females looking for a potential mate. My work consists of extracting song samples from field recordings using Syrinx software and cataloguing them for later analysis. I isolate and identify novel song types (holotypes) that compose an individual's repertoire and use them to group together the samples. We then proceed to analyze the spectrographs for performance quality based on factors such as trill rate and frequency bandwidth. With this data we can construct performance constraint graphs to determine physical limits in the production of these sexual signals, which are an essential piece for our future experiments. With our research we hope to pave the way for future behavioral ecologists to continue building upon this field.

INVESTIGATING POTENTIAL DRUG LEADS FROM THE MARINE RED MACROALGAE PEYSSONNELIA SP.

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Abstract

The marine red macroalgae genus *Peyssonnelia sp.* has been an ideal candidate for the discovery of novel chemical compounds that demonstrate unique biomedical characteristics. Recent studies have been able to point out novel sesquiterpene hydroquinones active against cancer cell lines and sterol glycosides active in kind against the latter. It has been suggested that this species may generate diverse chemical compounds of biomedical importance, specifically compounds active against human microbial pathogens. Unfortunately, few studies have been completed to support this hypothesis. In the following experiment, we generated organic solvents from a Fijian red alga *Peyssonnelia sp.* and evaluated biomedical activity against *Candida albicans* and *Staphylococcus aureus*, two drug resistant human pathogens. Our experiment showed that no extracts inhibited the growth of wild-type or amphotericin-resistant *C. albicans*; however, two nonpolar fractions resulted in minimum inhibitory concentrations of 250 µg/mL and 500 µg/mL against *S. aureus*. These results suggest the presence of biomedically active chemical compounds, although further study is warranted to isolate the possible compounds.

Keywords: Macroalga, *Peyssonnelia*, Marine natural products, antimicrobial, *Candida albicans, Staphylococcus aureus*

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MANTENIMIENTO Y DIGITALIZACIÓN DE LA COLECCIÓN HERPETOLÓGICA

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Abstract

La colección herpetológica en el Departamento de Biología del Recinto Universitario de Mayagüez tiene a su haber unos 6,800 especímenes de anfibios y alrededor de 2,000 especímenes de reptiles. Para ambos taxones, la mayoría de éstos son especímenes alcohólicos (ranas, salamandras, renacuajos, reptiles), otros son preparaciones esqueléticas secas y muy pocas preparaciones esqueléticas completas de doble tinción en glicerol (huesos y cartílagos). Las áreas geográficas representadas son principalmente del Neotrópico (Puerto Rico, Venezuela, Colombia, Ecuador, Perú, Cuba, República Dominicana.) y Norteamérica. Especímenes representando taxones exóticos, obtenidos por medio de intercambio con otras instituciones, expanden el alcance geográfico de la colección a otras regiones (África, Europa, Asia, Australia). La colección cuenta con tipos y paratipos de varios taxones de anfibios y representantes de algunas especies ya extintas. Los especímenes de esta colección se utilizan tanto para la enseñanza como para la investigación, y en este último renglón se otorgan préstamos de los mismos a investigadores de otras instituciones. Siendo esta colección un repositorio de biodiversidad, la digitalización tanto de los récords de especímenes como de fotografías digitales de los mismos, provee la oportunidad de hacer disponibles en el espacio cibernético dicha colección para la comunidad científica. A corto plazo, la finalidad de este trabajo es la reorganización total del la colección herpetológica y la creación de una base de datos para hacerla disponible a la comunidad científica. A largo plazo, esta base de datos se integrará junto con fotografías digitales de especímenes selectos, al programa SPECIFY® que permite el acceso en el ciberespacio a otras colecciones herpetológicas.

MONITORING THE EFFICACY OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN A MURINE MODEL OF CUTANEOUS LEISHMANIASIS USING *L. MAJOR* EXPRESSING GFP

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Abstract

Cutaneous leishmaniasis (CL) is an infectious parasitic disease widely spread in the developing world, with 1.5 million new cases reported each year. Although numerous treatment modalities are available, there is no ideal therapy for CL. Photodynamic therapy (PDT) has emerged as a very promising and effective treatment for CL. Previous studies in this field have shown that to achieve optimal parasite eradication and improve the therapeutic outcome of the treatment, several subsequent PDT administrations are necessary. These findings led to the search for an effective and simple way of monitoring the outcome of PDT that could enable us to determine when subsequent treatment administrations would be more effective in achieving parasite eradication. We have developed a murine model of CL with green fluorescent protein positive (GFP+) L. major that enables the monitoring of parasitic load via measurements of GFP fluorescence intensity; thus allowing for a faster, more efficient, and cost-effective way of monitoring the clinical outcome of PDT as a treatment method for CL. In addition to this, the developed model may provide new insights on the phototoxic aspects involved in the process of PDT. Although PDT regimens may be somewhat different in humans, it is expected that the developed model will facilitate the optimization and clinical translation of PDT as a therapy for CL and the eventual development of topical PDT treatments for other granulomatous infections.

ISOLATION OF TOXIN COMPONENT INTERACTING PARTNERS USING T7 PHAGE DISPLAY

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Abstract

Since the terrorism attacks in 9/11 and the use of *Bacillus anthracis* as a biological weapon, the need for understanding the toxin mode of action and the development of biosensors for the microbe and/or toxin detection became a priority. Phage Display (PD) is a combinatorial chemistry technology that has matured to the point where it is now a powerful tool in the post-genome, been used for the detection of protein-protein interactions. T7 PD constructs involves the genetically fusion of a cDNA with a capsid protein gene, allowing the display of the translated fusion protein in the bacteriophage surface. The main focus of this project is to

use T7 PD technology to map and isolate interacting partners between human cDNA libraries with the *B. anthracis* Lethal Factor (LF). This is a metallo-protease component of the tripartite toxin secreted by *B. anthracis*, which inactivates regulators in the eukaryotic cell. T7 PD libraries expressing Human Colon cDNA, were used to isolate putative toxin interacting peptides after several rounds of affinity and selection (biopanning) using wild type and mutant lethal factor as targets. The cDNA present in the isolated bacteriophages which displayed the putative interaction partners was amplified by PCR, and the amplicons sequenced and analyzed in silico. A total of 38 putative interaction partners were isolated, 29 and 9 from mutant and wild type targets respectively. The higher number of isolates in the mutant target supports the fact of loss of function (cleavage) which maintains the interaction. The in silico data suggest consensus with proteins among others. Research is in progress to confirm the interactions (specificity tests). These Lethal Factor interacting partners represent a molecular source to better understand the toxin function/mechanism, as well as their potential used as a vaccine and/or biomarker.

