BIOLOGICAL SCIENCES DIVISION

BSD-1

GENETIC CHARACTERIZATION OF PILI (*Canarium ovatum* Engl.) FROM ALBAY, CAMARINES NORTE AND CAMARINES SUR, PHILIPPINES THROUGH ISOZYME ANALYSIS

Merlyn S. Mendioro*, Ma. Genaleen Q. Diaz,
Vera Marjorie E. Velasco, Maylynn C. Alcaraz, Roselle C. Lalamunan,
Karen G. Amoloza and, Luisa Villamael

Genetics and Molecular Biology Division,
Institute of Biological Sciences
College of Arts and Sciences, University of the Philippines
Los Banos College, Laguna
Tel: 049 5363368; Fax: 049 5362517; Email: msmendioro@yahoo.com

Based on esterase (EST), acid phosphatase (ACP), alkaline phosphatase (ALP), genetic variability was noted in nineteen accessions of pili (*Canarium ovatum* Engl.) from Albay, Camarines Norte, and Camarines Sur and in 11 accessions of unknown origin. Seven presumptive loci were determined. Two presumptive loci were observed in EST (EST1 and EST2), ACP (ACP1 and ACP2) and three for ALP (ALP1, ALP2, and ALP3). Percent polymorphism was 100%. Using a similarity coefficient of 0.60, the thirty accessions were divided into five clusters. Accessions of different origin grouped together which would indicate that variability exists in the accessions considered. To further prove that genetic diversity exists in pili, twenty five accessions collected from Oas, Albay were also subjected to isozyme analysis. Eighteen presumptive loci were determined: three for glucose-6-phosphate dehydrogenase (G6PD), two each for EST, phosphogluconate dehydrogenase (PGD), malate dehydrogenase (MDH), ACP, ALP, and phosphoglucomutase (PGM), and one each for glutamate oxaloacetate (GOT), phosphoglucoisomerase (PGI), and alcohol dehydrogenase (ADH). Only ALP2, G6PD2 and G6PD3 were monomorphic. The observed heterozygosity for ACP1, ACP2, ALP1, EST1, EST2, and PGD2 was higher compared to the expected heterozygosity. Fifteen of the presumptive loci were polymorphic (83.33%). Considering a similarity coefficient of 0.70, four clusters were obtained although the 25 accessions were collected only from a single location, Oas, Albay. This would indicate that accessions were genetically different. Pili being dioecious, is an obligate cross-pollinating crop. Genetic variability observed can be explained through recombination occurring during sexual reproduction.

Keywords: *Canarium ovatum* Engl., isozyme analysis, pili, polymorphism, genetic variability

BSD-2

GENETIC DIVERSITY OF *Brassica rapa chinensis*, *B. rapa parachinensis* AND *B. oleracea alboglabra* USING SIMPLE SEQUENCE REPEATS

Stephanie U. Celucia[1], Neilyn O. Villa[1*] and Robert dela Peña[2]

[1]Genetics and Molecular Biology Division, Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna, Philippines
Tel: 0918 628 3949; Email: neilyn24@yahoo.com
[2]Asian Vegetable Research and Development Center, Taiwan

The genetic diversity of 39 accessions of *Brassica rapa chinensis*, 28 accessions of *B. rapa parachinensis*, and 29 accessions of *B. oleracea alboglabra* was studied. Twelve primers were used and produced 122 scorable bands in which 77 were polymorphic. The average of polymorphic loci was 71.08%. Genetic diversity among the species was very high indicating great genetic differences within each species. Geographical origin of the species greatly affects genetic diversity. The phylogenetic tree showed that *B. rapa chinensis* and *B. rapa parachinensis* are genetically closely related to each other. Also, greater genetic diversity was shown within *B. oleracea alboglabra* species compared to *B. rapa chinensis* and *B. rapa parachinensis* species. Theses results provide useful information for crop improvement and in properly selecting parents of crosses to produce agronomically improved lines.

Keywords: genetic diversity, SSR, microsatellite markers, *Brassica rapa chinensis*, *B. rapa parachinensis*, *B. oleracea alboglabra*

BSD-3

LIQUID NITROGEN-FREE EXTRACTION PROCEDURE FOR *Penicillium chrysogenum* and *Saccharomyces cereviseae* GENOMIC DNA ISOLATION

Franco G. Teves[1*], Elma B. Quiao[1], Shiela Dagondon[1], and Lydia M. Bajo[2]

[1*] Molecular Genetics and Microbial Technology Laboratory, Department of Biological Sciences, Telefax: (063) 351 6131; Email: franco_teves@yahoo.com
[2] Department of Chemistry, College of Science and Mathematics,
MSU-Iligan Institute of Technology, 9200 Iligan City

Nucleic acid extraction and purification are crucial steps in molecular biology experiments. Disruption of eukaryotic cells with cell walls, or tissues, is usually initiated using liquid nitrogen which quickly freezes samples, protecting nucleic acids from being degraded by non-specific nucleases, and making cells or tissues brittle, paving the way for mechanical breakage of cell walls. Other reagents are then added to prevent nucleic acid degradation, especially for ribonucleic acid (RNA), which easily undergoes digestion by very stable contaminating ribonucleases.

A genomic DNA isolation protocol was modified, originally designed for isolating plant genomic DNA for the isolation of fungal genomic DNA without liquid nitrogen. By eliminating this reagent in the modified DNA extraction procedure, genomic DNA from *Penicillium chrysogenum* and *Saccharomyces cereviseae* was obtained with comparable quality and concentration when compared to using liquid nitrogen. The essential modifications include freezing and/or cooling of all materials and reagents prior to use, use of modified lysis buffer, and the utilization of acid-washed sand for cellular disruption. The quality of the DNA has been shown to be at par with DNA obtained with the aid of liquid nitrogen, when run in agarose gel electrophoresis and used in cloning experiments. Liquid nitrogen is a very scarce chemical especially in Third World countries, including the Philippines, requires great care in storage and handling, and is relatively expensive. Thus, an additional benefit of using this protocol is cost reduction in generating genomic DNA for various molecular biology applications.

Keywords: nucleic acid, liquid nitrogen, genomic DNA, *Penicillium chrysogenum*, *Saccharomyces cereviseae*, agarose gel electrophoresis

BSD-4

ONTOGENETIC PATTERN OF DOUBLE-STRANDED FREE NUCLEIC ACID (DS-FNA) FROM THE WATER OF COCONUT (*Cocos nucifera* L.)

Marni E. Cueno*, Karlo Mari C. Paulino and Rita P. Laude

Institute of Biological Science, College of Arts and Sciences
University of the Philippines Los Baños, College, Laguna 4031
Tel: 495362893

Previous reports have indicated that the mRNA transcripts involved in fatty acid synthesis in coconut are expressed ontogenetically in the coconut solid endosperm. In an attempt to determine whether a similar ontogenetic pattern of mRNA transcripts occurs in coconut liquid endosperm or coconut water, free nucleic acid (FNA) samples from leaf rank (LR) 14-22 drupes were extracted and concentrations of each FNA samples were also quantified. Initially, the presence of double stranded free nucleic acid (DS-FNA) was established using FNA samples obtained through ammonium acetate extraction. DS-FNA was detected in LR 15 but absent in LR 20. DS-FNA from crude coconut water of LR 14-22 were extracted and showed that only LR 19 sample gave the highest concentration estimated to be 1ng/iL. To properly establish the ontogenetic pattern of DS-FNA, concentrated coconut water from LR 14-22 were used. A gradual increase in DS-FNA was observed from LR 14 to LR 19, with an estimated amount of 0.4 ng/iL and 9.0 ng/iL, respectively. After reaching its peak (LR 19), a sudden decrease at LR 20 (0.4 ng/iL) was noted but no DS-FNA was detected in LR 21-22. These novel results indicate for the first time that DS-FNA is present in coconut water and the amount of DS-FNA is affected by the ontogenetic development of the drupe.

Keywords: free nucleic acid (FNA), double stranded-free nucleic acid (DS-FNA), coconut water, ammonium acetate extraction, *Cocos nucifera* L..

BSD-5

CORONA AND POLLINARIA DIVERSITY IN PHILIPPINE HOYAS

Simeona V. Siar, Edwin E. del Rosario and Jennelyn M. Carandang

Crop Genetics and Plant Breeding Division,
Crop Science Cluster-Institute of Plant Breeding,
College of Agriculture, University of the Philippines Los Baños,
College, Laguna 4031,
Tel: 049 5363304; Fax: 049 5363438; Email: moninasiar@yahoo.com

The Philippines possesses one of the richest and most diverse range of hoya species in the world. Following the 2003 Angiosperm Phylogeny Group, hoya belongs to family *Apocynaceae* (Gentiales, Asterids). The plant is characterized by shiny waxy leaves, hence the common name 'wax plant'. Most of the hoya species have a climbing or viny habit, however some species are short and bushy. The flowers are so striking and beautiful resembling a star with sweet lemony fragrance especially at night.

Thirteen (13) Hoya species were characterized for genetic diversity in terms of some floral traits. Mature inflorescence were collected from each species and examined under the microscope. Sample flowers were measured for the following characters: corona length and width, pollinarium length and width and retinaculum length and width. These floral traits are used by taxonomists and botanists in identifying and delineating hoya species.

Results showed wide variation as well as close relationship among species in terms of the parameters tested. *Hoya pubicalyx* and *Hoya coriacea* had the largest values for corona, pollinium and retinaculum measurements. The lowest values for most traits were observed in *Hoya obscura*. A dendogram was generated illustrating the clustering of hoya species into distinct genetic groupings. *Hoya coriacea* and *Hoya obscura* were the most genetically distinct pair of Hoya species, while close genetic similarity was observed between *Hoya bicolor* and *Hoya crassicaulis*. The results have provided additional information for research workers and hobbyists useful for breeding, taxonomic identifications and future investigations.

Keywords: *Apocynaceae*, hoya, genetic diversity, variation, cluster analysis, dendrogram

BSD-6

ELLIPTIC FOURIER SHAPE ANALYSIS OF THE LEAVES AND LEAFLETS OF THE FAMILY LEGUMINOSAE: IMPLICATIONS IN THE SYSTEMATICS AND EVOLUTION OF THE SPECIES

Cesar G. Demayo, Mark Anthony J. Torres, and Muhmin Michael E. Manting

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Leaf and leaflet shape variability was assessed in a total of forty-one (41) species of legumes, belonging to twenty-four genera and three subfamilies using the method of Elliptic Fourier Analysis. Digital images of the leaves were used, which were converted to black and white using an image processing software. The outlines of the leaves and leaflets were then summarized using chain codes. Mathematical algorithms were applied on the chain code data to generate a total of eighty shape variables which were used to reconstruct the mean leaf and leaflet shapes for all species. On the other hand, the extent and degree of leaf shape variability within and between species belonging to the same subfamily was assessed using the method of Principal Component Analysis. Results showed that the major shape variations observable within all species of legumes were associated with changes in the length-to-width ratios and curvatures of the middle part of the leaves and leaflets among others. Ordination of the different species showed that some of the families are composed of species with varying leaf and leaflet shapes such as that of the subfamily Caesalpinioideae. This result is in congruence with previous studies asserting that this subfamily is a heterogeneous unit and is not a proper phylogenetic unit. This is because some species have leaves and leaflets that are similar in shape with those of particular members of one of the other subfamilies, than they have with the other members of this subfamily. The results of this study are further discussed in the light of the diversification and evolution of this group of plants as well as with the taxonomic classification of already known and unknown species included in this study.

Keywords: Elliptic Fourier analysis, legumes, evolution, Systematics

BSD-7

APPLICATIONS OF FRACTAL DIMENSIONS IN ASSESSING VARIATIONS AMONG TWENTY-FIVE SPECIES OF LEGUMES FROM ILIGAN CITY, PHILIPPINES

Cesar G. Demayo, Mark Anthony J. Torres and Muhmin Michael E. Manting

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

In this study, the geometry of the leaves of twenty-four (24) species of legumes including three unknown species collected from Iligan City, Philippines were compared and quantified through Fractal analysis using the box counting method. Using this method, images of the leaves and leaflets were covered by a sequence of grids made of squares of ascending sizes. For each grid, two values were recorded: the number of squares intersected by the image, $N(s)$, and the side length of the squares, s. The regression slope (D) of the straight line formed by plotting $\log[N(s)]$ against $\log(1/s)$ indicating the degree of complexity, or the Fractal Dimension (FD), the values of which range from 1 to 2. The FD values of the different species were then compared through box-and-whisker plots and analyzed using Principal Component Analysis. Results showed interspecific differences in the geometry of the leaves and leaflets as explained by Principal Component 1. The results of this study showed that the different species have varying levels of interspecific and intraspecific morphological diversity.

Keywords: Fractal Dimension, legumes, box-counting, Principal Component Analysis.

BSD-8

LEAF VENATION ARCHITECTURE OF TWO VARIETIES OF *Lantana camara* L. FROM CAMIGUIN ISLAND, PHILIPPINES

Mark Anthony J. Torres, Cesar G. Demayo, Muhmin Michael E. Manting, Edmar Taray, Shiela D. Obnimaga, Jennifer Dinganon, Joanne Aberilla, Ajquizar T. Baser, and Angela Marie Cariaga

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

The leaves of two varieties of *Lantana camara* L. vary in shape even within a single plant. This feature could have a direct influence on the venation architecture of this species. Owing to its importance for systematic classification, attention is paid largely in this study to the architectural properties among leaves of this species that have different shapes. Such studies are important as venation geometry and density influences the mechanical stability of the leaves and may affect such properties as susceptibility to herbivory. Thus, the aim of this study is determine the extent of intra- and intervarietal variation in venation patterns as well as phenotypic plasticity of some venation properties, such as venation density, vein angles and intervein distances. To do this, leaves from two varieties of *L. camara* L. that are common in an area located several kilometers uphill from Catarman and in Balintawak in Camiguin Island, Philippines were collected. The samples were scanned and several aspects of leaf venation patterns were measured such as the angle between the 1° and 2° veins and intervein distances using the ImageTool software. The data were treated with multivariate methods of statistical analysis such as Principal Coordinate Analysis (PCo) to determine how different the venational properties of the leaves are. The results showed no populational differences in leaf architecture indicating that geographic distance is not a factor affecting the leaf venation geometry. The results of this study, however, showed a high extent of individual variation. The number of secondary veins also differs between leaf samples among the leaves of a single plant which also influences intervein distances. Moreover, certain venational features are shown to be associated with particular leaf shapes. The results of this study are interpreted in the context of ecophysiology and the possible relationship between form and function of the leaf venation system of this species of plant.