BIOMASS OF *DIOSCOREA BULBIFERA* AND *MUCUNA PRURIENS* AS RAW MATERIAL FOR THE ANAEROBIC BIOTRANSFORMATION INTO A RENEWABLE ENERGY SOURCE

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Abstract

Methane is an important energetic gaseous product obtained by the degradation of biomass in anaerobic environments. This biogas can be a useful renewable energy source given its calorific value of 55 kJ/kg surpass only by Hydrogen. Climbing vines, an abundant and diverse group of plants, can be a future material for bioconversion into this renewable energy source. Vines represent 18 to 24% of plants in a hectare of Subtropical or Tropical Forest, with a growth period of eight to twelve months a year. Using anaerobic microcosms containing 1.5 g of individually blended biomass from *Mucuna pruriens*, *Dioscorea Bulbifera* and its tubers,

we determined the capacity of each microbial community from three different natural habitats to biotransform the biomass into methane. The products of metabolism, methane and volatile fatty acids were quantified using a GC and a HPLC to determine the extent of degradation and compare which natural sediment harbor the best microbial community for the bioconversion. Our preliminary results indicate that all the sediments harbor microorganisms that are capable of producing the biogas from the biomass in question, but the inoculated with sediments from a rice-paddy field degraded the climbing vines biomass and tuber regardless of species at a faster rate than any other. The tuber produced by *D. bulbifera* was degraded preferentially by the microorganisms in all natural sediments but yielded the most methane when inoculated with rice paddy. In addition, the biomass from climbing vines and tuber regardation and bioconversion was from *D. bulbifera*. In conclusion, the biomass from climbing vines and their tuber could be used as a raw material for the generation of renewable energy source but the selection of biomass and the particular microbial community needed to carryout the efficient transformation requires further studies.

THE EFFECT OF sTLT-1 ON BACTERIA-ACTIVATED NEUTROPHILS

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Abstract

It is well known that platelets regulate blood coagulation, however, recently they have been shown to play a major role in immune regulation. TLT-1 (Triggering Receptor Expressed in Myeloid (TREM)–like transcript-(TLT)-1 is a receptor found in the α -granules of platelets. Characterization of the tremI1-/-mouse has shown that they bleed after an immune challenge. These results suggest that TLT-1 may be an important link between the inflammatory and coagulatory compartments thru the regulation of innate immune function. Moreover, recent publications demonstrate that activated platelets release large amounts of the soluble form of TLT-1 (sTLT-1) into the blood, although sTLT-1's role in coagulation or inflammation is not understood. Determination of sTLT-1 function is critical for a better understanding of the link between hemostasis and inflammation. As a preliminary step to this understanding, we devised a in vitro system using flow cytometry to measure neutrophil activation. Neutrophils were isolated thru density gradient centrifugation and incubated with E.Coli in the presence or absence of sTLT-1. Neutrophils activation was measured at 2 hours using degranulation (CD66b and CD35) and adhesion (CD 11b) markers by flow cytometry. Our results demonstrate a down-regulation of the expression of CD66b and CD35 on activated neutrophils. There was no apparent regulation of any sort in the expression of CD11b. These results suggest that sTLT-1 has an inhibitory effect on the degranulation of bacteria-activated neutrophils, which may provide insight into the immune bleeding phenotype of the *treml1-/-* mouse. Our studies suggest that TLT-1 may preemptively regulate neutrophil function as a mechanism to control bleeding. Further studies of the interaction between the sTLT-1 and neutrophils will allow us a broader understating of the physiological processes occurring in the hemostatic and immunological systems of a patient during sepsis.

ACTINOBACTERIA ASSOCIATED TO THE YEAST AGRICULTURE ANT CYPHOMYRMEX MINUTUS

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Abstract

The fungus-growing ants have a symbiotic association with basidiomycetous fungi that serve as their food source. This fungal cultivar is attacked by pathogenic fungi in the genus *Escovopsis* (Ascomycota). To protect their cultivar from pathogens the ants use Actinobacteria, well known for their ability to produce antibiotics. In the Caribbean, little information about the interaction between the yeast agriculture ant Cyphomyrmex minutus and their symbionts is available. The purpose of this study was to isolate Actinobacteria associated with C. minutus. We sampled during the rainy season in the Cambalache Forest in Arecibo. We collected a total of 17 nests from which 74 different bacteria morphotypes were isolated. Cyphomyrmex minutus ants were collected from the field with sterile forceps and placed in sterile containers for transport to the laboratory. We mashed 3 to 4 ants and washed them in NaCl solution (0.7%) and then 300µL of the supernatant were plated into Petri dishes with chitin media (CHA). This medium is nutrient poor with osmotic restrictions that prevent fast-growing bacteria to develop, thus favoring slowgrowing actinobacteria that can degrade complex carbon sources. Colonies were further purified and maintained in Yeast Malt Extract Agar (YMEA). Preliminary classification of isolates was done based on morphology using light microscopy. In conclusion, we have found a diverse community of Actinobacteria associated to C. minutus nests. Further identification will be assessed through 16S rDNA sequencing. Also, we seek to understand the possible role of the Actinobacteria in this community and how it affects fungi that are potentially pathogenic for the ant nest.

GROWTH AND BIOCOMPATIBILITY STUDIES OF OSTEOBLAST CELLS (hFOB1.19) CULTERED ON TITANIUM Alloys WITH HYDROXYAPATITE

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Abstract

Biomaterials are frequently used as implants on bone tissue lesions. Use of titanium alloys is very common for the construction of implants due to their high strength, low weight and excellent corrosion resistance, yet they suffer from low fracture toughness and poor wear properties. In order to fulfill its function a biomaterial must osseointegrate, Previous studies have shown that calcium phosphate such as hydroxyapatite (HA) is biocompatible, non toxic and capable of binding to the bone, promoting osseointegration. Hydroxyapatite in block form has shown a low resistance fatigue, when applied to a metal substrate by plasma spray. In vitro and in vivo studies have shown that human osteoblast cells attach to titanium alloys with no apparent side effects. The main objective of this research is to study in vitro osteoblast cell growth and biocompatibility of hFOB 1.19 line cells cultivated on Titanium alloys with deposits of Hidroxiapatite at 5mT, 10mT and 15mT. MTT and SEM evaluated adhesion and proliferation guantitatively and gualitatively respectively. Preliminary Scanning Electron Microscope (SEM) data has shown that a monolayer of osteoblast cells proliferated on the surface of the alloys maximum growth and biocompability was observed in the 15mT HA deposits. The MTT assay also demonstrates viability and biocompatibility, in which the 15mT HA sample had the highest optical density and highest cell number. Results of SEM and MTT Assay suggest that coating of HA in Titanium alloys promote cell adhesion and proliferation.

DETECTION OF MICROBIAL BIOPROSPECTS FROM THE PHYTOTELMATA OF THE PITCHER PLANT NEPENTHES SP

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Abstract

Some carnivorous plants such as the genus Sarracenia and Nepenthes have an unusual and specific environment present in their pitchers known as phytotelmata. This aquatic microenvironment could serve as a habitat for bioprospecting microbes to unravel new activities with diverse applications. The main focuses of this project is the isolation and characterization of microbes (bioprospects) from Nepenthes' pitchers, and test their Biotechnological potential. Pitchers' fluid from Closed (C), Opened (O) and Semi-opened (SO) was collected and spreaded onto enriched solid media with a pH similar to the respective pitcher's fluid. The samples were incubated at 25°C and 37°C for a week. The pitcher's fluid analysis showed to be acidic, pH from 4.0 - 5.0. After three days of incubation, there was microbial growth only in the fluid coming from the opened pitchers, suggesting that there was not cultivable endemic microflora in the fluid, at the media and parameters used. The isolates were analyzed microscopically, microbiologically, and physiologically. Three basic morphotypes were found in the sample, with an optimum growth temperature of 25°C and variable pH growth intervals. Morphological features, genomic DNA extraction and molecular analyses confirm that isolates belong to fungi kingdom. To determine their specific taxonomic classification and phylogenetic relationship ribosomal region 18S rDNA, internal transcribed spacer regions and intergenic spacer regions have been amplified and sequenced for in silico analysis. Preliminary Basic Local Alignment Search Tool (BLAST) has link some isolates to high-level mannosylerythritol lipids producer yeasts, a group that have been used for ecological restoration on polluted soils.

BIOMECHANICAL PROPERTIES OF BOVINE INTERVERTEBRAL DISCS UNDER

COMBINED COMPRESSIVE AND

TORSIONAL LOADINGS

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Abstract

The importance of the human spine lies in its functions as body upright support while keeping a flexible range of motion, and as a protective structure of the spinal cord. Backbone conditions such as scoliosis and degenerative diseases such as osteoporosis jeopardize the correct functionality of the spine. Moreover, a diseased vertebral column causes abnormal stresses and reactions on the intervertebral discs (IVD) causing severe damage to the soft tissue. This study is expected to contribute to a better understanding of the deformations of fresh frozen bovine IVD under combined compression and torsional loading, with the objective of determining the elastic and shear moduli of the IVDs in the thoracic-lumbar region. It has been previously shown that calf spine is a suitable substitute for the human spine in specifically conditioned *in vitro* tests. For this study, vertebrae from T6 to T13, and from L1 to L6 were retrieved and samples were prepared without dissecting the IVD. Using custom-made equipment, measurements of angular deformation along both the compression axis and one bending axis, and vertical

displacement along the compression axis were obtained. From the resulting data, through stressstrain models, it was possible to approximate the visco-elastic properties within the linearly elastic region. The preliminary findings showed that there is a complex array of cross-linked visco-elastic fibers on the outer disc annulus, while a soft gelatinous core characterizes the disc nucleus.

ABUNDANCIA DE LA ALMEJA ASIATICA, CORBICULA FLUMINEA (MÜLLER), EN DIFERENTES SEDIMENTOS DEL EMBALSE GUAJATACA

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Abstract

La almeja asiática, *Corbicula fluminea* (Müller), especie proveniente del sureste de Asia, se introdujo en América del Norte a principios del siglo XX. El establecimiento de la almeja asiática en los sistemas acuáticos la ha convertido en una especie béntica dominante. En Puerto Rico, *C. fluminea* fue reportada por primera vez en 1998, para el río Cayey; desde entonces, no se han monitoreado las poblaciones de este organismo. El propósito de este estudio es determinar si la composición del sedimento afecta la abundancia de la almeja en el embalse Guajataca. Se establecieron nueve estaciones y se tomaron muestras, con una draga Ekman, a una profundidad de 1-3 m. Las muestras fueron cernidas con un tamiz de 0.05 mm. En el laboratorio, se contabilizaron las almejas y se determinó la composición de los sedimentos de cada estación. Los resultados preliminares sugieren que los sedimentos con mayor densidad de *C. fluminea* fueron los de composición arenosa, y que los sedimentos arenosos proveen mejor hábitat para el crecimiento y desarrollo de este organismo.

DISTRIBUTION OF INTEGRONS AMONG DIFFERENT ENVIRONMENTS IN PUERTO RICO

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Abstract

The extensive and abusive use of antibiotics over the years has created resistance to these drugs, especially in bacterial pathogens. This phenomenon has become the most important problem of infectious pathology and human health. The main concern is that the pool of genes responsible for antibiotic resistance is using a horizontal transferring mechanism to move through the environment. One of the mechanisms responsible for the transferring of genes is the mobile genetic element called integrons. With the discovery of integrons, a new field of research is open to determine antibiotic resistance on the free environment. According to this, different locations in Puerto Rico (including impacted and non-impacted zones) were sampled during the year. The samples were processed using serial dilution methods and then inoculated on TSA and R2A media with the antibiotics Cyclohexamide, Sulfadiazine and Kanamicine and incubated at 25 °C. Genomic DNA from resistant colonies was used as template for the detection of integron-based elements by PCR using universal primers. Positive strains will be further screened to detect the type of integron present by PCR. Preliminary results demonstrate that integron-based antibiotic resistance is widespread in the environment and it is not limited to impacted areas.

BIODEGRADABLE NANOCARRIERS FOR PHOTODYNAMIC THERAPY

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Abstract

Photodynamic therapy (PDT) is an increasingly used method for the treatment of oncological, cardiovascular, and ophthalmic diseases, using the concept that photosensitizers (PS) can produce singlet oxygen and other reactive oxygen species (ROS) when exposed to light in the presence of oxygen. However, the clinical use of many PS has been limited by their non-specific damage to normal tissues, by their degradation into an inactive form, by their hydrophobicity, and by other problems. Methylene blue (MB) an attractive PS candidate for PDT because of its high quantum yield of singlet oxygen generation. However, clinical use of MB is limited since it isomerizes, losing its photodynamic activity. An alternative is the use of nanocarriers loaded with PS, such as the biodegradable co-polymers consisting of PLGA and PCL. The goals of this project are to: Synthesize the nanocarriers and characterize their morphology and photodynamic activity for future applications in drug delivery for breast cancer.