Keywords: venation, *Lantana camara*, vein angle, intervein angle

BSD-9

SEX DIFFERENCE IN THE SIZE AND SHAPE OFTHE WINGS IN THE DAMSELFLY, *Pseudagrion Pilidorsum Pilidorsum*

Mark Anthony J. Torres, Cesar G. Demayo and Russel T. Yu

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Patterns of distribution of damselflies differ by sex, with males clumped in or near water bodies and the females usually found away from the water body. Previous studies on other species of damselflies have shown that these differences are reflected as differences in the sizes of the wings between the two sexes. In this study, differences in the size and shapes of the wings between the two sexes of the damselfly *Pseudagrion pilidorsum pilidorsum* was assessed using image analysis and landmark-based analysis. To do this, images of the wings were scanned at uniform dpi. Then, the Cartesian coordinates of twenty-five landmarks from around the wing were extracted using an image analysis and processing software. These coordinates were then subjected to two different analyses. Euclidean Distance Matrix Algorithm was applied on the data to generate linear distances between the landmarks. The resultant interlandmark distances were used to compare the sizes of the wings between the sexes. On the other hand, the raw coordinate data was also Procrustes-fitted to generate shape variables. These shape variables were analyzed using Relative Warps analysis to determine sex-differences in the shapes of the wings. Results showed statistically significant differences in the size of the wings between males and females. Also, tests for significant differences of the Relative scores between the sexes revealed dimorphism in wing shapes related to differences in sex. The results of this study are discussed in relation to differences in the flight ecologies of the males and females of this species.

Keywords: Euclidean Distance Matrix Algorithm (EDMA), damselfly, *Pseudagrion pilidorsum pilidorsum* relative warps, interlandmark distances

BSD-10

PARTIAL WARP ANALYSIS OF VARIABILITY IN CLAW MORPHOLOGY IN AN ADULT POPULATION OF THE LAND CRAB, *Cardisoma Carnifex*, FROM EL SALVADOR, MISAMIS ORIENTAL

Cesar G. Demayo, Mark Anthony J. Torres and Abner Paulo Sendad

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

This study was conducted to determine morphological disparities in the shapes of the claws of the land crab, *Cardisoma* canifex from a single adult population. The method of partial warp analysis was employed in this study using landmarks sets that are placed on homologous structures in the claws. Then, descriptors of the shapes of the crabs were generated via a Procrustes-fitting algorithm applied on the landmark data. Scatter plots were generated using the partial warp scores calculated from the Procrustes-fitted coordinate data to be able to determine variations in the shapes of the claw. Transformation grids were also generated to visualize patterns of claw shape variations within this population of crabs. Results revealed the existence of two morphs within the population that differ in the shape of the claws. The result of this study is discussed in the light of differences in the feeding habits of these two morphotypes.

Keywords: Partial Warp Analysis, Transformation grids, land crabs, *Cardisoma* canifex, morphotypes

BSD-11

SHAPE AND COLORMORPHISM IN THE SWORDTAIL FISH, *Xiphophorus Helleri* (CYPRINODONTIFORMES: POECILIIDAE): ANY EVOLUTIONARY CONNECTION?

Cesar G. Demayo, Mark Anthony J. Torres and and Edgar Vincent Quitos

Department of Biological Sciences, College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

This study was conducted to determine the extent of morphological variation in the common freshwater fish *Xiphophorus helleri* using landmark-based analysis of the truss network. A total of fifteen landmarks were chosen, the Cartesian coordinates of which were collected using an image analysis software. The fish truss networks were aligned using Procrustes fitting which eliminated size and rotational translation. The shape residuals left after the Procrustes fitting were then subjected to thin-plate spline grid analysis which allowed comparison of the mean shapes of the male and female fishes. The samples collected from Lawis, Burrun, Iligan City showed the existence of two adult color morphs, namely green and red. Thus, shape variation between these two color morphs was also assessed. Ordination of the fish samples was also done using the relative warp scores and partial warp scores computed using the shape residuals. Sizes of the fishes were also measured and compared using the Euclidean distance matrix algorithm which returned a matrix of interlandmark distances. Result showed overlapping of the convex hulls in the shape space after ordination of all samples. However, when the shape and size residuals were separately subjected to multivariate analysis of variance, the result showed significant differences between the males and the females and between the two color morphs. Subsequent tests for significant mean differences using Hotelling's t^2 test and discriminant function analysis also gave concordant results. The results of this study revealed sexual dimorphism both in shape and size in this species of fish. Morphological disparity between the two colormorphs is discussed in the light of evolution.

Keywords: procrustes, ordination, discriminant analysis, *Xiphophorus helleri*

BSD-12

LANDMARK-BASED ANALYSES OF SEXUAL DIMORPHISM IN SELECTED SPECIES OF *Sardinella*

Cesar G. Demayo, Mark Anthony J. Torres and Jay B. Mag-usara

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Landmark-based analysis of the fish truss network in three species of *Sardinella* was performed to identify sexually dimorphic features. A total of ten landmarks that are distributed along the length of the fishes were identified. Differences in the shapes of the two sexes were analyzed over the procrustes-fitted data using thin-plate spline grids, relative warps and partial warp scores. Sexual size dimorphism was also assessed using Euclidean distance matrix algorithm. Multivariate methods of statistical analyses were also performed to supplement the different analyses. Results showed that only a few landmarks are sexually dimorphic. These are the landmarks associated with the caudal fin, dorsal fin and the snout. Also, results of the EDMA analyses revealed sexual size dimorphism among the three species. These results are consistent with the results of other studies arguing for ecological divergence between the two sexes.

Keywords: Sexual dimorphism, EDMA, Procrustes, *Sardinella*

BSD-13

PHENOTYPIC PLASTICITY, SEXUAL SELECTION AND THE EVOLUTION OF MALE COLOR PATTERNS IN A POPULATION OF GUPPIES FROM STO. NIÑO COLD SPRING, CAMIGUIN ISLAND, PHILIPPINES

Mark Anthony J. Torres, , Alfand Dy Closas, Hanna Daud
Jessa Mampao, Lois Lumontod, Daryl Sencil and Cesar G. Demayo

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

The guppy, *Poecilia reticulata*, is a live-bearing fish and is one of the most popular freshwater aquarium fish species in the world. There is a great deal of variation in the color patterns in male members of this species across geographic populations. The most common observation is that male guppies that live in populations saturated with predators tend to be dull and subdued in color while those that live in populations with fewer predators are more colorful. This is because two selection regimes operate on populations of guppies – vividly colored guppies are favored by sexual selection while vaguely colored guppies are favored by natural selection through predation.

A pool of cold spring water sprouting from a sandy stratum located 4 kilometers north uphill of Catarman, Camiguin in the Philippines hosts a population of guppies of diverse coloration. The pool which measures 25 m x 40 m makes the population of guppies in it isolated from the rest of the water system. Visual census also showed that the fish community is dominated by guppies. Thus, this reproductively isolated population offers opportunity to study the relative importance of sexual selection through female choice on male guppy coloration.

A total of ninety-two male guppies were examined and the color pattern of which were quantified. Each spot was measured for its size and area and assigned to a color category. Other complementary measures of color pattern were also assessed such as the total number of spots of a given color, the total area of the body covered by spots of a given color, and the mean relative area of each spot color (MRE). Results showed that male color patterns in this population of guppies vary in color, size and position. The colors fall into three basic categories: carotenoids pigments, melanic pigments and structural colors. Both yellow (MRE of 2.73 mm^2; 32.05%) and black (MRE of 3.50 mm^2; 41%) colors are dominant among the males indicating that female mate choice in this population of guppies has favored the appearance of such colors. Cluster analysis showed that no two males have exactly the same color pattern which suggests that a mechanism must exist in the population that helps maintain a diverse color pattern among the males.

Keywords: guppy, *Poecilia reticulata*, phenotypic plasticity, sexual selection, color patterns

BSD-14

EGG AND PREZOEAL STAGES OF A SAND CRAB, *Emerita* sp. FROM MAGOONG, LINAMON, ILIGAN CITY

Mark Anthony J. Torres, Siegfried Hector Razo and Cesar G. Demayo

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

The egg and prezoeal stages of sand crabs, Emerita sp., from a population in Magoong, Linamon, Iligan City were collected from gravid females and examined using a stereomicroscope at 3X magnification. Gravid females were held in a large aquarium supplied with salt water and filled with sand collected from the study area. Photographs of samples that were taken from different adults and represented different stages of prezoeal development were examined. Results showed that females are able to produce egg masses that may contain several viable eggs. As the young (larvae) develop, the egg passes through different color stages. The eggs appear orange in color apparently because of the presence of yolk. Then the eggs swell as cells begin to divide and changes from orange to dark brown and finally gray. Several prezoeal stages were found and described. The size and shape of the antennae, endopodites, maxillules, maxillae and second maxillipeds were also documented.

Keywords: Sand crab, *Emerita*, prezoeal, egg

BSD-15

SOME OBSERVATIONS ON THE CARAPACE AND TELSON MORPHOLOGY OF A POPULATION OF MOLE CRAB, *Emerita* Sp. FROM MAGOONG, LINAMON, ILIGAN CITY

Mark Anthony J. Torres, Sigfried Razo, Joey Rey Cartajenas, Emmanuel Boniao, Karen Coronado, Vicky Rodriguez, Kate Villanueva, Sittie Rohanna Pundogar, Kathleen Podiotan, and Cesar G. Demayo

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

The systematics and taxonomy of the mole crab, *Emerita sp.*, a putative bioindicator from Magoong, Linamon, Iligan City is unclear. The problem stems from the widespread variation in carapace color patterns amongst individuals in this population and the lack of literature regarding these species of organisms in the Philippines. Also, correct classification of these organisms is necessary before it can be used as bio-indicator. Thus, mole crabs were collected from the study site and morphometric differentiation among individuals was quantified. Patterns of morphometric variation were examined using geometric morphometrics and multivariate statistics, respectively. Principal component analyses of the carapace shape characters revealed that some gravid mole crabs have morphologically distinguishable carapace and telson shape showing possible relationship between the shapes of the carapace and telson and fecundity. Further genetic studies must be conducted to determine genetic structuring on a micro or macro-geographic scale to confirm the taxonomic distinctiveness of the mole crabs and to determine if variation in the carapace and telson shape possesses some genetic basis or only a product of allometry.

Keywords: Systematics, mole crab, *Emerita* sp., geometric morphometrics, carapace, telson

BSD-16

ELLIPTIC FOURIER ANALYSIS OF SHAPE VARIATIONS WITHIN AND AMONG SIX SPECIES OF BIVALVES COLLECTED FROM MURCILLAGOS BAY

**Mark Anthony J. Torres, Cesar G. Demayo, Geneveve Podiotan, Tyrone Enoc,
Christian Noel Saporno, Donel Tulog, Hosea Manda,
Jun Rey Quinto and Jonathan Sobremonte**

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Elliptic Fourier Analysis was used in this study to determine the extent and degree of morphological diversity within and among six species of bivalves collected from Murcillagos Bay. These include *Circe scripta, Anadora inequevalves, Codakia tigerina, Meretrix meretrix, Mytilus smaraginous* and *Pitar* sp. In order to do this, the bivalves were scanned at uniform dpi and the resulting images were binarized using SCIONIMAGE, an image analysis and processing software. The contours of the bivalves were then summarized as chain codes. A mathematical algorithm was used to compute for a total of 20 harmonic modes, with each variable consisting of four shape variables. These shape variables were then used as morphometric variables for Principal Component Analysis. Also, the mean shapes of each species were reconstructed. The extent and degree of variations within each species were then computed and shown as positive and negative standard deviations from the mean shapes. Results of this study showed variations in the shapes of the calcareous valves within each species of bivalves. Ordination of the various species also revealed morphological disparities among the species. The results of this study clearly indicate the usefulness of outline analysis in determining shape variations in bivalves.

Keywords: bivalves, elliptic fourier analysis, ordination, harmonic modes, *Circe scripta, Anadora Inequevalves. Codakia tigerina. Meretrix meretrix, Mytilus smaraginous* and *Pitar* sp

BSD-17

GEOMETRIC MORPHOMETRIC ANALYSIS OF THE TRUSS NETWORK IN SEVERAL SPECIES OF BIVALVES

Cesar G. Demayo, Mark Anthony J. Torres and Romme Ray Sullano

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Variations in shape of the area formed between the anterior and posterior muscle scar, umbo and ligament in the valves and the distances between them were assessed based on the landmark data, which were subjected to various geometric morphometric analyses. A total of 95 samples of bivalves were collected composed of *G. melanaegis* (n=22), *G. tumidum* (n=10), *A.. squamosa* (n=24), *C. orbicularis* (n=16), and *Curbicularia sp.* (n=23) which were subjected to geometric morphometric and statistical analyses. Procrustes fitting of the landmark points allowed for the comparison of truss network of the bivalve, eliminating size and rotational translation. Thin-plate spline grids (TPS) were used to summarize the shapes of the biological structures. Partial warp analysis (PA) was used to identify the position of specific landmarks that vary considerably among taxa. Principal Component (PCA) Discriminant Function analysis (DA) were used to confirm or reject the hypothesis that the shape of the area defined by the anterior and posterior adductor muscle scars, umbo and ligament as defined by the landmark points could be used to discriminate the species of bivalves used in this study and the patterns of size differences across all species were determined by comparing the interlandmark distances between the landmark points generated using Euclidean Distance Matrix Algorithm (EDMA). The results of this study clearly imply that the shape of the area formed between the anterior and posterior muscle scar, umbo and ligament in the valves and the distances between them could be used as a taxonomic character in the classification of bivalves and indicates the usefulness of the various geometric morphometric methods in shape differentiation and variations in selected species of bivalves.

Keywords: truss network, Euclidean Distance Matrix Algorithm, Procrustes

BSD-18

MORPHOLOGICAL DISPARITY OF THE PELVIC GIRDLE AMONG FIVE SPECIES OF BATS: IMPLICATIONS FOR LAUNCH

Cesar G. Demayo, Mark Anthony J. Torres and Gianhope T. Silao

Department of Biological Sciences
College of Science and Mathematics
MSU-Iligan Institute of Technology, Iligan City

Previous studies have shown that patterns of skull shape variation among several species bats are related to their feeding ecology. In this study, the shapes of the pelvic girdles of one insectivorous and four frugivorous bats were compared using geometric morphometric methods. In order to do this, the pelvic girdles of the bat samples were scanned at 800 dpi. Then the x and y coordinates of a total of 18 landmark points and 185 outline points were collected from around the contour of the bones, separately, using an image analysis and processing software. For the landmark analyses, the 18 landmark coefficients were used as morphometric variables for multivariate statistical analysis such as partial warps analysis (PWA) and hierarchical cluster analysis in order to assess its shape. The size component was also evaluated by subjecting the raw data to Euclidean Distance Matrix Algorithm. The data obtained were then subjected to the appropriate geometric morphometric analyses. For the outline analyses, the 185 sample points were subjected to Elliptic Fourier Analysis. EFA of these coordinates returned a total of 40 coefficients that were used to reconstruct the shapes of the pelvic bones of each bat sample. The EFA coefficients were also used as morphometric variables for multivariate statistical analysis such as principal component analysis (PCA) and discriminant function analysis. The results echo the morphological disparity between the two groups. The results were also correlated to the feeding ecology and launch mechanism employed prior to flight. The morphological disparity in the shape of the pelvic girdles observed in this study may suggest important differences in the locomotor architecture between the two groups of bats.

Keywords: *frugivorous, locomotor architecture, EDMA, Elliptic Fourier Analysis*

BSD-19

PRELIMINARY SURVEY AND ELEMENTAL CHARACTERIZATION OF LICHENS AS POTENTIAL BIOMONITOR FOR ATMOSPHERIC POLLUTION

Leni L. Quirit[1], Preciosa Corazon B. Pabroa[2], William Gruezo[3] and Karen N. Hernandez[1]

[1]Natural Sciences Research Institute, University of the Philippines Diliman
[2]Philippine Nuclear Research Institute, Diliman, Quezon City
[3]Institute of Biological Sciences, University of the Philippines
Los Baños, Laguna

The two approaches for collecting atmospheric pollution samples are (a) direct collection of airborne particulate matter, precipitation and total deposit, and (b) the use of air pollution biomonitors. Using the first approach is costly (in terms of equipment, personnel and power). The second approach is cheaper (i.e. no expensive technical equipment is needed) and simpler (sampling is relatively easier, generally relying on the common and permanent availability of the bio-species in the field).

Sampling for potential biomonitors was initially done on six sites — the Arroceros Forest Park in Manila; Parks and Wildlife (PW), Quezon City Memorial Circle (QCMC), Natural Sciences Research Institute and the Institute of Chemistry in Quezon City; Mauban, Quezon (a relatively unpolluted forest site) and the Batangas Coal-Fired Thermal Power Plant (BCFTPP) or Calaca Power Plant (CPP) in Calaca, Batangas. Ferns, mosses and lichens were collected during the preliminary surveys but because lichens were found to be the most abundant and ubiquitous, sample collection was later limited to lichens only. Sampling sites for elemental analysis (but not for lichen species identification) were eventually narrowed down to just the PW and CPP sites due to logistics limits.

Identification of the collected lichen samples was done by Dr. William Gruezo of the the Institute of Biological Sciences, UP Los Baños. Nine different species (or genera, for those which cannot be identified down to the species level) were identified. *Pyxine cocoes (Sw.) Nyl.* species was found predominant for all the sites. Lichen diversity comparison among the sites seemed to indicate level of pollution exposure

Due to its abundance and ubiquity, samples identified as *Pyxine cocoes (Sw.) Nyl.* were chosen for elemental analyses. Preliminary levels, trends and correlations in elemental findings will be presented, and a comparison of the two methods used, Inductively Coupled Plasma (ICP) and X-ray Fluorescence (XRF) Spectrometry.

The Zn and Pb correlation was the most interesting, with PW lichens levels generally higher than CPP lichens and the ash samples levels for these two elements. The correlation between these two elements are also much better for the PW lichens compared to the other two types of samples (r^2 correlation coefficients **0.24** for CPP, **0.85** for PW and **0.42** for the ash samples).

Graphs show the better Zn and Pb correlations in the PW samples, with the plots for the CPP ash and lichen samples showing a large scatter of data points. Studies on elemental compositions of air particulate filter samples from 2001 to 2004, at the Philippine Nuclear Research Institute (PNRI), show that Zn and Pb are also well correlated in the air particulate filter samples of the DENR Quezon City ambient sampling site station (at the Manila Observatory). Comparison of the Pb vs Zn plots of the ambient site air particulate samples and the PW lichen samples show a striking similarity between the two types of samples.

Keywords: air pollution, biomonitors, lichens, XRF, ICP, air particulate filters

BSD-20

DETERMINATION OF THE FUNGAL POPULATION AT THE INDOOR ATMOSPHERE OF A SCHOOL BUILDING

Erwin P. Elazegui

College of Science, Technological University of the Philippines, Manila

Fungi are a diverse group of eukaryotic organisms. They reproduce sexually or asexually by means of spores. Fungal spores are an invisible part of our environment. This study was conducted to determine the fungal population at the indoor atmosphere of the College of Arts and Science building at the TUP Manila campus. Collection of samples was done by Gravitation Plate Method in randomly designated areas of the school building. Sabouraud Dextrose Agar (SDA) was used as growth media . Plates were incubated at 30 °C for 5 – 7 days . Isolates were identified based on their morphology by slide culture technique and cultural characteristics such as colony color and texture appearance on SDA. Fungal isolates that were observed are pathogenic opportunists and have an industrial significance. These includes *Aspergillus niger* , *Rhizopus sp.* , *Alternaria sp.* and *Penicillium sp.*

Keywords : fungi , fungal spores, gravitation plate method , pathogenic opportunists

BSD-21

PREVENTING BIOHAZARD DISASTERS IN PHILIPPINE SCIENCE LABORATORIES

Franco G. Teves

Molecular Genetics and Microbial Technology Laboratory, Department of
Biological Sciences, College of Science and Mathematics
MSU-Iligan Institute of Technology 9200, Iligan City
Telefax: 063 3516131; Email: franco_teves@yahoo.com

Biohazard and bioterrorism are basically unknown to many Filipino researchers in Philippine colleges and universities and generally very foreign to most students. The stiff competition for best science projects in national and international levels has led to a lop-sided trend in the students' choice of investigatory projects which require manipulation of microorganisms. Yet, most high schools do not have the basic facilities, or the expertise to undertake microbiological experiments, therefore placing high school students at risk of being exposed to potential pathogens for lack of necessary training and knowledge.

Roughly 70% of high school science investigatory projects submitted annually in the life sciences category at least in Northern Mindanao deal with microorganisms. A survey of microbial species commonly used include those with known pathogenic members or are potential pathogens, such as *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. These microbial isolates are usually purchased from culture collections of universities or research institutions by the science advisers, which are then either kept in the high school science laboratory or brought to a university where the experiments are conducted. Examination of the materials and methods written by the students generally reveal vague descriptions of aseptic techniques and decontamination procedures. Inability to answer questions during the actual science fairs corroborates the notion that most high school students undertake microbiological experiments without sufficient training and knowledge.

Measures such as policy formulation regulating microbiological experiments below the tertiary level, accreditation of microbiological culture collections and science advisers working with microorganisms, regulating the indiscriminate buying and selling of microbial cultures, and enhancing microbiological education for high school science teachers and students, will have to be in place to prevent an impending biohazard disaster in the country.

Keywords: biohazard, bioterrorism, pathogens, aseptic techniques, decontamination

BSD-22

IMPROVEMENT OF METHODOLOGIES FOR GREENHOUSE GAS INVENTORIES OF THE LUCF SECTOR IN THE SOUTHEAST ASIAN REGION

Damasa B. Magcale-Macandog

Institute of Biological Sciences, College of Arts and Sciences
University of the Philippines Los Baños

Greenhouse gas emissions inventories are vital for quantifying the release of greenhouse gases into the atmosphere and for assessing the impacts of increasing greenhouse gases in the changing patterns of climate change. Estimations of carbon emissions and sequestration from forest and land-use change are complicated, and often controversial, due to complex biological factors, lack of data or reliable data, and adverse and complex human impacts on forest resources.

A review of the national GHG inventory reports of the Philippines, Indonesia and Thailand revealed a great variability in the values of the annual biomass increment and aboveground biomass used in the different national GHG inventory studies. The major problems in the estimation of GHG emissions or removals from the LUCF sector in the three countries are the lack of activity data and the limited access to sparsely available data. There is a strong need to develop a database to improve the quality of activity data and emission factors. Creation of this database will entail compilation of all existing data and literature; updating of forest resources and land use inventory, and conduct of experimental studies, sampling, survey and measurement for the different activity data and emission factors.

Allometric regressions to predict aboveground biomass for different forest plantations including *Tectona grandis*, *Pinus merkusii*, *Swietenia macrophylla* in Java, *Tectona grandis* in Thailand and secondary forests in the Philippines were developed and improved. Information on wood density plays an important role in improving the estimates of tree biomass for the calculation of greenhouse gas emission and uptake. Information on wood density of Philippine trees was organized in a database. Currently, the database contains 542 entries from 243 species belonging to 59 families.

A geographic information system (GIS)-based model was developed to spatially predict the aboveground biomass of secondary forests in the Philippines. A database of the physical (soil type, slope, elevation) and climatic (agroclimate zone, annual rainfall) properties of the different administrative units (provinces) in the country was assembled from secondary data and existing maps. The relationship of the physical and climatic factors (independent or predictor variables) and the forest aboveground biomass (dependent variable) was determined through

multiple linear regression analysis. The resulting equation was used to predict potential aboveground biomass of secondary forests in the country. Overlaying the potential biomass map to the remaining secondary forest areas resulted to a map of the estimated aboveground biomass of secondary forests.

Keywords:

BSD-23

A PRELIMINARY STUDY OF BACTERIAL BIOFILMS IN WATER RESERVOIRS AND WATER DELIVERY LINES

Wilma T. Cruz

Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna
Email: wilmatc@yahoo.com

Water is an effective vehicle for the spread of pathogens and low numbers of opportunistic pathogens can be found in potable water. While chlorination is usually effective in eliminating fecal coliforms and most transient pathogens, bacteria in biofilms can withstand chlorination. This study examined the occurrence of biofilms in a random selection of water reservoirs and water delivery lines of establishments within the University of the Philippines Los Baños (UPLB) campus and determined the identity of the bacteria isolated from the biofilms.

Microscopic examination of glass slides that were submerged in the water reservoirs of two water pumping stations within the UPLB campus revealed the presence of biofilms. Bacteria were also recovered from the swabs taken from biofilms that formed inside running water faucets as well as the faucets and inside walls of water containers and dispensers located in various buildings. The identity of bacteria isolated from the biofilms were determined using the Analytical Profile Index (API) System. The bacterial isolates included both Gram positive (*Bacillus, Micrococcus, Staphylococcus*) and Gram negative (*Aeromonas, Citrobacter, Empedobacter (Flavobacterium), Escherichia, Klebsiella, Pantoea, Pasteurella, Pseudomonas* (*Brevundimonas*), *and Sphingomonas*) species. The predominant isolates were *Micrococcus* (21.4%), *Bacillus* (14.3%), *Pasteurella* (14.3%) and *Staphylococcus* (12%). The different enteric bacteria made up 21.4% of the isolates. Among the isolates were opportunistic pathogens.

Keywords: water delivery lines, water reservoirs, biofilms, opportunistic pathogens, enteric bacteria

BSD-24

METAL TOLERANCE AND ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED FROM RIVERS OF CAVITE

Yolanda A. Ilagan* and Joanne A. Agustin

Cavite State University
Indang, Cavite, Philippines
Email: yoly_ilagan@yahoo.com

One hundred and one bacterial isolates were obtained by plating samples of river water. These bacteria belong to the genera *Enterobacter, Alcaligenes, Staphylococcus, Micrococcus, Pseudomonas, Proteus, Acinetobacter, Bacillus, Enterobacter* and *Flavobacterium*. They were subjected to varying concentrations of metals and antibiotics.

The isolates exhibited growth decline as the concentration of heavy metals increased. Majority of them exhibited tri-tolerance and bi-tolerance . Iron-Lead-Zinc tolerance was the most observed pattern.

Bi- and tri-resistance were the most common observed patterns of resistance to antibiotic which are Ampicillin–Oxytetracycline and Ampicillin-Kanamycin-Clindamycin, respectively.

High percentage of isolates exhibited tolerance to lead. Combination of Clindamycin-Lead and Erythromycin-Lead resistance were also exhibited by most isolates.

Keywords: metal tolerance, antibiotic resistance

BSD-25

PARTICIPATORY INVENTORY AND DISTRIBUTION OF ENDANGERED, ENDEMIC AND ECONOMICALLY IMPORTANT PLANTS IN HAMIGUITAN RANGE WILDLIFE SANCTUARY, DAVAO ORIENTAL

Victor B. Amoroso, Reyno A. Aspiras* and Janece Jean A Polizon*

Central Mindanao University, Musuan, Bukidnon
088-222-5748; 0917-5495084
amorosovic@yahoo.com

Mt. Hamiguitan Range Wildlife Sanctuary in Davao Oriental is a protected area in Mindanao with pygmy forest in an ultramafic soil. With the participation of *Bantay Gubat* as local researchers, this study was conducted to inventory and determine the distribution of the endemic, endangered, and economically important flora in Mt. Hamiguitan Range and its environs. Transect walk and sampling plots revealed four vegetation types viz. agroecosystem, dipterocarp, montane and mossy forests. The mossy forest consisted of two subtypes, the typical mossy forest and mossy-pygmy forest. It further showed that Hamiguitan Range is a habitat of 843 species, 242 genera and 120 families of plants. Of these, 678 were angiosperms, 29 gymnosperms, 121 ferns and 15 fern allies. It is also the home of 8 endangered species, 27 rare species, 47 endemic species and 165 economically important species. Further assessment revealed nine (9) species considered as new record in the Philippines or in Mindanao. Inventory of trees and shrubs in sampled plots showed high species richness per unit area and high diversity values. Not only that Mt. Hamiguitan exhibited high species richness but it also contained many endemic, endangered, rare and economically important species of plants. The lowland dipterocarp and pygmy forests and their species at lower elevation are highly threatened due to overharvesting of forest products and road expansion for mining activities. The identified habitats of these threatened and endemic species should be given high priority for protection and conservation.

Keywords: species, diversity, assessment, vegetation types, Natural Park

BSD-26

ESTIMATES OF CARBON STOCKS IN THE PROPOSED MINE SITE IN MOUNTAIN ECOSYSTEM OF TAMPAKAN, SOUTH COTABATO, PHILIPPINES AND ENVIRONS

Jan Paolo T. Pollisco[1], Nelson M. Pampolina[1], Dixon T. Gevaña[2], and Jose Sebua[3]

[1]Department of Forest Biological Sciences, CFNR-UP Los Baños
[2]Environmental Forestry Program, CFNR-UP Los Baños
[3] Sagitarius Mines Incorporated, Tampakan, South Cotabato

Carbon stored in biomass of trees, intermediate layer, herbaceous plants and forest litter was estimated in grassland and forest ecosystems along the gradient in mountain ecosystem within the boundaries of three provinces under the proposed mine sites of the Sagittarius Mines Incorporated in Southern Mindanao. The amount of carbon produced during photosynthesis was computed based on biomass that accounts for approximately 45% of the plant total weight. The diameter of trees measured at breast height was used to compute biomass through allometric equations. The plant biomass was sampled from 10 sampling plots that were equally distributed alternately at 100 m interval along a kilometer transect each in low (400-799 m asl), medium (800-1,199 m asl), and high (1,200-1,600 m asl) elevation gradients. Each sampling plot measured 10x10 m for trees, 1x1 for herbaceous, and 0.3x0.3 m for necromass.

The lower and middle slopes, comprising mostly of grassland, agricultural crops, and patches of secondary forest in contrast to nearly intact primary forest in higher elevation, were relatively disturbed. A total of 52-67 plant species and 46-51 genera were identified in the lower and middle slopes, consisting of ecologically threatened species (*Shorea contorta, Parashorea malaanonan, Dillenia philipinensis, Alstonia macrophylla, Cinammomum mercadoi, Palaqium luzoniense,* and *Neolitsea vidalii*) and bio-invasive *Piper aduncum*. There were 49 species and 33 genera sampled towards the peak in the old growth forest that include endangered species (*Dacrycarpus elatum, D. imbricatus, Syzigium* and *Lithocarpus* spp). Estimated carbon stored in plant biomass aboveground from disturbed slopes varied from 74.9 ± 64.3 to129.9 ± 62.2 tons ha^{-1} compared to 608.1 ± 300.8 tons ha^{-1} in lush primary forest. Carbon stock in the undergrowth herbaceous however, was higher in open gradient than in secondary and primary forest. By contrast, stored carbon from belowground necromass was greater in primary and secondary forest than in open grassland ecosystem.