NUTRIENT ANALYSIS AND ALGAL CHARACTERIZATION AT

CARTAGENA LAGOON, LAJAS, PUERTO RICO

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Abstract

Water-quality analysis was performed in three different sites at Cartagena Lagoon, Lajas, Puerto Rico. The limnological parameters used for this evaluation were dissolved oxygen (DO, mg/L=ppm), temperature (°C), ammonia-nitrogen (ppm), and phosphate (ppm). The main purpose of this experiment was to evaluate the nutrient levels and physico-chemical conditions and correlate them with algae. The high algal and cyanobacteria biomass and nutrient concentrations present at the lagoon are indicators of eutrophication. This water body had low oxygen (0.50-2.47 mg/L), and moderately high ammonia-nitrogen (0.02-0.18 ppm) and phosphate levels (0.91-1.54 ppm). The range of temperature was normal (25.1-31 °C) for a wetland. Species differentiation was performed to study the algal species that could inhabit low-oxygen environments. The most abundant species in all three sites were the protists *Cryptomonas sp., Euglena sp.*, and *Navicula* (diatom), and the cyanobacterium *Oscillatoria sp*. This project was funded by BioMinds.

TLT-1 MEDIATES PLATELET-NEUTROPHIL INTERACTION

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Abstract

Platelets are involved in hemostasis and inflammation. Platelet α-granules are secretory granules that house adhesion receptor P-selectin. Upon activation P-selectin is exposed and mediates primary contact with neutrophils or monocytes through interaction with the P-Selectin Glycoprotein Ligand (PSGL)-1. Studies with the Psel-/- mice reveal increased hemorrhage in response to inflammatory challenge, and delayed neutrophil migration demonstrating that Pselectin mediates hemostasis as well as platelet regulation of neutrophils. Recently our laboratory has cloned the Triggering Receptor Expressed in Myeloid (TREM)-Like Transcript (TLT)-1, which is also found in the platelet α -granules. Expression of TLT-1 correlates with p-selectin expression (p< 0.996). Oddly enough, characterization of tremI-/- mice also shows a phenotype similar to the Psel-/- mouse suggesting that these receptors may have overlapping or tandem function. To test this hypothesis we developed a flow cytometric assay to measure platelet-leukocyte conjugates in wild type and trem11-/- mice. Mice were treated with lipopolysaccharides (LPS) nasally and blood was taken from the retro-orbital plexus at 0, 1, 2, and 12 hours and the amounts of platelet leukocyte conjugates were analyzed by flow cytometry. At base line, there was no significant difference in platelet-neutrophil conjugates (PNC) between wild type and treml-/- mice. However, after 1 and 2 hours of LPS treatment, PNC were significantly higher over base line in wild type mice compared to null mice. In conjunction with our previously published data, this data supports the hypotheses that platelet-leukocyte conjugates are affected by the presence of TLT-1, and suggest that TLT-1 and P-selectin work in tandem to mediate leukocyte function.

DESCRIPTION OF THE PROKARYOTIC COMMUNITY IN THE BIVALVE LUCINA PECTINATA IN MARINES WETLANDS OF CABO ROJO, PUERTO RICO

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Abstract

Lucina pectinata is a large tropical bivalve belonging to family Lucinidae that lives in marines wetlands deeply burrowed in black mud with high sulfide concentration; they are mostly found in the Caribbean area. From H2S and O2 the symbiont produced energy for CO2 fixation. To described the prokaryotic communities we used different procedures such as: extraction of the endosymbiont population by percoll cushion method, analyze the samples in Flow Cytometry (FCM) and in fluorescence microscopy, also we studied the molecular part of the clam, PCR amplification and sequencing of bacterial 16S rRNA genes from lucinid gills. After analyzing the data obtained through FCM, bacteria populations with a length of 3 and 6 micrometers in length were defined. Statistical analysis were conducted to determine if there were a related difference on the size of the bacteria's according to the shell size of the clams; determine if there were related differences on the prokaryotic community according to the shell size of the clams; and determine if the number of gills (1 or 2) used for the study and the amount of populations reported in the gill structures (1-3) had a statistical difference between one and another. The results support that the symbionts size and population are independent of the clam size and the number of gills used. Furthermore, the data support the hypothesis that the presented symbiotic patter is characterize to bivalves present in environments with relatively high concentrations of hydrogen sulfide and low concentrations of oxygen.

SEARCHING FOR A GENETIC TOOL: ISOLATION OF RHODOBACTER SPHAEROIDES BACTERIOPHAGES FROM WATER SAMPLES IN PUERTO RICO

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Abstract

Rhodobacter sphaeroides is a purple non-sulfur anoxyphototrophic (PNSAP) bacterium that has one of the most diverse and versatile metabolism known, being capable of growing in a wide variety of environments and physiological conditions. While this microbe has a great application biotechnologically and has been used as model to understand photosynthesis, there are no good genetic tools for its manipulation such as transfer of genetic material thru transduction. To date, there are different types of *R. sphaeroides* bacteriophages found in Europe, North America and recently in the southwestern area of Puerto Rico. The only viral particle analyzed from Puerto Rico. reported no transductional capability under the conditions tested. Recently, several water samples from the northern region of Puerto Rico were tested for the presence of R. sphaeroides phage particles without success. The main purpose of this project is to isolate and characterize "rhodophages" from other water reservoirs in Puerto Rico. R. sphaeroides 7001 strain was used as target due to its mutagenized restriction system, allowing the infection of any viral particle present in the environment. Water samples from eleven regions in Puerto Rico were collected, and combined for faster bacteriophage screening. The bacteriophages detection in the sample involved amplification of the viral particles by succession enrichment of the water sample with R. sphaeroides 7001. An overlay assay was used to detect the plaques after the enrichments. From a total of seventeen water samples, six of them showed the presence of viral particles seen as concurrent lysis. Individual testing of the water samples indicated that the samples from Mayaguez, San German, Trujillo Alto, Coamo, Orocovis and Dorado have bacteriophages capable of infecting R. sphaeroides 7001 strain. Also the isolates showed lack of a lipidic envelope, and resistance to organic solvents. Research is in progress to determine phage forming units, and target specificity.