Overall, the above results indicate the significant role of vegetation in carbon sequestration and the likely impact of denudation.

Keywords: carbon stocks, South Cotabato, Endomycorrhiza, mountain ecosystem, Allometric equation

BSD-27

ISOLATION OF ENVIRONMENTAL DNA AND ANALYSIS OF A PARTIAL LIBRARY OF THERMOPHILIC MICROORGANISMS FROM MT. MAKILING MUDSPRING, LOS BANOS, LAGUNA, PHILIPPINES

Nacita B. Lantican*, J. Jason L. Cantera, Ma. Genaleen. Q. Diaz and Asuncion K. Raymundo

Institute of Biological Sciences, College of Arts and Sciences
University of the Philippines Los Baños, College, Laguna, Philippines 4031
*Tel: (63-049) 536-3368; Email: nacitalantican@yahoo.com; nbl@gmail.com

The Mudspring at the University of the Philippines Los Banos is a naturally occurring, extremely hot environment located on the mountainside of Mt. Makiling, Laguna. It is a potentially rich source of thermophilic microorganisms with novel properties; but the present status of the diversity of the thermophiles in this environment is not well documented. The extreme environmental conditions and high temperature required for isolating and growing the microorganisms from Mudspring limited the cultivation of the prevailing microflora and its subsequent utilization. Thus, DNA-based approach became the method of choice for analysis and eventual utilization of the wide diversity of microorganisms from Mudspring. Several protocols and DNA extraction kits were tried and considerable amounts of DNA were extracted only from the settled pond water using the Bio 101 Fast DNA Spin Kit. Difficulty in direct DNA isolation was probably due to high sulfur content and low pH. The isolated DNA was digested with *Eco*R1 and ligated into plasmid pBluescript (pBS). A number of clones were obtained and sequenced. Blast results showed that the inserts were less than 1000 kb and most clones showed no significant homology to any known gene, suggesting that the extracted DNA contains unique sequences from uncharacterized microorganisms, thus the absence of significant hits in the databases. This is the first study on environmental DNA, and showed the potential of such kind of approach in the genetic analysis of the diversity of the microorganisms in Mudspring and in other extreme environments in the Philippines.

Keywords: Thermophiles, Environmental DNA, Mudspring

BSD-28

AN ASSESSMENT OF THE BENTHIC COMMUNITY STRUCTURE: IMPLICATIONS ON THE INTEGRITY OF LAKE PALACPAQUEN, SAN PABLO CITY, PHILIPPINES

Hazel Caasi, Jobe de la Torre, Teresita Perez and Claveria Rene Juna

Deapartment of Environmental Science
Ateneo de Manila University, Loyola Heights
Quezon City, Philippines

Lake Palacpaquen is the shallowest and second largest lake in the Seven Lakes of San Pablo City. The lake is threatened by sedimentation and water quality. The macroinvertebrate community composition of the lake was investigated. The effect of the lake's water quality on the benthic macroinvertebrates was evaluated using the Belgian Biotic Index (BBI) and the Family-Level Biotic Index (FBI). Samples were obtained from four stations in the lake: inlet, pool, periphery and outlet and *in-situ* water quality parameters were measured. Substrate varied from organic litter, to gravel and fine sediments. Dissolved oxygen concentrations and turbidity were generally lower in November 2005 while pH level, temperature and conductivity were lower in December 2005 following a series of heavy rainfall.

The dominance of mollusca genera *Melanoides*, *Tarebia*, *Bellamya* and *Angulyagra* was observed. Six other genera of mollusks were found, including the bivalve *Corbicula* and the foreign species, *Pomacea canaliculata*. Larvae of the Family Chironomidae were abundant in the pool.

Taxa diversity was highest in the periphery and the lake outlet in December 2005 and January 2006. Diversity of the entire lake obtained from composite samples was highest in January (H' = 0.834). BBI scores were lowest in November and highest in January for all sites. Based on the BBI, Lake Palacpaquen was moderately polluted in November and December, and was lightly polluted to unpolluted in January. FBI scores were lowest in January (6 = fairly poor), but this rating is still indicative of the possibility of substantial pollution.

Keywords : Biotic Index, Benthic Organisms, Water Quality, Pollution, Lake

BSD-29

DIVERSITY AND ENDEMISM OF VERTEBRATE FAUNA IN MT. MALINDANG, MINDANAO, PHILIPPINES

Olga M. Nuñeza, Apolinario A. Alicante, Marie Rosellyn C. Enguito and Fritzie B. Ates

Department of Biological Sciences
MSU-Iligan Institute of Technology, Iligan City
Email: olgamnuneza@yahoo.com

The rapid destruction of tropical rainforest in Southeast Asia that occurred in the 20th century drastically altered the environmental conditions to which many forms of vertebrate life had become adapted (Sodhi et al., 2004). Malindang range is one of the upland ranges where faunal diversity has been severely threatened due to forest loss. This study was geared towards the generation of knowledge on faunal resources through a participatory approach. The prospect would be that a better understanding of the faunal resource diversity, under shared responsibility would lead to better resource management.

Using a combination of mistnetting, trapping, line transect, and opportunistic methods, 257 species of vertebrates fauna including 114 endemic and 25 threatened species were recorded. Fifty-nine herpetofaunal species were recorded comprising 26 amphibians and 33 reptiles with 42% and 48% endemism, respectively. Of the 162 species of birds recorded, 66 (24%) are Philippine endemics. Nineteen species of bats and 17 species of nonvolant mammals (12 endemic, 4 threatened) were recorded. Percent endemism was highest in the mossy forest while the most number of threatened species was found in the submontane dipterocarp forest. TWINSPAN analysis showed that the mossy and montane forest cluster represents a vegetation type different from the other clusters. Translating the cluster data into a biodiversity map revealed that biodiversity value is low in the agroecosystem, moderate in lowland dipterocarp, mixed dipterocarp, mixed lowland dipterocarp and mossy forest, high in almaciga, and very high in montane and submontane dipterocarp forests.

The forest sites at higher elevation appear to be very important for the continued existence of faunal species with critical population. However, despite habitat loss, the lowlands are still able to support a considerable number of endemic species. These results need to be taken into account in the formulation of conservation and management plan for Mt. Malindang.

Keywords: vertebrate, fauna, threatened, endemic, species

BSD-30

DISTRIBUTION AND CONSERVATION OF THE AVIFAUNA OF AGUSAN MARSH, AGUSAN DEL SUR, PHILIPPINES

Milagros P. Sucaldito and Olga M. Nuñeza*

Department of Biological Sciences, College of Science and Mathematics
Mindanao State University-Iligan Institute of Technology, Iligan City
Email: milagros_sucaldito@yahoo.com

Agusan Marsh is identified as the 1009th RAMSAR site as Wildlife Sanctuary which serves as wintering ground for migratory and wetland birds from Southeast Asian regions and Western countries during the winter season. It is considered as one of the most ecologically significant wetland ecosystems in the Philippines. In this study, four habitat types were assessed to determine the distribution and conservation of the birds in the marsh.

Sago, terminalia, mixed swamp and peat swamp forests in Agusan Marsh were surveyed using a combination of mist netting (3,337 net days) and transect walk methods. One hundred twenty-six species of birds were identified consisting of 26 migrants, 69 residents, and 31 endemics (23.81% endemism) of which five species are categorized as threatened. The mixed swamp forest had the highest species richness (S=92) while peat swamp forest had the highest number of migratory birds (S=26). Endemicity was found to be highest in the terminalia forest (20.63%) while sago forest had the least number of bird species. Canonical correspondence analysis (CCA) of the birds' local distribution in relation to environmental variables showed 80.7% variance of species abundance and distribution. The migratory species, Siberian Ruby throat (*Luscinea cyane*) which was recorded only in Luzon as accounted by Kennedy et al., 2000 was found also in Agusan Marsh indicating that this bird may not only be confined to Luzon but could be found in other places in the Philippines.

Five species of birds which include two migratory species were reported to be of socioeconomic importance. Changes in the structural and floristic composition brought about by forest degradation such as conversion of forest into agriculture were seen as threats to the birds in Agusan marsh. Conservation of the different habitats in the marsh appears to be very necessary for the retention of the broadest avifaunal diversity.

Keywords: avifauna, distribution, conservation, endemism, species richness

BSD-31

DISTRIBUTION, DIET AND ENDOPARASITES OF AVIFAUNA IN MT. SAMBILIKAN, DIWATA RANGE, AGUSAN DEL SUR, PHILIPPINES

Rowena B. Caro and Olga M. Nuneza

Department of Biological Sciences, College of Science and Mathematics
MSU-Iligan Institute of Technology, Tibanga, Iligan City
Email: rwn_caro@yahoo.com

Mt. Diwata is one of the Important Bird Areas of the Philippines. This study investigates the distribution of avifauna in Mt. Sambilikan, using a combination of mist-netting (932 net days) and transect walk methods. Assessment of the Mixed Lowland Dipterocarp Forest, Mixed Dipterocarp Montane Forest and Mossy Dipterocarp Forest showed 97 species belonging to 13 orders and 37 families. Of these, 56 are endemic (39 Philippine Endemic, 9 Mindanao Faunal Region Endemic, 8 Mindanao Island Endemic). The high percentage endemicty (58%) indicates that the area provides a good habitat to a large number of avian species. Sixteen threatened species were recorded (one critically endangered, seven vulnerable and eight near threatened).

Mixed Dipterocarp Montane Forest had the highest species diversity (H'=2.83). Species richness (S = 72) was recorded to be highest in the Mixed Dipterocarp Montane Forest (670 – 750 masl) while the lowest species richness (S=33) was recorded in the Mossy Dipterocarp Forest (1,000 – 1, 050 masl).

Fourteen samples of *Hypsipetes philippinus* and eight samples of *Macronous striaticeps* were dissected to examine diet composition as well as endoparasites. Knowledge on the diet and endoparasites could be helpful in the management of birds in the wild. Results showed that the diet of *Hypsipetes philippinus* includes digested fruit pulp and fibers, seeds, and insects of family hemiptera. The gut of *Macronous striaticeps* yielded seed and fruit pulp of *Ficus minahassae* and seeds. Segmented endoparasites were found in the intestine of birds, however, percent occurrence of endoparasites was higher in *M. striaticeps* than in *H. philippinus.*

Despite the kaingin system of farming and hunting of birds for food, Mt. Sambilikan remains to be the home of many endemic and threatened birds. Proper management and conservation measures appear to be necessary to protect the bird species and other forms of biodiversity in the area.

Keywords: Avifauna, Diversity, Endemism, Distribution, Species Richness

BSD-32

DIVERSITY, DIET, AND ENDOPARASITES OF SNAKES IN MT. SAMBILIKAN, DIWATA RANGE, AGUSAN DEL SUR

Amy G. Ponce and Olga M. Nuñeza

Department of Biological Sciences, College of Science and Mathematics
Minadanao StateUniversity Institute of Technology, Iligan City
Email: jade_tazz@yahoo.com

Mt. Sambilikan, with an elevation of 1,050 masl is part of the Diwata range in Agusan del Sur which is one of the key conservation sites in the Philippines. A field ecological study on snake fauna was conducted utilizing the opportunistic method, in order to come up with a reliable species assessment including species richness, species endemicity, distribution and abundance. Diet composition as well as endoparasites of the snake species were also examined. Ten snakes were recorded comprising seven species under Family Colubridae, one species under Family Elapidae and two species under Viperidae. Highest diversity (H'=1.039) was recorded in the mixed dipterocarp montane forest while lowest diversity (H'=0.062) was documented in the mossy dipterocarp forest. A relatively even distribution was observed for all sites sampled. *Tropidonophis dendrophops* and *Trimeresurus flavomaculatus* were the only endemic species (20%) recorded..

Diet examination results showed that snake species of Family Colubridae generally feed on lizards and frogs. Viperidae was found to feed on rodents and bats while Elapidae even feeds on snakes, besides rodents. Endoparasites belonging to Order Ascarida were the parasitic nematodes found infesting the digestive tract of the snake species.

No threatened snake species according to IUCN criteria was recorded in Mt. Sambilikan but according to what was locally observed all snake species were threatened due to the local people's practice of killing the snakes immediately when encountered. Some local residents also utilize snakes for food and medicine. The fast rate of human settlement in the area is seen as a threat to the snakes in the area. Enhancing awareness and knowledge of the local people on the importance of the snake species and implementation of conservation strategies could be an important step towards the conservation of the snakes in Mt. Sambilikan, Diwata range.

Keywords: snake, diversity, diet, endoparasite, endemic

BSD-33

DIET AND DISTRIBUTION OF BAT SPECIES IN MT. SAMBILIKAN, DIWATA RANGE, AGUSAN DEL SUR

Berna Lou L. Aba and Olga M. Nuñeza

Department of Biological Sciences
MSU-Iligan Institute of Technology, Iligan City
Email: lou_heaven@yahoo.com

The Philippines is richly diverse in important flora and fauna species. Mt. Sambilikan of the Diwata Range is home to one of the important fauna, the Chiroptera. Thus, the investigation of the diet, species composition, biodiversity indices, species similarity and environmental variables of bats was done in Mt. Sambilikan, Agusan Del Sur from October 24-Novenber 21, 2006. Three sampling sites with three subsites differing in elevation and vegetation were established. Mist netting and harp trapping were done for a total of 768 net and trap nights. Sixteen species of bats were recorded: nine pteropodids, 1 rhinoliphid, 1 vespertilionid, and five hipposiderids. Eleven species were Philippine Endemic, and five non-endemics. Most of the species captured were of the least concern status except for *Haplonycteris fischeri* that is rated vulnerable (VU) based on IUCN Red List.

Forty guts from *Ptenochirus jagori*, *Rousettus amplexicaudatus* and twenty from Insectivorous bats were examined. Food items or prey units were categorized into four: Follicle/Cillia/Hair-like substances (F/C/H), partially digested matter (PDM), digested matter (DM), and insect debris (ID). Ten percent of *Ptenochirus jagori* examined appear to have endoparasite. Eighty percent of insectivorous bats contained follicles, cilia, or hair-like substances (F/C/H), and 75% of partially digested matter (PDM). Ninety percent of *Rousettus amplexicaudatus* contained digested matter (DM). Sixty five percent of insect bats contained insect debris (ID). Species composition and abundance among the three sampling sites was not significant probably due to the nearly similar vegetation in the subsites. Instead, the species abundance between subsites was highly significant (0.001) probably due to the variation of habitat per subsite. The species composition was more or less evenly distributed between sampling sites for there was no dominance in any species. Environmental factors such as elevation and vegetation appear to have an effect on the species distribution and diet.

Keywords: Bat, diet, distribution, relative abundance, endoparasites

BSD-34

A PHYLOGENETIC STUDY OF PHILIPPINE SPECIES OF TOXOGLOSSATE MOLLUSCS USING mtCOI GENE SEQUENCES

Glaiza F. Narvacan[1], Angela Clarissa DR Cantalejo[1], Francisco M. Heralde III[1,2], Virginia D. Monje[1]

Medical Biotechnology and Genome Research Laboratory
National Institute of Molecular Biology and Biotechnology, College of Science
[2]Marine Science Institute, College of Science
University of the Philippines, Diliman, Quezon City 1101

The members of the Toxoglossa Superfamily (*Conidae, Turridae and Terebridae*) possess a specialized feeding mechanism that may involve toxins[1]. Some *Conidae* species have toxins which revealed pharmaceutical potentials[2] which may also be present in their close relatives that can be identified in phylogenetic analyses. In this study, the marker utilized is the mitochondrial cytochrome oxidase I gene (mtCOI). It is popularly used because of its large size and its protein sequences that contain highly conserved functional domains and variable regions[3] DNA from each snail was extracted either by the Xanthogenate-SDS DNA extraction protocol or by the DNA Easy Extraction Kit. The mtCOI gene was amplified by PCR. The gene was directly sequenced and homologous sequences to the ones obtained were searched for using BLAST. Sequence and phylogenetic analyses were performed using the Molecular Evolution Genetics Analysis (MEGA) version 3.1 software[4]

Presently, partial mtCOI gene sequences of two *Turridae* species, *Turridrupa prestoni* and *Lophiotoma acuta*, and one *Terebridae* species, *Terebra babylonia*, were obtained. BLAST results indicated that the obtained mtCOI sequences were homologous mostly to those of other gastropod snails. *Turridrupa prestoni* was at the base of the phylogenetic tree, constructed using the Minimum Evolution Method with Bootstrap Analysis. Moreover, the position of *Lophiotoma acuta* was next to it, indicating the possibility that the *Turridae* family is more ancient than the other two toxoglossate families. Also, *Terebra babylonia* appeared to be more related to *Conidae* species than to *Turridae* species. This may mean that the *Conidae* is more related to the *Terebridae* (and vise versa) than to the *Turridae*. This implies that the biological characteristics of the species within the *Conidae* are more similar to those of the species within the *Terebridae*. Thus, search for toxin-derived drugs using Terebra may be just as promising, if not more, as the Conus snails.

Acronyms Used:
mtCOI – mitochondrial cytochrome oxidase I
PCR – polymerase chain reaction
BLAST – Basic Local Alignment Search Tool
MEGA version 3.1 – Molecular Evolution Genetics Analysis Version 3.1

Keywords: Toxoglossa Superfamily (*Conidae, Turridae and Terebridae*), molluscs. Conus snails, phylogenetics, mtCOI.

BSD-35

TRANSLOCATION OF COPPER IN NON-MYCORRHIZAL AND MYCORRHIZAL TARO (*Monochoria sp.*) IN MINE SOIL AND GARDEN SOIL AMENDED WITH INCREASING LEVELS OF COPPER

Nelly S. Aggangan[1], Richard Kenneth V. Ching[2], Allan Vincent C. Miñon[2] and Juveneil Eissyd J. Perez[2]

[1]National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños, College, Laguna 4031
Tel/Fax: 049 536 0563; Email: nea@laguna.net
[2]Pedro Guevara Memorial National High School
Sta. Cruz, Laguna

Taro or commonly known as gabi (*Monochoria* sp.) is a very popular root crop in the Bicol region for the famous "laing" recipe. Gabi is planted in backyard, farms and even in mine waste dumpsites. Mine wastes contain heavy metals that are detrimental to plants, animal and human health. Most of the mining companies in the Philippines mine for Cu. This study aimed to determine the absorption and translocation of Cu by gabi growing in mine waste soil and in Cu-amended garden soil. Soil samples from mine sites in Paracale, Camarines Norte were collected and found to contain 3.51 mg Cu/kg soil. Gabi plants were planted in pots filled with mine soil and garden soil amended with 100, 200 and 400 mg/kg Cu. These were either uninoculated or inoculated with a commercial mycorrhizal inoculant Mykovam. Mykovam consists of different species of endomycorrhizal fungi. The experiment was done in a screenhouse following a Randomized Complete Block Design with ten replicates. Gabi planted in mine sites were also collected and analyzed for Cu.

Results show that inoculated gabi exhibited greater total plant dry weight than uninoculated ones. The corms gave the greatest dry weight compared to other plant parts. Cu tolerance of mycorrhizal plants is 200 mg/kg while that of non-mycorrhizal plants is 100 mg/kg. Cu concentration of twelve-week old non-mycorrhizal gabi was well distributed in the leaves, roots, and corm and greatest in their stalk. On the other hand, inoculated counterpart had high Cu concentration in the roots. This observation concurred previous findings that Cu is concentrated in the roots of mycorrhizal plants indicating the ability of the latter to filter out heavy metals into the upper portion of plants particularly the edible parts. In the case of gabi, all parts except the roots can be eaten by human. Cu concentration in the stalk of non-mycorrhizal gabi ranged from 53–70 mg/kg, which are beyond the maximum acceptable limit of 36 mg/kg by the Dutch Standards. Mycorrhizal plants had Cu concentrations (from corm to leaves) that are far below the maximum acceptable limit. Gabi grown in mine sites also accumulated high amount of Cu in the edible portions. With these observation, it is highly recommended that studies be conducted to verify the results and determine the accumulation of other heavy metals.

Keywords: copper, gabi, heavy metal, mycorrhiza, taro, mine soil, garden soil

BSD-36

THE EFFECT OF PREPARED DIET ON THE SOMATIC AND GONAD GROWTH PERFORMANCE OF THE SEA URCHIN *Tripneustes gratilla* (LINNAEUS, 1758)

Facundo B. Asia*, Joji Grace Villamor and Jogel C. Faylogna

MMSU College of Aquatic Sciences and Applied Technology
Currimao 2903, Ilocos Norte, Philippines
Telefax: (077) 793-6916; Email: dong_asia@yahoo.com

Somatic growth and gonad growth and quality of the sea urchin *T. gratilla* fed with prepared diets based on *Sargassum sp.* were studied *in vitro* using plastic basins from February to June 2006. It consisted of three treatments with three equal replications arranged in CRD as follows: I-Fresh *Sargassum sp.* (control), II-Dried pellets, and III-Fresh Extruded pellets.

No significant variations were observed in the somatic growth of *T. gratilla* among the feeding treatments indicating that prepared diet is comparable with fresh diet. Highest growth rates were observed during the first culture month decreasing towards the end of the study. The fresh natural food gave better gonadosomatic index and gonad color than the prepared diets but not for granularity. However, in a follow-up study (Asia, 2006) to optimize feed ration of the organisms, the effect of natural food and prepared *Sargassum sp.* diet at 4.0 to

5.0% BW/day feeding ration on gonadosomatic index and gonad color were comparable ($p<0.05$). Observed water parameters were within the favorable ranges for growth and survival of the organism.

The successful introduction of prepared diets for *T. gratilla* opens opportunities of incorporating gonad color enhancers in the diet that improves quality of the organism for market and consumption. The study likewise demonstrated the viability of land-based culture of the organism using both the fresh natural food and prepared diets important in sustaining a year-round harvest and broodstock source for hatchery and seed stock production.

Keywords: *Tripneustes gratilla*, prepared diet, somatic growth, gonad growth and quality

BSD-37

ASPECTS ON THE BIOLOGY AND FISHERY MANAGEMENT OF *Siganus fuscescens* IN PUJADA BAY

Bernadette J. Nanual[1] and Ephrime B. Metillo[2*]

[1]Department of Natural Sciences, Davao Oriental State College of Science and Technology, Mati 8200, Davao Oriental; Email: dettenanual@yahoo.com;
[2]Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Ave., Iligan City 9200; Tel: (063) 221 4050 up to 55 local 137; E-mail: ovcre-ebm@sulat.msuiit.edu.ph; ipemetillo@yahoo.com

The white-spotted spinefoot rabbitfish, *Siganus fuscescens*, is an important but overfished species in Pujada Bay, Davao Oriental. In this study, sex ratio, body size, fecundity and gonadosomatic index (GSI) of *S. fuscescens* were determined for a year from three sites in Pujada Bay. The generated population structure and reproductive patterns database became input into the fishery management plan of the species. Sex ratio was generally 1:1. Mean standard lengths ranged from 9.1-12.9cm, significantly much smaller than the expected length at maturity of 20cm and maximum body length of 40cm (FishBase 2005). However, relatively larger individuals were found in the less fished marine reserve site. Mean fecundity was lower than expected (FishBase 2005) and ranged from 10,981.8-39,791.6, and, again, individuals from the less fished site contributed higher values. Spawning occurred from February to April and peaked in March. The species employs an opportunistic life-history strategy (high reproductive effort, early maturity, frequent spawning), but our data suggest that extreme overfishing may have driven the species to mature much earlier and spawn at a much smaller body size with a much lower fecundity. The less fished site, however, seems to allow individuals to mature at a larger body size and spawn higher number of eggs. Hence, some degree of protection from overfishing may help in the sustainability of populations and fishery of *S. fuscescens* in Pujada Bay. This study has recommended to the local government that the less fished site be declared as a marine reserve.

Keywords: fish resources, spinefoot rabbitfish, *Siganus fuscescens*, Pujada Bay, fecundity, gonadosomatic index, fishery

BSD-38

MONSOONAL INFLUENCE ON THE DISTRIBUTION, ABUNDANCE AND ASSEMBLAGE STRUCTURE OF ZOOPLANKTON IN ILIGAN BAY, NORTHERN MINDANAO, PHILIPPINES

Anelyn L. Dapanas and Ephrime B. Metillo*

Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Ave., Iligan City 9200
Telephone: (063) 221 4050 up to 55 local 137
Email: ovcre-ebm@sulat.msuiit.edu.ph, ipemetillo@yahoo.com

We conducted a systematic sampling of physico-chemical factors and zooplankton in the upper 25-m surface layer across a 24-station grid in Iligan Bay to determine whether the assemblage structure and abundance of zooplankton differ across stations and between northeast and southwest monsoon months, and to establish correlations between zooplankton parameters and physico-chemical factors. Abundance was estimated by stereomicroscopy, and assemblage structure was analyzed using multivariate and univariate routines available in the E-PRIMER v.5 software (Warwick and Clarke 2001). Abundance across stations and between months was higher during the southwest monsoon months. Assemblage structure were similar in terms of taxonomic composition and dominant taxa (>67% of the total abundance: calanoid copepods, copepod nauplii and copepodites, and tintinnids). Change in assemblage structure were largely attributed to shifting in ranks of taxa (bivalve veligers, ostracods, gastropod veligers, fish eggs, larvaceans, chaetognaths, and cyclopoid copepods) that contribute <7% relative abundance. Assemblages consist of high, moderate, and low levels of relative abundance, and taxonomic richness and diversity, but the very crucial are those that had low diversity and abundance in the southeastern sector of the bay during the northeast monsoon, and those with low diversity but high abundance in the southwestern sector during the SW monsoon. The former assemblages could be associated with areas with pollution stress while the latter with a possible micro-upwelling event. Salinity, temperature, and total dissolved phosphorus and nitrogen were weakly correlated with zooplankton spatio-temporal variations, but contrasting monsoonal patterns and associated hydrodynamic processes are also important in structuring zooplankton assemblages in Iligan Bay.

Keywords: zooplankton, disturbance, northeast monsoon, southwest monsoon, multivariate analysis, Iligan Bay

BSD-39

SPECIES IDENTIFICATION AND GENETIC DIVERSITY ANALYSIS OF YEAST ISOLATES FROM PHILIPPINE RICE WINE STARTERS BY DNA FINGERPRINTING

Elaine Y. Lim[1], Vivian Panes[1], Gabriel O. Romero[2*]

[1]Biology Department, Ateneo de Manila University, Quezon City
[2]Plant Breeding and Biotechnology Division, Philippine Rice Research Institute
Science City of Muñoz, Nueva Ecija Email: goromero@philrice.gov.ph

In the Philippines, considerable variation in rice wine quality is often observed among and within wine producers. Microbial composition of rice wine starters is critical to maintaining the wine quality. To better understand the microbial ecology, yeast strains were isolated from starters obtained from three areas in the Ifugao province for morphological and molecular characterization. DNA analysis was done using modified RAPD using 20-mer SRILS Uniprimers. Three of 12 SRILS primers revealed the highest level of polymorphism of DNA bands among the yeast isolates. SRILS 1 was the most discriminatory primer, differentiating between closely related *Saccharomyces cerevisiae* strains 2103 and 2104. With SRILS 9, isolate K2 from Kiangan showed identical pattern with *Hansenula anomala* strain 2063, while both SRILS 6 and 9 revealed that isolates B3 from Banaue and K3 were very similar to *Saccharomycopsis fibuligera* strains 2076, 2077, 2081 and 2109. The unknown isolates and the corresponding genetically similar reference strains also showed comparable morphological characteristics. These results demonstrate the utility of RAPD with SRILS primers in yeast species identification and classification, and present a major step towards achieving a better control of starter constitution and rice wine quality.

Keywords: *bubod*, NTSYS, polymerase chain reaction, SRILS, tapuy

BSD-40

ISOLATION AND SCREENING FOR BACTERIA FROM ROOTS OF SELECTED EPIPHYTIC ORCHIDS FOR GROWTH ON NITROGEN-DEFICIENT MEDIUM, PLANT GROWTH-PROMOTION, AND ANTIBIOTIC PRODUCTION

Mary Grace Melendres Palayan and Noel G. Sabino*

Microbiology Division-Institute of Biological Science, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna

Epiphytic bacteria were isolated form the roots of five orchid species, three of which belong to the genus *Dendrobium*, one under *Phalaenopsis*, and the other under *Cymbidium*. Out of the 50 isolates observed growing on Nutrient Agar (NA) and Burk's Medium (BM) plates, 22 were Gram positive and the rest were Gram negative. Only 11 of the 20 bacterial isolates growing on Burk's Medium, a medium without nitrogen, were shown to grow on Nitrogen-Deficient Combined C (NDCC) medium and considered as potential nitrogen fixers. All isolates were screened for plant growth promotion by coating monggo (*Vigna radiate* var. *pag-asa7*) seeds with bacterial cell suspension, and then allowing them to germinate in water agar medium. Increase in height of the seedlings was used as index of growth promotion. Among the 50 isolates, only 3 isolates namely, De$_1$BM8, DE$_3$SsBM4, and De$_3$IBM7, showed statistically significant growth-promoting activity. All bacterial isolates were screened for antibiotic production. Isolates De$_2$IBM6 and De$_3$SsNA5 consistently yielded a 1-mm diameter zone of inhibition against the test organism *Bacillus subtilis*. None of the isolates had the ability to produce an antimicrobial substance against the test organism *Escherichia coli*. Preliminary characterization of the isolates capable of growing on NDCC medium and/or promoting plant growth suggest that the aerobic Gram negative con-endospore-forming rod-shaped isolates namely, De$_1$SsBM6, De$_1$SsBM7, De$_1$IBM8, De$_2$SsBM3, De$_2$IBM5 and CySBM1, may be tentatively placed under the Family Psedomonadaceae, Azotobacteriaceae, or Rhizobiaceae. Isolate De$_1$SBM1 which is a Gram positive non-endospore-forming aerobe, on the other hand, can be tentatively classified as a member of either the genus *Kurthia, Renibacterium, or Caryophanon*. Isolate PhSsBM4, a budding Gram positive non-endospore-forming obligate aerobe, may be a species of the genus *Hyphomicrobium*. Whereas the Gram positive endospore forming rods namely, De$_3$SsBM4 and De$_3$IBM7, may be tentatively identified as members of the genus *Bacillus*.