EFFECT OF pH MEDIA ON BRYOPHYTE GROWTH: A STUDY FOR PROTOCOL OPTIMIZATION

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Abstract

Anthropogenic disturbance has affected greatly the diversity and richness of bryophytes. In situ and ex situ plans have helped in the restoration and conservation of endangered species. With the ex situ technique, it is unknown how nutrient, illumination, sterilization and pH may affect Neotropical species. Since pH can influence the germination of spores or asexual propagules and the dedifferentiation of gametophytic modules; therefore, we studied the effect of medium pH (4, 5, 6) on spores of Octoblepharum albidum and Vesicularia vesicularis var. rutilans), gametophyte of Neckeropsis disticha, Pilotrichidium antillarum, and Pottiaceae sp. and asexual propagule of Calymperes afzelii for optimal growth according to species requirements. Secondly we studied if these species had a more specific pH requirements (4-5, 5-6). Samples were inoculated in petri dishes (according to pH treatment) in sterile medium (1/2MS), solidified with 1% phytagel and seal with parafilm. At 5-6 weeks, variation in these modules were measured and compared for each species. Species showed different patterns of growth. Among treatments, low survival limited the production of modules on the gametophyte of the Pottiaceae sp. Meanwhile, N. disticha and P. antillarum grew, but did not vary among the different pH treatments. O. albidum & C. afzelii preferred a pH of 6, while growth in V. vesicularis var. rutilans augmented at a pH of 4. On specific requirements, we observed that O. albidum grew best when pH was 5.0, and N. disticha and P. antillarum when pH was of 6.0. C. afzelii had no preference on pH 5-6. We observed difference in substrate pH and in vitro growth preference. Plant grew at all pH but optimal were obtained for only a few (V. vesicularis. & C. azelii) and were those that pH ranges in study were similar to those of substrate, but poor when substrate pH was far from the in vitro treatments.

PROYECTO ADOPTE UN GEN

Pérez D., Tascón E., Adorno V., Andujar B., Ascencio L., Bermúdez R., Cabán N., Colon K., Colon L., Crooke J., Delgado P., Fabre C., González E., Hernández Z., Irizarry L., Lithgow J., Martínez I., Ortiz D., Ortiz S., Rivera K., Rodríguez G., Rodríguez J., Shokooh S., Torres M., Torres M., Vargas K., Vélez A., Albertorio L., Paredes D., Martínez M., Plaza E., Collado X. and Curbelo F.

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Abstract

El proyecto Adopta un Gen está siendo trabajado por 30 estudiantes en el laboratorio a cargo del profesor Martínez Cruzado. El objetivo es usar información recopilada sobre los polimorfismos causantes de las enfermedades más comunes en Puerto Rico, para la síntesis de un chip para diagnósticos de éstas en neonatos puertorriqueños. Para lograrlo, los estudiantes escogemos un gen utilizando las herramientas bioinformáticas disponibles como por ejemplo OMIM, Genome Browser, dbSNP, ALFRED, Genetics Home Reference, Blast, entre otros, y artículos científicos. Cada estudiante escoge un gen, estudia su ontología, e identifica por lo menos un polimorfismo asociado a una enfermedad que se crea que pueda ser tratable en recién nacidos. El estudiante diseña un par de iniciadores (primers) que amplifique un segmento de un tamaño tal que permita secuenciarlo en una sola reacción de Sanger. El fragmento amplificado debe incluir el polimorfismo identificado y otros polimorfismos que puedan dar información sobre su origen continental. El análisis se lleva a cabo sobre muestras no relacionadas entre sí, y recolectadas por los estudiantes en todos los municipios de Puerto Rico. Las primeras amplificaciones se están realizando durante el mes de abril. Los datos en conjunto darán información sobre la frecuencia de cada uno de estos polimorfismos, su distribución geográfica y su posible origen en Puerto Rico.

CARACTERIZACIÓN DE LA DIETA DEL DIABLITO ROJO, AMPHILOPHUS LABIATUS (ACTINOPTERYGII), EN EL EMBALSE GUAJATACA

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Abstract

Amphilophus labiatus es un pez cíclido nativo de los lagos Nicaragua y Managua. Esta especie habita las zonas bénticas de cuerpos de agua dulce y tiende a ser un depredador voraz. Se alimenta mayormente de peces pequeños, caracoles, larvas de insectos, gusanos y otros organismos bénticos. El primer reporte de *A. labiatus* en Puerto Rico fue en el río Loíza para el año 2006. Hasta el momento, en la Isla, no se han estudiado los hábitos alimenticios de esta especie. El propósito de este estudio es caracterizar los organismos que forman parte de la alimentación de *A. labiatus* en el embalse Guajataca. Diecisiete individuos capturados en torneo de pesca deportivo fueron disecados. Los sistemas digestivos fueron analizados bajo microscopio y se clasificó su contenido en cinco categorías: caracoles, peces, crustáceos, insectos y otros. Se utilizó el método volumétrico para determinar el volumen total para cada categoría. Los resultados preliminares indican que *A. labiatus* se alimentará en su mayoría de caracoles, en especial *Tarebia granifera* y *Pyrgophorus* spp., los cuales son la presa béntica más abundante en este embalse.

ASSESSMENT OF PARASITISM IN *PTEROIS VOLITANS* FROM COASTAL WATERS OF PUERTO RICO

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Abstract

Pterois volitans is a Pacific species recently introduced to the western north Atlantic. The first confirmed record of this invasive fish was 1985 in the Florida Peninsula. However, during Hurricane Andrew in 1992, 6 specimens escaped from a sea-side aquarium in south Florida. After this, lionfish dispersion was confirmed (Courtenay, 1995). Pterois. volitans prey on twenty-one families of teleost fishes including Gobiidae, Labridae, Gramatidae, Apogonidae and Serranidae; and also on crustaceans (Morris, et.al., 2009). Although it has been twenty-six years since their appearance, studies about parasitism on this fish are scarce. Previous work reported monogeneans, copepods, leeches and a myxosporidean from lionfishes but only from the Pacific (Morris, et.al, 2008). The objective of this research was to determine the presence of parasites on P.volitans from the coasts of Puerto Rico. Gills, stomachs and intestines of twenty-eight lionfishes provided by the Department of Natural and Environmental Resources, collected by fishermen from the west coast of Puerto Rico, were examined. The most frequently found parasites were digeneans in the stomach and intestine. In addition, one leech from the gills and one nematode in a stomach were found. These findings made us ask further questions about lionfish parasitism including: Are *P.volitans* acquiring their parasites by opportunistic parasitism or are the parasites coming from the lionfish's prey?; Are their Pacific parasites the same species as the ones in the Atlantic? These questions are being answered in the second part of this research. A deeper research may give us an insight about food habits, health of the parasitism of these fishes in Puerto Rico and the Atlantic. We thank the DNER Fisheries Laboratory especially Ms. Noemí Peña for providing specimens and the University of Puerto Rico, Mayaguez for use of laboratory facilities and materials.