Keywords: epiphytic bacteria, orchids, plant growth promotion, antibiotic production, nitrogen fixation

BSD-41

EFFECT OF PLANT PRESERVATIVE MIXTURE (PPM) ON CONTAMINATION RATE AND GROWTH OF *Vanda sanderiana* Reichb.F. SEEDLINGS (ORCHIDACEAE) IN VITRO

Alexander B. Quilang*, Norberto R. Bautista, Gale B. Taylan and Rachel F. Madera

Plant Biotechnology Project, Research & Development Center
Rizal Technological University, Boni., Ave., Mandaluyong City
Tel: 534-8267 loc 123; Fax: 534-9710; Email: plantbiotech_rtu@yahoo.com

Plant Preservative Mixtureä (PPM), a proprietary broad-spectrum preservative or biocide was studied to determine its effectiveness and optimum level/ concentration in reducing microbial contamination in orchid culture bottles. Different levels of PPMä (0, 0.125, 0.25, 0.50, 1.0, 2.0 and 4.0 ml/l media) were added to Knudson C. Unsterilized *Vanda sanderiana* seeds were inoculated on the media for each treatment. Contamination was observed for each treatment and results were converted to percentile. Percentage contamination decreased as preservative mixture concentration in basal media increased. Therefore, the preservative mixture appeared to be effective in minimizing microbial contamination in culture bottles.

Keywords: *Vanda sanderiana*; orchids; PPM; biocide; anticontaminant

BSD-42

MYKOVAM AND PLANT GROWTH PROMOTING RHIZOBACTERIA FOR GROWTH ENHANCEMENT OF *Jatropha curcas.*

Jocelyn T. Zarate* and Lilia M. Fernando

National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños, College, Laguna
Email: joytzarate@yahoo.com and limafe226@yahoo.com

Jatropha curcas, a non-edible oil bearing and drought-hardy shrub is a potential renewable alternative source of biodiesel. Massive planting is currently on-going in many areas of the country. Most of these areas are acidic, infertile and unsuitable for good plant growth. The economics of the industry depends significantly on production yields that should not only rely on application of expensive, imported chemical fertilizers. The effect of mycorrhizal inoculant "Mykovam" and plant growth promoting Rhizobacteria (PGPR) on the growth of Jatropha seedlings grown in unsterilized field soil was investigated.

Results showed that inoculation with Mykovam and PGPR at transplanting time, either singly or combined, significantly promoted taller height, wider stem diameter, larger leaf area, longer root length and heavier biomass of Jatropha seedlings after three months in the nursery. Root length and total leaf area were significantly longer and larger when both biofertilizers are present, as compared to when inoculated singly. Similarly, nitrogen and phosphorus uptake of seedlings was higher with both biofertilizers present. The phosphorus and nitrogen nutrition of the seedlings was greatly enhanced, due to the synergistic interaction of the Rhizobacteria and mycorrhizal fungi. Survival and growth of seedlings in the field are being monitored to determine if this good growth in the nursery will translate to early plant maturity and increased seed yield.

Keywords: *Jatropha curcas*, Mykovam, plant growth promoting bacteria, mycorrhiza

BSD-43

EVALUATION AND CHARACTERIZATION OF OIL CELLS IN SEEDS OF *Jatropha curcas* L.

Vivian S. Tolentino*, Patricia Cruz, Carlo Santos, Vivian Panes, Wilberto Monotilla

Department of Biology, Ateneo de Manila University
Loyola Heights, Quezon City
Telefax: (632) 4261034; Email: vtolentino@ateneo.edu

The oil from *Jatropha curcas* L., commonly called "tubang bakod" is a promising alternative source of energy, specifically biodiesel. However, efficient extraction of oil is yet unattainable due to the undetermined location of oil cells. This study focuses on the characterization oil cells in seeds at different developmental stages based on cell type, cell wall, form and shape. Determining the exact location of cells in the different tissues of the seed may aid in efficient extraction and cost effective production of oils. Preliminary test for the presence of oils was done by determining the percent composition of the different tissues in the seed This was done in order to ascertain which tissue in the seed may possibly contain the greatest volume of oil as may be correlated in the number of oil cells. It was observed that the outer seed coat and endosperm greatly composed the seed weight. The outer seed coat and endosperm were later pounded using a pneumatic clicker and applied on oil control films to test oil yield. It was observed that the outer seed coat was devoid of oil while the endosperm has oil. Middle-aged and mature endosperms were also sectioned with a sliding microtome, and stained with Sudan rot B and viewed under a CH-20 Olympus light microscope and CK-2 Olympus inverted microscope. It was observed that oil cells appeared red with Sudan Rot B. The endosperm of the seeds showed oil cells interspersed with circular dark staining parenchyma cells, with foamy cytoplasm and a three-layered cell wall, outer and inner cell wall and a middle suberin layer.

Keywords: oil cells, Sudan Rot, parenchyma, suberin, cell wall

BSD-44

ISOLATION, SCREENING AND IDENTIFICATION OF SULFATE-REDUCING BACTERIA AND EVALUATION OF THEIR ABILITY TO PRECIPITATE COPPER FROM WASTEWATER SOLUTION

Lorele C. Trinidad [1]* and Rhea G. Abisado[2]

[1]BIOTECH and [2]Institute of Biological Sciences
University of the Philippines Los Baños, College, Laguna 4031

Concern for health and environmental hazards posed by heavy metal contamination led to the elucidation of the potential use of sulfate-reducing bacteria (SRB) as bioremediation agents in copper-contaminated water, soil or sediments. Eight H_2S-producing bacteria were isolated from an abandoned mine site in Mogpog, Marinduque. Among the eight bacterial isolates, RA-4 exhibited the highest (14.72 mg/100ml) H_2S production which was quantified using the iodometric titration method. A decline in H_2S production was observed for all isolates after nine days of incubation except for isolate RA-3 with 12.13 mg/100 ml H_2S. Partial morphological and biochemical characterization of the local isolates temporarily placed them under subgroup 2 or subgroup 3 of the dissimilatory sulfate-reducing bacteria.

Flame atomic absorption spectrophotometric (FAAS) analysis of copper-containing sulfate reducing medium (SRM) inoculated with the selected isolates showed that isolate RA-4 exhibited the highest amount (75.85%) of copper precipitated from solution. This proves that indigenous SRB could be used as an effective agent for bioremediation for copper-contaminated wastewaters.

Keywords: Sulfate-reducing bacteria (SRB), bioremediation, copper precipitation

BSD-45

OFF BOTTOM CULTURE OF *Caulerpa lentillifera* AGARDH IN THREE DIFFERENT WATER LEVELS IN THREE DIFFERENT SITES USING DIFFERENT CULTURE MEDIA IN THE MARINE WATERS OF SAN FRANCISCO, CEBU, PHILIPPINES

Serapion N. Tanduyan, Ricardo B. Gonzaga, and Virginia D. Bensig

Cebu State College of Science and Technology- Fishery and Industrial College
6050 San Francisco, Cebu Campus
Telefax: 032-4970318; Email: standuyan@yahoo.com

Caulerpa lentillifera has been reared traditionally in bottoms of fishponds. This was first reared in Mactan Island, Cebu Philippines in ponds and considered as one of the export commodities because of its high demand. Conversion of mangroves into ponds has been banned in the Philippines and the off bottom culture of this alga was studied in order to find its growth in an open and natural body of waters cultured at three different water levels and different culture sites.

This study used the Randomized Block Design (RCBD) with four culture media as treatment. Treatment 1 used plastic screen cage: Treatment 2, tubular plastic screen: Treatment 3 nylon screen cage and Treatment 4 tubular nylon screen. The culture media were placed in a bamboo raft where this was divided into three layers representing the surface, midlayer and the bottom layer and placed in different sites which are muddy, rocky and sandy bottom. Sampling was done every15 days for 3 months taking wet weight of the plant as factor.

Results show that as to the efficiency of each culture medium based on the water level it was found out that on the surface level the *Caulerpa lentillifera* placed in plastic screen cage has the highest growth rate in Site 1 (muddy site) and tubular nets ranked first in site 2 (rocky) and site 3 (sandy).

For the middle layer based on the mean weight of the plant it showed that tubular nets has the highest in site 1 (muddy) and plastic screen cage got the highest in site 2 (rocky) and 3 (sandy).

For the bottom layer of the marine area the tubular plastic screen dominates the highest growth rate of all the culture medium used from site 1, 2 and three.

Analysis of Variance (ANOVA) revealed that there is no significant difference on the growth rate of *Caulerpa lentillifera* placed in plastic screen cage, tubular nets with the different water levels in the three culture sites.

Keywords: *Caulerpa lentillifera*, off bottom culture, Water levels and San Francisco, Cebu Philippines.

BSD-46

ASSOCIATED BACTERIA IN *Kappaphycus alvarezii* THALLI EXHIBITING "ICE-ICE" AND NORMAL PHENOTYPES FROM THE KOLAMBUGAN AREA OF PANGUIL BAY, LANAO DEL NORTE

**Wanda Andriene D. Aguja, Franco G. Teves*, Faith B. Amorado
and Henry I. Rivero**

Molecular Genetics and Microbial Technology Laboratory,
Department of Biological Sciences,
College of Science and Mathematics, MSU-Iligan Institute of Technology
9200 Iligan City
*Telefax: (063) 351 6131 Email: franco_teves@yahoo.com

"Ice-ice" is a coined term describing the white, icy appearance of seaweeds affected by a disease of still undetermined etiology. Panguil Bay in Northern Mindanao, which is formed by shorelines extending from the coastal areas of Lanao del Norte to areas in Misamis Occidental, has been the site of recent "ice-ice" devastation, endangering its dollar-earning industry.

Bacterial profile of both "ice-ice" affected and normal *Kappaphycus alvarezii* thalli were examined by comparing total bacterial load and bacterial genera that grow under aerobic conditions using nystatin-supplemented nutrient agar prepared with sterile filtered seawater.

Results show apparent higher average bacterial load in thalli with "ice-ice" phenotype (2.58×10^5 colony-forming units/ gram sample, CFU/g) compared to those with normal phenotype (8.5×10^3 CFU/g) from three sampling periods.

Bacterial genera present in the healthy thalli include *Bacillus*, *Listeria*, and *Lampropedia*, while those present in thalli with "ice-ice" symptoms are *Pseudomonas*, *Staphylococcus*, *Proteus*, *Listeria*, *Erwinia*, and *Xanthomonas*. The presence of *Bacillus* sp. most probably indicates soil contamination. The presence of *Listeria* sp., *Staphylococcus* sp., and *Proteus* sp. gives some indication of contaminants from human or animal sources. The genus *Lampropedia* indicates the presence of high organic content of the aquatic environment. Both *Erwinia* and *Xanthomonas* have species that are plant pathogens.

Although it is difficult to assign a particular bacterial group as the etiologic agent of "ice-ice", the bacterial profile obtained already provides vital information on the hydrologic conditions in the environment where the seaweeds are cultivated. However, the possible direct pathologic activities of *Pseudomonas*, *Erwinia*, and *Xanthomonas* isolates could not be ruled out, since members of these Gram-negative genera are known to be plant pathogens.

A logical proactive approach to seaweed cultivation therefore would be to ensure good water quality in shallow water seaweed farming, or to adopt deep water farming techniques for seaweeds, where hydrologic physicochemical parameters are comparatively better.

Keywords: "ice-ice", *Kappaphycus alvarezii*, bacterial profile, colony-forming units, hydrologic conditions

BSD-47

MYCORRHIZA AND BACTERIA AS GROWTH ENHANCER AND AS BIOLOGICAL CONTROL OF NEMATODES IN TISSUE-CULTURED BANANA VAR. LAKATAN

Paul Jemuel S. Tamayao[1], Nelly S. Aggangan[2] and Teodora O. Dizon[3]

[1]UP Rural High School, Paciano Rizal, Bay, Laguna
[2]National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños, College, Laguna 4031
Tel/Fax: 049 536 0563; Email: nea@laguna.net
[3]Plant Pathology, Institute of Plant Breeding, Crop Science Cluster
University of the Philippines Los Baños, College, Laguna 4031

This study was conducted to determine the potential of mycorrhizal fungi and nitrogen fixing bacteria as growth promoter and as biological control against nematodes in tissue-cultured banana var. Lakatan under screenhouse conditions. This experiment was conducted following a Randomized Complete Block Design with nine treatments and 10 replicates per treatment. Meriplants were potted in cups filled with sterile soil sand mixture, immediately upon arrival. Mycorrhizal inoculant "MYKOVAM" containing mixture of *Glomus* and *Gigaspora* was placed in contact with the roots during potting after the roots were dipped in slurry of Bio-N containing N-fixing bacteria *Azospirillum* spp. Nematodes *Radopholus similis* and *Meloidogyne incognita* solutions were poured into the soil, two months after inoculation with mycorrhiza at concentrations of 1,000 and 5,000 larvae or eggs per seedling, respectively. Height, diameter, leaf length and leaf width were taken every two weeks until four months.

Results show that mycorrhiza and mycorrhiza + bacteria inoculated seedlings grew better than those of the control treatment. Growth of plants treated with *R. similis* alone and *M. incognita* alone was comparable with the control. The effect of *R. similis* was not so pronounced as the root lesions were just starting unlike the effect of *M. incognita* where numerous galls were found in the roots. Neither

R. similis or *M. incognita* suppressed the colonization of mycorrhizal fungi in the roots of Lakatan seedlings. By contrast, the two nematodes decreased the bacterial colonies obtained from roots of mycorrhiza + bacteria plants. *M. incognita* gave higher reduction in the number of colony forming units of *Azospirillum* than *R. similis*. In terms of the plant growth, mycorrhiza with or without bacteria + *M. incognita* were taller, had bigger diameter and larger leaf area than the control and the other treatments. This implies that mycorrhizal plants were tolerant to nematodes. There were no significant differences between growth of plants with mycorrhiza alone or mycorrhiza + bacteria. More importantly, mycorrhizal plants gave the highest fine and coarse root dry weights. This is a very critical factor since roots are the water and nutrient absorbing organs of plants that is directly correlated to plant growth and health.