ASSESSMENT OF GENETIC DIVERSITY IN SWEETPOTATO *IPOMOEA BATATAS* L. GERMPLASM COLLECTION IN PUERTO RICO – PRELIMINARY STUDY

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Abstract

Sweetpotato *lpomoea batatas L.* ranks seventh in annual production among the food crops in the world. Sweetpotato is an important crop for its nutritional value and for its ability to adapt to different environmental conditions. It also has the potential to reduce food supply problems in the future. Sweetpotato grown in Puerto Rico is poorly understood. But due to its importance is necessary to increase the knowledge of the local varieties in Puerto Rico. A sweetpotato collection consisting of 23 samples is being cultivated at Gurabo Experimental Station, Puerto Rico. Currently this collection is been introduced to tissue culture. However, a study that analyzes the genetic diversity of this germplasm has not been conducted. In the present study, simple-sequence repeat (SSR) markers using an influorescent PCR technique will be utilized for the genetic diversity assessment of this collection. At the moment we have optimized the DNA extraction protocol in order to obtain DNA of good quality and quantity while also minimizing the time required to complete the extraction procedure. The SSR marker assessment is ongoing. Genetic diversity estimators will be analyzed after the completion of the marker amplifications. With this work we expect to expand the knowledge of the sweetpotato germplasm in order to improve the conservation management programs of this resource.

PROBING FOR INTEGRON-ENCODED ANTIBIOTIC RESISTANCE AMONG FECAL INDICATOR BACTERIA

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Abstract

Traditionally fecal bacteria have been used as indicators of water quality. Based on their detection, it is inferred that fecal contamination has occurred and that potential harmful microorganisms might be present at the tested site. However with this culture-based approach, it is not possible to evaluate inconspicuous risks to human health posed by the presence of transmissible genetic determinants that increase the pathogenic potential of their hosts. Recently, integrons, a genetic system carried by bacteria, have been implicated in the establishment and dispersal of antibiotic resistance (ABR) traits which include protection mechanisms against the most important antibiotics used for the treatment of infection in humans. To evaluate the presence of integron-encoded resistance among indicator bacteria, axenic cultures presumptively characterized as coliforms (n= 66) and enterocci (n=66) were recovered from coastal environments with a history of wastewater impact. These were submitted to an integron-targeted PCR assay for the retrieval of antibiotic resistance genes. Six coliform isolates and six enterococci strains were positive for the presence of ABR genes encoded by class 1 integrons. DNA sequencing analyses revealed the presence of genes 99-100% identical to determinants conferring resistance against beta-lactam antibiotics, trimethoprim, and guaternary ammonium compounds. Our results indicate that exposure to environments impacted by wastewater contamination may constitute a risk for human health as resistance mechanisms which compromise the effectiveness of treatment options for bacterial infections were found in viable bacteria outside healthcare settings.

MICROBIAL FUEL CELLS AS ENRICHMENT DEVICES FOR ELECTRICIGENIC MICROBES

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Abstract

Devices known as microbial fuel cells operate based on the ability of enviromental bacteria to oxidize organic matter and mediate the transfer of electrons through electrodes. These bacteria are commonly associated with anoxic sediment environments. Nevertheless, knowledge on their diversity and function is still limited. To our knowledge, electricigenic microbes have not been described in Puerto Rico and the broad biodiversity associated with tropical environments holds great promise for uncovering novel species with the potential for developing alternative energy sources. We have prepared a prototype microbial fuel cell to asses its usefulness as an enrichment device for environmental electricigens. The prototype was constructed with graphite electrodes and loaded with sediments from the Guanajibo River estuary. Voltage readings ranging from 30 to 47mV during an incubation period of 100 days were detected. Furthermore, the design of the device was optimized to minimize manipulation and facilitate handling for evaluating function during long term experiments. We expect to characterize the community associated with the anodes using DNA based methods to obtain a comprehensive vision of the dominant groups and their relationship to known electricigens.

MAGNETIC NANOPARTICLES FOR CANCER THERAPY

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Abstract

Magnetic nanoparticles represent an option to overcome limitations associated with targeting and drug delivery efficiencies in conventional chemotherapies. The goal of this research is to functionalize magnetic iron oxides with anticancer agents and/or photo-sensitizers for oncologic applications. As a first-stage, iron-oxide nanoparticles were synthesized via co-precipitation method using Sodium Hydroxide and Ammonium Hydroxide as precipitating agents. Assynthesized nanoparticles were functionalized with Oleic acid and Mercaptopropionic acid (MPA). X-Ray Diffraction measurements indicated an average crystallite size in the 7 to 12 nm range and Infrared and Raman analyses evidenced the functionalization of the magnetite surface by Oleic acid aroups and Mercaptopropionic acid (MPA). Room-temperature magnetization measurements showed that the maximum magnetization for an applied magnetic field of two Tesla varied between 37 and 67emu/g. Iron Oxide nanoparticles under 15nm and with high magnetization values have been synthesized and successfully functionalized with Oleic acid and MPA. Ongoing efforts include optimizing functionalization protocols to proceed with the development of platforms for Magnetic Drug Targeting and Photodynamic Therapies.

COMPARATIVE GENOMIC STUDIES OF INDELS IN HUMANS AND TWO ASIAN PRIMATES: PONGO ABELII AND MACACA MULATTA

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Abstract

Indels, missing or additional fragments discovered by sequence alignments between closely related species or between individuals within populations are common in the genomes of primates. Preliminary computational analysis of human (*Homo sapiens*), macaque (*Macaca mulatta*) and orangutan (*Pongo abelii*) reference genomes from NCBI revealed 84 indels in two pair wise comparisons (human to macaque, and human to orangutan). The objective of this study is to validate the existence of these fragments *in vitro*. Specifically, we aligned homologuous flanking regions of 400 base pairs using MUSCLE alignment program, and designed primers nested in the conserved sequences within these regions. Finally, we amplified fragments under investigation and visualized them on agarose gels. From a total of 84 indels, 77 were from human-macaque comparison, of which 38 were confirmed, 12 were not validated, and 27 are still in progress; all six indels for orangutan have been confirmed.

These results suggest that computational analysis alone is not sufficient for the discovery of insertions and deletions, and laboratory validation is required. The discovered indels will be good candidates for the search of disease variants in humans. Most importantly, these features most likely are part of what makes humans different from primates. The next step in this study will be to look for ancestral and derived polymorphisms in human populations and in other primate species.



Undergraduate Research Symposium 2011 Department of Biology UPR-Mayagüez



Search, Isolation and Characterization of Radiation Resistant Bioprospects from Extreme Environments in Puerto Rico

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Abstract

Since the discovery in 1956 of the bacterium Deinococcus radiodurans, this polyextremophile has been studied due to its ability to survive in extreme environments, and has served as a model in the study of other radiation resistant microorganisms. These studies could be key to develop new biotechnological strategies, such as bioremediation of extremely contaminated and highly toxic environments, and new therapeutic techniques for cancer and other serious illnesses. This research project involves the search for UV radiation resistant microbes from hypersaline and mineral rich soils, and microbial mats. The samples were serially diluted and spread on culture media. The patched isolates were exposed to UV-C radiation for periods ranging from 5 to 45 minutes. A total of 18 radiation-resistant-candidates (RRCs) were obtained from the plates exposed to 10 and 15 min. A total of 16 bioprospects were isolated from the hypersaline soil samples, and 2 from the mineral rich soil samples. Gram staining performed to some of the isolates showed the presence of gram-positive and gramnegative rods. Further exposure to UV-C radiation confirmed the radiation resistance of the isolates, and DNA extraction and 16SrDNA amplification was performed to molecularly characterize the RRCs. The In-silico analysis showed that the RRCs belong to Pseudomonas, Enterobacter and Shewanella genera. The presence of endospores and tests for resistance to UV-A and UV-B radiation are the next steps using spore staining and a solar simulator. The isolation of radiation resistant microorganisms that are found in extreme environments such as hypersaline and mineral rich soils could indicate that these microorganisms are also polyextremophiles.

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DETERMINACION DE LA PRESENCIA DE TLT-1 EN EL CEREBRO DE RATON

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Abstract

El receptor activador expresado en células mieloides como transcripto 1 (TLT-1), es una proteína presente en los alfa gránulos de plaquetas en reposo y en la superficie de plaquetas activadas. TLT-1 desempeña un importante papel en la homeostasis vascular. El objetivo de este proyecto es determinar la presencia de TLT-1 en cerebro de ratones puesto que ratones nulos para esta proteína presentan un grado de agresividad mayor, lo que sugiere que la expresión de TLT-1 debe desempeñar una función importante en la conducta de los ratones. Se extrajeron los cerebros de ratones nulos y silvestres, se cortaron las diferentes regiones de los cerebros y cada una de estas regiones fue lisada. Para comprobar la presencia de TLT-1 en estas regiones se realizó western blot y se utilizó mTLT-1 como anticuerpo específico. Se encontró que TLT-1 está presente en todas las regiones del cerebro de ratón, encontrándose en mayor concentración en el cerebelo y la medula.

SONG SHARING IN TWO NEIGHBORHOODS OF ADELAIDE'S WARBLERS (DENDROICA ADELAIDAE)

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Abstract

Young birds usually learn songs by copying neighbors and other adults. As a result, many territorial birds share at least a part of their repertoire with their neighbors. Generally, there is a negative correlation between distance and degree of song sharing between territories. As a preliminary study to determine the adaptive advantages of this phenomenon, we compared the extent of song sharing within and between two neighborhoods of Adelaide's Warblers (*Dendroica adelaidae*) in the Cabo Rojo Wildlife Refuge, Puerto Rico. We recorded songs from three birds in each neighborhood to measure song type sharing. We predicted that song sharing would be greater within neighborhoods than between neighborhoods, and that it would fall off with linear distance.

EFFECTS OF FIRE ON LEAF PHENOLOGY OF BUCIDA BUCERAS, A NATIVE DRY FOREST TREE IN PUERTO RICO

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Tropical dry forests are one of the most threatened forest types worldwide. These unique ecosystems have a history of disturbance due to human activity, invasive species and fire. Periodic human-caused forest fires have both direct and indirect negative effects on dry forests. Fires decrease native tree cover, kill native seedlings, alter nutrient dynamics in the soil and increase dominance of invasive grasses. However, in some dry forest sites that experience frequent human-caused fires, a few large individual native trees are able to persist and survive repeated fires. No previous work has addressed the effect of these periodic fires on the long-term longevity and health of trees inhabiting these sites. We sought to determine whether the frequent, periodic fires of a disturbed site dominated by an invasive grass can have a significant effect on leaf phenology, longevity and productivity of the native tree Bucida buceras (Combretaceae), úcar, found in the Guánica Dry Forest in Puerto Rico. We haphazardly selected 10 trees from a burned area of the forest in which frequent fires occur (every 1-2 years) and another 10 trees from an unburned area that, although also dominated by invasive grasses, had not burned in >20 years. We then tagged five branches in each of these trees and on these branches counted new and old leaves during February and March 2011. The timing of new leaf production differed between burned and unburned sites. New leaf production in the burned sites began up to a month earlier in the burned site when compared to the unburned. This difference in phenology resulted in trees in the burned sites producing leaves earlier in the dry season which may subject these trees to longer periods of drought stress and potentially could affect their wellness and survival.

THE EVOLUTION OF VOCAL DUETTING IN PERCHING BIRDS

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Abstract

Vocal duetting in birds is taxonomically widespread, but still poorly understood. It occurs in over 400 species, representing 40% of bird families. A barrier to understanding the evolutionary causes of duetting is that previous studies have focused on only one species at a time. Although this technique can teach us a lot about the current function of duetting, it does not provide a compelling answer to the question, "under what ecological conditions will duetting evolve?" We use the comparative method to address this question in the perching birds (Order: Passeriformes). This involves identifying passerine species that duet, pairing each of them with related non-duetting species, and using the computer program Mesquite to reconstruct ancestral traits and analyze evolutionary hypotheses. This final step involves correlating the evolution of duetting with ecological variables that evolve simultaneously with this trait, such as latitude. I will discuss our goals, demonstrate our methods, and detail our progress so far in this long-term collaborative project.

DEVELOPMENT OF ANAEROBIC MICROCOSMS CAPABLE OF BIOTRANSFORMING ALGINATE INTO THE ALTERNATIVE RENEWABLE ENERGY SOURCE METHANE

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Abstract

The anaerobic digestion of organic matter including marine algae to obtain biogas as a biofuel is one of the possible alternatives to produce a clean, sustainable, and environmentally friendly energy source. This is extremely relevant for sub-Tropical islands, like Puerto Rico, that do not have fossil fuels and for those that are concerned with the ever limiting resources and the pollution associated with the fossil fuel industry. Using anaerobic microcosms containing 1% of blended marine algae mixtures or alginate as sole carbon and energy source we determined the capacity of each microbial community from three different natural habitats to biotransform this substrates into methane. The product of metabolism, methane, was quantified and monitored through time using a GC. Our results, after 56 days, indicate that all the sediments harbor microorganisms that are capable of producing biogas from alginate. The microcosms containing sediments from the mechanic shop produce the highest amount of methane followed by the sediments from a coffee residue oxidation pond and the microcosms with rumen as inoculum produced the least amount. The amount of methane produced by the microbial community from the mechanic shop sediments was 60 to 76% more than the produced by the other two microcosms respectively. In conclusion, the polysaccharide alginate could be used to produce a

renewable energy source but the particular microbial community needed to carryout the efficient transformations are not found everywhere in nature. More importantly, this research could result in the description of the first anaerobic bacterium that is capable to degrade alginate.