Keywords: Lakatan banana, mycorrhiza, nitrogen fixing bacteria, nematodes

BSD-48

DIFFERENTIAL ANGIOGENIC MODULATION IN THE CHICK EMBRYO CHORIOALLANTOIC MEMBRANE (CAM) ASSAY BY POLAR AND FRACTIONAL POLYSACCHARIDE EXTRACTS OF *Ganoderma applanatum*

Franco G. Teves[1*], Michael Cereno M. Bajo[1], Lydia M. Bajo[2]
Roberto M. Malaluan[3]

[1*] Molecular Genetics and Microbial Technology Laboratory, Department of
Biological Sciences, College of Science and Mathematics
Telefax: (063) 351 6131 Email: franco_teves@yahoo.com
[2] Department of Chemistry, College of Science and Mathematics
[3] Department of Chemical Engineering Technology
School of Engineering Technology
MSU-Iligan Institute of Technology, 9200 Iligan City

Angiogenesis refers to the formation of new blood capillaries, which occurs in both physiological and pathological processes in humans. It is an important factor in wound healing, growth of adipose tissue, and as a component of the female menstrual cycle. In tumor progression, angiogenesis is a requirement for neoplastic growth and survival.

Angiogenic modulating substances were obtained from the bracket fungus *Ganoderma applanatum* through fractional polysaccharide (FPS) and supercritical carbon dioxide extraction (SF-CO_2) protocols. Four crude polysaccharide extracts from the FPS and one polar extract from the SFE using high pressure CO_2 (300 atmospheres or atm) at 40æ%C, were tested for angiogenic modulation using the chick embryo chorioallantoic membrane (CAM) assay.

Three out of four crude polysaccharide residues showed significant inhibition on angiogenesis, whereas significant angiogenic stimulation was observed with the polar residue. This was statistically confirmed using a CRD factorial analysis of data obtained from the CAM assay. These results reveal a cocktail of diverse biologically active compounds in the fungal extract with biomedical potential. The documented anti-neoplastic effects of the polysaccharide residues reported in China and other Asian countries may be due to their anti-angiogenic properties. On the other hand, angiogenesis stimulation by the polar residue may be utilized in enhancing transplanted organ survival and as an adjunct treatment for paralysis caused by blood vessel damage.

This is the first study reported in the Philippines and elsewhere on the use of the CAM assay for determining the angiogenic modulation effects of *Ganoderma applanatum* extracts.

Keywords: angiogenesis, neoplastic growth, fractional polysaccharide, supercritical carbon dioxide extraction, chorioallantoic membrane assay, *Ganoderma applanatum*

BSD-49

OPTIMIZATION OF PRODUCTION AND PARTIAL PURIFICATION OF PEDIOCIN FROM *Pediococcus acidilactici* PNCM 10289

Sheila Mae N. Sagpao[1], Francisco B. Elegado[2]* and Agnes F. Zamora[1]

[1]Institute of Biological Sciences, College of Arts and Sciences
[2]National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Baños, College, Laguna 4031
*Email: fbelegado@hotmail.com

The influence of several nutritional and non-nutritional factors on the fermentative production of pediocin, an antilisterial peptide produced by *Pediococcus acidilactici* PNCM 10289, was studied. Initial results showed that bacteriocin activity of the medium supernatant was optimal (6,400 AU/mL) at 10 h in Tryptose-Glucose-Yeast Extract (TGE) broth incubated at 40 °C with an initial pH of 6.0.

Initial medium optimization, specifically carbon and nitrogen sources in the production of the bacteriocin, was also done. A maximum activity of 12,800 AU/mL was obtained in the base medium with 3% brown sugar as carbon-source and supplemented with 2% trypticase as nitrogen-source. Results also showed that a bacteriocin titer of 6,400 AU/mL, equal to that obtained using synthetic TGE medium, was obtained using the base medium supplemented with a cheaper N-source which was soybean meal (1%). A maximum bacteriocin activity of 12,800 AU/mL was obtained from the base medium using a cheaper C-source (2% molasses) instead of 3% brown sugar and supplemented with 1% soybean meal. Also, in this base medium, crude yeast extract obtained by boiling baker's yeast was utilized as a substitute for commercial yeast extract.

From 1 L of de Man, Rogosa and Sharpe (MRS) medium used for cell culture, bacteriocin activity of 51,200 AU/ml was recovered in a 50 ml semi-purified extract by adsorption of the bacteriocin on to the bacterial cells at pH 6.0 and desorption at pH 2.0. However, when the cells were grown in the formulated medium, the purification method gave less bacteriocin titer (6,400 AU/ml) due to adsorption on to other solid components of the medium. After the insoluble $CaCO_3$ was removed from the culture medium, a higher bacteriocin activity of the extract (12,800 AU/mL) was obtained.

Keywords: bacteriocin, *Pedicoccoccus acidilactici*, pediocin, substrate, optimization

BSD-50

PRODUCTION AND CHARACTERIZATION OF PECTINASES BY *Aspergillus niger* IN SOLID STATE FERMENTATION WITH COCONUT HUSK

Jennifer D. Saguibo[1], *Veronica C. Sabularse[2], and Chay B. Pham[3]

[1,3]National Institute of Molecular Biology and Biotechnology (BIOTECH)
[2]Institute of Chemistry, College of Arts and Sciences
University of the Philippines Los Baños, 4031, College, Laguna, Philippines
Telefax: (049) 536-2721; E-mail address: jen-saguibo@yahoo.com.ph

Pectinases are a group of enzyme that attack and depolymerize pectin by hydrolysis and transelimination as well as by de-esterification reaction which hydrolyze the ester bond between the carboxyl and methyl group pectin. They are produced by a variety of microorganisms but the filamentous fungi *Aspergillus niger* is most widely used.

Production and characterization of pectinases, particularly pectin transeliminase (PTE) and polygalactoronase (PG), by *Aspergillus niger* van Tieghem with dried coconut husk as a substrate was studied in solid-state fermentation system. Strain of filamentous fungi used in research was isolated from rotten young coconut husk and selected for their capacity to produce pectinase. The best fungal strain, MH 29, exhibited the highest pectinase under the optimum conditions: pH 6, 30°C and 80% moisture content. The result of SDS -PAGE showed that pectinase obtained from extract of fermented coconut husk contained both PG and PTE with 45 KD and 39.5 KD, respectively. Both enzymes exhibited an optimum pH of 4.0 and an optimum temperature of 50 °C. The activation energy of PG KJ/mol while that of PTE was 1.10 KJ/mol. Km and Vmax were 8.86 mg/ml and 0.027733 min/umole reducing sugar, respectively, for PG. PTE was found to have a Km of 5.71 mg/ml and Vmax of 0.01665 min/increase in absorbance at 235 nm.

Abbreviations: PG - polygalacturonase, PTE - pectin transeliminase, KD - kilodalton

Keywords: *Aspergillus niger*, solid-state fermentation, pectinase, polygalacturonase, pectin transeliminase

BSD-51

NATURAL FERMENTATION OF COFFEE PULP: A MICROBIOLOGICAL ANALYSIS

Yolanda A. Ilagan*, Joyce C. Mojica, Airene C. Panganiban, Randy Capala and Ven Michael U. Feraer

Cavite State University Indang, Cavite, Philippines
Email: yoly_ilagan@yahoo.com

Coffee pulp was supplemented with forages, ipil-ipil and molasses and allowed to ferment without bacterial inoculum for three months. Proximate and chemical analysis of the fermented product showed that among the treatments, T1 (coffee pulp and molasses) had the highest moisture content, total sugars and reducing sugars; T2 (coffee pulp, molasses and napier grass) gave the highest ash and phosphorus content, sufficient crude fat and lowest pH; T5 (coffee pulp, molasses and ipil-ipil) had significantly high crude protein, calcium and nitrogen free extract; T8 (coffee pulp, molasses, para grass and ipil ipil) had the highest crude fiber content T4 (coffee pulp, molasses and paragrass) gave the highest lactic acid concentration.

Microflora of naturally fermented coffee pulp included 11 bacterial genera, *Bacillus, Propiniobacterium, Staphylococcus, Micrococcus, Enterococcus, Planococcus, Enterobacter, Erwinia, Citrobacter, Pseudomonas* and *Alcaligenes*; seven mold genera, *Aspergillus, Acremonium, Penicillium, Cephalosporium,*

Helminthosporium, Fusarium, Cladosporium and the yeast genus *Candida*. Lactic acid bacteria were also isolated but the species were not identified.

In another experimental set-up, coffee pulp plus molasses and ipil-ipil was inoculated with different bacteria (*Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus pentosaceus* and *Pediococcus cerevisiae*) prior to fermentation. Statistical analysis showed insignificant differences ($P>.05$) among treatments in terms of ash, moisture, crude fat and crude fiber but significant differences ($P< .05$) in terms of crude protein and nitrogen free-extract. The organisms isolated included the bacteria *Bacillus, Pseudomonas, Enterobacter, Staphylococcus* and *Micrococcus* and lactic acid bacteria; molds, *Aspergillus, Acremonium, Fusarium, Penicillium, Helminthosporium, Goetrichum Alternaria* and yeasts, *Hansenula and Candida.*

Keywords: natural fermentation, coffee pulp, proximate analysis, lactic acid

BSD-52

CHARACTERIZATION OF BACTERIOCINOGENIC LACTIC ACID BACTERIA ISOLATED FROM PHILIPPINE FERMENTED FOODS

Vanessa Marie T. Lim[1], Andrea Therese R. Gervasio[1],
Marilou R. Calapardo[2], Jennifer D. Saguibo[2], Marilen M. Parungao[1]
and Francisco B. Elegado[2]*

[1]Department of Biology, College of Arts and Sciences
University of the Philippines Manila, Padre Faura, Manila
[2]National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Baños, College, Laguna
*Email: fbelegado@hotmail.com

One hundred sixty putative isolates of lactic acid bacteria (LAB), obtained from twenty fermented foods purchased from public markets in Central Luzon, were screened for bacteriocin production. Boiled and pH-neutralized culture supernatants of the LAB isolates were spotted on to agar plates using *Listeria monocytogenes* (Lm) or *Enterococcus faecium* (Ef) as indicator microorganisms. Thirty LAB isolates showed bacteriocin activities, eight of which had high activity against Lm (e" 6,400 AU/ml) and good activity against Ef (e" 1,600 AU/ml).

The nature of bacteriocin encoding genes was determined through polymerase chain reaction (PCR) assay using primers for pediocin, plantaricin, nisin and lactococcin. The template DNA were aqueous extracts from cells of LAB isolates added with HPLC water and treated in boiling water bath for 10 min.

Results showed that five were positive for pediocin gene, two for plantaricin gene and two for nisin gene. None encoded for lactococcin gene and it was presumed that the remaining bacteriocins were of other types.

Two high bacteriocin-producing LAB isolates, namely strains 3G3 and 4B1, were selected and tested for acid and bile resistance. They were identified as *Lactobacillus pentosus* and *L. fermentum* using API 50CHL id kit, respectively. Both strains were relatively stable upon exposure for 3 hr to pH 3 artificial gastric juice (AGJ) with 0.2% pepsin. At pH 2, both gave more than 60% viability after 3 hr in AGJ. Further incubation for 3 hr in simulated intestinal fluid (SIF), containing 0.3% Oxgall (bile), gave 45% and 72% viability for 3G3 and 4B1, respectively. *L. fermentum* 4B1 had both good acid and bile tolerance. This particular LAB has very good probiotic potential with added high bacteriocin production, properties that are very attractive in the development of new probiotic food and drinks.

Keywords: *bacteriocin, polymerase chain reaction (PCR), probiotic lactic acid bacteria*

BSD-53

EXTRACTION AND EVALUATION OF KAPPA CARRAGEENAN FROM *Kappaphycus alvarezii* (SACOL VARIETY) AS SUBSTITUTE FOR AGAR IN MICROBIOLOGICAL MEDIA

Lorna T. Enerva[1], Monica Marie A. Jocson[2], and Rolando S. Pispis[2]

[1]SciTech R and D Center
[2]College of Science, Polytechnic University of the Philippines
Email: lorna enerva@yahoo.com

Kappa carrageenan was extracted from *Kappaphycus alvarezii* (Sacol variety), red seaweed that grows widely in almost all parts of the Philippines. Twenty grams of the seaweed was soaked in 700 mL of hot distilled water for one hour, boiled for 1.5 hours, filtered and 95% isopropyl alcohol was used as the precipitating agent. The moisture content 11.89%, ash 25.83% and acid-insoluble ash 0.92% conformed to the specifications set by USPXXII (1990). The value for the gelling property of 5.87 sec is necessary for a gelling agent in microbiological agent. The obtained molar ratio for 3,6 anhydrogalactose and galactose signify a regular structure of the products although the result for the determination of the sulfate content of 11.73 was low. The IR spectrum showed similar absorption curve with the known idealized Kappa Carrageenan. The UV-Vis spectrum showed maximum wavelength at 198 nm with absorbance of 1.458.

A microbiological media was prepared with 2.0% of kappa carrageenan and evaluated by using a reference media which is a commercial bactoagar. Kappa carrageenan showed little difference between the gelling and melting temperatures compared to the big difference shown by agar. The performance of the kappa carrageenan media gave a better acceptability for not exceeding the + or -30.00% difference between the sample and the agar (standards) in the culture of *Escherichia coli* and *Staphylococcus aureus* using the streak plate method and swabbing method.

Keywords: microbiological media, seaweed, streak plate method, swabbing method

BSD-54

DEVELOPMENT OF AN ANTIBODY ASSAY FOR *Blastocystis hominis* INFECTION

Korina Ada D. Tanyu[1] and Windell L. Rivera[1,2*]

[1]Institute of Biology, College of Science, University of the Philippines Diliman
[2]Molecular Protozoology Laboratory, Natural Sciences Research Institute,
University of the Philippines Diliman
Tel No. 920-5471; Email address: wlrivera@up.edu.ph

Blastocystis hominis is a common intestinal protozoon found in humans and animals. However, its pathogenicity is still questionable. This study aimed to develop an antibody assay for *B. hominis* using the indirect fluorescent antibody test (IFAT). Stool and serum samples were collected from public hospitals in Metro Manila. The presence of *Blastocystis* in the stool samples were checked using microscopy and polymerase chain reaction (PCR). Results show that not all PCR- or microscopic-positive samples are positive for serum antibodies using IFAT. It is possible that the infection is fairly recent for individuals that were positive for microscopy, PCR, or both but negative for IFAT. There were microscope-negative samples that were positive for IFAT due to the presence of the persisting IgG even after infection is gone. The average titer is 1/22. There were 7 samples with ¼ titer, 7 samples with 1/16 titer, and 4 samples with 1/64 titer. Individuals from this study may be asymptomatic as titers obtained were not high. Chronic exposure to the organism is required to elicit a strong humoral response. *B. hominis* affects individuals regardless of their status as no relationship is observed among age, sex, and microscopic and immunological diagnoses.