ABUNDANCIA Y RIQUEZA DE MOLUSCOS ASOCIADOS A BOSOUES DE SPATHODEA CAMPANULATA EN ZONA ALUVIAL Y KARSTICO

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La agricultura y el desarrollo industrial en Puerto Rico provocaron cambios en los usos de terrenos y en la ecología de la isla. Los cultivos de la caña de azúcar en suelos aluviales y del café y los cítricos en suelos kársticos son ejemplos de cambios en los terrenos. Con el desarrollo industrial estos cultivos fueron abandonados permitiendo así la colonización de especies como *Spathodea campanulata* y surgiendo nuevos bosques secundarios. La contribución ecológica de estos bosques ha sido evaluada para plantas vasculares, briófitos y algunos invertebrados. Sin embargo, no conocemos si estos bosques secundarios proveen un hábitat para otros organismos como los moluscos. En el centro de la isla muestreamos dos localidades, Cibuco y Pugnado, con diferente uso de terreno: suelo aluvial y kárstico. En cada localidad se trazó un transecto en el interior y otro en el exterior de 20 m x 1 m y se documentó todo individuo en esta área. Encontramos 11 especies para ambos bosques; siendo *Caracolus sp., Polydontes sp. y Megalostoma sp.* los que presentaron la mayor cantidad de individuos. En el área muestreada en Pugnado encontramos 346 individuos y en Cibuco 222; sin embargo no es estadísticamente diferente. Cibuco mostró mayor riqueza en el exterior que en el interior mientras que Pugnado fue a la inversa. Entre los bosques, el kárstico (Pugnado) mostró mayor riqueza que el aluvial (Cibuco). Bosques de *Spathodea campanulata* regenerados a partir de plantaciones de café y eítricos sostienen mayor riqueza de las plantaciones de caña de azúcar.

EFFECT OF sTLT-1 ON R848-ACTIVATED NEUTROPHILS

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Abstract

Toll-like receptors (TLR) are receptors that play an important role in innate immunity by recognizing molecular patterns found in pathogens. TLR7 and TLR8 are key receptors that recognize single-stranded RNA viruses. Following viral stimulation, the TLRs induce polymorphonuclear neutrophils (PMN) to produce inflammatory cytokines promoting the immune response, which may lead to tissue injury if not properly regulated. TREM-like transcript-1 (TLT-1) is a receptor localized in the alpha-granules of platelets that relocates to the surface upon platelet activation and regulates the interface of inflammatory and hemostatic responses. Platelets also release a soluble fragment, sTLT-1 upon activation. The exact role of sTLT-1, however, is not known. In a clinical study conducted in Puerto Rico, patients diagnosed with viral syndrome (dengue, west nile, etc) demonstrated a correlation between high neutrophil counts and elevated soluble TLT-1 (sTLT-1), suggesting that sTLT-1 may affect neutrophil function. The aim of this project is to determine if sTLT-1 inhibits TLR7 activation of neutrophils. Human peripheral blood neutrophils were stimulated with imidazoquinoline resiguimod (R-848), a synthetic agonist of TLR7/TLR8. Neutrophil activation was determined by measurement of established neutrophil activation markers such as CD66B, CD35, CD11b, and IL-8 by flow cytometry. We expect that sTLT-1 will inhibit the expression of these TLR7 induced markers in neutrophils suggesting that sTLT-1 is an important factor in decreasing the neutrophilic response against viral infections. Here we show the status of these ongoing studies.

IDENTIFICATION OF FUNGI ASSOCIATED WITH Nasutitermes TERMITE NESTS

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Abstract

Termites are insects that have shown to be highly susceptible to fungi, for example Aspergillus and *Pecilomyces* species found in termite nests are thought to be entomophagous, and there is a possibility that some might also be entomopathogenic. Preliminary studies in Nasutitermes nests have shown that Aspergillus species compete with other fungal species and bacteria that may inhibit fungal growth. Our goal is to find which species of fungi are present in Nasutitermes nests in a tropical environment and what ecological role they play in them. Samples of three termite nests from Miradero, Mayagüez were cut using a handsaw sterilized with 70% ethanol and placed in sterilized plastic containers. Once in the laboratory, the samples were diluted with sterilized distilled water in three tubes (one for each nest), which were later plated in Potato Dextrose Agar (PDA) medium. In other three media plates (for each nest) of PDA, pieces of the termite nest were scattered. After a period of 5-10 days of incubation, the isolated fungi were purified by transferring to PDA and Malt Extract Agar (MEA) until there were no contaminants. For purposes of this project we focus only in one sampled nest. We isolated a total of 34 fungal cultures. Our results show that fungi in the genera Aspergillus and Penicillium are predominant in our isolations from the termite nest alongside other fungi to be yet identified. After completing isolation of fungi from all sampled nests, identification through light microscopy and characterization by ITS rDNA gene sequencing will be performed; as well as bioassay confrontations in vitro to established potential ecological roles.

CLASS SIZE AND INFESTATION RELATIONSHIP BETWEEN ENTEROBRYUS HALOPHILUS (OPISTOKONTA: ICHTHYOSPOREA) AND MOLECRAB EMERITA PORTORICENSIS (CRUSTACEA: ANOMURA)

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Abstract

In Puerto Rico, the symbiont protist Enterobryus halophilus (Opistokonta: Ichthyosporea) has been reported associated to the hindgut of the molecrab Emerita portoricensis (Crustacea: Anomura). A study of prevalence in populations of *E. halophilus* is being conducted to determine if a class size dependent infection exists. We collected samples in Mani Beach, Mayagüez, Puerto Rico, which extends between Añasco River and Punta Algarrobo for about 4.6 km. The beach is located near the port of Mayagüez and it has been affected directly by pollution. Emerita portoricensis lives in aggregations, which appear as groups in the intertidal zone. Recruits (3-4 mm males, 3-5 mm females) are often found 5 meters away from the surf zone receiving only some of the swash. Adults are found living in the surf zone, where they filter-feed in the currents caused by the impact of incoming waves. A class size dependent infection is expected due to the fact that adults are exposed to protist spores floating in the environment for longer periods of time comparing to the juveniles. Emerita portoricensis individuals (39) were collected through a standardized random sampling method. A 7 meter transect was divided into 3 sampling zones, starting at 7, 5 and 0 meters from the surf zone. Each molecrab was sexed, measured and, dissected to establish the presence of E. halophilus. The hindgut was mounted in slides with distilled water and later fixed by infiltrating lactophenol-cotton blue 0.5%. Presence/absence of the symbiont was studied under light microscopy and recorded. After 5 weeks of sampling, preliminary data indicate that there is a prevalence of female adults in the overall population (of that which was sampled). Adults have a prevalence of 73% while juveniles present 17%. The current data is not sufficient for a statistical analysis yet. We will continue collecting data to validate the results of this study.