Keywords: *Blastocystis hominis*; protozoa; indirect fluorescent antibody test (IFAT); polymerase chain reaction (PCR)

BSD-55

COLONY GROWTH OF PHILIPPINE ISOLATES OF *Blastocystis hominis* IN SIMPLIFIED SOFT AGAR MEDIUM

Ezra M. Valido[1] and Windell L. Rivera[1,2*]

[1]Institute of Biology, College of Science
[2]Molecular Protozoology Laboratory, Natural Sciences Research Institute
University of the Philippines Diliman
Tel No. 920-5471; Email address: wlrivera@up.edu.ph

Blastocystis hominis is a protozoan that is often observed in the human gut and in various animal hosts such as reptiles, amphibians, and some mammals. It is a polymorphic organism both morphologically and genetically. Agar cloning technique of *B. hominis* has been observed in both solid and semi-solid agar using Iscove's modified Dulbecco's medium (IMDM). In this study, Philippine isolates of *B. hominis* were grown by pour plate method in semi-solid agar using Locke's solution. Inoculated plates contained 0.7% agar, 10% horse serum and 0.1% sodium thioglycollate. Plates were incubated at 37 °C in microaerophilic jar for 7-10 days. Biconvex disk-shaped colonies were seen and abound at the bottom half of the medium. Colonies growing at the agar-glass interface were flat and consisted of thin layers of cells. From these colonies, large amoeboid-cells were frequently seen on the periphery while smaller cells were concentrated at the core. Analysis of the SSU rDNA genetically established the identity of the clones to be *B. hominis*. This is the first report on agar cloning of *Blastocystis* in a compound medium.

Keywords: *Blastocystis hominis*; protozoa; colony growth; agar cloning

BSD-56

BIOACTIVE COMPOUND/S FROM ETHANOLIC EXTRACTS OF *Cajanus cajan* (Linn.) Mill sp. (Pigeon Pea)

Manilyn R. Rodriguez*, Agapito D. Castillo,Jr.*,Joyve Charisma E. Dapito*, Darlene A. Macaraig* and Teofila O. Zulaybar**

*Batangas State University, Batangas City
** National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Banos, College, Laguna, Philippines
Telefax No. (063-049) 5362724:E-mail-teofilazulaybar@yahoo.com

Natural products, mainly of plant origin, have been used for the treatment of diseases. World Health Organization estimated 80% of the world's population rely mainly on traditional medicines for primary health care. In the Philippines, more than 300 medicinal plants were traditionally used as substitutes for modern cures in healing illnesses. One of the medicines which had been used as remedy for variety of ailments such as cough, sore gums, bronchitis is *Cajanus cajan*. Different plant parts the roots, stem and leaves were extracted with ethyl alcohol (95%). Preliminary screening of the crude extracts was done by paper disc method using *Staphylococcus aureus* 1823, *Bacillus subtilis* 1514, *Escherichia coli* 1824, *Salmonella typi* 1756, *Candida albicans* 2219 and *Aspergillus parasiticus* 3055 as test organisms. Streptomycin (1000 ppm) was used as positive control for bacteria, Nystatin (100,000 units/ml) for yeast, cycloheximide (1000 ppm) for molds and ethyl alcohol as negative control. The active extract was dried in rotary evaporator and diluted with ethyl alcohol at 10000 ppm concentration. MIC and bioautography were done using *S. aureus* and *B. subtilis* as test organisms.

Paper disc assay showed that only crude leaf extract is effective against *S. aureus* and *B. subtilis* (16 mm and 18.7mm dia. zone of inhibition, respectively) which is significantly different against the control antibiotic (22.0 and 32.7 mm, respectively) by DMRT but not effective against the other test organisms. Leaf extract also showed inhibitory activity against *S. aureus* and *B. subtilis* with MIC of 62.5 and 31.25 ppm, respectively. Several spots were observed on TLC viewed under 366nm UV. Bioautography showed 1 very big active spot against *S. aureus* and *B. subtilis*. Results showed that the leaf extract of *Cajanus cajan* can be a potent source of bioactive compound that may replace some of the synthetic drugs being used today.

Keywords: *Cajanus cajan*, *S. aureus*, *B. subtilis*, mic,tlc

BSD-57

PCR-BASED DETECTION OF BANANA BUNCHY TOP VIRUS IN *Pentalonia nigronervosa* Coquerel

Kristine Ace F. Quirante, Maria Armila D. Ruiz, and Vermando M. Aquino*

National Institute of Molecular Biology and Biotechnology
University of the Philippines - Diliman, Quezon City
Tel: (632) 927-7516; Email: vmaquino@up.edu.ph

Pentalonia nigronervosa or banana aphid is the insect vector for the banana bunchy top virus (BBTV), which infects members of the Musaceae family such as banana and abaca. A reliable and sensitive detection system for the virus in its insect vector is important in the study of virus replication and movement. This study reports on the development of a polymerase chain reaction (PCR)-based detection system for BBTV in whole and dissected components of *P. nigronervosa*. Specific primers were designed based on the movement protein gene of BBTV and tested using several PCR profiles. Upstream conditions, such as total DNA extraction, were also varied in order to improve the amplification of the target sequence. Aphids were dissected and DNA extraction and PCR amplification were performed separately for the head, thorax, and hemolymph. Using the optimized conditions of DNA extraction and PCR primer-profile combination, PCR was able to detect BBTV in the whole and dissected components of the aphid.

Keywords: aphid, banana bunchy top virus, movement protein, *Pentalonia nigronervosa*, polymerase chain reaction

BSD-58

OPTIMIZATION OF ABTV DNA-4 EXPRESSION IN *E. coli*

Maria Armila D. Ruiz and Vermando M. Aquino*

National Institute of Molecular Biology and Biotechnology
University of the Philippines Diliman, Quezon City
Tel: (632) 927-7516; Email: vmaquino@up.edu.ph

Abaca bunchy top nanovirus (ABTV) is the most important viral pathogen of abaca. The virus causes stunting and significant reduction in fiber yield. ABTV is an 18-20 nm isometric virus that has a multicomponent genome of at least six circular single stranded DNA. In this study, heterologous expression of the DNA-4 component that codes for the movement protein was optimized. Isolation and characterization of the component were performed. Total DNA was obtained using the CTAB extraction method. PCR amplifications produced a 380-bp fragment which was cloned in pEXP5-NT/TOPO vector and expressed in *Escherichia coli* BL21(DE3) strain. Conditions such as IPTG concentration, incubation period, and temperature were varied. The expression of an 11.8-kDa movement protein was analyzed through SDS-PAGE. Expression has been observed to increase with increasing IPTG concentration and incubation period. Optimum expression was obtained at 30 °C.

Keywords: abaca, abaca bunchy top virus, *E. coli*, heterologous expression, movement protein

BSD-59

ANTAGONISTIC STUDY OF *Agaricus blazei* MURILL IN PATHOGENIC FUNGAL ISOLATES

Roma May D.G. Garcia[1], Maria Marah L. Rodriguez[1]
and Angelita P. Medalla[1,2]*

[1]Biology Department, College of Arts and Sciences,
[2]Mushroom Biotechnology Laboratory, Research and Development Center
Rizal Technological University
Boni Avenue, Mandaluyong City 1550
Tel No.: 534-8267 loc 123; Fax No.: 534-9710
Email: angie_p_medalla@yahoo.com

Solid and submerged culture conditions were used to investigate the antagonistic properties of *Agaricus blazei* Murill against five test fungi namely *Aspergillus niger, Fusarium moniliforme, Trichoderma spp., Penicillium spp., and Rhizopus spp.* The cultural and morphological characteristics of the microorganisms; the development of mycelia and spores; and the color and appearance at different incubation times were observed. Interaction of the five test fungi was evaluated using the method of Bell et al. (1982). The incubation period was observed on the seventh and fourteenth day of competing. Minimum Inhibitory Concentration (MIC) and Mycelial Free Culture (MFC) determination in 24 and 48 hours were also tested. Mycelial free culture fluid (MFCF) was used to test the antagonistic activity of *A. blazei* in submerged culture condition. A parallel comparison was compared having three incubation periods in determining antagonistic property of *A. blazei* in five test fungi. From the different culture conditions that have been acquired, test fungi had overgrown *A. blazei* in both Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) in fourteen days of incubation periods. In MFC Fluid, the growth of test fungi in MEA was negative compared with the positive growth in PDA. Generally, *A. blazei* was colonized by the test fungi in two culture conditions tested while a negative zone of inhibition were observed in all the techniques used. Beta D-glucan in the mycelium of *A. blazei* was found to be not as active as those in the fruiting bodies. Hence, the use of fruiting bodies is recommended.

Keywords: antagonistic activity, *Agaricus blazei* Murill, Minimum Inhibitory Concentration, MIC, Mycelial Free Culture, MFC, Malt Extract Agar, MEA, beta D-glucan

BSD-60

A RANDOM AMPLIFIED POLYMORPHIC DNA MARKER AS SOURCE OF PRIMER SEQUENCES FOR PCR-BASED DETECTION OF *Escherichia coli* O157:H7

Maria Teresa M. Perez* **and Susana M. Mercado**

National Institute of Molecular Biology and Biotechnology
University of the Philippines Los Banos
College, Laguna 4031
Telefax: (049) 5362721; Email: mtmperez14@yahoo.com.ph

Escherichia coli O157:H7 is considered the most virulent strain among the enterohemorrhagic strains of *E. coli* (EHEC). It has been isolated from fecal matter of cattle, wild birds and domestic animals. Infection of humans arise from fecal contamination of milk products and the water supply, contact of intestinal contents with meats in slaughterhouses, contamination of fresh produce with animal manure, and direct contact with an infected animal or human. Early identification of contaminated foods, water and beverages will ensure disease prevention, thus, the importance of a reliable, speedy and sensitive means of detecting the strain.

The study aimed to test the applicability of a single 0.85 kb DNA marker obtained from amplification of *E. coli* O157:H7 by a random 10-base primer, BIOTECH code 12, as source of specific primer sequences for polymerase chain reaction (PCR)-based detection of the pathogen. Three *E. coli* O157:H7 reference strains, four confirmed rectal fecal isolates from cattle, two enteropathogenic *E. coli* (EPEC), two enteroinvasive *E. coli* (EIEC), two non-pathogenic *E. coli*, two *Shigella* sp., four *Salmonella* sp., three *Staphylococcus aureus*, one *Enterobacter aerogenes*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Proteus vulgaris*, and *Bacillus cereus* were subjected to PCR using primer BIOTECH code 12 and DNA hybridization analysis to determine homology of the 0.85 kb *E. coli* O157:H7 sequence with other bacteria tested. The 0.85 kb amplicon generated from the three reference strains were submitted for DNA sequencing and the partial sequence information subjected to DNA homology analysis using the Basic Local Alignment Search Tool (BLAST). Significant alignments of *E. coli* O157:H7 (10084) and (TWO2302) partial sequences showed 92% and 93% homology respectively, with O157:H7 complete genome. Two forward primers and one reverse primer were designed and tested in genomic DNA of *E. coli* O157:H7, EPEC, non-pathogenic *E. coli* and *Shigella* sp. Primer sets Ec-07f / Ec-08r and Ec-09f / Ec-08r each amplified a single 350 bp and 300 bp amplicon in all seven *E. coli* O157:H7 strains tested at 52°C annealing temperature. The randomly amplified DNA marker generated PCR primers that could specifically detect *E. coli* O157:H7.

Keywords: *E. coli* O157:H7, polymerase chain reaction, pathogen detection, designed primers, random amplified polymorphic DNA, sequence analysis

BSD-61

OVER- EXPRESSION OF THE PHOSPHOLIPASE C GENE IN *Ralstonia solanacearum* AND ITS IMPLICATION TO VIRULENCE

Jocelyn T. Zarate,[1] Asuncion K. Raymundo[2*] and Caitlyn Allen[3]

[1]National Institute of Molecular Biology and Biotechnology (BIOTECH)
Email: joytzarate@yahoo.com
[2]Institute of Biological Science, College of Arts and Sciences
University of the Philippines Los Baños, College, Laguna
Email: akr@laguna.net
[3] Department of Plant Pathology, University of Wisconsin- Madison, USA
Email: cza@plantpathwisc.edu

Bacterial wilt disease caused by *Ralstonia solanacearum* is a serious and devastating disease affecting more than 200 plant species including tomato, potato, and banana. Disease control has been difficult due to its heterogeneity and the emergence of hypervirulence in existing virulent strains.

Phospholipase C was studied as it is one of the many virulence factors secreted by animal pathogens that may also contribute to plant pathogenesis, through its effect on intracellular signaling. The *R. solanacearum* wild type strain T523 was transformed with a plasmid containing *plcN* gene to determine if the introduction of such gene will result to an increase in virulence. The mutant strain (T523PlcN) with over-expressed *plcN*, grew as well as the wild type in culture, both in rich (CPG) and in minimal media (BMMG). Phospholipase C activities were determined by growing the bacteria in low phosphate medium (Hepes medium) for three days until stationary phase. The release of the yellow chromogen, p-nitrophenol after hydrolysis of the synthetic substrate p nitrophenylphosphorylcholine (NPPC) was monitored. Phospholipase assay showed that the wild type strain produced 1.85×10^{-12} U PlcN/cfu, while the mutant strain gave 27 fold higher PlcN activity at 4.93×10^{-11} U PlcN/cfu. Virulence testing of the wild type and mutant strains using direct petiole infiltration showed that strain T523PlcN was slightly less virulent than the wild type on susceptible tomato variety Bonny Best but both were not able to wilt the resistant variety Hawaii 7996. Phospholipase C may play a role in the signaling response of the host to the pathogen and this needs further elucidation.

Keywords: *Ralstonia solanacearum*, bacterial wilt, phospholipase C, virulence factors

BSD-62

PHILIPPINE ACTINOMYCETES AGAINST *Erwinia carotovora*

Irene A. Papa*, Teofila O. Zulaybar and Bernardo C. Mercado

National Institute of Molecular Biology and Biotechnology (BIOTECH)
University of the Philippines Los Baños (UPLB), College, Laguna 4031
*Email: iwapapa@yahoo.com

Major diseases of potatoes like blackleg and soft rot are caused by bacteria of the genus *Erwinia (Pectobacterium)*. Since chemical pesticides are not successful against these diseases, a good alternative is the use of biological control agents to control plant pathogens. This method has gained increasing interest as a result of public concern about the ill-effects of continued chemical use in the environment and the need to find alternatives to chemical treatment.

The potential of locally isolated actinomycetes as biocontrol agents against *Erwinia carotovora* was investigated. A total of 348 actinomycetes isolated from different soil samples were screened against *E. carotovora* NRRL B 134 (BIOTECH 1752) using agar plug method. Fifty six (56) actinomycetes showed inhibition against the growth of *E. carotovora*. The ability of these isolates to produce the bioactive compounds was evaluated in two fermentation media, yeast malt broth (YMB) and Croatian fermentation medium (CFM) by cylinder cup assay. Among these isolates, Actinomycetes 63, 9B and 67, all cultured in CFM exhibited the highest zone of inhibition (ZOI) of 30.45 mm, 26.60 mm and 26.45 mm against *E. carotovora*, respectively, compared with the positive antibiotics used (erythromycin, 15 ug/ml and kanamycin monosulfate, 1000ppm) with 8.30 mm and 14.5 mm ZOI, respectively. Actinomycetes 37B, 9B and AC 43 grown in YMB, on the other hand, showed high growth inhibition of 22.20 mm, 21.60 mm and 20.25 mm, respectively. These actinomycetes with higher ZOI than the control antibiotics have the potential as biocontrol agents against *Erwinia carotovora*.

Keywords: Actinomycetes, *Erwinia carotovora*, agar plug assay, cylinder cup assay, biological control agents